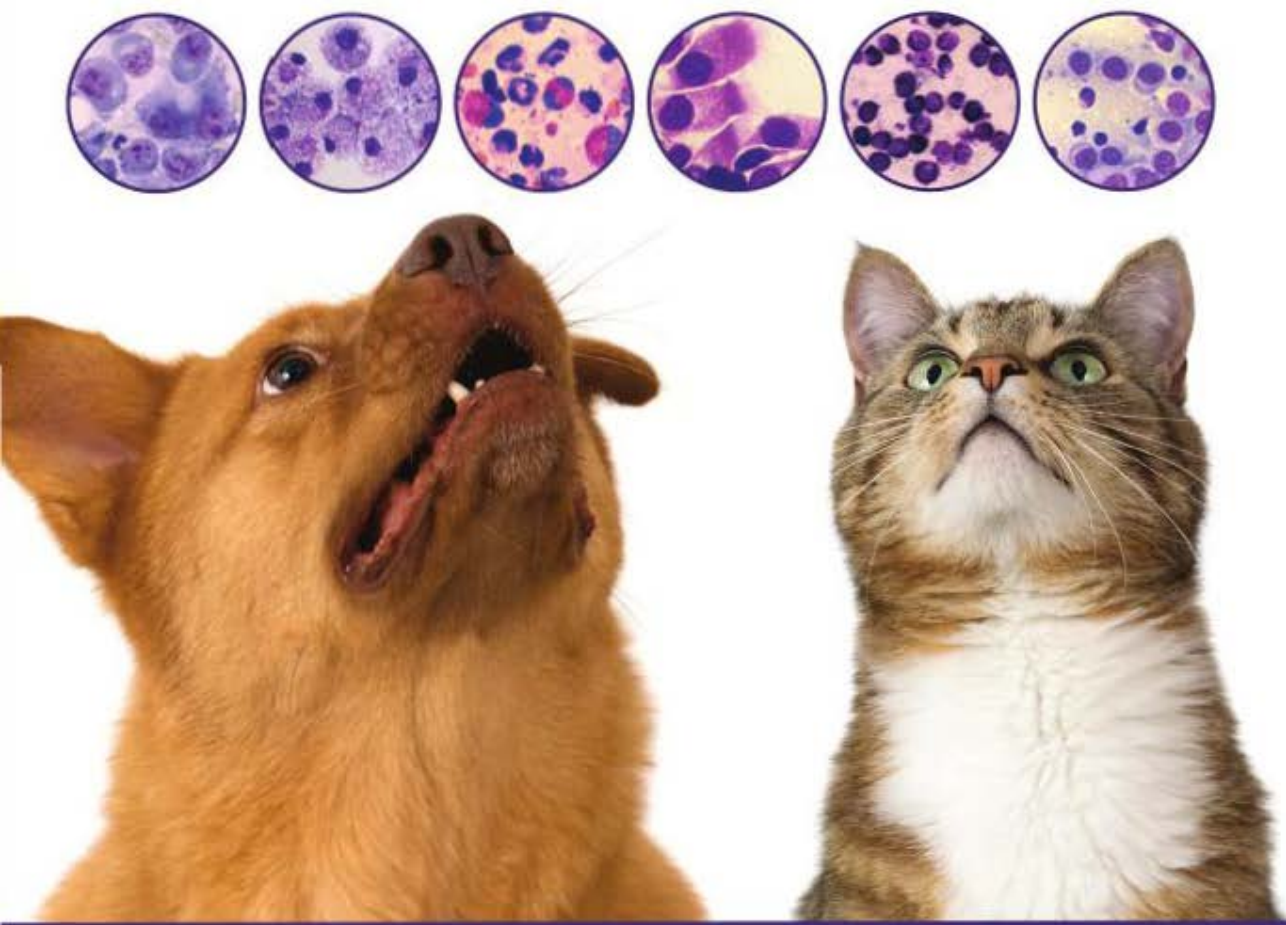
Manual of Diagnostic Cytology of the Dog and Cat

Edited by **John Dunn**



WILEY Blackwell

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Edited by

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Axiom Veterinary Laboratories Ltd
Newton Abbot
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Preface

During the last decade, diagnostic cytology has become an increasingly important and frequently used tool in the veterinary practitioner's diagnostic armamentarium. *Manual of Diagnostic Cytology of the Dog and Cat* is aimed primarily at small animal clinicians and undergraduate veterinary students who wish to enhance their knowledge in this particular discipline, although clinical pathology residents should also find this manual useful.

The initial concept for this project evolved from the numerous requests received from veterinary clinicians in first opinion practice for a user-friendly and easily accessible reference source. The intention, therefore, has not been to compete with the numerous more comprehensive reference textbooks currently available on the market.

To this end, this manual reviews the techniques for obtaining diagnostic specimens and the general principles of cytological interpretation. It describes in a clear and concise manner the most common lesions and related disorders encountered in a practice setting. Considerable emphasis has been placed on the use of high-quality colour images. A conscious attempt has been made to describe the lesions in the figure legends rather than in the text thereby minimising the unnecessary repetition of facts. References are provided only where considered necessary. Readers are instead directed to the list of reference textbooks and articles for more in-depth information regarding each of the body systems.

Finally, I am indebted to the authors, all highly qualified experts in their field, for their excellent contributions. Thanks are also due to Nick Morgan and the editorial team at Wiley Blackwell, most notably Jessica Evans and Justinia Wood, for their forbearance in seeing this project through to its conclusion.

John Dunn

Editor's Note

Because the final size of cells and other structures in digital photographs is variable in many cases due to cropping and resizing of photos, the magnification factors in the photographs throughout this manual are not given. The size of infectious agents and other cells can be compared to that of adjacent erythrocytes and leukocytes (see Figure 15.1).

The following stains have been used to stain the cytology specimens depicted in the following chapters unless stated otherwise in the figure legend:

Chapter1: May–Grünwald–Giemsa stain

Chapter 2: Modified Wright's stain

Chapter 3: May–Grünwald–Giemsa stain

Chapter 4: Wright's–Giemsa stain

Chapter 5: Wright's–Giemsa stain

Chapter 6: Wright's–Giemsa stain

Chapter 7: Wright's–Giemsa stain

Chapter 8: Wright's–Giemsa stain

Chapter 9: Modified Wright's stain

Chapter 10: Wright's–Giemsa stain

Chapter 11: Modified Wright's stain

Chapter 12: May–Grünwald–Giemsa stain

Chapter 13: Wright's–Giemsa stain

Chapter 14: Pappenheim stain

Chapter 15: Wright's stain

1 Cytological Collection Techniques and Sample Preparation

Natali Bauer

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Acquisition of a fine-needle aspirate for cytological examination is a fast and easy, minimally invasive technique which can be performed in every practice or clinic. The advantages are that generally no anaesthesia or sedation is required and the risk of haemorrhage is minimal while the technique provides an excellent evaluation of single cell morphology. In contrast to histology, however, it has to be kept in mind that the tissue architecture is not preserved and cannot be evaluated. Histopathological examination of biopsy specimens allows the assessment of growth patterns and the margins of the lesion can be visualised if necessary, but surgical biopsy is associated with a higher risk of haemorrhage and anaesthesia (local or general) is necessary.

Adequate techniques of sample preparation and staining are mandatory for the optimal interpretation of cytological specimens. Moreover, correct interpretation of any cytological specimen requires correct microscopic examination and recognition of common artefacts. This chapter describes the practical approach to optimal sampling, routine staining techniques and the systematic microscopic evaluation and detection of common artefacts.

Sampling techniques

Fine-needle aspiration

Fine-needle aspiration cytology is a useful technique for the investigation of soft tissue masses (cutaneous lesions, lymph nodes, intra-thoracic or intra-abdominal masses) and effusions from body cavities. The technique can be easily performed in a practice setting. The following basic equipment is required:

- Glass slides with a frosted end which can be easily labelled.
- 5 ml syringe (if required also a 2 ml or 10 ml syringe; a 10 ml syringe might be advantageous for aspirating very firm masses),

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Figure 1.1 Fine-needle aspiration using a needle and syringe.

- 20–22 G needles.
- A pencil for labelling the slides with the date and localisation of the lesion as well as the patient´s name. Note: Labels written with a ballpoint pen or marker may be washed away with alcohol-based stains (e.g. Diff-Quik, Wright's, May–Grünwald–Giemsa).

For organs such as the liver or spleen, longer needles are usually required especially in large dogs. Here, a spinal needle with a stylet is recommended to avoid contamination by tissues adjacent to the mass or organ (with softer tissues smaller needles and syringes can be used).

Fine-needle aspirates can be taken with an 'aspiration technique' or a 'non-aspiration technique'. The non-aspiration technique is preferred for sampling of all masses or organs which are highly vascular (e.g. spleen, liver) in order to minimise blood contamination. Overall, the sampling procedure should take no longer than 5–10 s, and several smears should be prepared.

- Aspiration technique:
  - The mass or organ (e.g. a peripheral lymph node) is immobilised with one hand and the needle is inserted with the other (Figure 1.1). Wherever possible, fine-needle aspiration of abdominal organs or masses is best performed under ultrasound guidance.
  - The skin is disinfected as for venipuncture.
  - The needle with attached syringe is inserted into the lesion.
  - The plunger is withdrawn, and while maintaining negative pressure, the needle can be redirected to aspirate different regions of the mass or organ.
  - The needle with attached syringe is removed after releasing the plunger.
  - The syringe is filled with approximately 3–5 ml air and reattached to the needle to expel the aspirate gently on the glass slide.



Figure 1.2 Fine-needle aspiration using an aspiration gun, e.g. 'Zyto-Gun®' (Scil animal care company GmbH, Viernheim, Germany).



Figure 1.3 The non-aspiration technique using a 'needle-alone technique' is useful for obtaining samples from small lesions such as pustules or bullae.

- Note: To facilitate pulling the plunger, commercial aspiration guns may be useful when aspirating masses or organs which are difficult to immobilise since the vacuum can be easily maintained with one hand (Figure 1.2).

- Non-aspiration technique: Two methods of this technique can be used for sampling.
  - 'Needle-alone technique': The needle without the syringe attached is inserted into the lesion after disinfection of the skin (Figure 1.3). The needle is then rapidly moved back and forth in the tissue approximately ten times before it is withdrawn. A syringe already filled with 3–5 ml air ensures a rapid expulsion of the aspirated material onto the slide.



Figure 1.4 The non-aspiration technique with the syringe attached to the needle is used here to sample the spleen of a dog with ascites and icterus under ultrasonographic control. Note the syringe is prefilled with air and is held between the thumb and forefinger.

- Alternatively, the needle is inserted with a syringe already filled with 2–3 ml of air attached (Figure 1.4). The needle and syringe are then rapidly moved back and forth in the tissue before the needle with syringe attached is removed. The aspirated material is then ejected onto the slide, and smears are prepared immediately.

Cytological smears can be prepared using the blood smear (Figure 1.5) or squash preparation technique (Figure 1.6).

Impression smears/imprints

Imprints can be made from wet surfaces (e.g. biopsies, ulcerated or exudative skin lesions) as well as from dry skin lesions using Sellotape (Figure 1.7 and Figure 1.8). It may be necessary to blot away excessive blood or tissue fluids from the surface of a biopsy specimen with a clean, dry swab or paper towel before making the imprint onto a clean glass slide. The disadvantages of impression smears are that they only collect cells from the surface of the lesion and therefore may not be representative of underlying pathology, fewer cells are collected and bacterial contamination is more likely.

Scrapings

Scrapings may be useful for sampling extremely firm lesions which are less likely to exfoliate cells with the aspiration technique. After the lesion is cleaned and dried, a large scalpel blade (held at a 90° angle) is moved several times over the surface of the

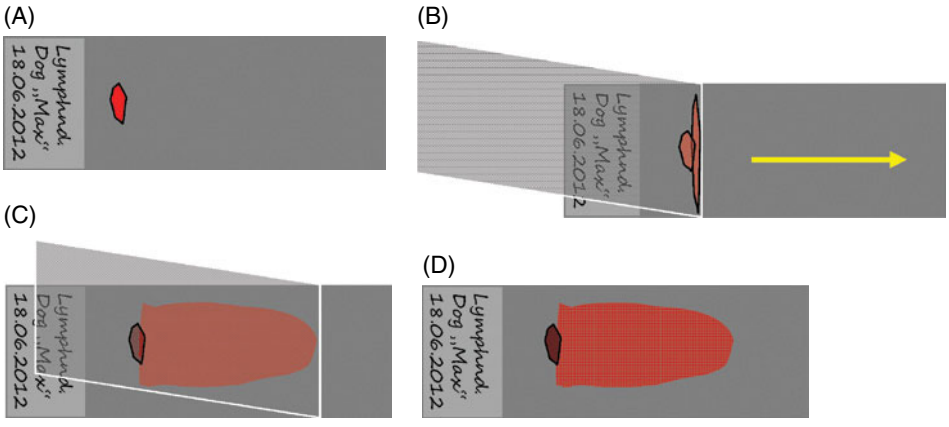


Figure 1.5 Preparation of the smear using a blood smear technique. (A) The aspirated material is deposited onto the glass slide by ejecting 3–5 ml air through the syringe and needle. (B) A second slide held at a 45° angle (for highly viscous fluids such as joint fluid, smaller angles of approximately 25° are recommended) is brought towards until it makes contact with the aspirated material. (C/D) The material is distributed along the width of the spreader slide which is then pushed forwards smoothly and rapidly.

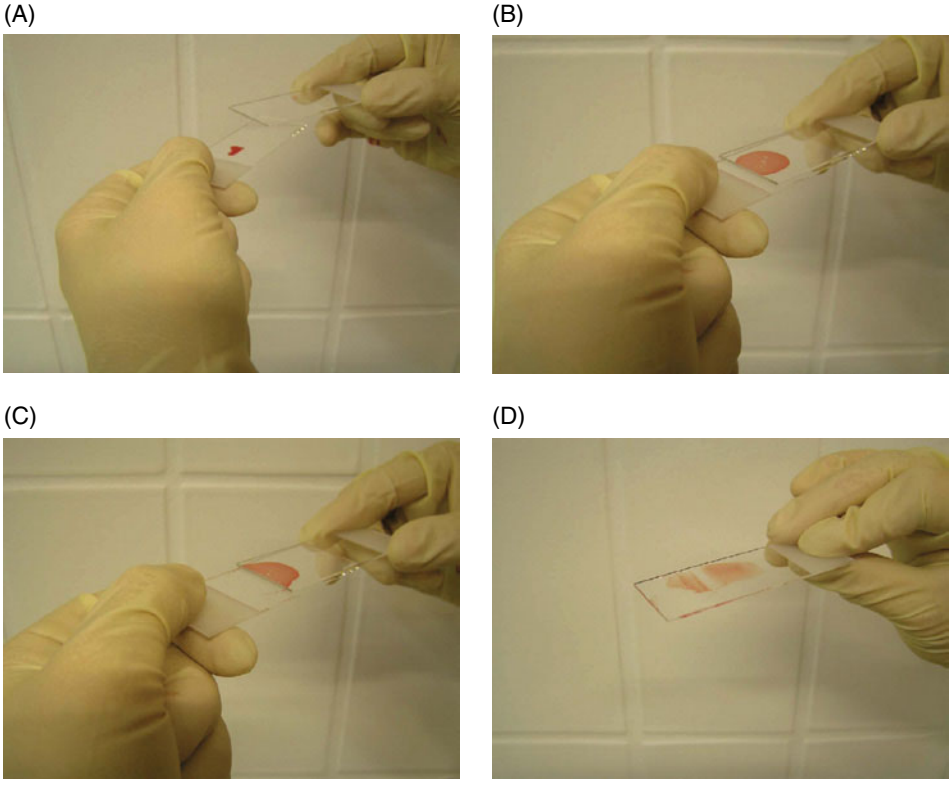


Figure 1.6 The squash preparation technique. (A) The fine-needle aspirate is placed on the glass slide by ejecting 3–5 ml air through the syringe and needle. (B) A second slide is gently placed on top of the first one. Capillary forces result in the slides adhering to each other. (C) The top slide is gently drawn over the bottom slide on which the aspirate has been deposited. (D) The top (spreader) slide is removed once it reaches the end of the bottom slide which can then be submitted for cytological examination.

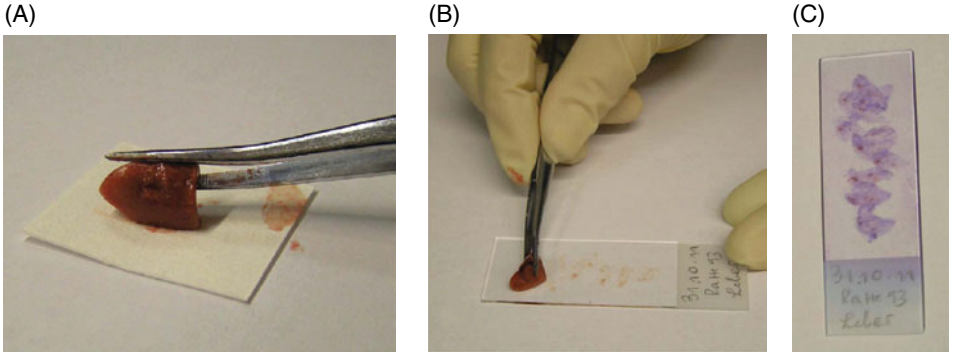


Figure 1.7 Impression smear of a liver biopsy. (A) Prior to preparing the impression smear, blood contamination is minimised by pressing the biopsy gently on a filter paper. (B and C) The imprint smear is prepared by touching the slide with the surface of the biopsy in several areas.

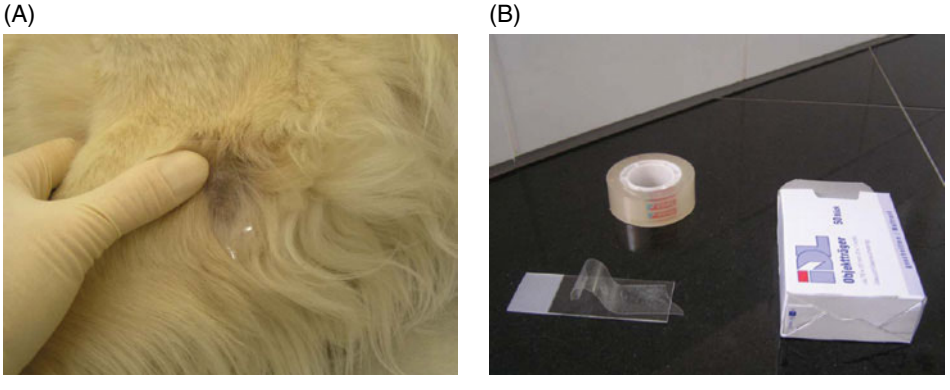


Figure 1.8 Sellotape imprint. (A) A strip of Sellotape is pressed several times onto the skin lesion. This technique is ideal to show micro-organisms (bacteria, yeasts) on the skin surface. For assessment of cells, however, other techniques such as evaluation of Romanowsky-stained skin scrapings are preferred. Parasites such as lice or Cheyletiella mites can also be detected with an unstained Sellotape preparation. (B) For preparation of a Romanowsky-stained Sellotape imprint, the piece of Sellotape is firstly put on the slide like an upturned 'u' (sticky side down), and the imprint can then be stained without fixation. After staining (and also for preparation of an unstained Sellotape imprint), the strip of Sellotape is put flat on the slide and can be then evaluated microscopically.

lesion in the direction of the person taking the sample. The scraped material is then transferred to a slide and is distributed evenly with the scalpel blade, or a second slide may be used to prepare a squash preparation using the technique described previously.

Swab smears

Swab smears are especially useful for preparing smears from fistulous tracts, the vagina or the ear canal (Figure 1.9). They are less useful for tumour diagnosis (the disadvantages are similar to those described for impression smears).

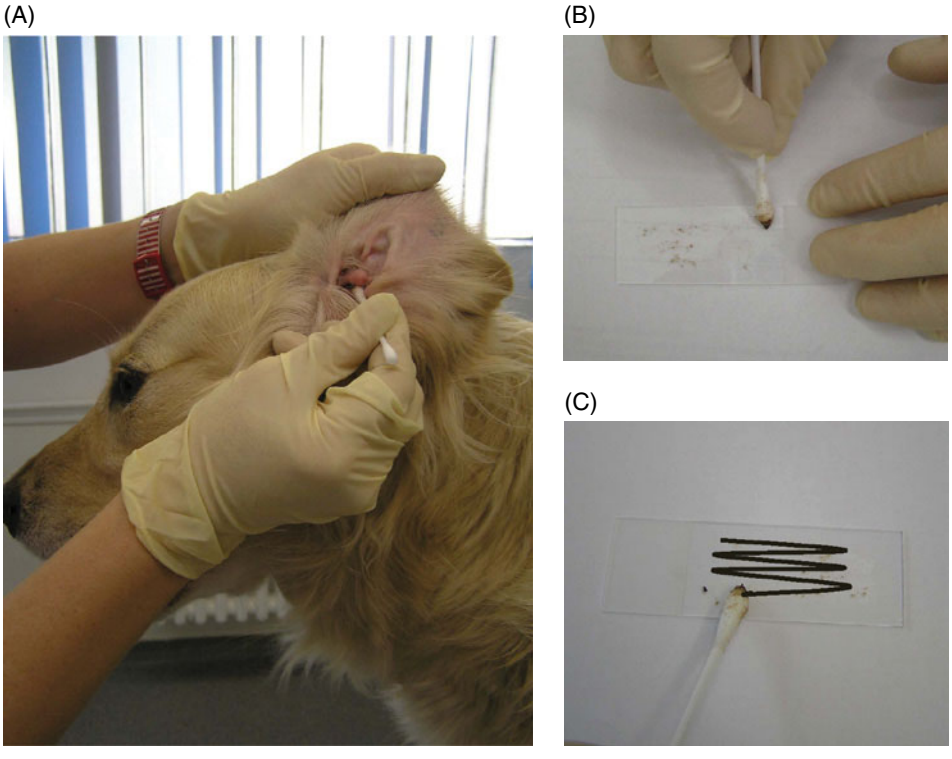


Figure 1.9 Collection of an ear swab for cytological investigation. (A) The cerumen or discharge is collected with a cotton bud. (B) The material is then rolled out onto a slide. (C) Note the meandrical movement of the cotton bud.

Brushings

Brushings are taken with a cytobrush (Figure 1.10) and have the advantage that they are more representative of the deeper layers of the lesion than lavage fluids, imprints or swabs. They are commonly taken from the conjunctiva, respiratory tract or vagina.

Collection and handling of fluid samples for cytological examination

The hair at the site of fine-needle aspiration is clipped and the skin is disinfected. If abdominocentesis is performed without ultrasonography, samples are taken on the *linea alba* 2 cm behind the umbilicus. Thoracocentesis without ultrasonographic guidance is performed at the ventral thoracic wall between the sixth and eighth ribs. The needle is inserted cranial to the rib to avoid injury to nerves or blood vessels. If larger amounts of fluid are aspirated, a three-way stopcock should be attached to the hub of the syringe to avoid creating a pneumothorax. Fluid specimens should be routinely collected into EDTA (or a plain tube if bacteriology is required). Smears should be prepared within 30 min of sampling to avoid artefacts due to sample aging.



Figure 1.10 Acquisition of a conjunctival cytobrush preparation. The material collected with the cytobrush is rolled out on a slide. Note: The dog is pretreated with anaesthetic eye drops prior to sampling.

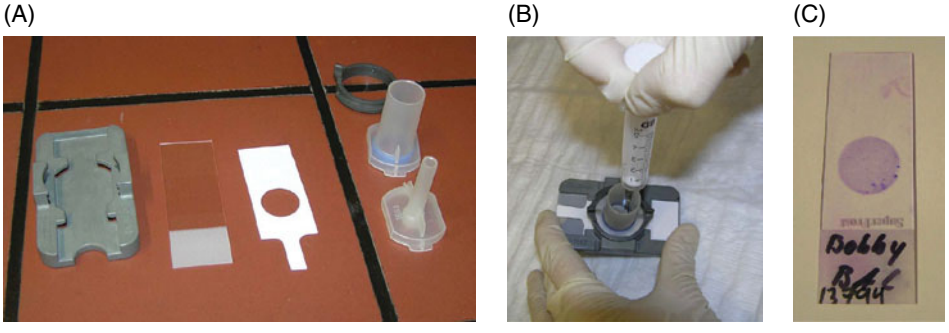


Figure 1.11 (A) This photograph demonstrates the slide holder apparatus, a glass slide, the sample chamber and filter cards for preparation of a cytospin preparation using a cytocentrifuge. (B) The cytospin chamber is filled with the fluid and is centrifuged at 18*g* for 3 min. The supernatant is then removed by aspiration with a syringe and attached needle before the cytospin chamber is removed and the smear is dry-centrifuged at 90*g* for 1 min. (C) A stained cytospin preparation.

For hypocellular fluids (e.g. bronchoalveolar lavage samples, cerebrospinal fluid), a cytospin preparation is preferred (Figure 1.11). For body cavity fluids (abdominal and thoracic effusions, synovial fluid), the cellularity can be estimated from a direct smear. If the cell count is low (i.e. < 10.0×10^9/L), a cytospin preparation is recommended in addition to the direct smear to facilitate detection of cell populations present in low numbers. When, as will often be the case in a practice setting, a cytospin centrifuge is not available, a sample chamber for preparing an 'in-house' sediment smear can be prepared (Figure 1.12).

Although the results tend to be of lower quality than the in-house sedimentation or cytospin techniques, a sediment smear can also be prepared by centrifuging

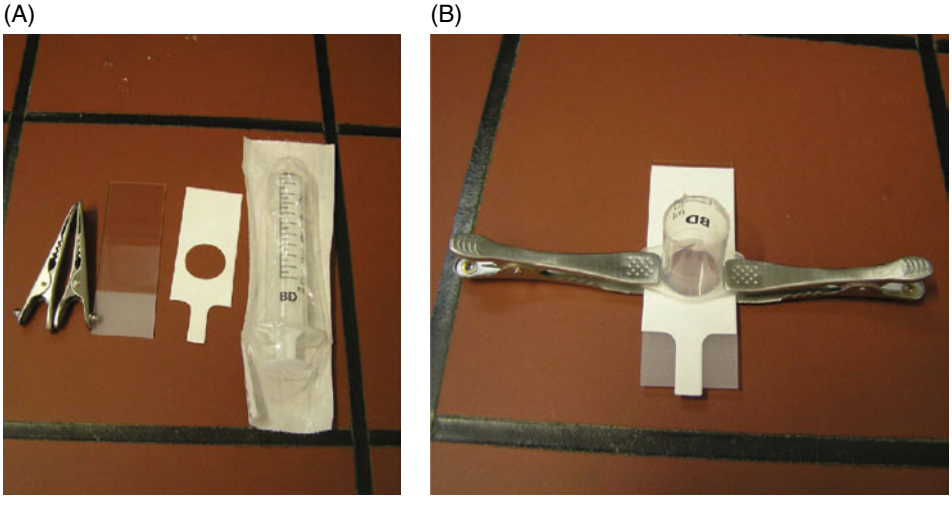


Figure 1.12 (A) If a cytocentrifuge is not available, an in-house sample chamber for preparing a sediment smear can be fabricated using the cut-off barrel of a syringe (a 10 ml or 1 ml depending on the volume of fluid obtained), ECG clamps and filter paper (if not available, you can use three layers of coffee filter paper in which an appropriate round hole is cut which is slightly larger than the inner diameter of the syringe). (B) The cut-off syringe barrel is attached to the slide and filter paper with the ECG clamps and is then filled with the fluid (50–200 µl). The cells are allowed to sediment for 1 h. The supernatant is then removed by aspiration with a syringe and attached needle, and the sample chamber is removed.

the specimen for 5 min at 1000 *g*. After centrifugation, the supernatant is removed (decanted into the sink or aspirated with a pipette) so that a small amount (one to two drops) is left in the tube. The sediment is then resuspended in the residual supernatant, and smears are prepared using a blood smear or line concentration technique.

Staining techniques

Several stains are available which can be used alone or in combination. Prior to staining, the smears are air-dried; further fixation is generally not necessary.

Routinely used alcohol-based Romanowsky stains (Figure 1.13) include:

- May–Grünwald–Giemsa stain
- Wright's stain
- Diff-Quik stain (Siemens Diagnostics Healthcare GmbH)

New methylene blue (NMB) stain (e.g. Accustain Reticulocyte Stain, Sigma Diagnostics, St. Louis) can be used to show up the nuclear and nucleolar structure (e.g. the nuclear chromatin) in greater detail. Typical staining characteristics of Diff-Quik stain, May–Grünwald–Giemsa stain and NMB stain are shown in Figure 1.14.

(A)



(B)



(C)



Figure 1.13 Examples of staining devices and stains. (A) Large round glass vessels used for staining in laboratories/practices with a high caseload. (May–Grünwald–Giemsa stain shown here). (B) Cuvettes with Diff-Quik staining solutions. (C) 'Cuvettes' for Diff-Quik stain. These 'cuvettes' are slide holders filled with the staining solutions. These can be used in veterinary practices with a low cytological caseload to avoid using large amounts of the expensive staining solutions. The slide is dipped in every vial five times over a period of 5 s, and then it is flushed with pure distilled water to remove excess stain. Note that fluids with a higher cellularity and protein concentration require a longer time to stain properly.

A simple staining technique using stains other than Diff-Quik (e.g. NMB stain) is as follows:

- The slide is placed on a tissue paper. The staining solution is added to the smear, e.g. with a micro-capillary.
- A cover slip is placed on top of the droplets of staining solution.
- Gentle pressure is applied to the cover slip, and the tissue paper is folded to absorb excess staining solution.
- The stained smear can be now evaluated under the microscope with a cover slip lens.

Microscopic examination of cytological specimens

Microscopic evaluation of the smears should always be performed in the same sequence:

After a macroscopic ('eyeball') evaluation of the smear to detect potential areas of interest (Figure 1.15A), the smear is scrutinised at low magnification with a

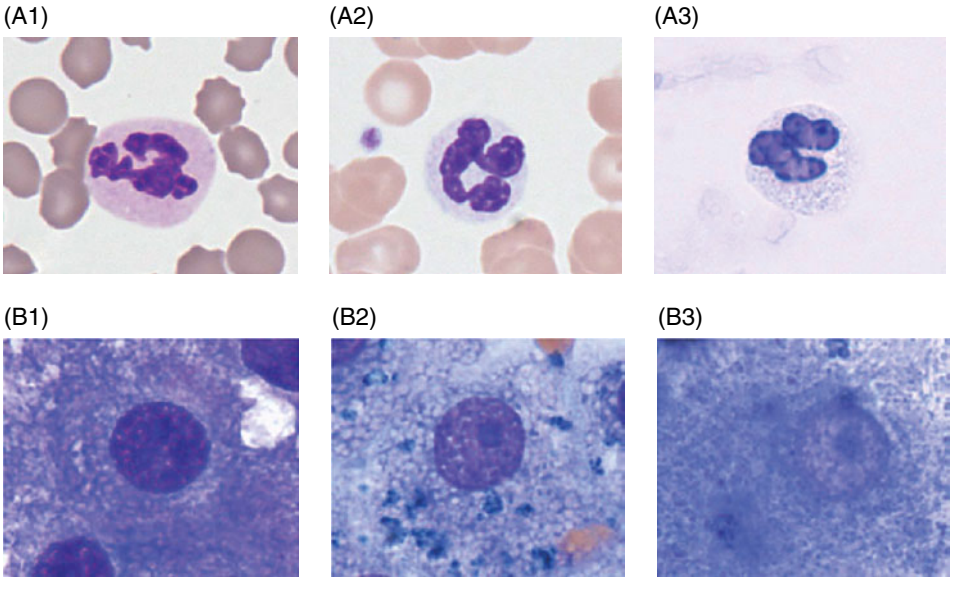


Figure 1.14 Staining characteristics of neutrophils (A) and hepatocytes (B) with Diff-Quik stain (1), May–Grünwald–Giemsa stain (2) and NMB stain (3). Diff-Quik and May–Grünwald–Giemsa stains show fairly similar results; however, chromatin structure of the nuclei and nucleoli can be seen in greater detail with the May–Grünwald–Giemsa stain. Moreover, mast cell granules do not always stain with Diff-Quik stain so that they may be misclassified as macrophages. Note that erythrocytes are clear in the NMB stain.

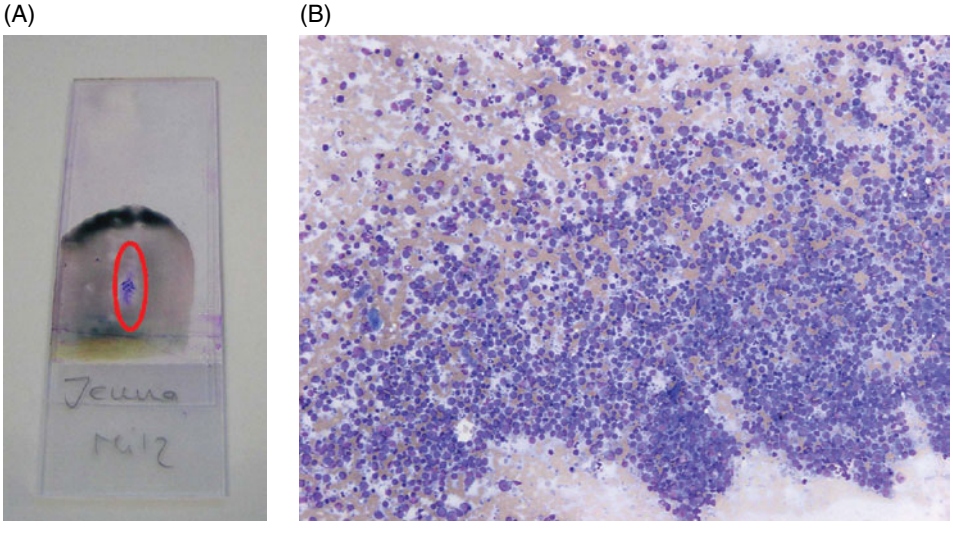


Figure 1.15 Microscopic examination of a smear (example is a splenic aspirate from a dog): (A) Step 1: Screen the smear macroscopically to detect areas with the most cellular material (circled). (B) Step 2: Screen the smear under low magnification using a 10× or 20× objective lens. In order to locate an area where cells are set in a monolayer and detect focal lesions (clusters of tumour cells, parasites such as microfilaria) Note: Large cellular populations tend to be found at the margins and at the feathered edge of the smear.

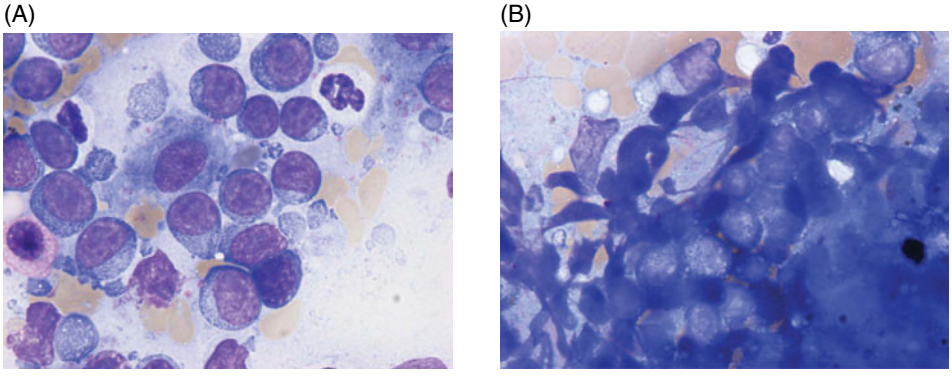


Figure 1.16 Microscopic examination of a smear (example is a splenic aspirate from a dog): (A) Step 3: When a suitable area is identified, cellular morphology is assessed under 1000× magnification (using a 100× oil immersion objective lens). Here, medium-sized macro-nucleolated lymphoid cells predominate consistent with a diagnosis of marginal zone lymphoma. (B) The same smear in an area where cells are not set in a monolayer. Single cell morphology cannot be assessed and cells are incompletely stained.

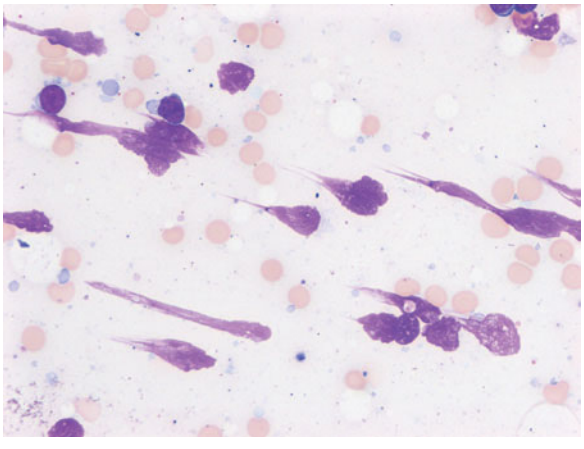


Figure 1.17 Crushing artefact (lymph node aspirate dog). Note the streaks of nuclear protein which are set in the same direction indicative of an artefact.

10× or 20× objective lens to detect areas in which cells are arranged in a monolayer and also areas which are hypercellular or have different staining characteristics (Figure 1.15B). These areas are then examined under higher magnification (with a 100× oil objective lens; Figure 1.16).

Recognition of artefacts

The recognition of artefacts is essential for correct interpretation of cytological findings. Artefacts may occur due to suboptimal sample preparation (e.g. crushing artefact results in smudged cells, 'bare' nuclei or cytoplasmic strands; Figure 1.17),

sample aging or contaminants such as stain precipitate (Figure 1.18), ultrasound gel (Figure 1.19), starch powder from surgical gloves (Figure 1.20), tissue paper or plant fibres (Figure 1.21) or pollen grains (Figure 1.22). Focusing and unfocusing will help to differentiate between contaminants and intracellular structures.

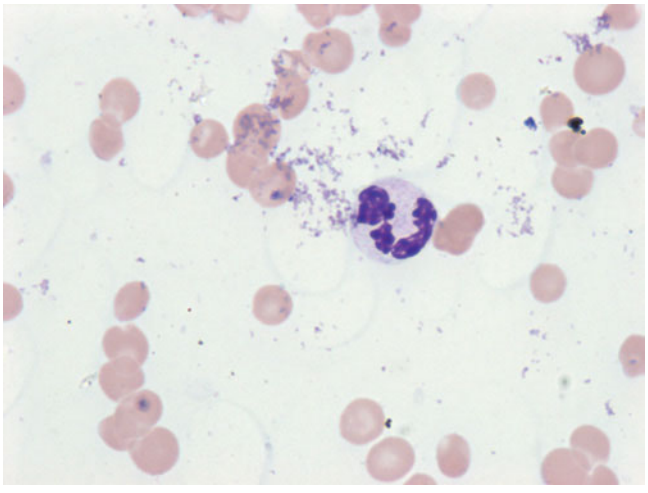


Figure 1.18 Precipitated staining solution (blood smear dog). Note the finely stippled azurophilic material on top and between the cells. Precipitated staining solution may be confused with *Haemoplasma* spp.; however, it can also be seen between the erythrocytes, and focusing up and down will confirm that the same material is on top of the erythrocytes.

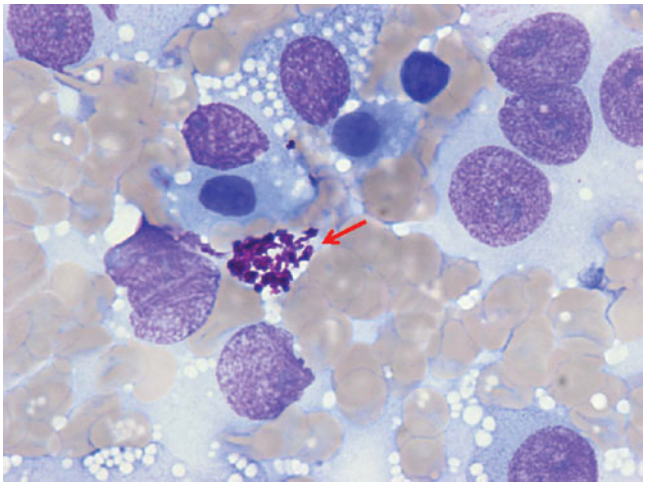


Figure 1.19 Contamination of the smear with ultrasound gel (renal carcinoma, dog). Note the irregular lilac granular material in the centre of the photograph.

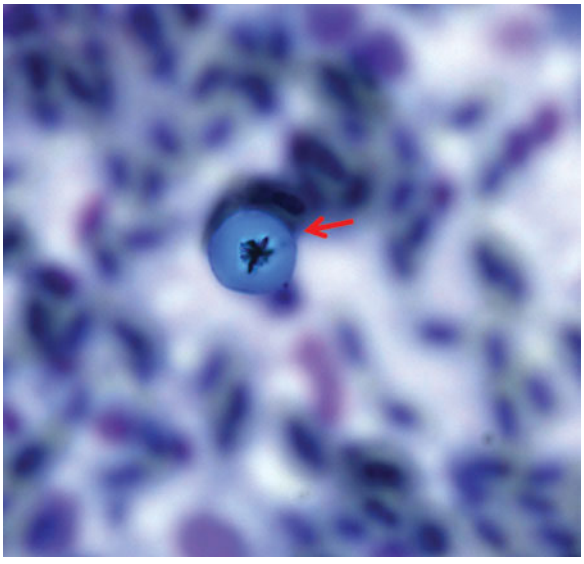


Figure 1.20 A starch powder grain (arrow) from surgical gloves is a contaminant in this fine-needle aspirate of a mass on the head of a grey parrot. Focusing and unfocusing reveals the typical 'cross' to y-shaped structure in the centre of the starch powder granule. Note: The starch powder grain is in focus, while the erythrocytes in the background are out of focus, indicating the contaminant is lying on top of the smear.

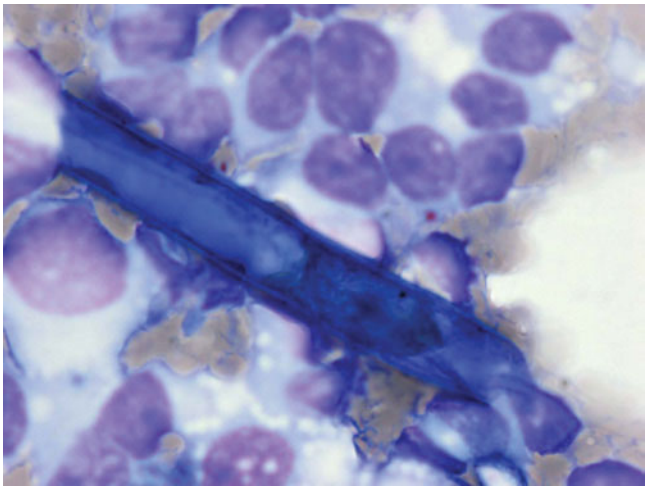


Figure 1.21 A plant fibre can be seen in this fine-needle aspirate of a renal carcinoma of a dog. This material might possibly be mistaken for fungal hyphae, but clear septation is missing, and focusing and unfocusing reveals that the material is placed on top of the cells.

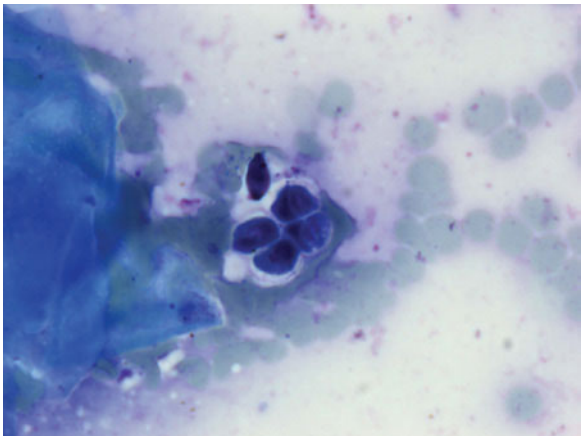


Figure 1.22 Pollen grains (centre of the figure) are present in this skin scraping from a dog.

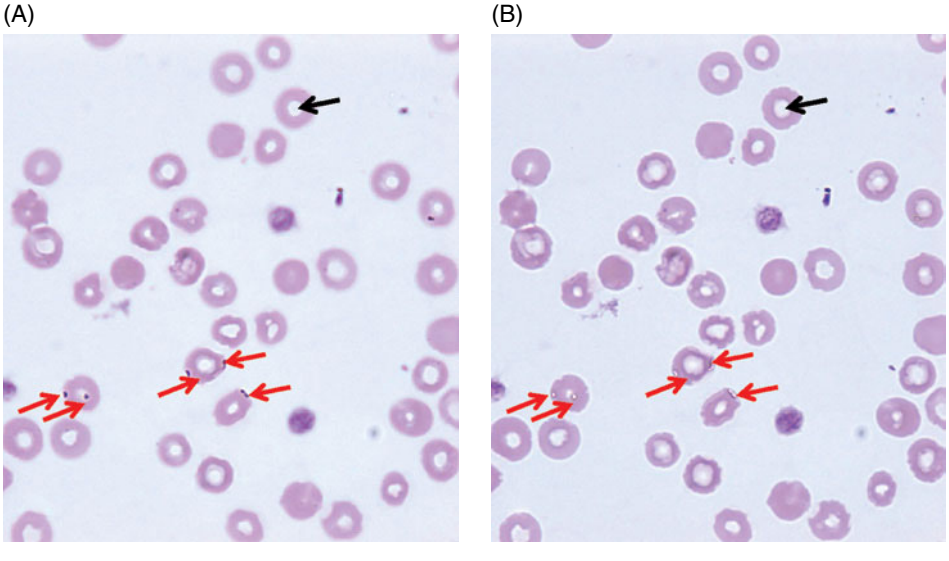


Figure 1.23 Drying artefacts on a blood smear of a cat. (A) Bluish rod-like structures which could be mistaken for *Haemoplasma* sp. can be seen at the margin of the erythrocytes (red arrows). Erythrocytes with 'punched-out holes' (torocytes, black arrows) resemble hypochromic erythrocytes; however, true hypochromasia is characterised by a gradual change of colour rather than this punched-out appearance. (B) Focusing up and down reveals that the bluish structures seen in (A) appear as refractile dots, hence confirming that this is a drying artefact.

The same applies for recognition of drying artefacts affecting erythrocytes which may appear as bluish intracytoplasmic structures resembling Haemoplasma organisms (Figure 1.23A); however, after unfocusing, they appear as refractile dots (Figure 1.23B).

2 General Principles of Cytological Interpretation

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Initial assessment of the sample: Is the preparation of diagnostic quality?

The sample should be examined under low power (10×) to assess cellularity and the sample background and to identify areas of particular interest. At this stage some samples will be recognised as compromised or inadequate for a definitive diagnosis. Some of the more common problems include:

1. Complete absence of cells. In the complete absence of nucleated cells, a definitive diagnosis generally cannot be made.
2. Cell rupture/distortion. Cells stripped of cytoplasm may appear abnormal and should not be assessed.
3. Poor staining.
4. Contamination of the sample with exogenous substances such as lubricant or ultrasound gel.
5. Exposure of cytology to formalin fumes alters staining characteristics and gives a 'washed-out' lightly basophilic appearance to the smears with most Romanowsky-type stains.
6. Occasionally the tissue aspirated is not the expected type, e.g. a presumed submandibular lymph node aspirate which yields normal salivary epithelial cells.
7. Sample too thick for assessment of individual cells (Figure 2.1).

Inflammation

Samples which contain only inflammatory cells are the easiest to interpret when screening cytology samples 'in-house'.

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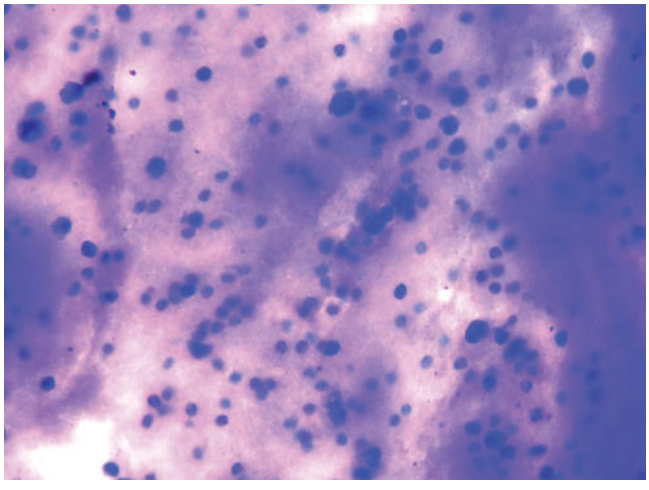


Figure 2.1 Avoid areas of the smears where the cellularity or the background is too thick to assess. Cells condense and the internal detail becomes impossible to assess.

The general approach to inflammation is to (i) identify the predominant cell type present in order to classify the inflammatory response into a broad category, (ii) identify any morphological abnormalities and (iii) attempt to establish any obvious cause for the inflammatory response.

Neutrophilic inflammation

A neutrophilic inflammatory response is one where 70% or more of the nucleated cells are neutrophils (the term ***suppurative*** is sometimes used when the number of neutrophils exceeds 85%).

Neutrophils may be further described as *non-degenerate* (Figure 2.2) or *degenerate* (Figure 2.3) based on morphology. Non-degenerate neutrophils are essentially similar to those in peripheral blood, suggesting that the environment is not directly damaging to them. These are seen most commonly in sterile inflammatory responses such as immune-mediated meningitis or polyarthritis.

Karyolysis is characterised by swelling of the cell and nucleus in response to a damaging or toxic insult to the neutrophil. Causes include exposure to bacterial toxins or irritant material, e.g. free urine, bile or pancreatic enzymes. As the nucleus swells, it becomes more rarefied, stains less intensely and appears more ragged and open. When karyolytic neutrophils are identified, it is important to actively search for causative organisms or substances. Septic neutrophilic inflammation is diagnosed by demonstrating organisms (most frequently bacteria) inside a neutrophil's cytoplasmic vacuoles. In contrast, *karyorrhexis* or *pyknosis* is characterised by the presence of rounded, densely staining remnants of nuclear material within the cell (Figure 2.4). This type of degenerative change is more often associated with the age-related death of non-degenerate neutrophils.

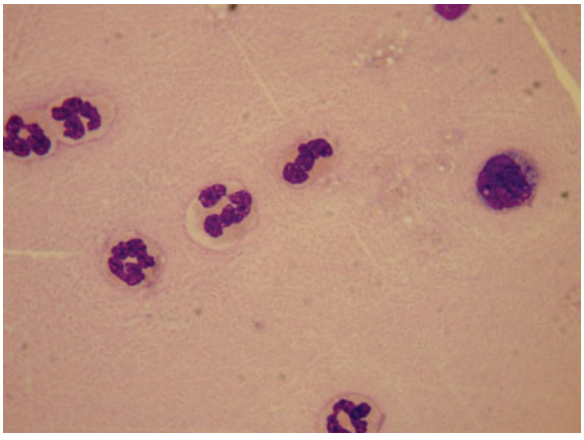


Figure 2.2 Non-degenerate neutrophils in synovial fluid from a dog with immune-mediated polyarthritis. The nuclear chromatin is condensed and the nuclei have smooth well-defined margins. Some are hypersegmented.

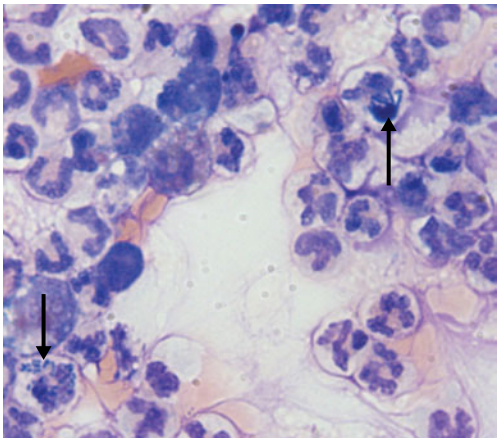


Figure 2.3 Degenerate neutrophils from a pyothorax in a cat. Although still recognisable, the nuclei are swollen and ragged. Intracellular bacterial rods are present (arrowed).

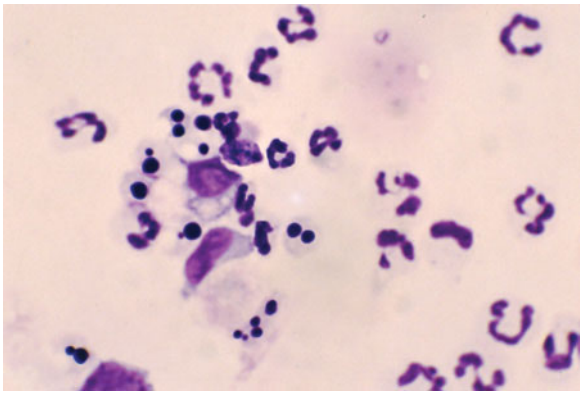


Figure 2.4 Pyknosis and karyorrhexis in neutrophils. Source: Dunn and Villiers (1998a). Reproduced with permission of BMJ Publishing Group Ltd.

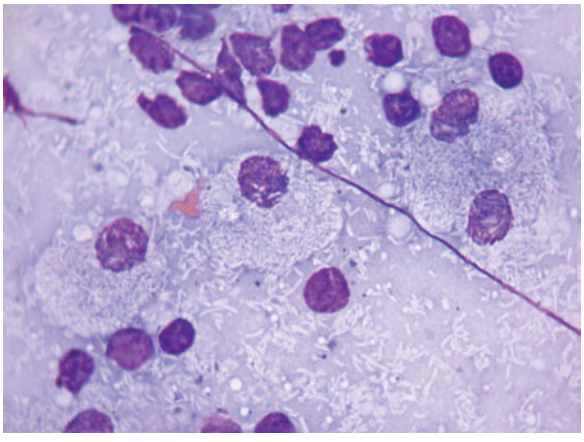


Figure 2.5 Macrophage-rich inflammation. In this case, the cytoplasm is packed with clear nonstaining rods (*Mycobacteria* spp.).

Macrophage-rich or granulomatous inflammation

A predominance of macrophages suggests a more insidious or chronic inflammatory process. Certain organisms are also associated with macrophage-rich inflammation (e.g. *Mycobacteria* spp.) and in some cases can be seen within phagocytic macrophages (Figure 2.5). Multinucleated macrophages or 'giant cells' can also be noted, especially when the inflammation is ongoing or foreign bodies are present. A mixture of macrophages and neutrophils is referred to as 'pyogranulomatous' inflammation and has similar causes.

Eosinophilic inflammation

This is often associated with hypersensitivity or parasitic reactions, although eosinophil numbers may also increase as a paraneoplastic response to certain tumour types (mast cell tumours, lymphoma). An increased number of eosinophils can also be aspirated from eosinophilic granuloma complex lesions and from organs, e.g. spleen, involved in hypereosinophilic syndrome (Figure 2.6).

Lymphocytic/plasmacytic inflammation

This type of inflammatory response may be associated with immune stimulation, e.g. at vaccine site reactions. It may also be identified in some other inflammatory conditions such as rhinitis and lymphocytic–plasmacytic inflammatory bowel disease. The lymphoid cell population may be relatively mixed, but a predominance of small lymphocytes may help to distinguish the lesion from lymphoma.

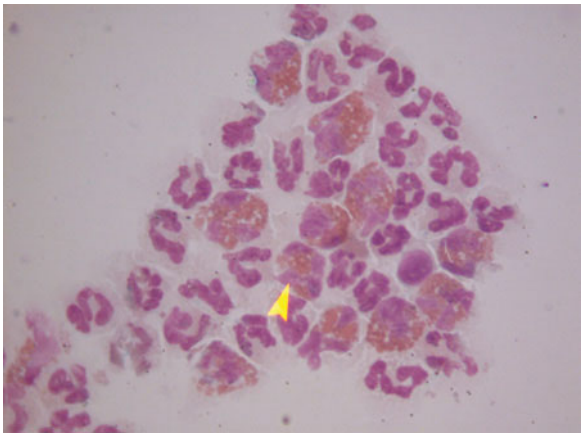


Figure 2.6 Mixed eosinophilic (one eosinophil is arrowed) and neutrophilic inflammation in a tracheal wash from a dog with eosinophilic bronchopneumopathy.

Mixed inflammation

When no single inflammatory cell type is predominant, the percentages of different cell types should be noted and, if possible, an underlying cause identified. In addition to the cell types described previously, mast cells may be noted in samples from some inflammatory lesions.

Aspiration of normal or hyperplastic tissue

In general, cells from normal tissues are very similar or indistinguishable from hyperplastic areas. Cells of a similar cell type and their nuclei should show minimal variation in size and shape (Figure 2.7). Most tissues have a cell turnover low enough such that morphological changes associated with proliferation are not common. Subtle variation in nuclear to cytoplasmic ratio, depth of cytoplasmic basophilia and signs of increased turnover such as the presence of nucleoli may occasionally be perceived in actively hyperplastic tissues, but generally the diagnosis is achieved by demonstrating cytologically normal cells from a mass or enlarged organ.

Neoplasia

In many cases, the aspiration of tissue cells without inflammation from an abnormal area is due to the presence of a neoplasm. Neoplastic populations may be benign (Figure 2.7) or malignant. With benign tumours the cytomorphologic features are more likely to be regular (in many cases, the changes are similar to the relatively mild ones described earlier for hyperplastic tissue).

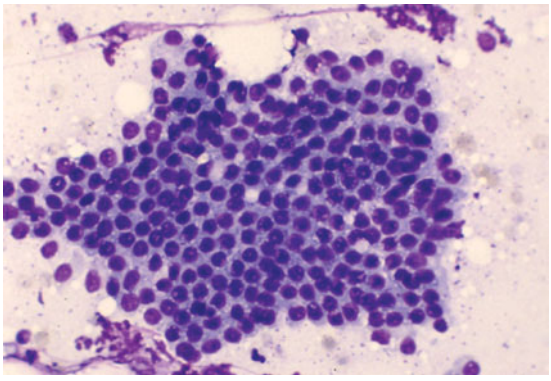


Figure 2.7 Benign prostatic hyperplasia. The cells and their nuclei are of relatively uniform size in this aspirate. Nuclear chromatin appears stippled. Tiny rounded nucleoli are present in some nuclei. Source: Dunn and Villiers (1998a). Reproduced with permission of BMJ Publishing Group Ltd.

Criteria for malignancy

Neoplastic transformation of cells often results in loss of growth inhibition and failure of internal quality control mechanisms. This may allow not only unfettered and uncontrolled growth in the transformed population but also the introduction and persistence of alterations in cell morphology.

The presence of any of following morphological characteristics, particularly the nuclear features, increases the index of suspicion that the cells are malignant:

- The presence of a population of cells in an abnormal location, e.g. anal sac epithelial cells in a sublumbar lymph node aspirate.
- Anisocytosis (variation in cell size).
- Anisokaryosis (variation in nuclear size).
- Variation in nuclear to cytoplasmic ratio (Figure 2.8). This is often increased (more nucleus to cytoplasm) in populations of neoplastic cells.
- The presence of one or more prominent nucleoli in many nuclei (Figure 2.9). Note that the presence of one or two small nucleoli is acceptable in cells from tissues with a high turnover (e.g. intestinal epithelial cells, hepatocytes).
- The presence of multinucleated cells especially if the nuclei vary in size within the same cell (Figure 2.10).
- Nucleolar abnormalities most notably the presence of macronucleoli (those larger than red cell size) and/or multiple nucleoli which vary in size and shape (Figure 2.9).
- Coarse or reticulated nuclear chromatin; cells which spend more time in the active proliferation phases tend to have coarser nuclear chromatin which may form strands (Figure 2.10).
- Nuclear moulding. In normal populations of cells, growth inhibition prevents the nuclei within the same cell or in adjacent cells from influencing the shape of each other (Figure 2.11).

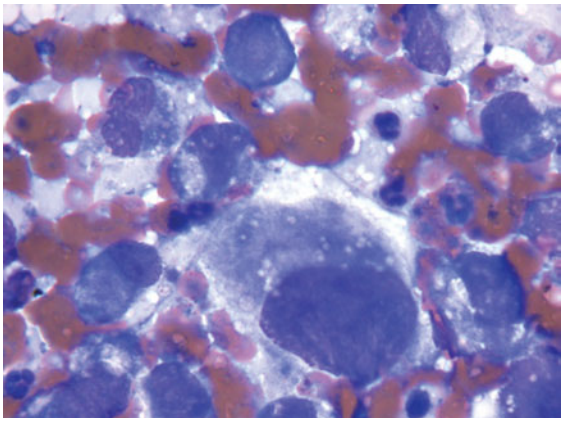


Figure 2.8 Anisocytosis (variation in cell size) and anisokaryosis (variation in nuclear size) in a malignant histiocytic tumour. A few neutrophils are also present.

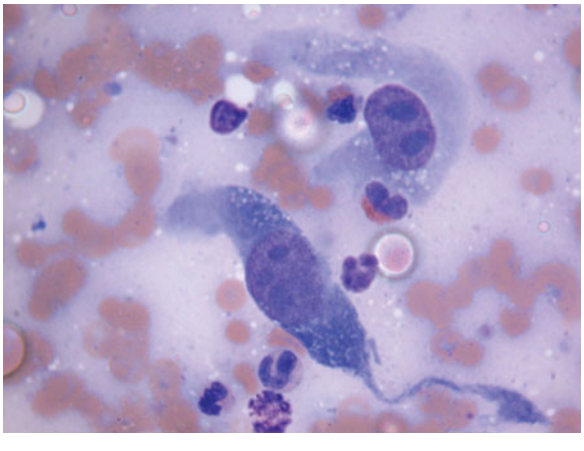


Figure 2.9 Multiple, large and variably sized nucleoli in a mesenchymal tumour. The lower cell in contains variably sized angular nucleoli.

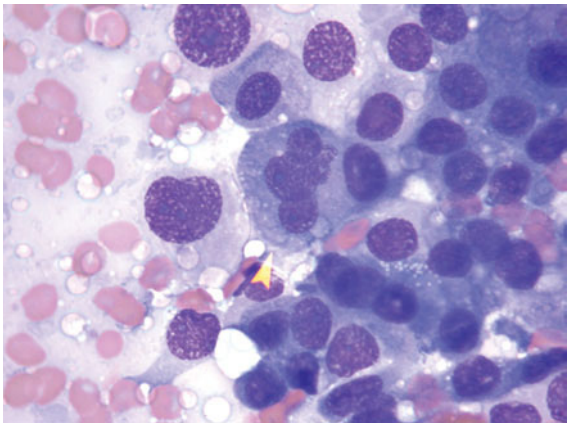


Figure 2.10 An example of abnormal multinucleation. The arrowed cell contains multiple nuclei which vary in size. In addition, the nuclei contain prominent nucleoli and coarse nuclear chromatin.

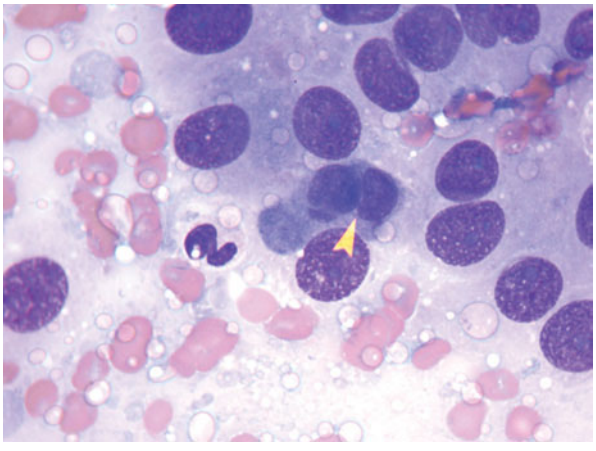


Figure 2.11 Nuclear moulding (arrowed).

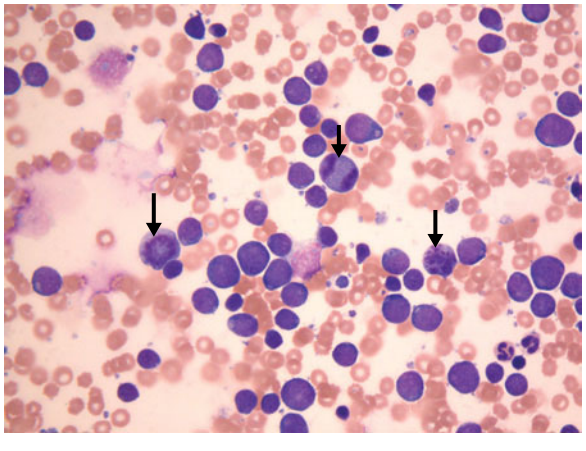


Figure 2.12 Increased numbers of mitotic figures. Three mitotic figures (arrowed) can be seen in this lymph node aspirate from a case of large cell lymphoma.

- A subjective increase in the number of mitotic figures or the presence of abnormal mitotic figures in which there is obvious chromosomal asymmetry or lag (Figure 2.12). Note: Mitotic figures can also be seen in hyperplastic tissue.
- Cytoplasmic basophilia. The increased content of mRNA in very active cells results in a darker, bluer staining cytoplasm with Romanowsky-type stains (Figure 2.13).
- Abnormal cytoplasmic vacuolation or cytoplasmic vacuolation in cells which are not normally vacuolated. Perinuclear vacuolation is of particular concern (Figure 2.13). Single large vacuoles which push the nucleus peripherally impart a 'signet ring' morphology to the cell. The presence of 'signet ring' cells is highly suggestive of carcinoma (Figure 2.14).

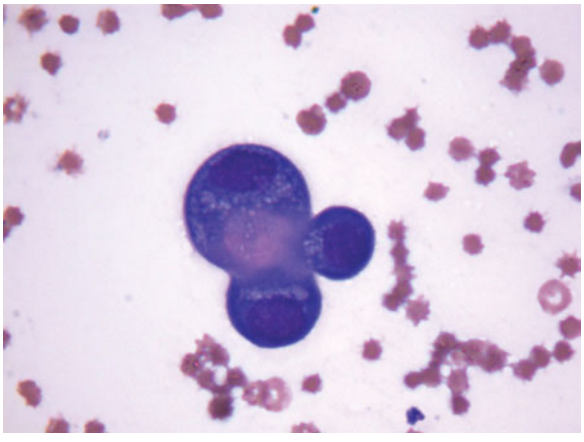


Figure 2.13 Cytoplasmic basophilia and perinuclear vacuolation in mesothelial cells which are either very reactive or possibly neoplastic.

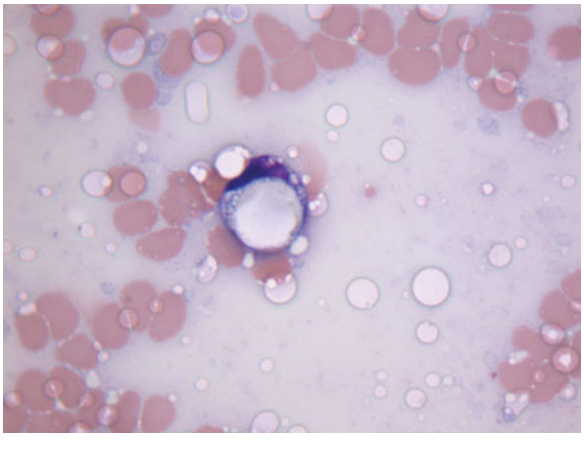


Figure 2.14 Carcinoma. Note the 'signet ring' morphology of the malignant epithelial cell.

Various combinations of the features described earlier can be identified in different neoplasms, and it should be noted that some malignant tumour types display very few of these changes (e.g. thyroid and anal sac carcinomas).

Dysplasia

When tissue is exposed to repeated or prolonged inflammation, infection or injury, dysplasia may occur, resulting in reversible morphological abnormalities which may mimic many of the criteria for malignancy listed previously (anisocytosis, anisokaryosis, variable nuclear to cytoplasmic ratio, multinucleation, presence

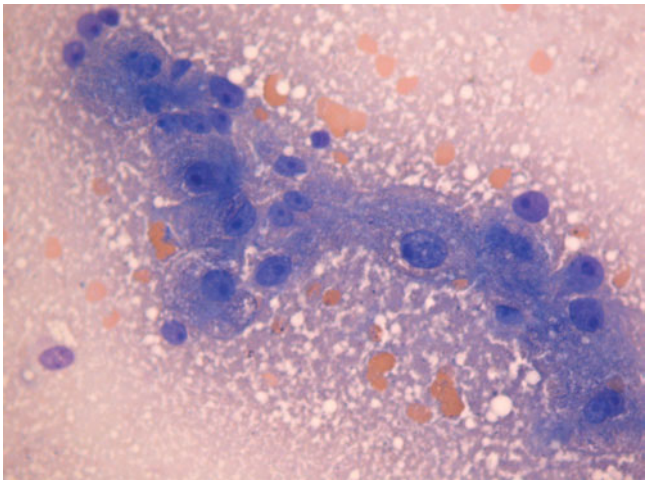


Figure 2.15 Dysplasia in a population of hepatocytes. There is evidence of anisocytosis and increased binucleation. Other nuclear features remain unremarkable.

of nucleoli and increased cytoplasmic basophilia; Figure 2.15). Although these morphological changes are often not as marked as those in a truly malignant process, there is a broad area of overlap.

The misdiagnosis of markedly dysplastic cell populations as malignant neoplasms and vice versa can be minimised by attempting to resolve any underlying inflammatory or infectious process or other injurious cause before re-sampling the lesion.

Characteristics of cell populations: Epithelial, mesenchymal or discrete round cell?

In some cases, tissue cells are so poorly differentiated that determining the tissue of origin is impossible based on morphology alone, but in most tumour aspirates the tissue can be subdivided into one of the following categories.

Epithelial

Cells of epithelial origin have often maintained their cell–cell bonds (desmosomes and hemidesmosomes) which results in aspiration of sheets or balls of adhered cells (Figure 2.16). The bonds themselves may appear as thin non-staining areas between cells. Cytoplasmic borders are well defined in individual cells, and cytoplasm is generally abundant, resulting in a relatively low nuclear to cytoplasmic ratio. Nuclei are frequently round. Epithelial cells of glandular origin may be arranged in a rosette (acinus) which may contain secretory product (Figure 2.17)

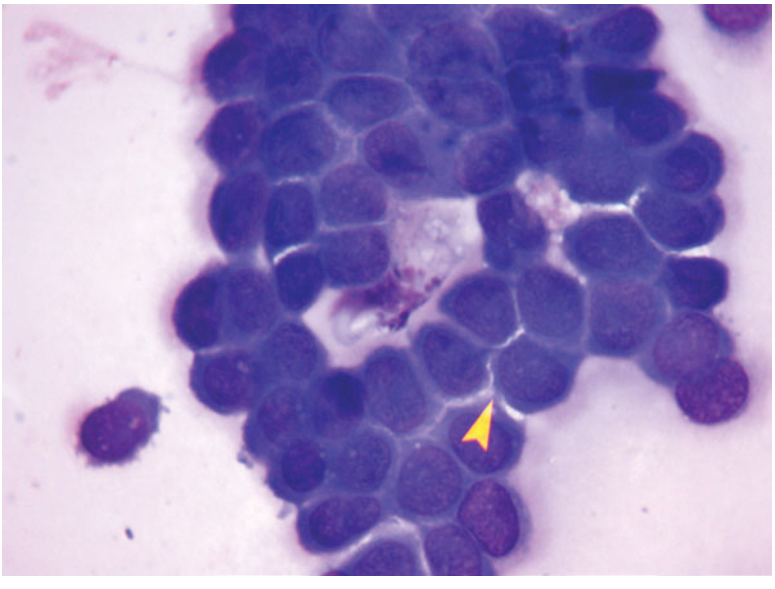


Figure 2.16 A cell-to-cell bond (arrowed) in a population of epithelial cells.

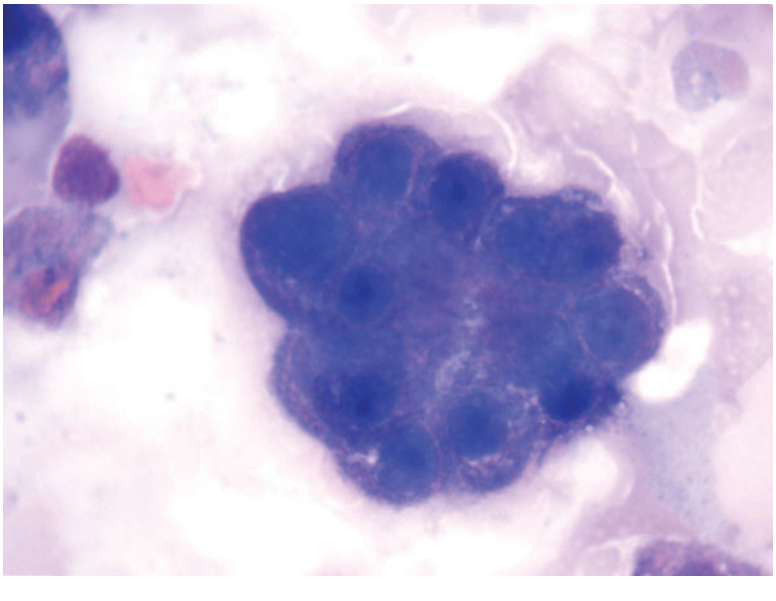


Figure 2.17 A rosetted (acinar) structure in glandular epithelium.

Mesenchymal

This category encompasses cells of connective tissue origin. Most have an oval to elongated nucleus and (with the exception of adipocytes) cytoplasmic margins which are poorly defined (Figure 2.18). Some cells may have the classic 'spindle' shape, but others may appear rounder and plumper. If the tissue type is productive, e.g. collagen from fibroblasts, chondroid from chondrocytes or osteoid from osteoblasts, the product may appear as an eosinophilic material associated with the cells, especially in more hypercellular areas (Figure 2.19). The presence of product, e.g. collagen, may anchor the cells in place and result in relatively low cell yields.

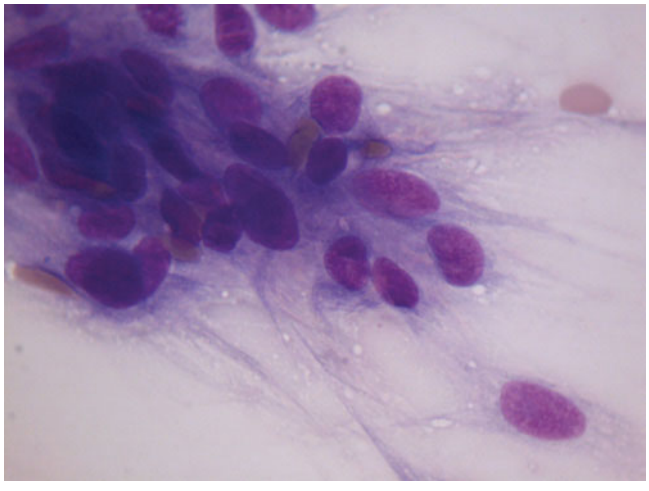


Figure 2.18 Mesenchymal cells with elongated to oval nuclei and wispy, indistinct cytoplasmic margins.

(A)

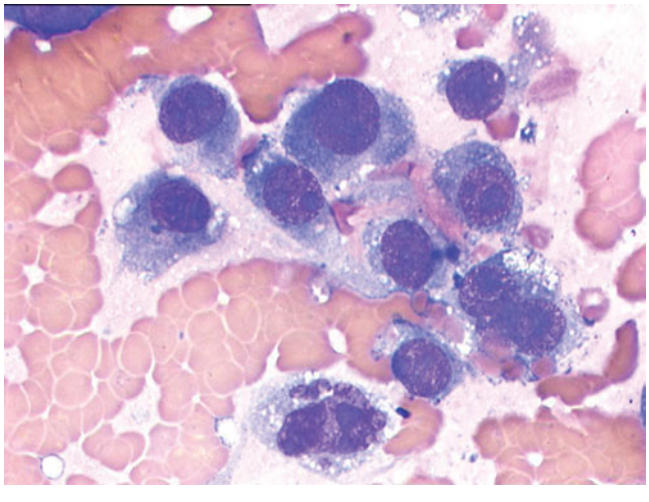


Figure 2.19 (A) Mesenchymal cells. Note the intracellular and extracellular eosinophilic stromal material which in this case is probably chondroid.

(B)

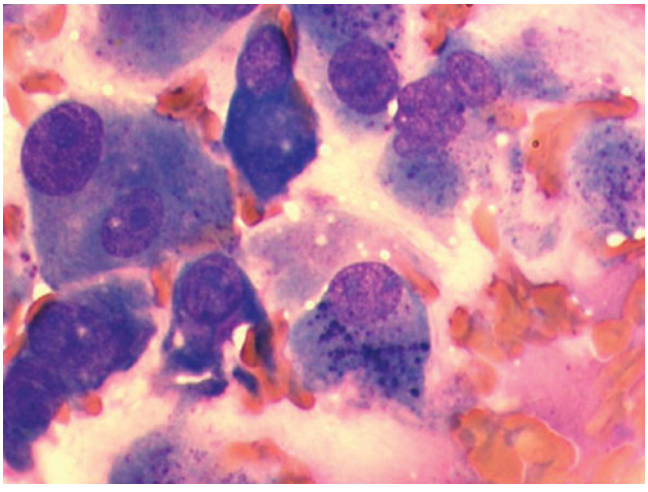


Figure 2.19 (*Continued*)
(B) Osteosarcoma (dog). These osteoblasts are showing a marked degree of anisocytosis, anisokaryosis and variation in nuclear to cytoplasmic ratio. The cells are surrounded by eosinophilic matrix material, probably osteoid. One large binucleated cell is present. The nuclei contain large prominent nucleoli which vary in size. Note also the cytoplasmic granules which may be responsible for producing the osteoid matrix.

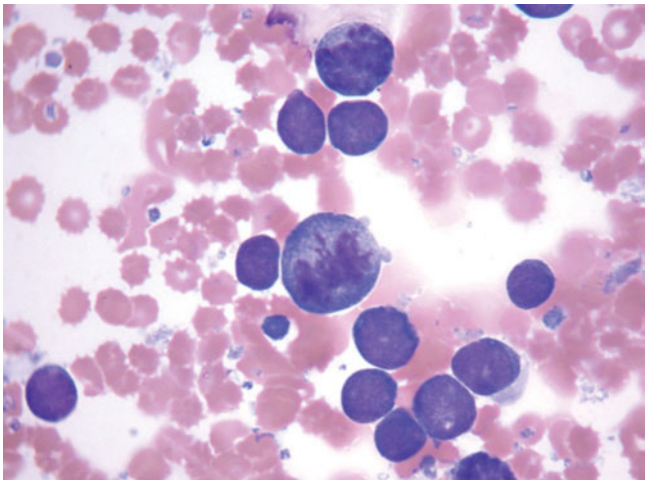


Figure 2.20 Large cell lymphoma. Note the individual cells with a high nuclear to cytoplasmic ratio. Two mitotic figures are also present.

Discrete round cells

There are relatively few cell types in this category. Round cell tumours tend to yield large numbers of individual cells which are not associated with a stromal product. Lymphoid tumours (Figure 2.20), plasma cell tumours (Figure 2.21), histiocytic

tumours (Figure 2.22) and mast cell tumours (Figure 2.23) are included in this category. Melanomas yield cells with round cell characteristics but they are usually included in the mesenchymal category (Figure 2.24).

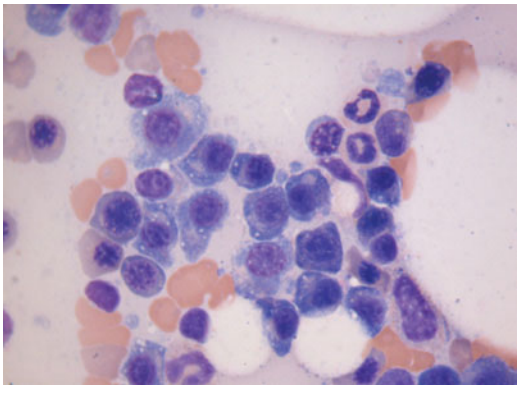


Figure 2.21 Plasma cells in bone marrow from a case of multiple myeloma. These are discrete cells with dark basophilic cytoplasm and a perinuclear clear zone ('halo').

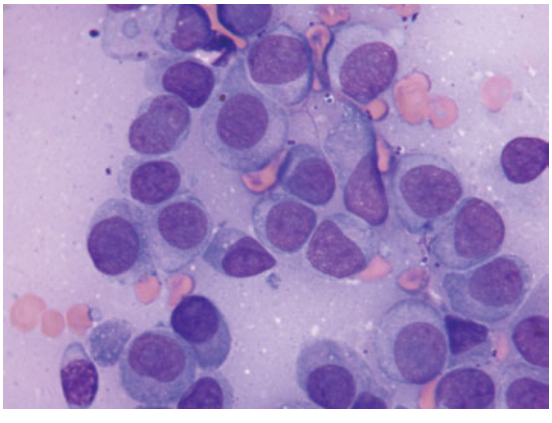


Figure 2.22 Histiocytoma. These discrete round cells have a moderate amount of basophilic cytoplasm, and a mild degree of anisocytosis and anisokaryosis is evident. Note also the indistinct nucleoli.

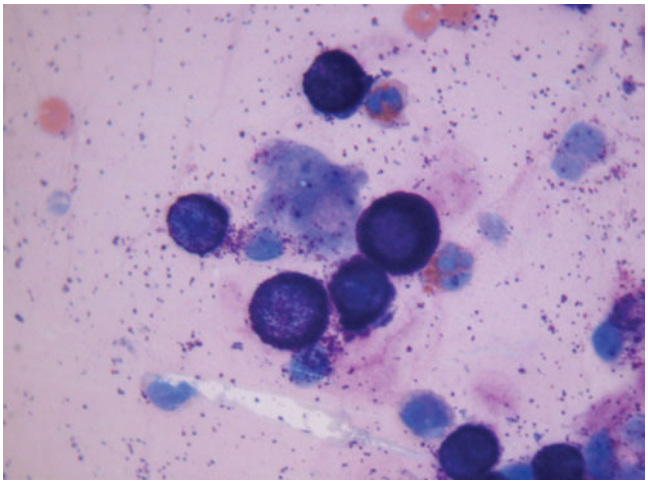


Figure 2.23 Mast cell tumour. These cells are packed with azurophilic cytoplasmic granules which obscure nuclear detail. Note also the cytoplasmic granules in the background from ruptured mast cells.

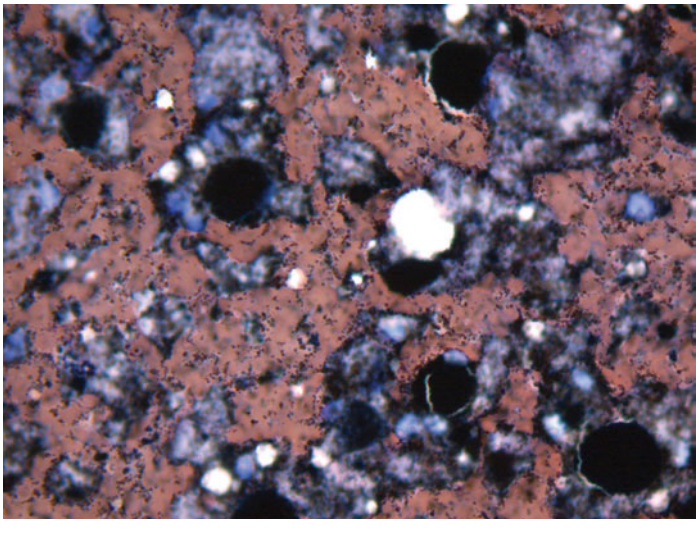


Figure 2.24 Melanoma. The cells contain black melanin granules which are obscuring all internal cellular detail. Free melanin pigment granules are also present in the background.

Cystic structures

High numbers of anucleated keratinised squamous epithelial cells may be found in a variety of cutaneous lesions (epidermal inclusion cyst, sebaceous cyst) as well as in some benign adnexal neoplasms (Figure 2.25). Aspirates from truly cystic structures within other organs (e.g. cysts in the kidneys of cats with polycystic disease) may yield only a proteinaceous fluid and very few or no cells. A definitive diagnosis may require histopathological examination of the cyst capsule in these cases.

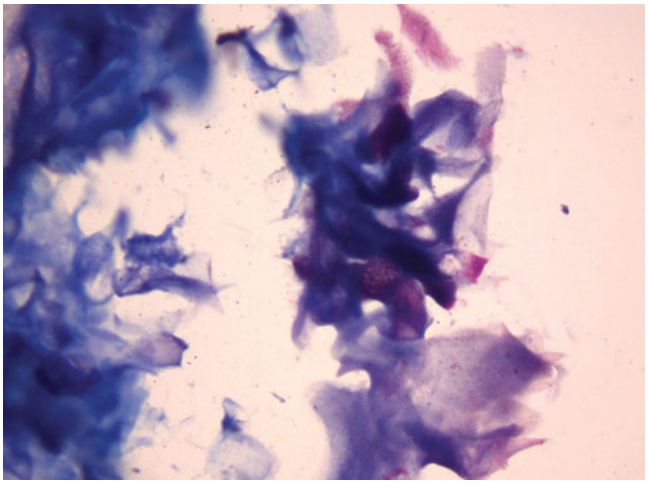


Figure 2.25 The presence of an abundance of anucleated keratinised squames and debris is consistent with an epidermal or a follicular inclusion cyst. Sebaceous cysts and some benign adnexal tumours may appear fairly similar.

3 Cytology of the Lymphoid Tissues

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The lymph node

Canine and feline lymph nodes are very similar in function and morphology to that of man and most domestic animals. The lymph node possesses a well-defined capsule containing elastic and smooth muscle fibres which continue in the node as a framework of septa and trabeculae. Most primary and secondary nodules are situated in the cortex, where they are surrounded by the paracortical regions and at least in part by the lymph sinus. Practically all paracortical lymphocytes are of T-cell origin, while those in the follicles are of B-cell origin intermixed with some T-lymphocytes. In the medulla, the lymphoid tissue consists of anastomosing cords of lymphocytes. Antigen-stimulated cells including plasma cells accumulate here. Histiocytic cells, including antigen-presenting cells, can be found in all compartments.

Normal cytological appearance and benign changes

Although normal lymph nodes are seldom aspirated, familiarity with the normal cytological appearance is necessary in order to recognise abnormalities. Mild antigenic stimulation takes place in the normal lymph node, and in principle all stages of B- and T-lymphocyte maturation can be identified. However, the majority (85–95%) of the cells are small B- and T-lymphocytes. These cells are characterised by little cytoplasm, round nuclei without nucleoli and often slightly rough chromatin structure (Figure 3.1). The size of these cells (about 10 μm in diameter) lies between that of erythrocytes and neutrophils.

Lymphoglandular bodies are cytoplasmic fragments which stain light blue with May–Grünwald–Giemsa (Figure 3.1). They are characteristic for lymphoid tissue, and their presence can be useful in differentiating lymphoid cells from those of an undifferentiated small cell carcinoma. Although not a sign of malignancy, their numbers are higher in high-grade malignant lymphomas.

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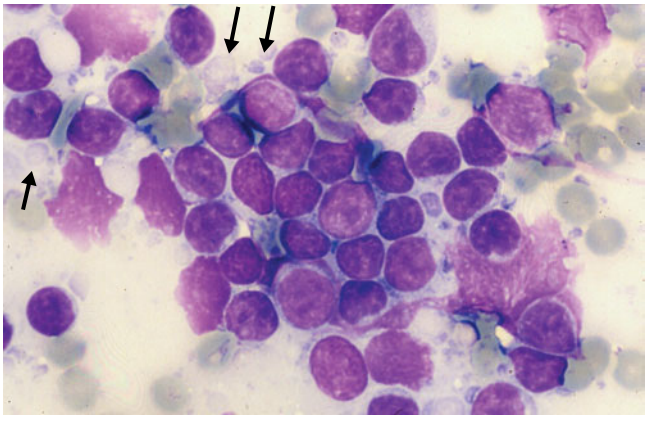


Figure 3.1 FNAB of a normal lymph node of a dog consisting of a monomorphic population of small lymphocytes, which are only little larger than surrounding erythrocytes. The pink material between cells is nuclear material from damaged cells. The small blue structures are lymphoglandular bodies (arrows).

A normal lymph node also contains other developing stages of the lymphoid series, e.g. medium-sized lymphocytes, immature blasts and plasma cells, but never more than 5–10% of the total number of lymphoid cells. Other non-lymphoid cells which are present sporadically in a normal lymph node include neutrophilic and eosinophilic granulocytes, macrophages/histiocytes, mast cells, erythrocytes and monocytes.

Non-lymphoid tissue

Normal or enlarged submandibular salivary glands are often mistaken for enlarged submandibular lymph nodes. Salivary gland cells are much larger than lymphoid cells, contain more cytoplasm and form acinar (gland-shaped) structures. Lymphocytes and lymphoglandular bodies are absent.

In obese animals, it is possible to have the wrong impression that the lymph node is enlarged because it is surrounded by a thick layer of fat. Fine-needle aspiration of these enlarged 'nodes' may yield only fat.

Reactive hyperplasia

One of the most common causes of a generalised lymphadenopathy is reactive hyperplasia, resulting from antigenic stimulation of the lymph nodes. Reactive hyperplasia is characterised cytologically by an increase in the number of medium-sized lymphocytes and large immature lymphoid cells, such as immunoblasts and centroblasts, in relation to the number of small, normal lymphocytes. There are also increased numbers of mitoses, lymphoplasmacytoid cells (cells which are intermediate between an immunoblast and plasma cell) and plasma cells (Figure 3.2).

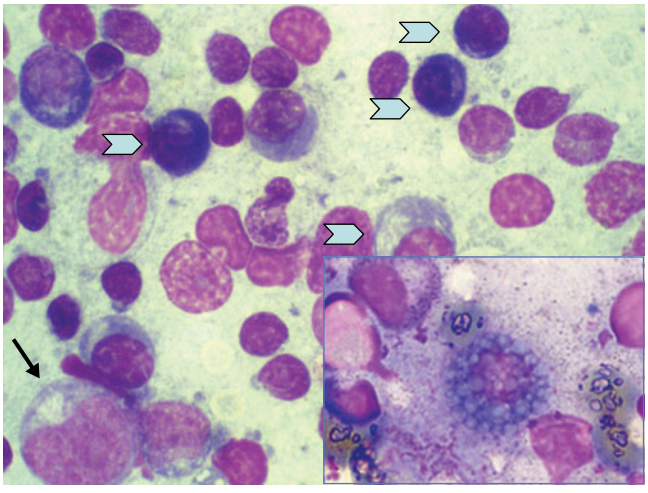


Figure 3.2 Aspirate of a hyperplastic lymph node. Several mature lymphocytes, an immunoblast (arrow), two neutrophils and several dark-blue plasma cells (arrowheads) are present. Inset: A Mott cell, i.e. a plasma cell with so-called Russell bodies in the cytoplasm.

Sometimes so-called Russell bodies are seen in the cytoplasm of plasma cells (Mott cells). These are vacuoles filled with immunoglobulins (Figure 3.2).

Depending on the underlying cause, there may also be increased numbers of other cell types, such as histiocytes (macrophages, dendritic cells, interdigitating cells), polymorphonuclear granulocytes and, especially in skin disorders, eosinophils and mast cells.

Lymphadenitis

The presence of many inflammatory cells in the lymph node is referred to as lymphadenitis. Differentiation into purulent and granulomatous lymphadenitis is made according to the predominant inflammatory cell type present.

Purulent lymphadenitis is characterised by an increased number of neutrophils, usually combined with a reactive lymphoid cell population and a few macrophages (Figure 3.3). Differentiation from reactive lymphoid hyperplasia may be difficult, but can also be quite clear, as in bacterial lymphadenitis which is characterised by an abundance of neutrophils and very few or a complete absence of lymphoid cells. There may be evidence of necrosis, and bacteria may be present. An increase in eosinophils is seen mainly in allergic dermatitis, some parasitic infections, but also in abdominal lymph nodes in cats with the hypereosinophilic syndrome.

Granulomatous lymphadenitis is also usually characterised by a slightly reactive lymphoid picture and increased numbers of macrophages, epithelioid antigen-presenting cells and multinucleated giant cells. Epithelioid cells are reticulum cells with an elongated oval nucleus, which is often indented and has a finely granular chromatin pattern. Epithelioid cells possess a variable (often minimal) amount of cytoplasm. These cells may be arranged in clusters which can resemble carcinoma

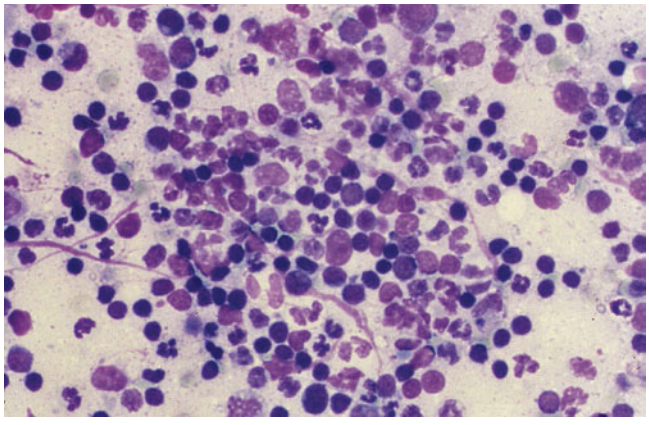


Figure 3.3 Purulent lymphadenitis. Mixture of small lymphocytes and many neutrophils. In the background are some 'threads' of degenerate nuclear material.

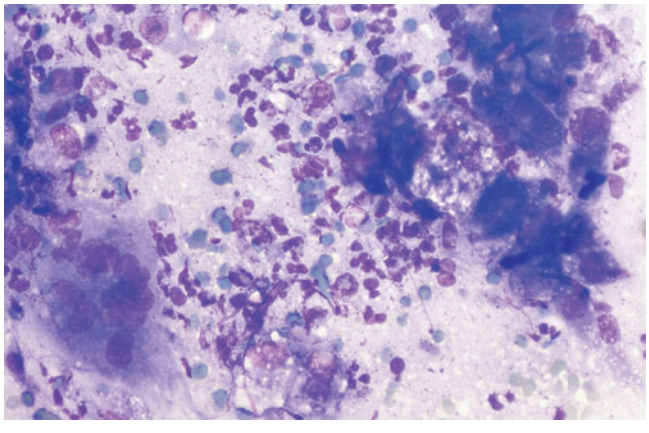


Figure 3.4 Pyogranulomatous lymphadenitis in a dog. Multinucleated giant cells, macrophages, degenerate neutrophils and lymphocytes are present. In the upper right quadrant, fungal hyphae can be seen.

metastases. Granulomatous lymphadenitis is seen in fungal and yeast infections (Figure 3.4 and Figure 3.5); protozoal infections, e.g. toxoplasmosis and leishmaniasis (Figure 3.6); and certain bacterial infections (e.g. infections with *Mycobacterium* spp.) (Figure 3.7).

A *dermatopathic lymphadenopathy* is a granulomatous lymphadenitis which occurs with skin disorders in which pruritus, scaling of skin and skin damage are prominent. The cellular picture is characterised by the presence of many brown-black melanin granules and a few eosinophils. Interdigitating cells are also encountered. These are elongated histiocytes with a characteristic indentation of the nucleus with a reticulated chromatin pattern (Figure 3.8). They can often only be identified as naked nuclei.

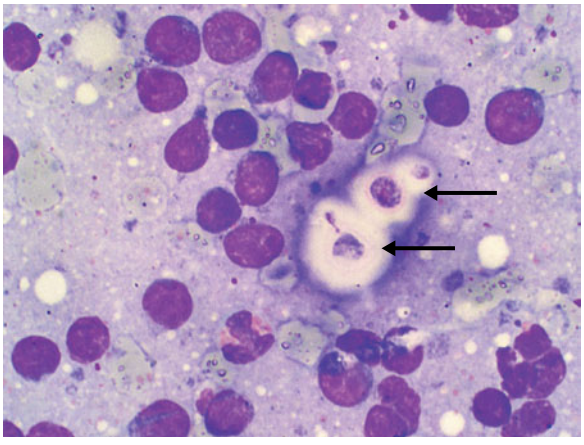


Figure 3.5 Lymph node aspirate from a cat with cryptococcosis. Among the lymphocytes, plasma cells and the occasional eosinophil two *Cryptococcus neoformans* yeasts are visible (arrows). Note the non-staining thick capsule and the thin double cell wall.

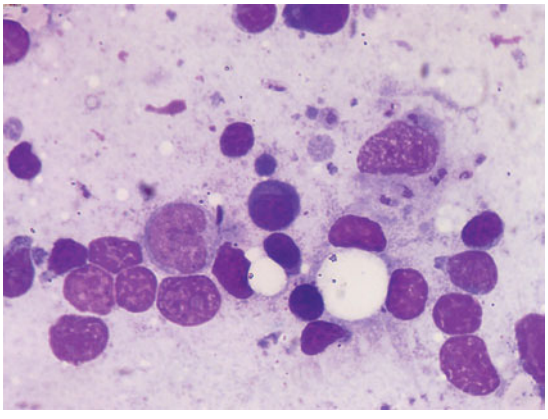


Figure 3.6 Dog with leishmaniasis. The amastigotes are present in the cytoplasm of the macrophages but can also be seen individually in the background of the smear. A few lymphocytes and plasma cells are present.

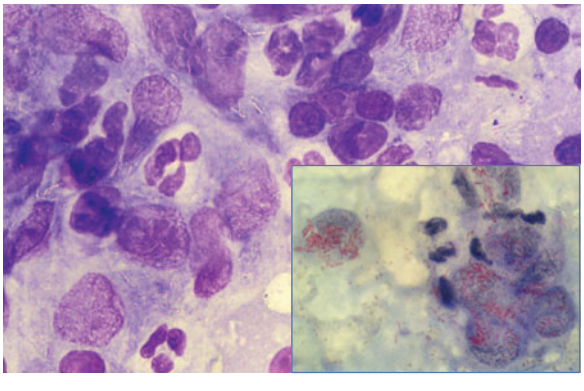


Figure 3.7 Granulomatous lymphadenitis due to mycobacterial infection in a cat. Several large histiocytic cells containing negatively stained bacterial rods can be seen. Inset: The same organisms stain acid-fast after restaining of slide with Ziehl–Neelsen.

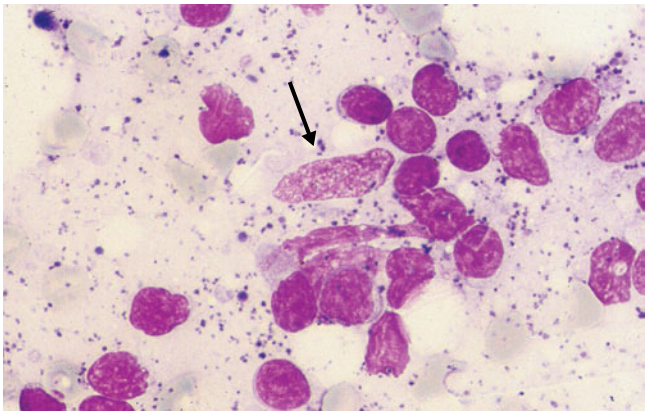


Figure 3.8 Dermatopathic lymphadenopathy in a dog. There are many brown-black melanin granules in the background, several mature lymphocytes and a large 'bare' nucleus of an interdigitating cell (arrow).

Neoplasia

Metastatic malignancies

In principle, all malignant tumours can metastasise via the lymphatic system. Some types of tumour metastasise earlier than others to the regional lymph node. Sarcomas generally spread by the haematogenous rather than by the lymphogenous route. Carcinomas (Figure 3.9), melanomas (Figure 3.10) and mast cells tumours (Figure 3.11) often metastasise to the lymph node, although this also depends upon the histological sub-type.

A cytological preparation from a lymph node which contains cells that are not normally present should raise suspicion of metastatic malignancy. It is important to note, however, that a lymph node aspirate may occasionally harvest cells from adjacent tissue. Hence it is necessary to first evaluate the 'foreign' cells for malignant characteristics before a diagnosis of tumour metastasis is made. It is also possible that a single aspirate may occasionally contain cells from both the primary tumour and the adjacent lymph node, e.g. if a normal or reactive supramammary lymph node and a caudal neoplastic mammary gland are aspirated simultaneously, an erroneous diagnosis of tumour metastasis may result.

In addition to the presence of metastatic cells, lymph node cytology also undergoes benign changes as a result of the immune response to the tumour cells. The number of macrophages, plasma cells and younger larger lymphoid cells is often increased. A few mast cells are always identified in a normal or hyperplastic lymph node. According to the literature, however, the number should not exceed 3%. An increased number of mast cells is suggestive of mast cell tumour metastasis or even mast cell leukaemia.

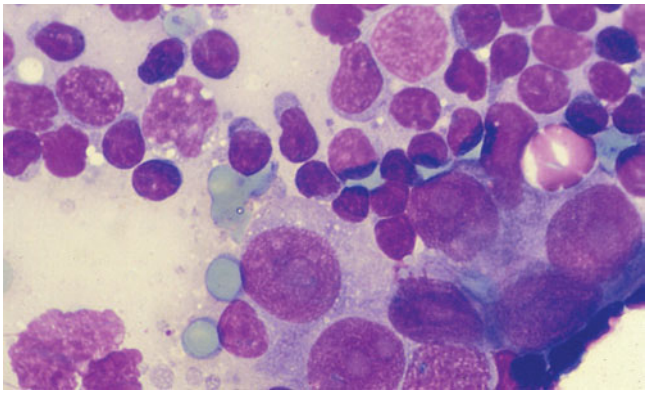


Figure 3.9 Adenocarcinoma metastases of a mammary gland tumour in a prescapular lymph node of a dog. The large nuclei of the tumour cells contain prominent nucleoli.

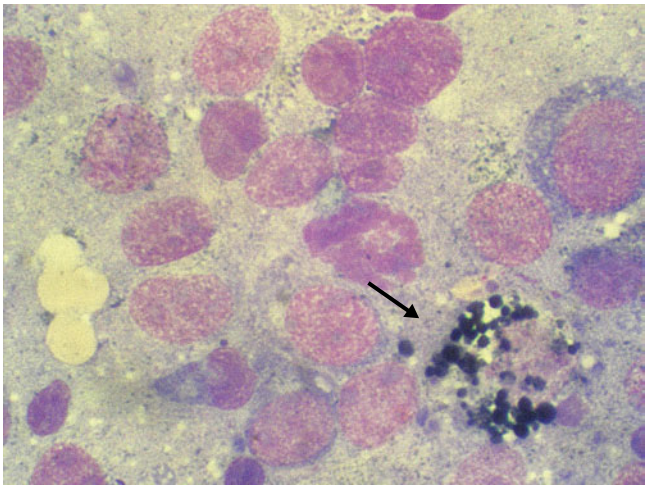


Figure 3.10 Melanoma metastasis in a canine lymph node. The majority of cells are tumour cells, although only a few contain intracytoplasmic fine black melanin granules. A melanophage containing clumped melanin pigment is also present (arrow).

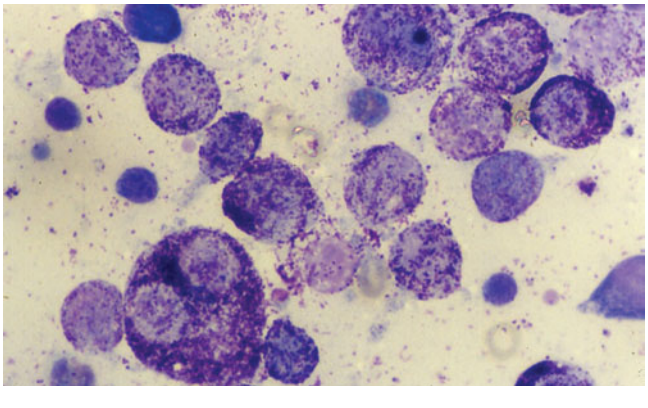


Figure 3.11 A lymph node with mast cell tumour metastases. The mast cells vary in size and have prominent nucleoli. A binucleated cell can also be seen.

Lymphoid malignancies

The cytological appearance of malignant lymphoma varies. A lymphoid cell can become malignant during each stage of its development, either by an arrest in further differentiation or by autonomous proliferation of a particular cell type (Figure 3.12). The cell types which are encountered in malignant lymphoma thus do not differ in appearance from normal lymphoid cells. The cytological differentiation rests on the presence of a monomorphic cell population; while in a non-lymphomatous lymph node, all different maturation stages of the lymphoid series are visible.

Various classification schemes have been developed for non-Hodgkin's lymphomas in man. The Kiel classification system is based entirely on the transformation scheme for normal lymphocytes and is very suitable for cytological purposes (Table 3.1 and Figure 3.12). In the literature, this Kiel classification has been applied successfully to malignant lymphomas of dogs. There is as yet no information about the applicability of this classification for malignant lymphomas of cats. From a practical viewpoint, the diagnosis of malignant lymphoma and its subclassification into low- or high-grade lymphoma is usually sufficient and has prognostic clinical significance. The classification into low- and high-grade lymphoma has a fair to good inter-observer agreement, but further sub-classification into the various sub-types has a much lower inter-observer agreement. It should also be emphasised that the classification into the Kiel classification sub-groups is based on cytomorphology and that a reliable prediction of immunophenotype is not possible on cytology alone. Additional immunophenotyping into B- or T-cell lymphomas can be performed on unstained direct smears or cytospin preparations (Figure 3.13).

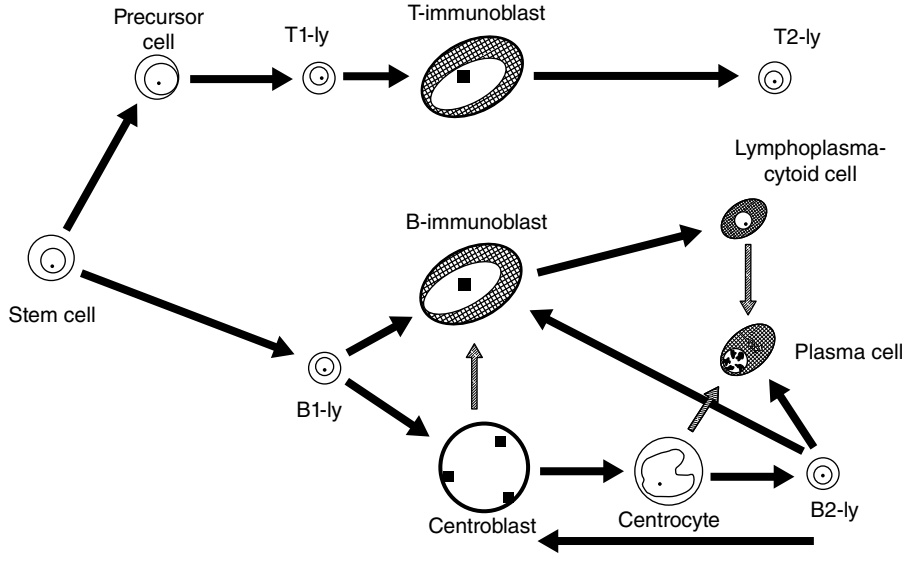


Figure 3.12 Transformation scheme of normal lymphocytes. Source: after Lennert, K. and Feller, A.C. (1990) Die Kiel-Klassifikation. In *Histopathologie der Non-Hodgkin-Lymphome (nach der aktualisierten Kiel-Klassifikation)*, 2nd edn, Springer-Verlag, New York, pp. 13–50. © Springer Science+Business Media; adapted from: van Heerde, P. (1984).

Table 3.1 Simplified classification of malignant lymphomas by cell type, based on the Kiel classification

Low-grade malignant lymphoma
Lymphocytic
Immunocytic
Plasmacytic
Centrocytic
Centroblastic/centrocytic
High-grade malignant lymphoma
Centroblastic
Pure centroblastic
Anaplastic centrocytic
Polymorphic centroblastic
Lymphoblastic
Immunoblastic
Other
Histiocytic
'Multilobated' cell

Source: Data from Lennert and Mohri (1978).

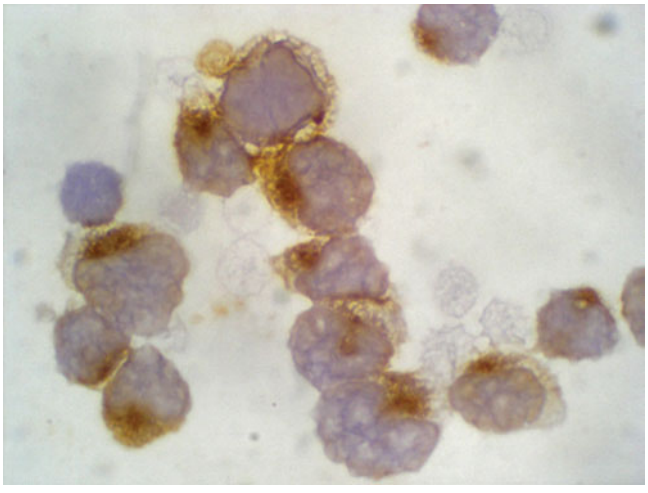


Figure 3.13 Immunostaining with CD79a antibody of a cytospin preparation from an aspirate of a canine malignant lymphoma, demonstrating the B-cell origin of the tumour.

A diagnosis of lymphoma is relatively straightforward if the aspirated cell population consists mainly of blasts. However, there are also forms of lymphoma, for example, lymphocytic and centrocytic lymphomas, in which the tumour cells more closely resemble mature lymphocytes. Also the diagnosis may be more

difficult when the lymphoma contains more than one cell type, as in the immunocytic or centroblastic/centrocytic sub-types. In many cases, the presence of enlarged lymph nodes and the non-reactive appearance of the lymphoid cell population may be decisive, especially if the cell population is monomorphic. When plasma cells or other inflammatory cells are present, the diagnosis may be in doubt, and histopathological examination of a surgical biopsy may be required for a definitive diagnosis.

Morphology of canine lymphomas classified by the Kiel classification

Lymphocytic lymphoma

This type of lymphoma consists of a monomorphic population of small, mature lymphocytes. In the new Revised European American Lymphoma/World Health Organization (REAL/WHO) classification, this type is included in the same group as chronic lymphocytic leukaemia. The cytological appearance is often difficult to differentiate from a normal non-reactive lymph node. If the lymphocytic lymphoma consists of B-lymphocytes, which can only be confirmed with certainty by immunophenotyping, the cells have a narrow rim of cytoplasm and the nuclei tend to be round with coarsely clumped chromatin pattern. This is in contrast to the T-lymphocytic lymphoma, in which the nucleus is slightly indented and has a dense chromatin pattern. Both types of lymphoma exhibit little cell multiplication. Lymphocytic lymphoma occurs infrequently in dogs (Figure 3.14).

Lymphoplasmacytoid/immunocytic lymphoma

The most important cell type here is the immunocyte, a small lymphoid cell with more cytoplasm than the cells of a lymphocytic lymphoma. The round nucleus may be eccentrically located, and an inconspicuous nucleolus may be present. This cell type has developed a little further in the direction of the plasma cell. This lymphoma sub-type is classified as a lymphoplasmacytoid lymphoma (Figure 3.15). The term immunocytic lymphoma is used when the majority of the cells are small lymphocytes but centrocytes, immunoblasts and plasma cells are also present (Figure 3.16).

Peripheral T-cell lymphoma consists of a mixed population of medium-sized and large cells. This type of lymphoma is difficult to differentiate from other lymphoma sub-types because reactive plasma cells may be intermixed between the tumour cells. The finding of the so-called ‘hand-mirror’ forms is suggestive for T-cell lymphoma (Figure 3.17).

Plasmacytic lymphoma

Plasmacytic lymphoma is extremely rare (Figure 3.18). This type of lymphoma is characterised by the presence of atypical plasma cells in varying stages of development.

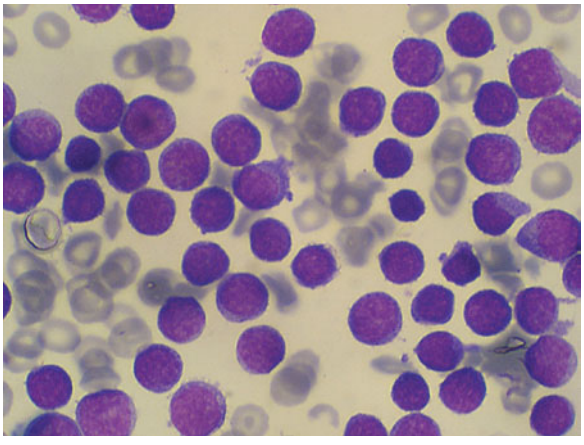


Figure 3.14 A monomorphic population of small, mature lymphocytes in a dog with lymphocytic lymphoma.

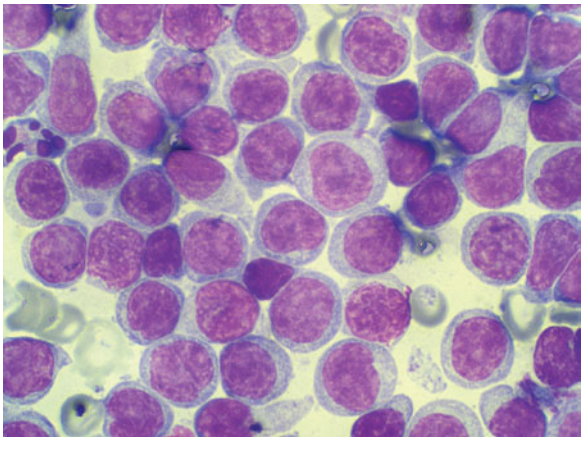


Figure 3.15 Lymphoplasmacytoid lymphoma in a dog. Note the monomorphic population small- to medium-sized lymphoid cells with abundant cytoplasm. The nucleus is sometimes eccentrically placed.

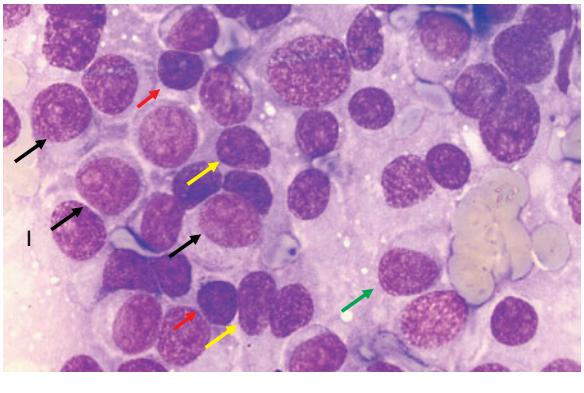


Figure 3.16 In this immunocytic canine lymphoma, a mixture of lymphoplasmacytoid cells (green arrow), centrocytes (yellow arrows) and immunoblasts (black arrows) can be seen next to some small lymphocytes (red arrows).

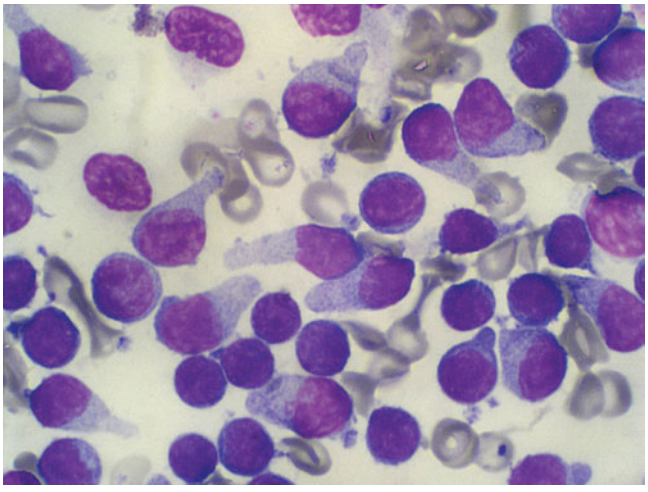


Figure 3.17 Small cell lymphoma in a dog demonstrating so-called 'hand-mirror' forms. The cytoplasmic protrusions are called uropods and are suggestive of T-cell origin.

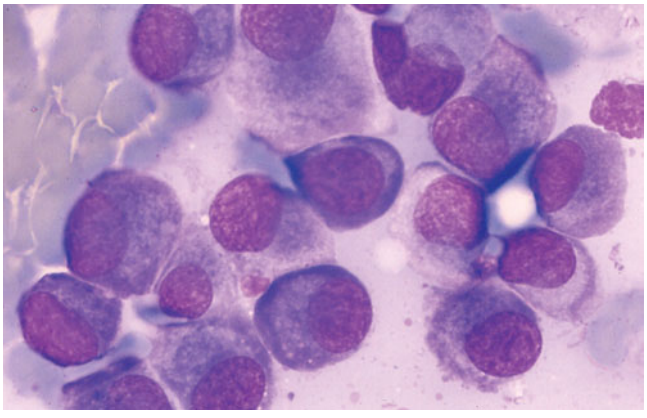


Figure 3.18 Plasmacytic lymphoma in a dog demonstrating large, atypical plasma cells.

Centrocytic lymphoma

This lymphoma consists primarily of centrocytes. Centrocytes are small cells with an irregular, sometimes indented nucleus (Figure 3.19). The cytoplasm is often absent or is very pale. The chromatin pattern is fine and there are usually no visible nucleoli.

Centroblastic/centrocytic lymphoma

As the name indicates, this type of lymphoma consists of both centrocytes and centroblasts (Figure 3.19). Centroblasts have a rim of dark basophilic cytoplasm and a large, round nucleus with multiple nucleoli that often lie adjacent to the nuclear

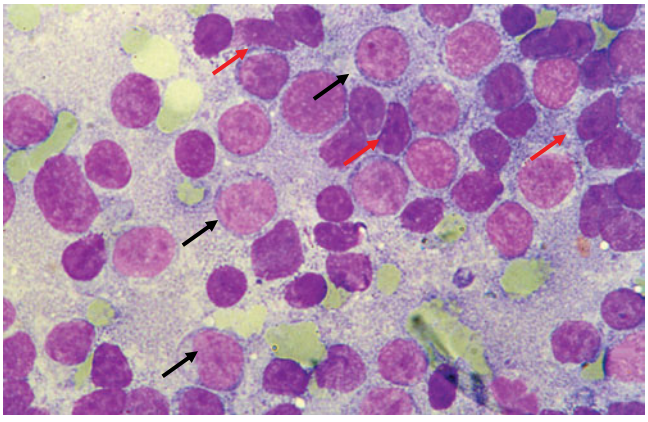


Figure 3.19 Centroblastic/centrocytic lymphoma. A mixed population of large centroblasts (black arrows) and smaller centrocytes (cleaved cells; red arrows) is present.

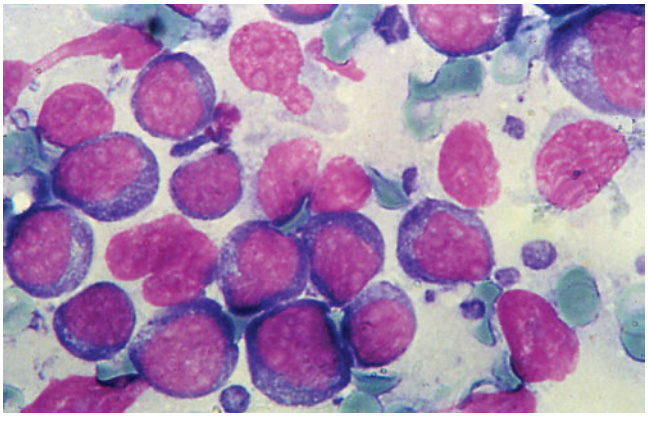


Figure 3.20 Aspirate of a centroblastic canine lymphoma. Large cells with dark-blue cytoplasm and large nuclei containing multiple small nucleoli are present.

membrane. Many mitoses are present. If the percentage of centroblasts is higher than 30–50%, the lymphoma is classified as centroblastic (Figure 3.20).

Centroblastic lymphoma

The primary cell type is the centroblast, but a few centrocytes will often be present. There are two forms of this sub-type. If immunoblasts are present in addition to centroblasts, the tumour is called a 'polymorphic centroblastic' lymphoma (Figure 3.21). If there are more than 50% immunoblasts, the tumour is referred to as an immunoblastic lymphoma (see following text). The other form is the 'anaplastic centrocytic' (centrocytoid) lymphoma (Figure 3.22). Anaplastic centrocytes are cells which morphologically resemble centrocytes, but they have a larger and irregularly shaped nucleus with a coarser chromatin pattern and, in contrast to

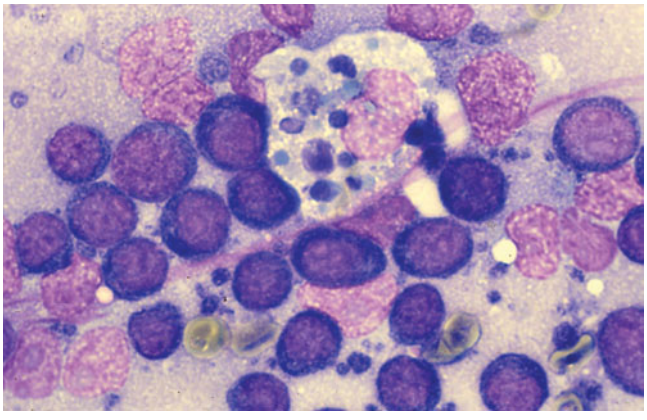


Figure 3.21 Polymorphic centroblastic malignant lymphoma. A monomorphic population of round centroblasts (with multiple small nucleoli). In addition, a few immunoblasts with a single large nucleolus are present. A large 'starry sky' macrophage containing cellular debris ('tingible bodies') is also visible.

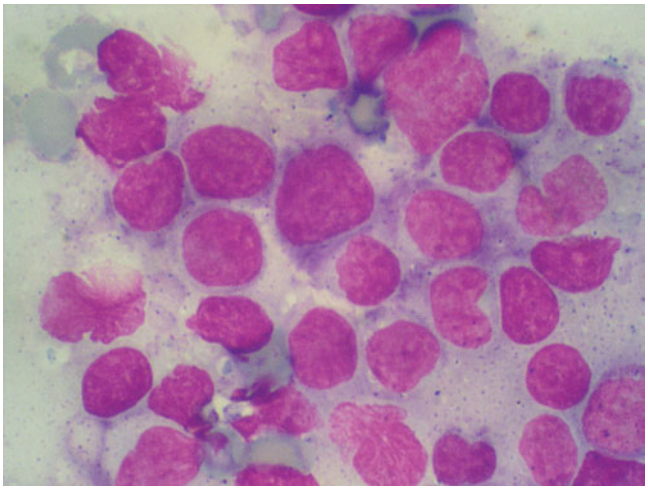


Figure 3.22 Anaplastic centrocytic or centrocytoid canine lymphoma characterised by large cells with irregularly shaped nuclei, no visible nucleoli and a small amount of pale cytoplasm.

centrocytes, a minimal amount of pale cytoplasm. In the dog, the centroblastic, polymorphic centroblastic and anaplastic centrocytic sub-types are the most frequently occurring types of lymphoma.

Lymphoblastic lymphoma

Lymphoblastic lymphoma occurs infrequently in the dog. The lymphoblast is a medium-sized, round to oval cell with a thin rim of light to moderately basophilic cytoplasm that is sometimes vacuolated. The nucleus has a fine chromatin pattern

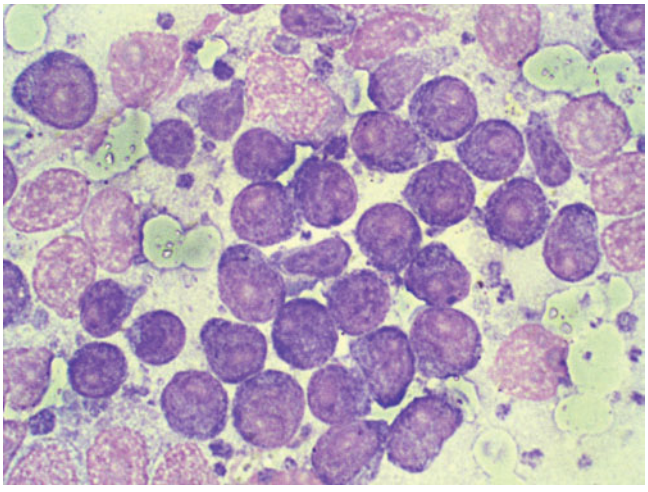


Figure 3.23 Immunoblastic lymphoma (dog). Apart from some naked nuclei in the background, a monomorphous population of immunoblasts characterised by a large centrally located nucleolus is present.

with a few small nucleoli. Many mitotic figures can be present. In humans this type of lymphoma sometimes contains 'starry sky' macrophages (Figure 3.21), which are thought to be characteristic of a certain sub-type, the so-called Burkitt's lymphoma. In the dog, however, this type of large, vacuolated macrophage that has phagocytised cellular debris can be identified in various types of lymphoma.

Immunoblastic lymphoma

If at least 50% of the cells in a preparation are immunoblasts, a diagnosis of immunoblastic lymphoma is made (Figure 3.23). Immunoblasts are large cells with moderate amount of basophilic cytoplasm and a large, round often eccentrically placed nucleus containing a single large, centrally located nucleolus. In the dog, in addition to these large immunoblasts, there is also a smaller type of immunoblast with a smaller centrally placed nucleus. In common with the larger immunoblasts, these cells have a large centrally located nucleolus.

Multilobated lymphomas

This sub-type is characterised by the presence of a large number of blasts with multilobulated nuclei (Figure 3.24).

Feline malignant lymphoma

In principle, the same sub-types of lymphoma can also be seen in the cat; however, the majority of feline lymphomas are more pleomorphic, making a diagnosis more difficult. Of particular note is the very anaplastic large cell sub-type (Figure 3.25)

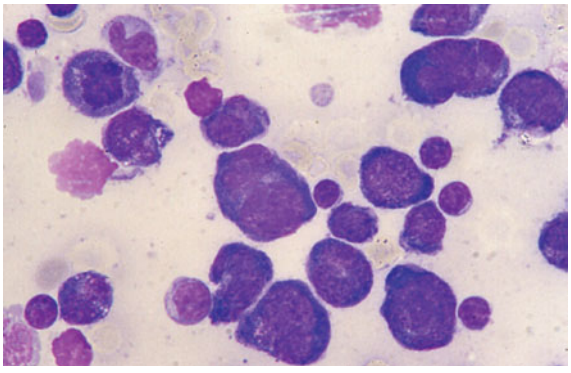


Figure 3.24 Multilobated lymphoma (dog). A population of large cells with irregular, sometimes multilobulated nuclei can be seen. In the background, several small lymphocytes are present.

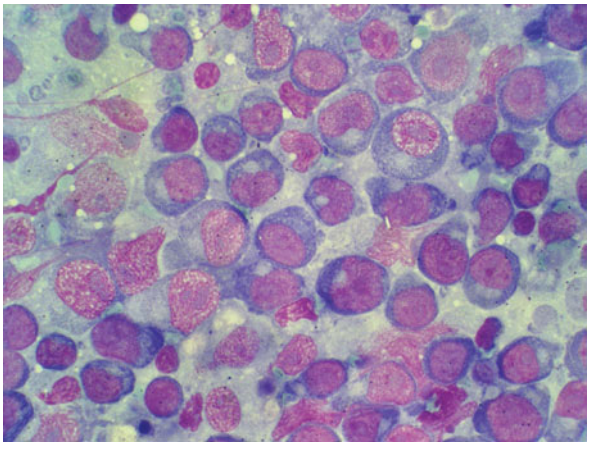


Figure 3.25 Large cell lymphoma in a cat. The nuclei are large and sometimes irregularly shaped and have a very prominent centrally located nucleolus, suggesting immunoblastic origin.

and the large granular lymphocyte (LGL) lymphoma (Figure 3.26). The latter is thought to be of T-cell (natural killer cell) origin.

Histiocytic sarcoma

The tumour cells are large cells with abundant, sometimes vacuolated cytoplasm. The nuclei are large and extremely pleomorphic (kidney-shaped, ring-shaped or 'donut'-shaped forms may be seen and some cells may be multinucleated; Figure 3.27). The nucleoli may be extremely large and prominent. Some tumour cells may show evidence of phagocytosis; however, this is not a pathognomonic feature.

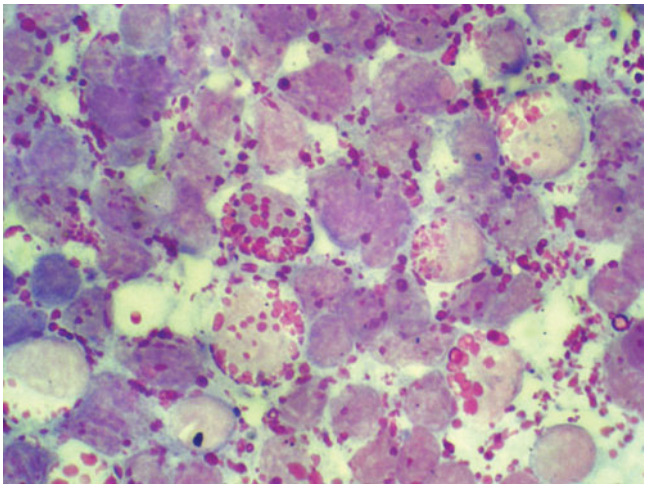


Figure 3.26 Aspirate of an abdominal lymph node in a cat with a LGL lymphoma. Note the large azurophilic cytoplasmic granules which can also be seen free in the background.

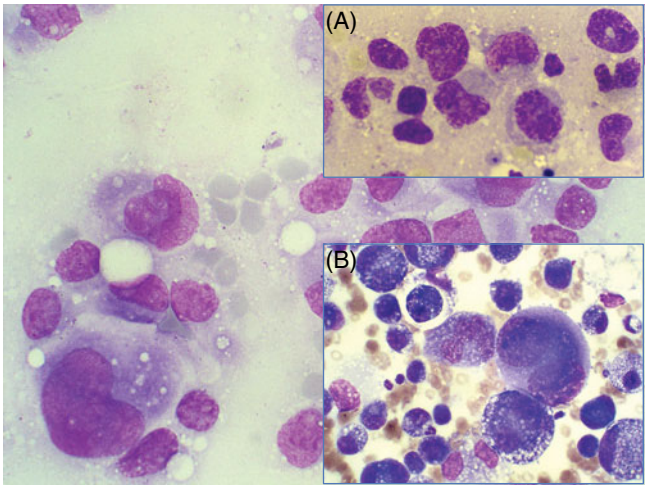


Figure 3.27 Histiocytic sarcoma in a canine lymph node. The tumour consists of large cells, with irregular, sometimes kidney-shaped, nuclei and abundant grey-blue cytoplasm with a few small vacuoles. Inset: (A) A ring-shaped or 'donut' cell. (B) A multinucleated cell.

The spleen

Splenomegaly and localised abnormalities identified by radiography or ultrasound are indications for fine-needle aspiration. The spleen is the largest lymphoid organ. It has no afferent lymphatic vessels, and its sinuses are filled with blood instead of lymph. The spleen is surrounded by a capsule from which many

trabeculae infiltrate the organ. The parenchyma is divided into white pulp and red pulp. The white pulp is lymphatic tissue which forms nodules, and the red pulp consists of erythrocytes. The spleen plays an important role in both the immune system and haematopoiesis, and the cytological content is therefore influenced by both systems.

Fine-needle aspiration is best performed under ultrasound guidance. Both the aspiration and non-aspiration techniques have been advocated (see Chapter 1). The latter technique is preferred in order to minimise blood contamination.

Benign changes

The reactive patterns are similar to those that occur in lymph nodes. A *reactive or hyperplastic spleen* (Figure 3.28) is therefore characterised by increased numbers of plasma cells and immature medium-sized or large lymphocytes. In addition, there may also be increased numbers of neutrophils, eosinophils, mast cells and macrophages. *Erythrophagocytosis*, with or without haemosiderosis, can be seen in immune-mediated or neoplastic disease (Figure 3.29). *Extramedullary erythropoiesis* or *haematopoiesis* is the most commonly encountered splenic abnormality and is characterised by the presence of red and white cell precursors and large megakaryocytes (Figure 3.30). Stromal components, e.g. connective tissue and capillaries, may also sometimes be aspirated (Figure 3.31)

Splenitis can be infectious and non-infectious in origin. It is characterised by increased numbers of neutrophils and/or eosinophils. As in the lymph node, histiocytic inflammation or hyperplasia can be caused by protozoal infections, e.g. leishmaniasis or fungal infections such as cytauxzoonosis and cryptococcosis.

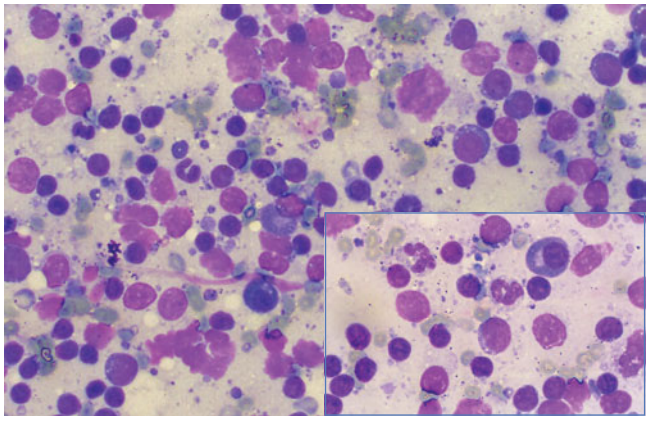


Figure 3.28 Splenic hyperplasia (dog). A mixed population of small- and medium-sized lymphocytes and some plasma cells are present. Note the streaks of pink nuclear material from damaged cells. Inset: High power magnification of the same aspirate.

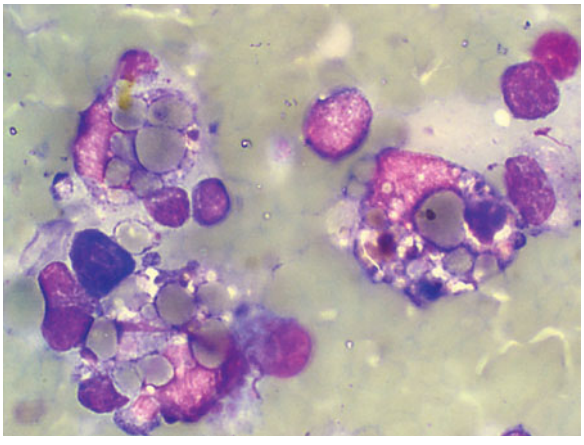


Figure 3.29 Aspirate of enlarged spleen in a dog with immune-mediated haemolytic anaemia. Several macrophages demonstrate erythrophagocytosis.

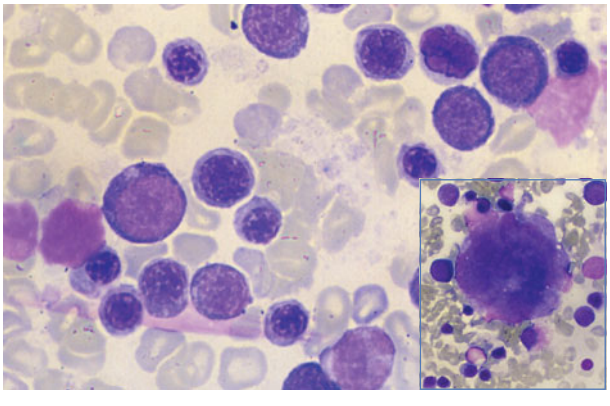


Figure 3.30 Splenic aspirate from an anaemic dog. Several erythrocyte precursors, including erythroblasts and normoblasts, are present indicating extramedullary haematopoiesis. Inset: A large megakaryocyte and erythroid precursors are present in this splenic aspirate.

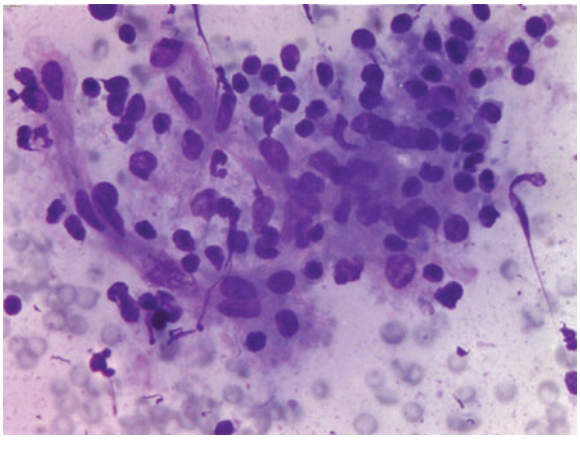


Figure 3.31 Splenic aspirate (dog). Connective tissue containing capillaries, lymphocytes and plasma cells can be seen.

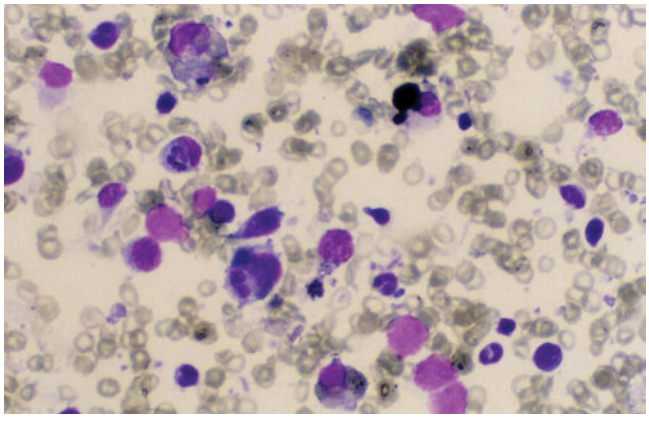


Figure 3.32 Splenic aspirate of a dog with the haemophagocytic syndrome. Several macrophages which have phagocytosed red cells and neutrophils are present.

Haemophagocytic syndrome refers to a non-neoplastic polyclonal proliferation of activated histiocytes in different organs, e.g. bone marrow and spleen. It can be associated with immune-mediated, infectious and neoplastic or myelodysplastic conditions. It also occurs as an idiopathic condition. It is characterised by the presence of bicytopenia or pancytopenia in the blood and many haemophagocytic macrophages in the bone marrow or spleen (Figure 3.32).

Neoplasia

The same haematopoietic tumours that can involve lymph nodes may affect the spleen, i.e. *malignant lymphoma* and *histiocytic sarcoma* (see the section on lymph nodes for detailed descriptions of these tumours). In addition to lymphoma, the spleen may be secondarily involved in animals with *leukaemias* (Figure 3.33). Differentiation of lymphoid leukaemias from primary lymphoma is often difficult.

Other haematopoietic tumours which can involve the spleen include *plasma cell tumours* (*extramedullary myelomas or plasmacytomas*) (Figure 3.34) and *mastocytosis* (Figure 3.35); the latter is more often seen in cats. Extramedullary myelomas and mastocytosis usually result in diffuse enlargement of the spleen and total replacement of the normal lymphoid cell population by neoplastic plasma cells and mast cells, respectively.

Mesenchymal tumours form by far the largest proportion of non-haematopoietic tumours (Figure 3.36). More than 90% of these are malignant and haemangiosarcomas are the most common. A definitive cytological diagnosis is difficult as is often the case with mesenchymal tumours. In addition, cell yield is often poor, and interpretation is hampered by severe blood contamination. Other cytological abnormalities such as reactive lymphoid hyperplasia, erythrophagocytosis and extramedullary haematopoiesis can occur concurrently. Metastatic tumours

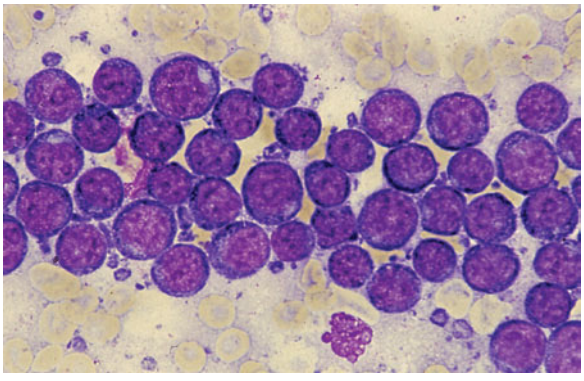


Figure 3.33 Monomorphous population of blast cells in the spleen of a dog with acute lymphoblastic leukaemia.

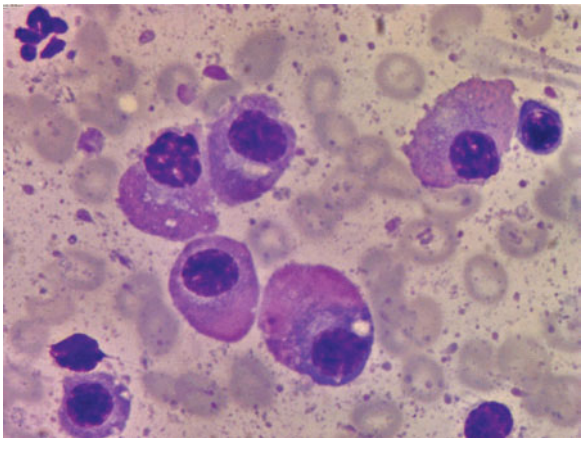


Figure 3.34 Splenic aspirate of a dog with an extramedullary multiple myeloma. Many plasma cells have the unique cytomorphologic appearance of so-called flame cells with basophilic cytoplasm bordered by a magenta rim.

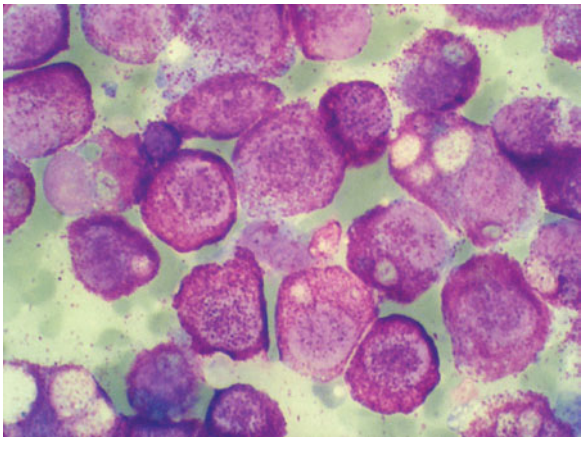


Figure 3.35 Mastocytosis of the spleen in a cat. A monomorphic population of mast cells with very fine purple cytoplasmic granules, characteristic for cats, is present.

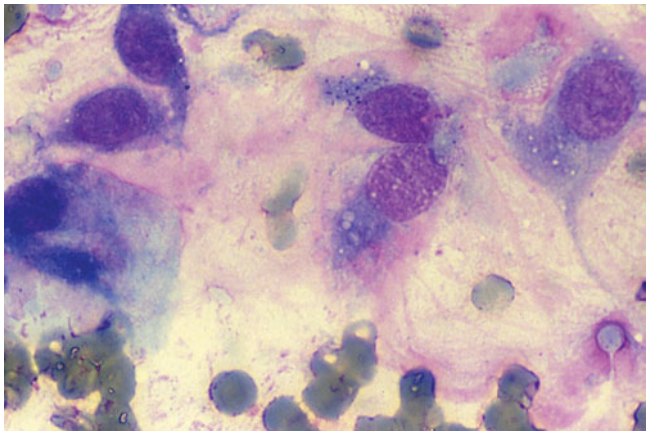


Figure 3.36 Splenic fibrosarcoma. The fibroblasts are located in a pink amorphous extracellular material.

involving the spleen are rare (metastases of carcinomas or melanomas are occasionally found in the spleen).

Thymus

The thymus varies in size and development with the age of the animal. The thymus consists of two lobes united by connective tissue. Each lobe is composed of numerous lobules, each containing cortical and medullary components. The cortex contains densely packed small lymphocytes within a network of epithelial reticular cells. In the medulla, the lymphocytes are less numerous and the reticulum is more prominent. The lymphocytes have more variation in nuclear size and cytoplasmic volume. Other cell types, e.g. mast cells, eosinophils and Langerhans cells, are also present. If biopsied, in principle the same cell population as in the lymph node can be found in the thymus.

Pathology of the thymus is usually tumour related, although rare cases of hyperplasia in the cat and thymic haemorrhages and cysts have been reported. Two types of primary neoplasia can occur in the thymus, namely, malignant lymphoma and thymoma. The malignant lymphomas are similar to those described already earlier in this chapter. Thymomas are rare tumours which originate from the epithelial cells within the thymus. Although benign and malignant thymomas have been described, the term malignant refers more to the clinical behaviour of the tumour rather than its cytological or histological appearance. Three different sub-types of thymoma can be recognised. In the *epithelial thymoma*, the reticular epithelial cells predominate, and only a few small lymphocytes may be present. The epithelial cells have large open vesicular nuclei. The cytoplasm is often absent or poorly defined. In predominantly

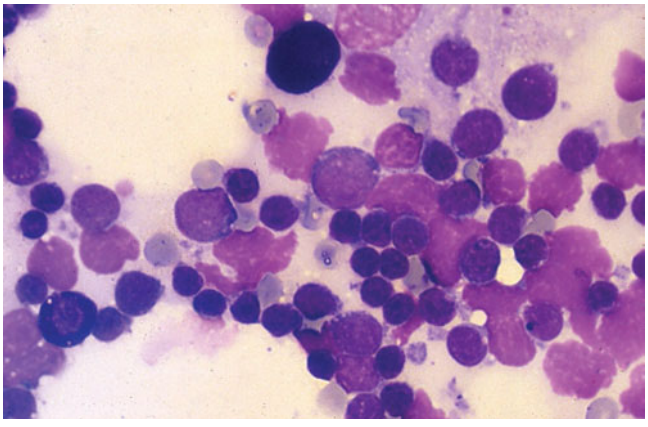


Figure 3.37 Lymphocytic thymoma (dog). A population of predominantly small lymphocytes is present. Two mast cells can also be seen.

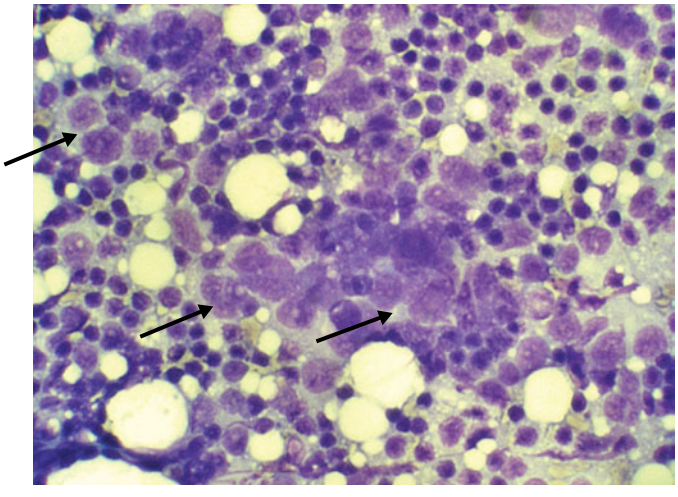


Figure 3.38 Mixed epithelial–lymphocytic thymoma in a dog. Small lymphocytes predominate, but epithelial cells with large vesicular nuclei are also present (arrows). The cytoplasmic margins of these cells are poorly defined.

lymphocytic thymomas (Figure 3.37), there are very few recognisable epithelial cells present. In *mixed epithelial–lymphocytic thymomas* (Figure 3.38), both cell types are present although the lymphocytic component is more prominent. Mast cells may also be present in some of these thymomas.

Acknowledgement

This chapter is based on: Teske, E. (2009) Clinical cytology of companion animals: Part 3. Cytology of the lymph node. *European Journal of Companion Animal Practice* **19**(2), 117–124. Reproduced with permission of FECAVA.

4 Cytology of Cutaneous and Subcutaneous Lesions

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Introduction

Most cutaneous and subcutaneous lesions can be classified sufficiently on a cytological basis to provide meaningful clinical guidance. Specimens for cytological examination are usually obtained by fine-needle aspiration or, if the lesion is ulcerated, by making impression smears (imprints).

The requirement for concurrent histological examination depends on the type of lesion under investigation. Most mast cell tumours and histiocytomas can be diagnosed definitively on the basis of cytological examination alone (although histology is usually required to grade mast cell tumours (MCT)). In contrast, most mesenchymal tumours require histological confirmation to precisely define tissue type.

Normal cell types

Normal skin is composed of several layers of squamous epithelial cells. Basal epithelial cells are round, are deeply basophilic and have a high nuclear to cytoplasmic ratio. The cells in the most superficial layer become cornified (keratinised), and their nuclei become pyknotic or disappear completely.

Aspirates from cutaneous or subcutaneous lesions may contain variable numbers of cornified squamous epithelial cells or anucleated keratinised squames (also referred to as keratin scrolls or bars; Figure 4.1). Sebaceous epithelial cells and adipocytes may also be aspirated from perilesional sebaceous glands and adipose tissue. Impression smears or scrapings of ulcerated lesions may contain only superficial cells, and secondary inflammation and/or infection may result in dysplastic changes which can mimic those associated with neoplasia (some impression smears may therefore not be truly representative of underlying pathology).

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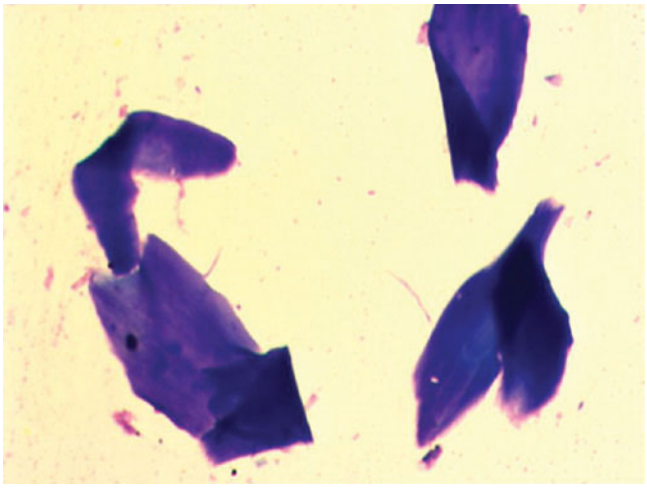


Figure 4.1 Anucleated keratinised squames (keratin scrolls).

Non-neoplastic lesions

Haematomas are large blood-filled masses usually resulting from trauma. An aspirate from a recently formed haematoma has the appearance of blood except that platelets are usually absent. As healing progresses, fibrocytes or fibroblasts and reactive macrophages may be seen (Figure 4.2).

Sialocoeles often form in the submandibular region or under the tongue. They contain clear or blood-tinged fluid which appears viscous (Figure 4.3).

Cutaneous abscesses are common in cats and are often the result of a bite wound. Anal sac abscesses commonly occur in the dog. Cytological examination reveals a large number of extremely degenerate neutrophils, many of which may contain intracellular bacteria (Figure 4.4). *Mycobacteria* spp. can cause deep-seated abscesses especially in cats. Large numbers of negatively stained rods can be seen within the cytoplasm of macrophages on a Romanowsky-stained smear (these organisms stain positively with Ziehl–Neelsen stain (see Figure 2.5, Figure 5.11 and Figure 15.2E)).

A pyogranulomatous response, characterised by a mixed population of non-degenerate neutrophils and macrophages, can be seen in aspirates from **injection site reactions** or **lick granulomas** (Figure 4.5). Variable numbers of small lymphocytes, plasma cells and mesenchymal-type cells may also be seen.

Eosinophilic plaques, granulomas or ulcers (eosinophilic granuloma complex) are especially common in cats but may also occur in dogs. These ulcerated lesions can occur on the face, trunk and thighs and occasionally in the oral cavity. When located on the upper lip, the lesions are often called 'rodent ulcers' (Figure 4.6).

Epidermal or follicular inclusion cysts occur in middle-aged or older dogs. They are usually round and well circumscribed and are often located on the dorsum or tail head (Figure 4.7; see also Figure 2.25). Rupture of the cyst can induce a secondary localised pyogranulomatous response.

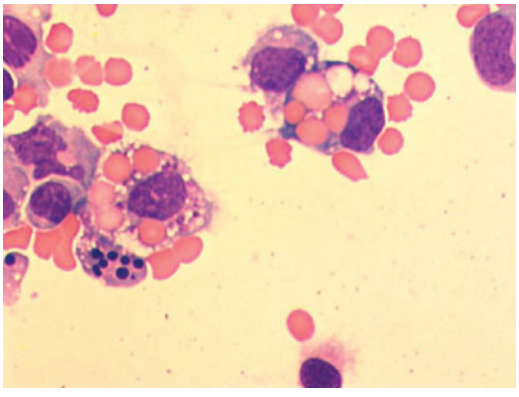
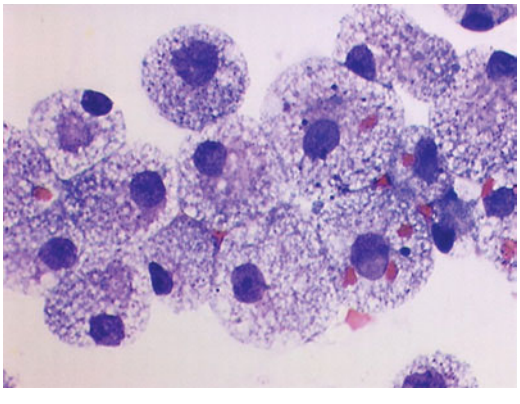


Figure 4.2 Haematoma. The reactive macrophages in this aspirate from an older lesion contain phagocytosed red cells. Source: Axiom Veterinary Laboratories Ltd.

(A)



(B)

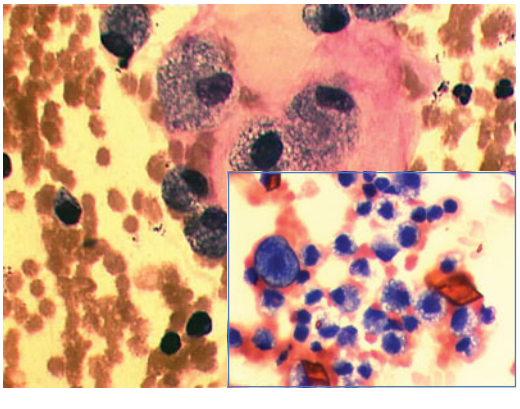


Figure 4.3 Sialocoele. (A) This aspirate contains a few red cells and large finely vacuolated mononuclear cells (these may be salivary epithelial cells or macrophages). (B) This aspirate also contains eosinophilic material which is probably saliva. Inset: Some cells contain rhombotic haematoidin crystals which represent haemoglobin breakdown product.

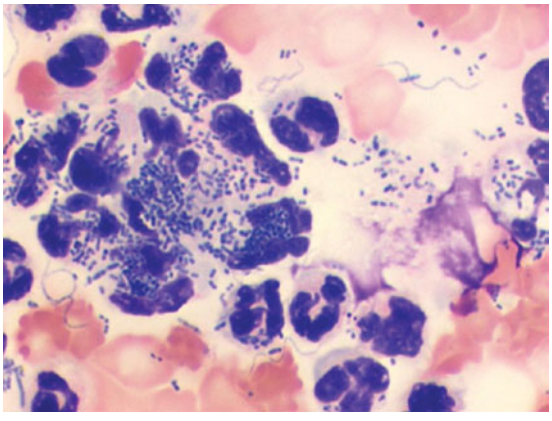


Figure 4.4 Anal sac abscess. Note the large number of degenerate neutrophils containing intracellular bacteria.

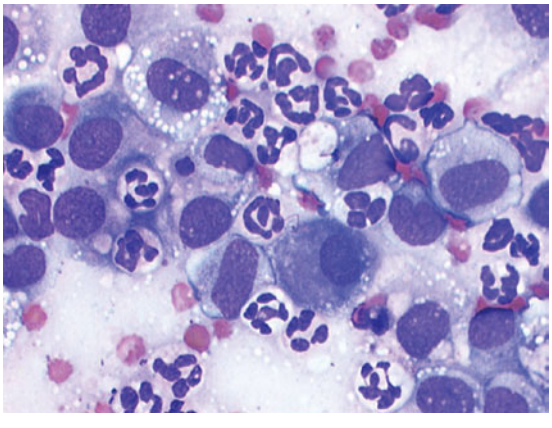


Figure 4.5 Lick granuloma. Note the mixed population of reactive macrophages and non-degenerate neutrophils consistent with a pyogranulomatous inflammatory response.

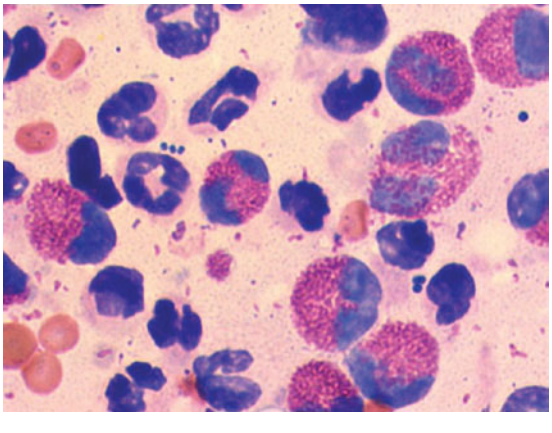


Figure 4.6 An impression smear of an eosinophilic granuloma ('rodent ulcer') on the upper lip of a cat. Eosinophils and neutrophils predominate and there is evidence of secondary bacterial infection. Although not present here, mast cells may also be seen.

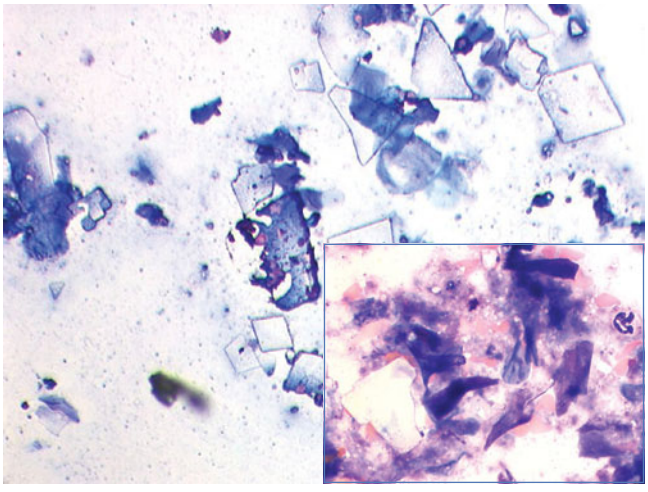


Figure 4.7 Epithelial inclusion or follicular cyst. This aspirate consists of keratin scrolls, anucleated keratinised squames and amorphous cellular debris. The large negatively stained structures are cholosterol cyrstals (these form from the degradation of cells within the cyst). Inset: Cholesterol crystals and amorphous keratinised debris. Source: Axiom Veterinary Laboratories Ltd.

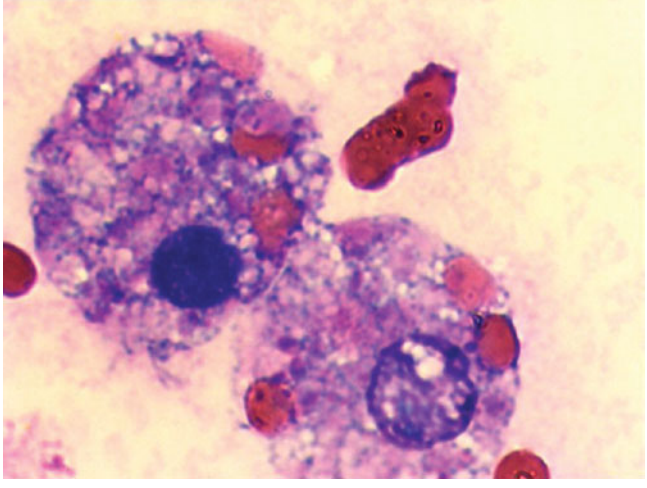


Figure 4.8 Hygroma. Red cells and two reactive macrophages containing phagocytosed red cells can be seen. Reactive fibrocytes may also be present.

Hygromas (Figure 4.8) are fluid-filled soft tissue swellings most often located on the elbows of large breed dogs. They result from repeated trauma or pressure. Aspirates from **seromas** appear similar but are usually less cellular.

Foreign body reactions (Figure 4.9) result from penetration of the skin by plant or some other inert material. The lesions are often fistulous and drain blood-tinged, sometimes purulent fluid. Cytological examination of swab smears may be useful.

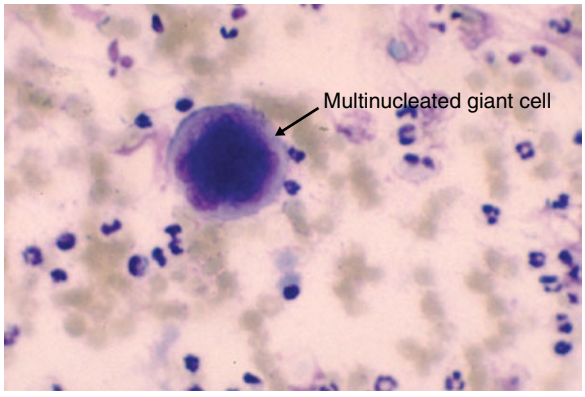


Figure 4.9 Foreign body reactions are characterised by a mixed inflammatory response consisting primarily of macrophages and lymphocytes with a variable number of neutrophils. A large multinucleated giant cell (arrow) and numerous neutrophils can be seen here. There may be evidence of a fibroblastic response and secondary infection is common.
Source: Dunn and Villiers (1998a). Reproduced with permission of BMJ Publishing Group Ltd.

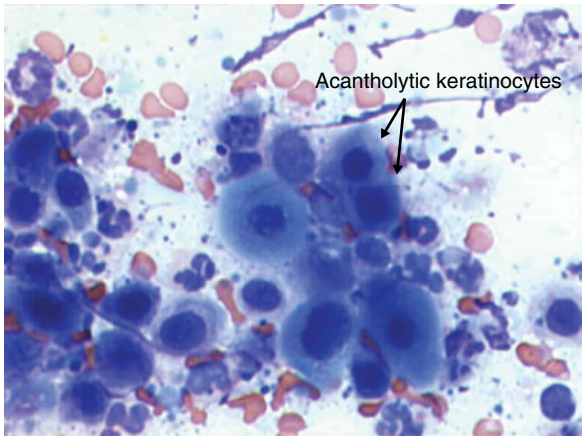


Figure 4.10 Pemphigus foliaceus. Aspirate from an intact pustule consisting of neutrophils and dark-staining acantholytic keratinocytes (arrows). Eosinophils may also be present.

Pemphigus foliaceus (Figure 4.10) is an immune-mediated skin disease characterised by the formation of pustules particularly on the head (nose and pinnae) and pads which subsequently rupture and form crusts.

Neoplastic lesions

Epithelial tumours

Basal cell tumours (**trichoblastomas**) are typically single well-circumscribed intradermal lesions which are usually benign. They are most frequently located on the head, neck and limbs. Basal epithelial cells usually predominate but there may also

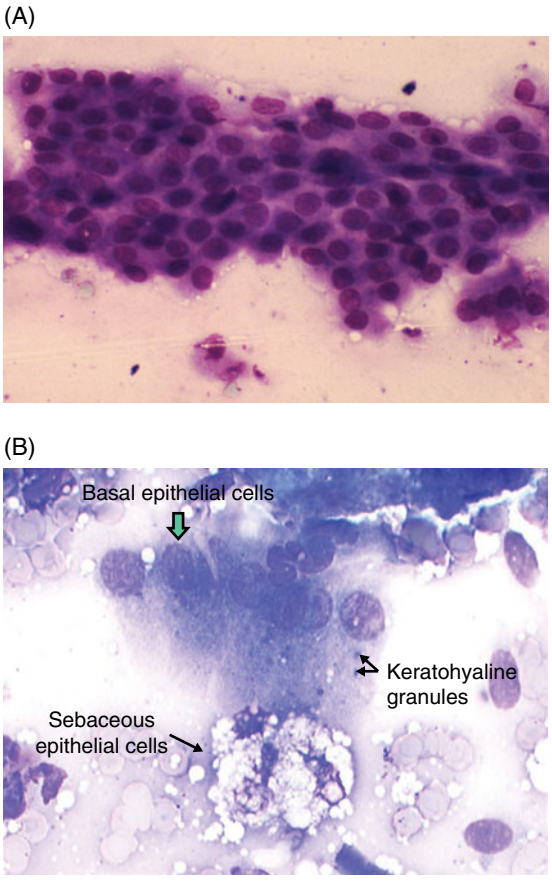


Figure 4.11 Basal cell tumour. (A) Shows a compact cluster of small basal epithelial cells. These cells are of uniform size and shape and have a high nuclear to cytoplasmic ratio. (B) Shows these cells when viewed side-on (green arrowhead). Note the keratohyaline granules (short arrows) and small cluster of sebaceous epithelial cells (long arrow). Source: Axiom Veterinary Laboratories Ltd.

be scattered sebaceous epithelial cells and keratinocytes, suggesting a degree of sebaceous or follicular differentiation (Figure 4.11). Basal cell tumours may therefore be difficult to differentiate from other adnexal tumours, and histopathology may be required. Some tumours appear pigmented if the cells contain melanin pigment granules.

Sebaceous adenomas (Figure 4.12) are often mistakenly referred to as 'warts'. They are small, usually multilobulated lesions which tend to occur most frequently on the head, neck and trunk of older dogs. **Sebaceous adenocarcinomas** are relatively rare. They may be ulcerated and poorly circumscribed and the sebaceous epithelial cells show obvious criteria of malignancy.

Squamous cell carcinomas (Figure 4.13) most frequently occur on the tips of the pinnae or nose of cats and the limbs, especially the digits, nail beds and pads, of dogs. These tumours tend to ulcerate, are locally invasive and may metastasise to the regional lymph node.

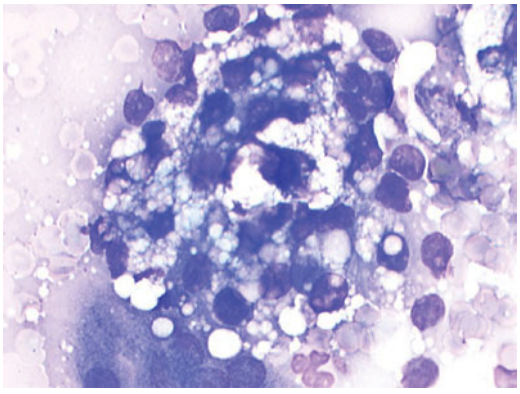


Figure 4.12 Sebaceous adenoma. These sebaceous epithelial cells contain a moderate amount of pale vacuolated cytoplasm and a poorly defined centrally located nucleus.

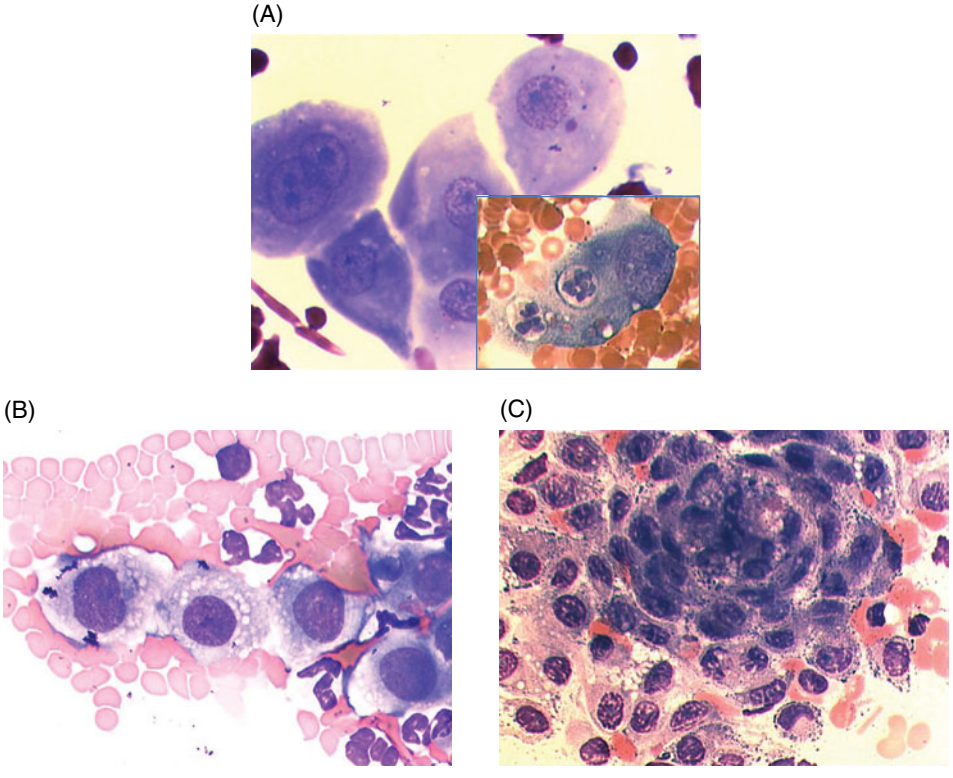


Figure 4.13 Squamous cell carcinoma. (A) This aspirate from a well-differentiated tumour consists of partially keratinised superficial squamous epithelial cells with angular cell margins. There is evidence of nuclear atypia (coarse nuclear chromatin and visible nucleoli). Inset: Some epithelial cells may contain other cell types, e.g. neutrophils (this is termed emperipolesis). (B) Less well-differentiated tumours show a more marked degree of anisocytosis and anisokaryosis, and the cells have a higher nuclear to cytoplasmic ratio and the nuclei contain visible nucleoli. Note the perinuclear vacuolation and neutrophils (squamous cell tumours often elicit an intense inflammatory response and, since the lesions are frequently ulcerated, secondary infection is common). Source: Villiers and Blackwood (2005). Reproduced with permission of BSAVA. (C) The cells may be concentrically arranged to form the keratin pearls seen on histology sections.

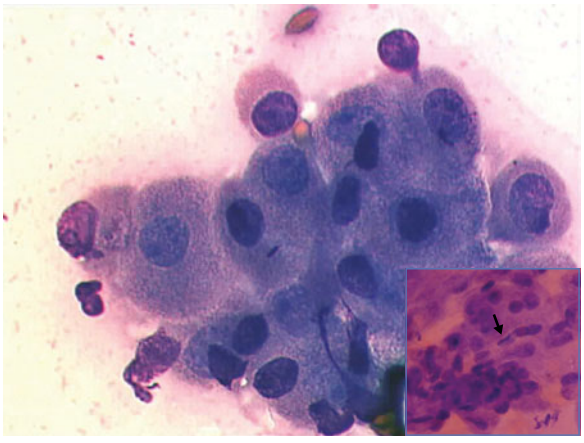


Figure 4.14 Perianal adenoma. The hepatoid cells have abundant granular pinkish/blue cytoplasm and a round nucleus containing one or more prominent nucleoli. Inset: Smaller basophilic reserve cells with a flattened nucleus (arrow) are present between the hepatoid cells.

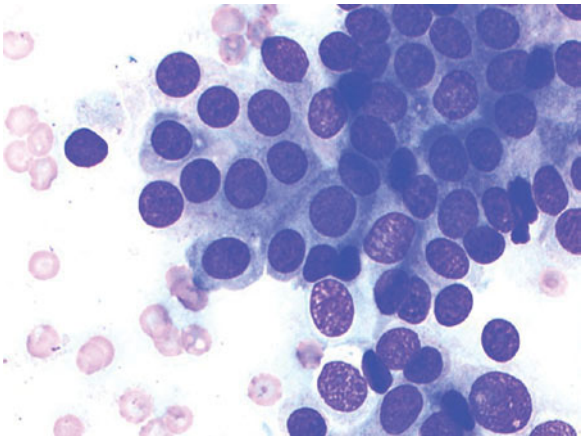


Figure 4.15 Apocrine gland carcinoma of the anal sac. Cells from these tumours usually exfoliate in large sheets or clusters, have a moderate amount of pale basophilic cytoplasm, a round or oval-shaped nucleus and a variable nuclear to cytoplasmic ratio. Cytoplasmic margins are poorly defined. Malignant characteristics are usually present but may not be as pronounced as with carcinomas involving other tissues. Source: Axiom Veterinary Laboratories Ltd.

Perianal adenomas (Figure 4.14) are usually seen in intact male dogs (occasionally in females), and most are located adjacent to the anus (occasionally on the tail, prepuce and perineum). The cells exfoliate in clusters and are hepatoid in appearance (i.e. they resemble hepatocytes). **Perianal adenocarcinomas** are rarely encountered. They tend to show more obvious criteria of malignancy, but some may appear well differentiated and cannot reliably be differentiated from adenomas on a cytological basis.

Apocrine gland adenocarcinomas of the anal sac (Figure 4.15) arise from the glands in the walls of the anal sacs. They have a greater tendency to occur in older female dogs. Adenocarcinomas of the anal sac are malignant and have often

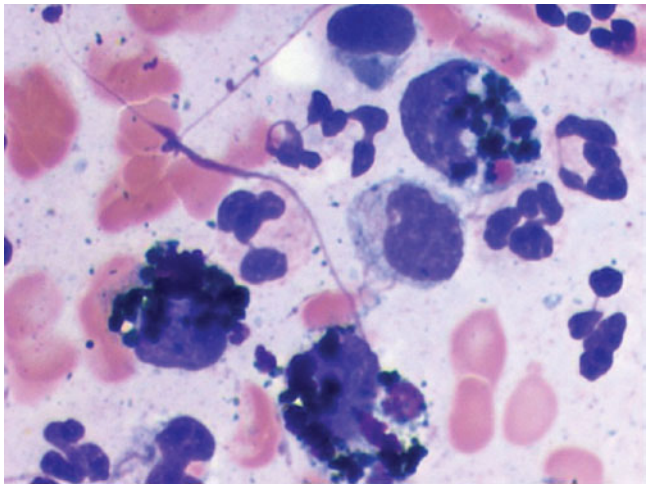


Figure 4.16 Ceruminous gland adenocarcinoma of the external ear canal. The neoplastic cells contain globules of black secretory material which obscure morphological detail. Neutrophils are also present.

spread to the sublumbar lymph nodes by the time they are diagnosed. For this reason it is important they are differentiated from perianal adenomas. More than 50% of cases have a paraneoplastic hypercalcaemia.

Ceruminous gland carcinomas (Figure 4.16) arise from the sweat glands in the external ear canal. They more commonly occur in cats. They are locally invasive and may metastasise to the regional lymph nodes.

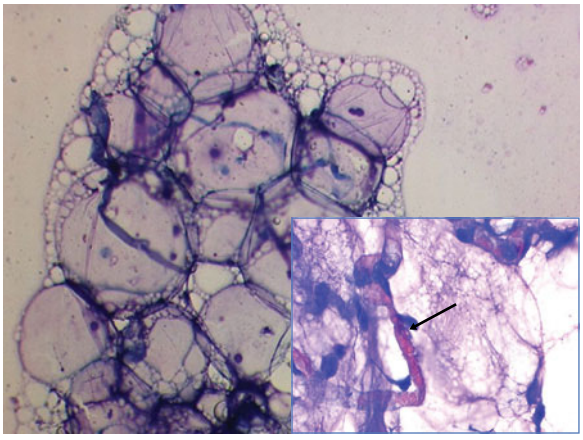
Mesenchymal tumours

Lipomas (Figure 4.17). Aspirates from lipomas have a greasy appearance and consist of numerous fat droplets which can be stained with Sudan red (these fat droplets disappear if alcohol fixative is used). **Liposarcomas** are rare tumours. They have been reported most frequently on the ventral abdomen. They are firm and poorly demarcated and adhere to underlying tissue. Some may ulcerate. The cells show obvious malignant features.

Haemangiopericytoma is an example of a *perivascular wall tumour* that arises from cells that line blood vessels (Figure 4.18). They occur in dogs and are most frequently located on the limbs. They are locally invasive and rarely metastasise but recurrence is common.

Cutaneous haemangiomas are benign tumours arising from vascular endothelial cells. They are more common in dogs than cats. Small elongated endothelial cells are often present in very low numbers and may be difficult to identify because of blood contamination. **Cutaneous haemangiosarcomas** (Figure 4.19) are rare tumours of older dogs and cats. Some appear well differentiated and cannot be reliably differentiated from haemangiomas. In contrast, the cells aspirated from anaplastic haemangiosarcomas show obvious malignant features.

(A)



(B)

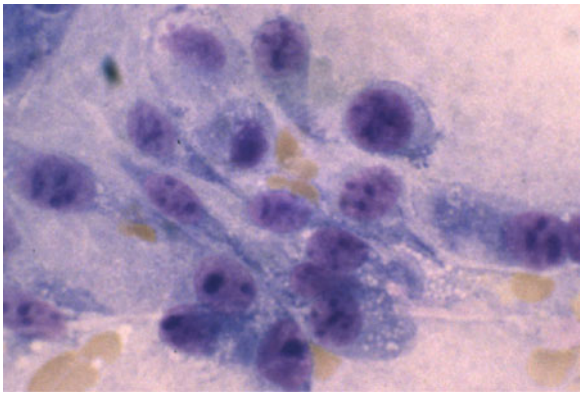


Figure 4.17 Lipoma. (A) Intact adipocytes may be aspirated either singly or in large clusters. Adipocytes have abundant clear cytoplasm and a small dark nucleus which is compressed on the periphery of the cell. Inset: Small blood vessels may also be seen (arrow). (B) Liposarcoma. Note the plump spindle-shaped mesenchymal cells with numerous variably sized cytoplasmic lipid vacuoles, a vesicular nucleus and multiple nucleoli. Source: Axiom Veterinary Laboratories Ltd.

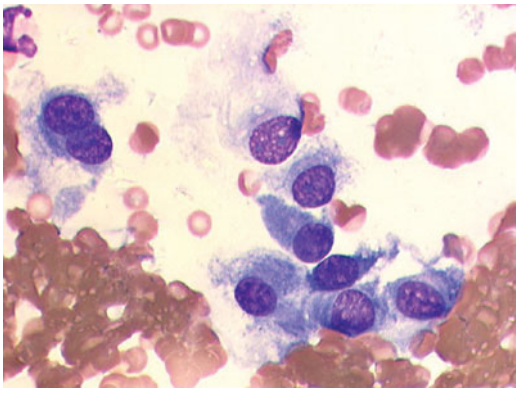


Figure 4.18 Haemangiopericytoma (perivascular wall tumour). These plump spindle cells have an oval-shaped nucleus and basophilic cytoplasm which may be vacuolated. Cytoplasmic margins are poorly defined and nuclear chromatin has a coarse stippled appearance. Source: Axiom Veterinary Laboratories Ltd.

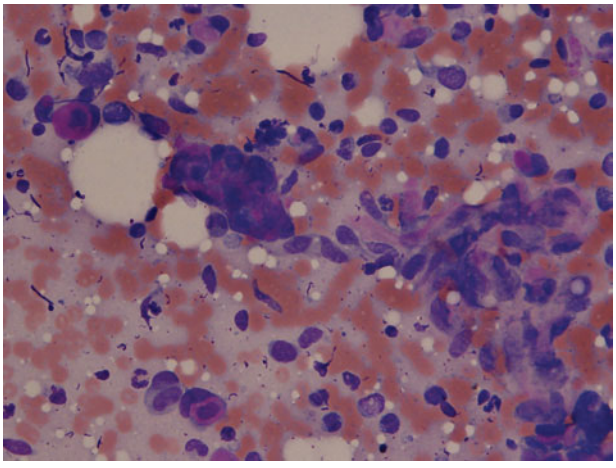


Figure 4.19 Cutaneous haemangiosarcoma. Note the spindle-shaped cells and eosinophilic matrix material. A moderate degree of anisocytosis and anisokaryosis is evident.
Source: Emma Dewhurst/Axiom Veterinary Laboratories Ltd. Reproduced with permission.

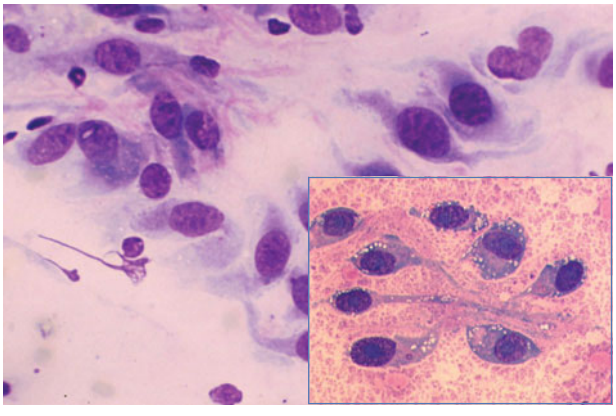


Figure 4.20 Fibrosarcoma. Cells from less well-differentiated fibrosarcomas are plumper and have a higher nuclear to cytoplasmic ratio. The nuclei possess multiple nucleoli and the cells are associated with eosinophilic collagenous material. Inset: Vaccine-induced fibrosarcoma in a cat. Source: Dunn and Villiers (1998a). Reproduced with permission of BMJ Publishing Group Ltd.

There may be evidence of erythrophagocytosis and/or chronic haemorrhage (e.g. haemosiderin-laden macrophages may be present).

Fibromas are relatively rare tumours and are composed of well-differentiated spindle cells with an oval-shaped nucleus and elongated cytoplasmic tails. **Fibrosarcomas** (Figure 4.20) are more common in the cat and have a predilection for the oral cavity, head and limbs. These tumours are poorly circumscribed and may ulcerate. Aspirates taken from reactive granulation tissue, fibromas and well-differentiated fibrosarcomas may appear remarkably similar, making cytological differentiation difficult. Feline sarcoma virus causes multiple fibrosarcomas in young cats. Vaccine-induced fibrosarcomas are locally invasive and slow to metastasise.

Melanoma (Figure 4.21). Benign and malignant melanomas occur more frequently in dogs than cats (most melanomas in the dog are benign).

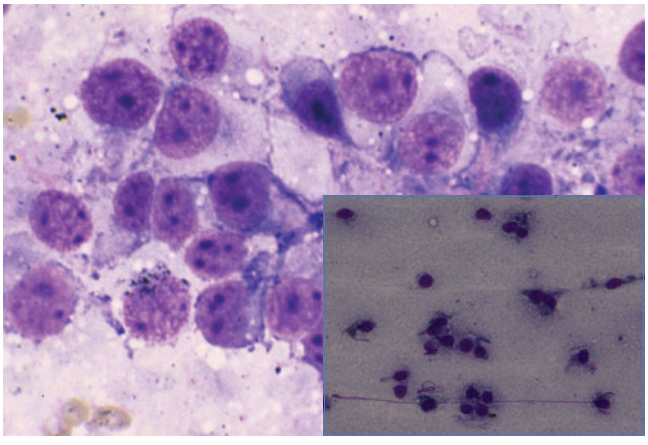


Figure 4.21 Malignant melanoma. Cells from poorly differentiated melanomas may contain relatively scant melanin pigment granules, and the cells usually have large nuclei containing several large irregular nucleoli. A moderate degree of anisocytosis and anisokaryosis is evident. Inset: These cells from a benign melanoma are of uniform size and shape. Note the abundant free melanin pigment granules in the background. Source: inset - Dilini Thilakaratne, James Cook University, Australia. Reproduced with permission.

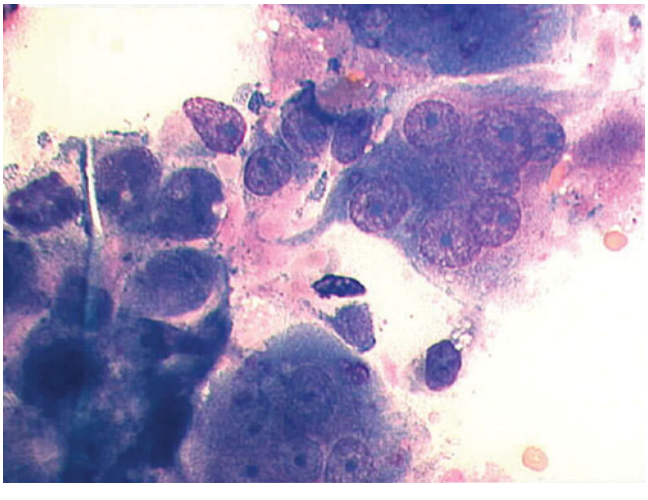


Figure 4.22 Anaplastic giant cell (soft tissue) sarcoma. Plump mesenchymal cells and large multinucleated 'giant' cells are associated with eosinophilic matrix material. These cells are showing multiple criteria for malignancy.

They appear as raised dark brown/black well-circumscribed masses. The cytological appearance of cells in aspirates from melanomas is variable (some may resemble those seen in discrete round cell tumours). Melanin pigment granules can usually be seen within the cells. Amelanotic melanomas are quite rare but with careful examination melanin granules can usually be seen in a few cells.

Anaplastic giant cell (soft tissue) sarcomas occur most frequently on the limbs of older dogs and cats and involve subcutaneous tissue or skeletal muscle (Figure 4.22). They are firm, poorly circumscribed and attached to underlying structures.

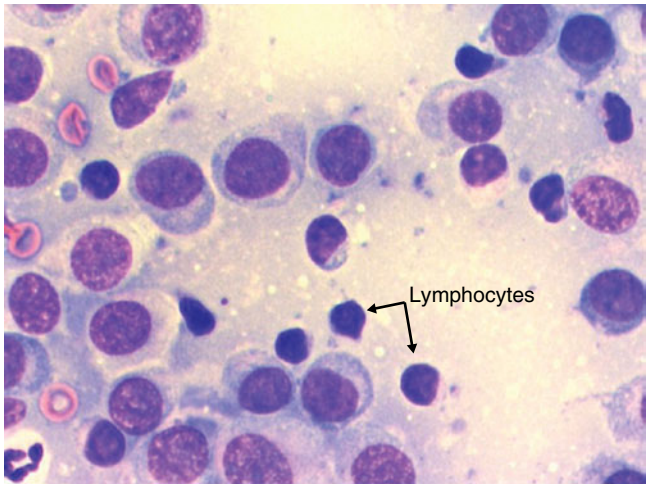


Figure 4.23 Canine histiocytoma. These cells have a moderate amount of pale basophilic cytoplasm. The nuclei are round, oval or indented and some nuclei contain one or more poorly defined nucleoli. Despite the fact that these tumours are benign, a mild to moderate degree of anisocytosis and anisokaryosis is evident. An increased number of lymphocytes (arrows) can be seen as these lesions start to regress.

Round cell tumours

Canine histiocytoma (Figure 4.23). Most histiocytomas occur in young dogs less than 5 years of age. They are small raised, well-circumscribed hairless tumours which can occur on the head (especially the pinnae), paws, limbs and trunk. Some may ulcerate and most regress spontaneously.

Other histiocytic neoplasms which can involve the skin include cutaneous histiocytosis, systemic histiocytosis and malignant histiocytosis (or histiocytic sarcoma). **Canine cutaneous histiocytosis** is characterised by the development of multifocal lesions similar to those described for cutaneous histiocytoma. **Systemic histiocytosis** occurs in young adult male Bernese mountain dogs but has also been reported in other breeds. The skin is consistently affected but regional lymph nodes and ocular sites, e.g. conjunctiva, may also be involved. The histiocytes in systemic histiocytosis do not show the malignant features seen in malignant histiocytosis. Aspirates may also contain moderate numbers of lymphocytes and neutrophils. **Malignant histiocytosis** is a rapidly progressive disease that occurs in older Bernese mountain dogs and less frequently in other breeds. It has also been reported in cats. Malignant histiocytes may infiltrate the spleen, lymph nodes, lung and bone marrow; the skin is only occasionally involved.

Canine Mast cell tumour (MCT) (Figure 4.24) occur most frequently on the trunk and limbs. Tumours located on the perineum, prepuce or digits tend to be more aggressive. MCT in cats tend to occur on the head, neck and limbs, and most solitary lesions are benign. Multiple 'histiocytic-type' MCT which may regress spontaneously have been reported in young Siamese cats. Histopathological examination is required to grade these tumours. A more accurate prognosis may

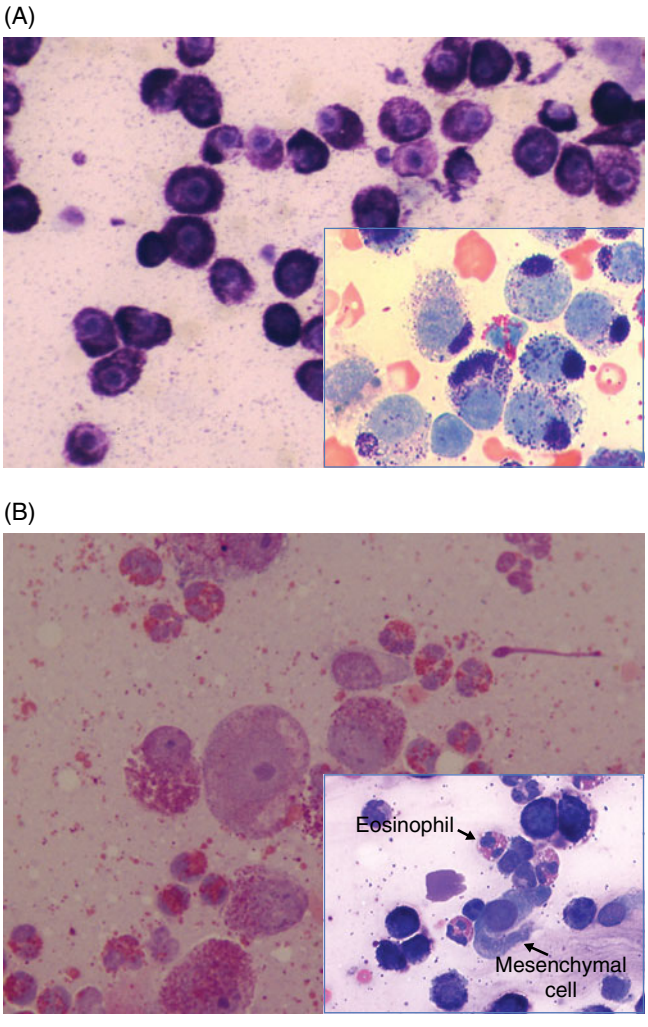


Figure 4.24 Mast cell tumour (MCT). (A) Mast cells contain azurophilic cytoplasmic granules which may obscure nuclear detail. The degree of cytoplasmic granularity is quite variable. Some rapid water-soluble Romanowsky stains may not stain mast cell granules. Inset: Occasionally, the mast cell granules are concentrated at one pole of the cell. (B) Mast cells from less well-differentiated MCT tend to contain fewer granules, and the cells demonstrate more obvious blastic features (note the prominent nucleoli and increased number of eosinophils). Toluidine blue can be used to stain the granules in these poorly differentiated cells. Inset: Aspirates from canine MCT often contain eosinophils and mesenchymal cells (arrowed). Source: Axiom Veterinary Laboratories Ltd.

be obtained by cell proliferation analysis (such a panel is performed by the Diagnostic Center for Population and Animal Health at Michigan State University; http://www.animalhealth.msu.edu). This panel evaluates three markers (Ki67 protein, proliferating cell nuclear antigen (PCNA) and argyrophilic nucleolar

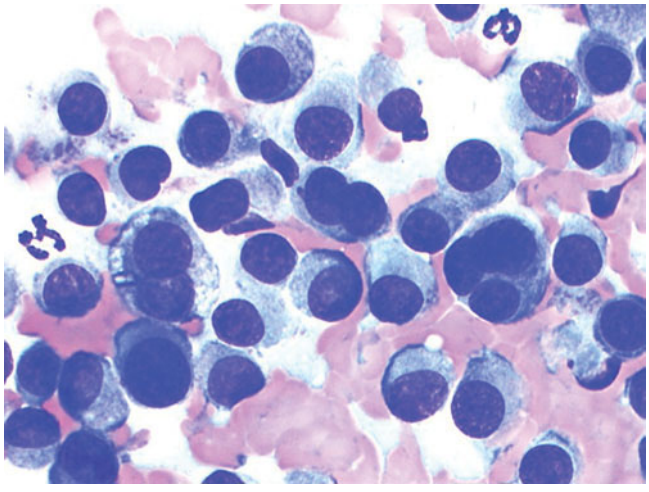


Figure 4.25 Plasmacytoma. The cells have a variable amount of dark basophilic cytoplasm and a round eccentrically placed nucleus with a coarse chromatin pattern. A moderate degree of anisocytosis, anisokaryosis and variation in nuclear to cytoplasmic ratio is evident. Binucleated and multinucleated cells are present. Pink amorphous material (amyloid) may be intimately associated with the plasmacytoid cells.

organiser regions (AgNORs)). It also includes a PCR test to check for the c-kit mutation in highly aggressive MCT which may respond to tyrosine kinase inhibitors, and KIT expression in the neoplastic mast cells is assessed by immunohistochemistry labelling.

Plasmacytomas have been reported on the skin (especially the digits and ears), oral cavity, gingiva and tongue of older dogs (these tumours are rare in cats). Although the cells can appear extremely pleomorphic, most plasmacytomas are benign (Figure 4.25).

Cutaneous lymphoma may arise primarily in the skin or occasionally as a manifestation of generalised lymphoma. Cutaneous lymphoma may present as solitary or multiple nodules or plaques or as a generalised pruritic exfoliative dermatitis. It can be classified as epitheliotrophic (also known as *mycosis fungoides*) or non-epitheliotrophic (differentiation requires immunocytochemistry; Figure 4.26). Epitheliotrophic lymphoma is the more common form and is usually of T-cell origin. The neoplastic cells infiltrate the epidermis and adnexa.

Transmissible venereal tumours (TVT) are most frequently seen in young roaming, sexually active dogs (Figure 4.27). In the United Kingdom they are usually only seen in dogs imported from temperate climates. The lesions are usually located on the external genitalia or oronasal area and are poorly circumscribed, ulcerated and haemorrhagic. Superficial secondary bacterial infection is common. The lesions may spontaneously regress.

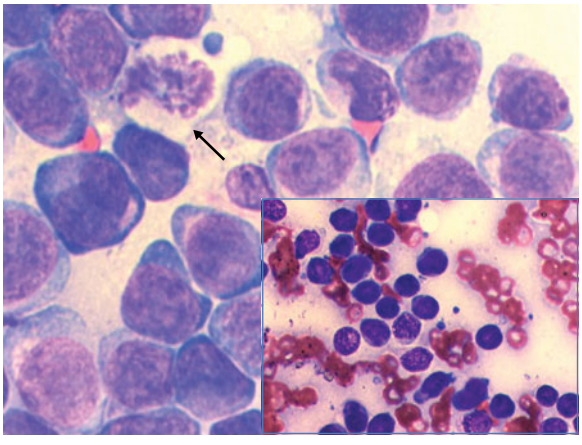


Figure 4.26 Cutaneous lymphoma (epitheliotrophic lymphoma). The neoplastic lymphoid cells vary in size from small to large with round, indented or convoluted nuclei which may contain visible nucleoli. The cells possess a variable amount of pale basophilic cytoplasm. Note the mitotic figure (arrow). Occasionally, in addition to generalised skin involvement, the animal may also be leukaemic with circulating T-cells in the blood (this is known as Sézary syndrome). Inset: The non-epitheliotrophic form is characterised by infiltration of the subcutis and dermis with lymphocytes with more typical morphology. Source: Axiom Veterinary Laboratories Ltd.

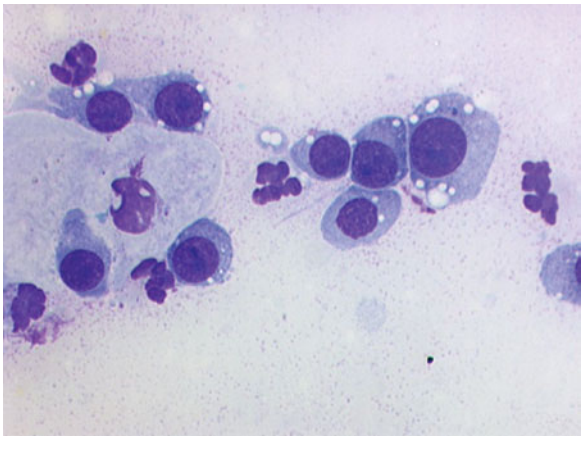


Figure 4.27 Transmissible venereal tumour. The cells from TVT have a moderate amount of pale basophilic cytoplasm. Note the small discrete cytoplasmic vacuoles. The nuclei are round with coarse nuclear chromatin and poorly defined nucleoli. Mitotic figures and a mixed inflammatory cell population may be present. Source: Axiom Veterinary Laboratories Ltd.

5 Cytology of the Respiratory Tract

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Introduction

Cytological interpretation of samples retrieved from the respiratory tract should take into account the history, clinical findings and any radiographic abnormalities. Failure to recognise a non-representative specimen, for example, one that is contaminated by oropharyngeal material during collection, may result in misleading cytological interpretation and provide meaningless culture results.

Collection techniques

Nasal flush

A nasal flush is performed under general anaesthesia. A soft rubber catheter is passed in a caudal direction through the external nares. Ten millilitres of sterile normal saline is flushed into the nasal cavity with a 20 ml syringe with alternating positive and negative pressure. At the same time the catheter is moved backwards and forwards to ensure maximum retrieval of tissue and cells. The aspirated fluid is transferred to an EDTA tube to preserve cell morphology. After centrifugation smears of the sediment are prepared for cytological examination (samples for bacteriology should be transferred to a sterile plain glass tube). A retrograde technique whereby a Foley catheter is passed over the soft palate into the nasopharynx has also been described (readers are referred to standard cytology texts for a fuller description of this technique).

Aspiration of suspected intranasal masses can be performed using a 6–10Fr polypropylene urinary catheter with the end cut at a 45° angle to provide a cutting edge, or alternatively a 20–22 G needle or Tru-Cut biopsy needle can be used if the mass can be visualised endoscopically. Care must be taken not to penetrate the cribriform plate (the correct catheter length can be gauged by measuring the distance from the external nares to the medial canthus of the eye). Once the catheter meets resistance, it is introduced into the mass, and negative pressure is maintained with the

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syringe in order to collect solid tissue fragments from which imprints or squash preparations are made (or tissue fragments can be submitted for histological examination).

In the author's opinion, cytological examination of nasal discharge is rarely helpful unless an obvious causative agent, e.g. *Aspergillus* sp. or *Cryptococcus neoformans*, is identified.

Transtracheal wash and bronchoalveolar lavage

Cytological examination of the trachea, bronchi and alveoli can be achieved by a tracheal wash (TW) and bronchoalveolar lavage (BAL). Interstitial lesions may not be diagnosed using these techniques. Transthoracic fine-needle aspiration may be a more appropriate technique for the investigation of interstitial masses identified by radiography or ultrasonography.

TW or BAL is indicated when there is radiographic evidence of tracheobronchial or pulmonary disease resulting in a chronic cough or respiratory distress. It may be necessary to perform a full cardiological investigation beforehand to exclude cardiogenic causes for the respiratory signs or radiographic lesions observed. TW and BAL are useful techniques for diagnosing hypersensitivity reactions, inflammation/infection (bacteria, fungi, parasitic larvae and protozoa) and neoplasia.

Both these diagnostic techniques induce a neutrophilic inflammatory response which peaks around 24 h after the procedure is completed. If the procedure has to be repeated, this should be done after 48 h has lapsed since any wash-induced inflammatory changes may complicate cytological interpretation.

Rare complications arising from these procedures include subcutaneous emphysema, pneumomediastinum, haemorrhage, hypoxia and needle tract infection.

Transtracheal wash

This technique is easier to perform in large dogs and usually does not require anaesthesia (light sedation may be necessary in small dogs and cats). The cough reflex remains intact and the sample is less likely to be contaminated by oropharyngeal material.

The skin is clipped and aseptically prepared. The subcutaneous tissue is then infiltrated with local anaesthetic. An 18 G jugular catheter or 14 G needle with 3.5Fr urinary catheter is inserted through the cricothyroid ligament, and 1–2 ml of normal saline/5kg BW is expelled from the attached syringe and aspirated immediately. The use of warmed saline may help to prevent airway constriction during sampling. The aspirated fluid should be collected into EDTA (cytology) and sterile plain tubes (bacteriology). A gauze wrap applied over the puncture site may help to prevent the formation of subcutaneous emphysema.

Bronchoalveolar lavage

This is the preferred technique for collecting a representative sample from the lower airways for cytological examination from small dogs and cats. The drawbacks are that general anaesthesia is required and there is a greater risk of oro-

pharyngeal contamination of the sample unless the endotracheal tube is passed directly into the trachea, avoiding contact with the oropharynx and arytenoid cartilages. If possible, the depth of anaesthesia should be such that it does not inhibit the cough reflex.

A long 14 G urinary catheter is passed down the endotracheal tube (the catheter must be longer that the endotracheal tube by at least 5–7.5 cm). One to two millilitres of normal saline/5kg BW is flushed through the catheter and aspirated immediately. A larger volume of saline, e.g. 1–3 ml/kg BW, may help to negate the catheter dead space which may be a significant factor particularly in small animals.

Similar samples can also be collected through a rigid or fiberoptic bronchoscope. This allows for visualisation of the bronchi and different collection sites, and biopsies can be collected if necessary.

Transthoracic fine-needle aspiration

Transthoracic fine-needle aspiration is a useful technique for the investigation of discrete and, to a lesser extent, diffuse interstitial lesions particularly if a mass is located adjacent to the thoracic wall although it may potentially give rise to more complications, particularly pulmonary laceration, haemorrhage and pneumothorax. The technique can be performed with the animal standing or in sternal recumbency preferably under ultrasound guidance. Sedation may be required if the animal is struggling or showing signs of severe respiratory distress.

Local anaesthetic is injected into the anterior edge of the intercostal space immediately caudal to the rib. With diffuse interstitial lesions the right diaphragmatic lung lobe is generally aspirated between the seventh and ninth ribs one-third of the distance down from the vertebral bodies to the costochondral junction.

Lesions close to the thoracic wall may be aspirated using a 22–25 G × 5 cm needle attached to a 5 ml or 10 ml syringe; deeper lesions may require a longer 22 G spinal needle to reach the site. The needle is inserted through the costochondral space at a 90° angle to the thoracic wall. Once the thoracic cavity has been entered, negative pressure is applied to the syringe and the needle is advanced into the lesion. The chances of obtaining an adequate sample are maximised if the needle is withdrawn and advanced two or three times in a slightly different direction maintaining negative pressure at all times. The needle is then withdrawn and the negative pressure is released just before the needle exits the thoracic cavity. Only a small amount of material may be present in the needle or the hub of the syringe, and this is expelled onto a glass slide. A smear is prepared in the usual way.

Cytological evaluation

In the author's experience, total and differential cell counts are of minimal diagnostic value. The cells are concentrated by centrifugation (5 min at 1000–1500 rpm). The sediment is re-suspended in a drop of supernatant and a smear prepared using the line concentration or blood film technique. A squash preparation may be helpful if the wash contains flocculent tissue flecks.

Nasal cytology

Normal cell types

Ciliated columnar epithelial cells predominate with smaller numbers of squamous epithelial cells (Figure 5.1). Clusters of mature lymphocytes originating from nasal associated lymphoid tissue may also be seen. Large numbers of bacterial organisms (cocci and rods) are usually present and constitute the normal microflora of the nasal cavity.

Inflammation/infection

Allergic rhinitis and lymphoplasmacytic rhinitis are allergic conditions characterised by increased numbers of eosinophils and lymphocytes and plasma cells, respectively. Primary bacterial rhinitis rarely occurs and more often is secondary to some other underlying pathological process, e.g. neoplasia, foreign bodies such as grass awns, dental disease and viral or fungal infection. In response to an intense inflammatory response, normal epithelial cells may show dysplastic changes which, if pronounced, can mimic those associated with neoplasia (Figure 5.2). Bacterial rhinitis, either primary or secondary, is characterised by large numbers of degenerate neutrophils, many of which contain ingested bacterial organisms. Fungal rhinitis can be either a primary or a secondary opportunist infection. The most common pathogens in the dogs are *Aspergillus* spp. (see Figure 15.3I) and *Penicillium* spp.; cryptococcosis is more common in cats (see Figure 15.3 F). Fungal infections can elicit a granulomatous or pyogranulomatous inflammatory response, but there is often evidence of a concurrent purulent or suppurative inflammatory component associated with secondary bacterial infection.

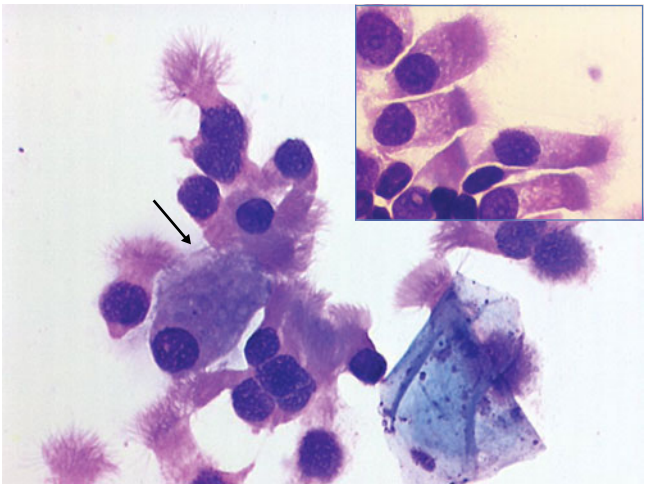


Figure 5.1 Numerous ciliated columnar epithelial cells. A goblet cell (arrow) and a single keratinised squamous epithelial cell covered in bacteria are also present. Inset: BAL (cat). Ciliated columnar epithelial cells. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

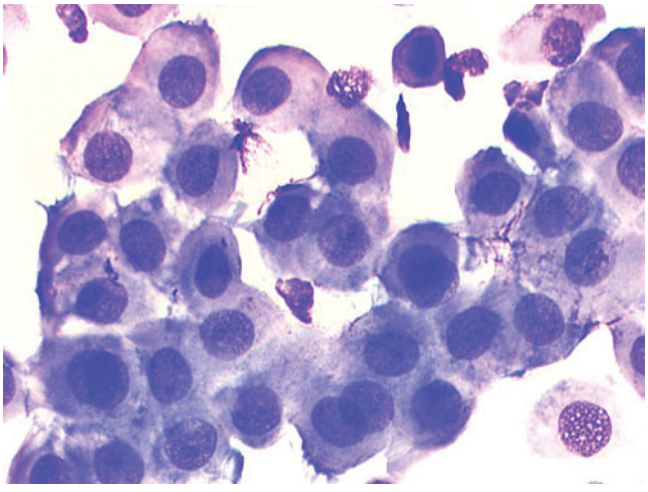


Figure 5.2 Nasal flush (canine). These dysplastic epithelial cells are of uniform size and shape, but nuclear chromatin has a coarse stippled appearance and the nuclei contain one or more nucleoli. Two binucleated cells are also present.

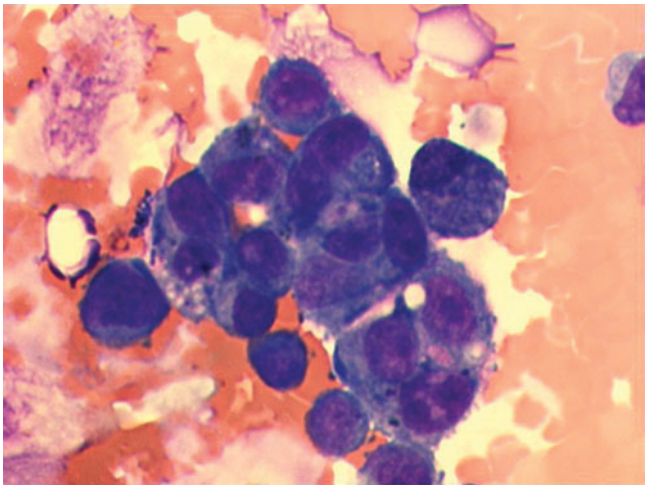


Figure 5.3 Nasal adenocarcinoma. The epithelial cells have dark basophilic cytoplasm. A moderate degree of anisocytosis, anisokaryosis and variation in nuclear to cytoplasmic ratio is evident. The nuclei contain large prominent nucleoli which vary in size and shape.

Neoplasia

Adenocarcinomas are relatively common in dogs and cats (Figure 5.3). Squamous cell carcinomas involving the nasal cavity are more common in cats. The cytological appearance of these tumours is similar to adenocarcinomas and squamous cell carcinomas located at other sites. Tumours of mesenchymal origin of the nasal cavity are rare (fibrosarcomas, osteosarcomas and chondrosarcomas occur occasionally). As

with most mesenchymal tumours, histological examination of a biopsy specimen is usually required to precisely define tissue type. The radiographic appearance and location of the lesion may also provide a few pointers. Lymphoma is the most common round cell tumour to involve the nasal cavity in dogs and cats. Again, the cytological appearance of nasal lymphoma resembles that seen at other sites. Anaplastic nasal carcinomas also have a round cell appearance, but the cells tend to be much larger.

Cytology of the trachea and bronchial tree

Normal cell types

Ciliated columnar epithelial cells line the trachea and bronchi (Figure 5.1). These often appear poorly preserved. Ciliated and non-ciliated cuboidal epithelial cells line the bronchioles. These may be present as single cells or in clusters. If visualised end-on the ciliated and columnar/cuboidal features of these cells may be difficult to appreciate (Figure 5.4).

Goblet cells are mucus-producing bronchial cells (Figure 5.5). Increased numbers can be seen with conditions resulting in chronic airway irritation and overproduction of mucus, e.g. chronic bronchitis and feline bronchial asthma.

Alveolar macrophages (Figure 5.6) may be the predominant cell type in BAL from clinically normal animals. The cytoplasm becomes vacuolated and may contain phagocytosed debris when these cells become activated.

Neutrophils normally constitute less than 5% of the total nucleated cell population. Increased numbers are seen with inflammation, infection or tissue necrosis. Eosinophils and lymphocytes are normally present in low numbers in dogs (<5% and 5–14% of the total nucleated cell population, respectively). In some healthy cats, the number of eosinophils may be as high as 20%. An increased number of eosinophils with variable numbers of other inflammatory cells may be seen with hypersensitivity reactions or

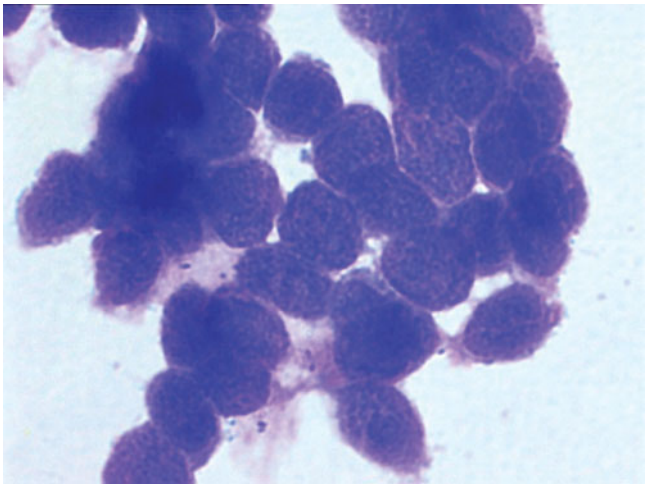


Figure 5.4 Ciliated or cuboidal epithelial cells viewed longitudinally. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

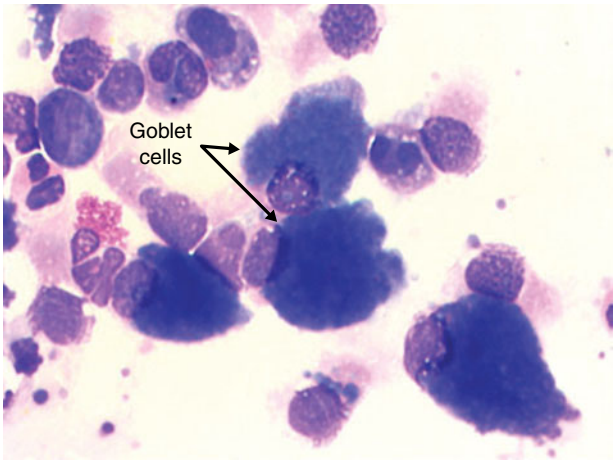


Figure 5.5 Goblet cells (arrows) contain large deeply basophilic mucin granules. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

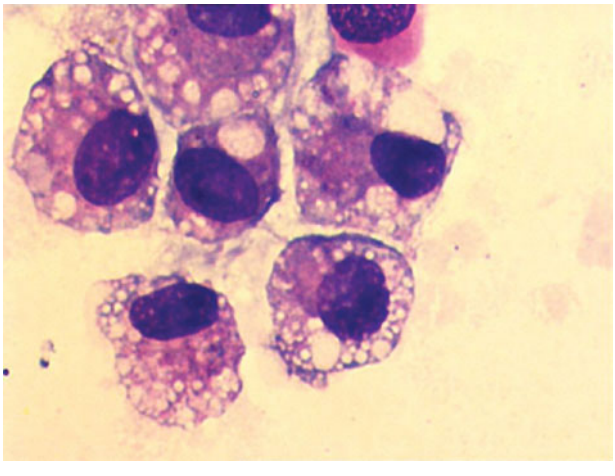


Figure 5.6 BAL (canine). Reactive alveolar macrophages with abundant basophilic vacuolated cytoplasm. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

parasitism. Increased numbers of lymphocytes can be seen with airway hypersensitivity and in response to antigenic stimulation, e.g. viral diseases and chronic infections. Some lymphocytes may transform into plasma cells. Mast cells are rarely seen in TW/BAL samples from healthy dogs and cats (<2% of the total nucleated cell population).

Mucus

Most washes/flushes, whether the sample is from the nasal cavity or lower respiratory tract, contain a small amount of mucus which stains as an eosinophilic background deposit. Increased amounts of mucus may be expected with inflammation

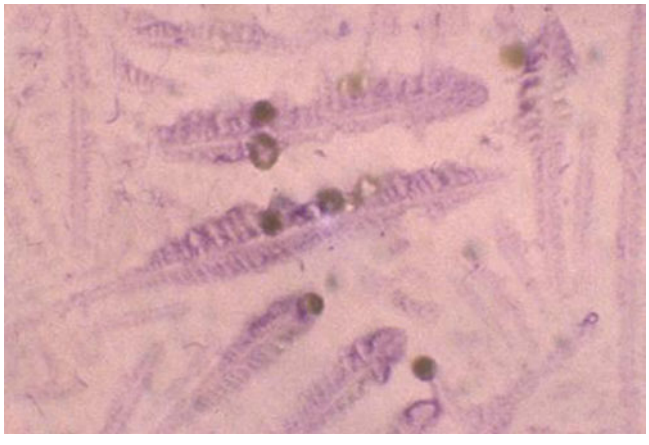


Figure 5.7 Mucus can assume a 'fern-like' appearance on a direct smear. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

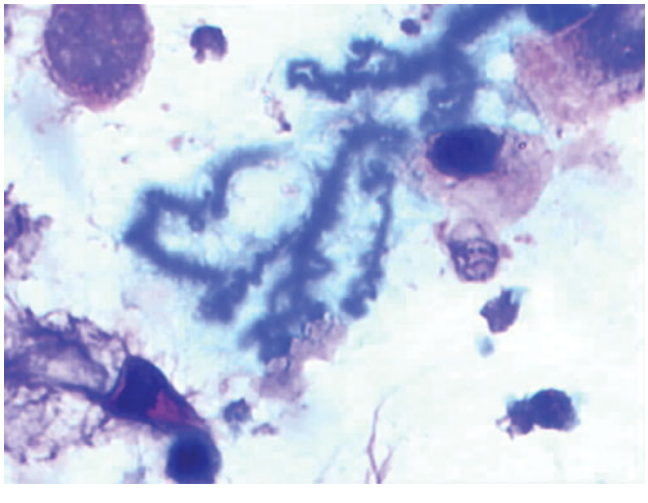


Figure 5.8 BAL (feline). Curschmann's spirals are frequently seen in conditions resulting in excessive production of mucus.

or infection (Figure 5.7). *Curschmann's spirals* are casts of inspissated mucus from the small bronchioles (Figure 5.8).

Oropharyngeal contamination

The presence of superficial squamous epithelial cells in a TW or BAL indicates oropharyngeal contamination. Bacteria (e.g. *Simonsiella* spp.) often adhere to the surface of these cells (Figure 5.9). A variable number of neutrophils may also be present.

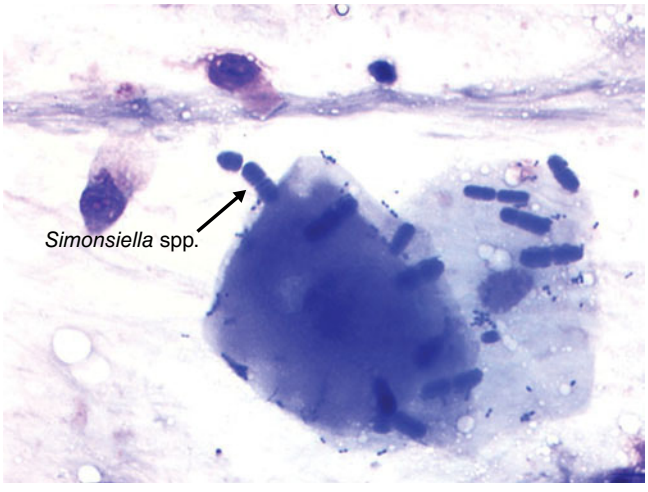


Figure 5.9 Oropharyngeal contamination. The keratinised squamous epithelial cells are covered in large *Simonsiella* organisms (arrow) and many cocci. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

Inflammation

Possible causes of a *neutrophilic (purulent) inflammatory response* (Figure 5.10) include tracheobronchitis (e.g. kennel cough), bronchopneumonia, inhaled bronchial/pulmonary foreign body, aspiration pneumonia, pulmonary abscess or a large necrotic tumour (e.g. primary lung tumours involving a single lung lobe). When the inflammatory response is associated with bacterial infection, the neutrophils appear degenerate and contain ingested organisms.

Mycobacterial infections, especially in cats (Figure 5.11), and mycotic or protozoal infections most frequently elicit either a *granulomatous or pyogranulomatous inflammatory response* characterised by an increased number of reactive epithelioid macrophages and a variable number of neutrophils. Large multinucleated giant cells, lymphocytes, plasma cells and eosinophils may also be present in low numbers.

BAL cytology from dogs and cats with chronic bronchitis tends to be less specific. The inflammatory response may be relatively mild, and there may be evidence of increased mucus production. A mixed inflammatory response may be evident although in many cases macrophages may predominate.

Eosinophilic inflammation

As a general guideline, an inflammatory response is considered to have a significant eosinophilic component if the number of eosinophils is greater than 10% of the total nucleated cell population. In some cases, the number of mast cells may also be slightly increased (Figure 5.12 and Figure 5.13). Differential

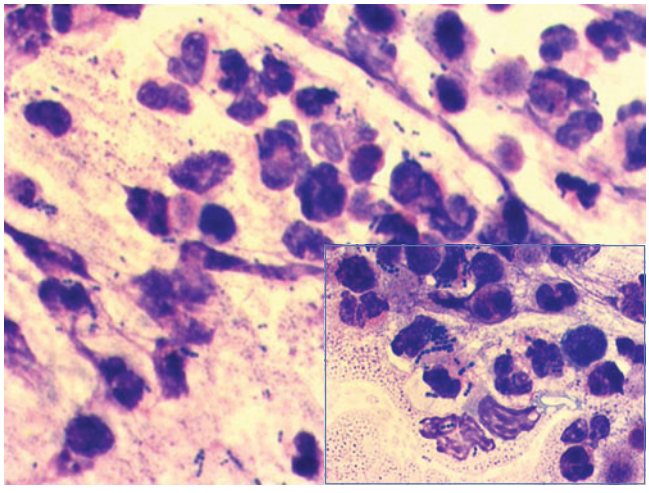


Figure 5.10 Bronchopneumonia. This hypercellular BAL consists of large numbers of degenerate neutrophils. Many contain intracellular bacterial organisms (inset). Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

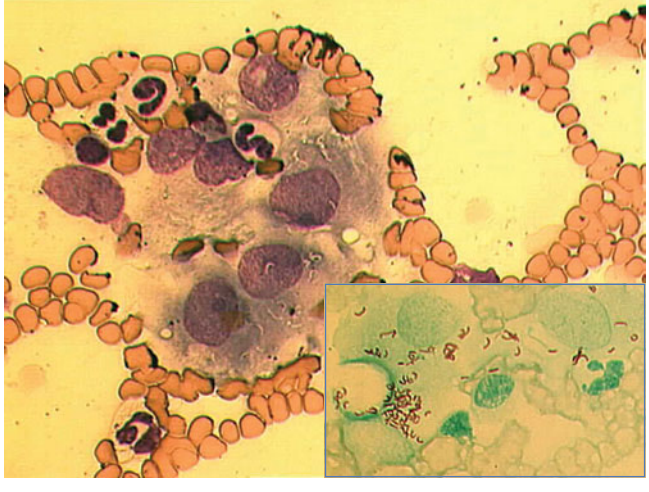


Figure 5.11 A cluster of macrophages containing phagocytosed negatively staining mycobacterial organisms. Inset: Same case. The mycobacterial organism stain acid-fast positive with Ziehl–Neelsen stain. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

diagnoses for consideration include feline bronchial asthma, pulmonary infiltrate with eosinophils (this includes allergic bronchitis, eosinophilic bronchopneumonia, pulmonary eosinophilic granuloma associated with *Dirofilaria immitis* infection) and neoplasia (e.g. lymphoma, mast cell tumours). Parasitism, e.g. *Oslerus osleri*,

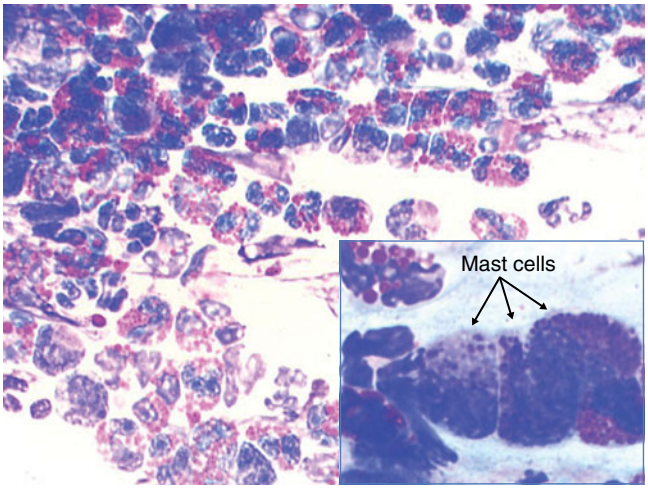


Figure 5.12 BAL (canine). Eosinophilic inflammation. At least 10% of the nucleated cells are eosinophils. Inset: Same case. Note the three mast cells (arrows). Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

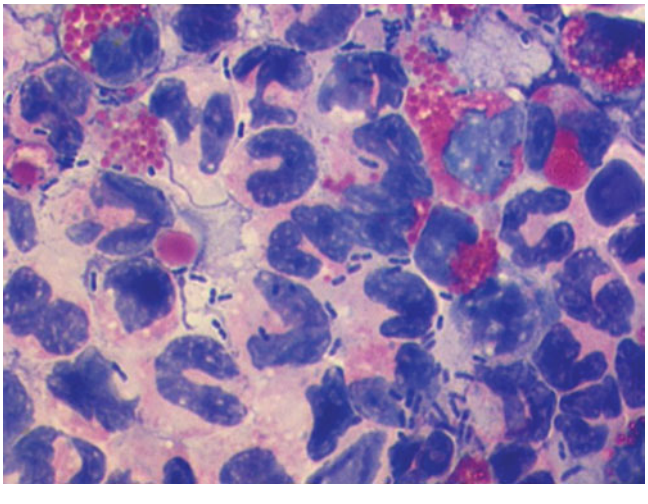


Figure 5.13 BAL (canine). Eosinophilic inflammation. There is evidence of secondary bacterial infection. Note the significant numbers of degenerate neutrophils and intra- and extracellular bacterial rods.

Angiostrongylus vasorum, *Crenosoma vulpis* and *Aelurostrongylus abstrusus* (cats), should also be considered (Figure 5.14). Some eosinophils may have a round or an oval-shaped rather than a segmented nucleus (so-called globoid eosinophils; Figure 5.15).



Figure 5.14 This canine BAL contains a first larval stage of a metastrongyle species. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

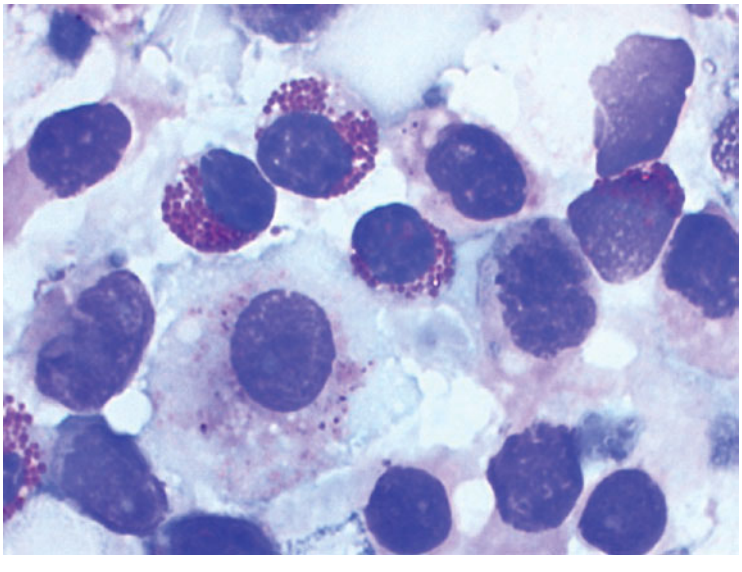


Figure 5.15 BAL (canine). Globoid eosinophils have a round rather than segmented nucleus.

Dysplastic epithelial cells

Columnar and cuboidal epithelial cells can become dysplastic in response to inflammation or chronic irritating stimuli, e.g. dust and smoke (Figure 5.2). Chemotherapy can also induce severe dysplastic changes. Differentiation of dysplastic from neoplastic cells may be difficult.

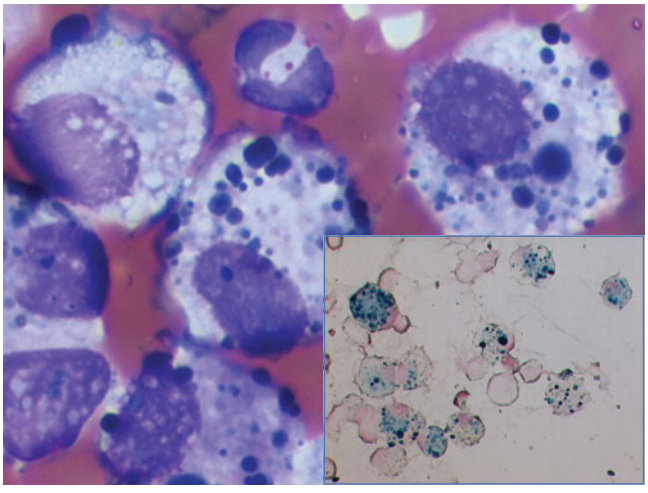


Figure 5.16 Haemosiderophages containing phagocytosed haemoglobin breakdown product. Inset: Haemosiderin stains as bluish-black deposit with Prussian blue stain. Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

Squamous metaplasia

Normal columnar or cuboidal epithelial cells may be replaced by stratified squamous epithelial cells. This represents an adaptive response of epithelial cells to chronic inflammation or irritation.

Intrapulmonary haemorrhage

The presence of macrophages containing red cells or haemoglobin breakdown products, e.g. haemosiderin (haemosiderophages) or haematoidin crystals, indicates previous intrapulmonary haemorrhage (Figure 5.16). Possible causes include trauma, pulmonary foreign body, infectious diseases (bacterial, fungal, protozoal, parasitic), lung lobe torsion, feline bronchial asthma, congestive heart failure, pulmonary thromboembolism, coagulopathies (e.g. dicoumarol toxicity) and neoplasia (primary/metastatic).

Neoplasia (primary or metastatic)

Cytological differentiation of pulmonary carcinomas is not possible. Some primary lung tumours, e.g. bronchogenic carcinomas, are more likely to involve the bronchial tree and as a result may exfoliate cells in TW or BAL specimens (Figure 5.17 and Figure 5.18). Haemolymphatic neoplasia, e.g. lymphoma or malignant histiocytosis (histiocytic sarcoma), may also involve the pulmonary parenchyma and can be diagnosed by BAL cytology. In contrast, metastatic lung tumours tend to produce interstitial lesions, and neoplastic cells are rarely seen. Fine-needle aspiration cytology of discrete pulmonary masses may be more rewarding in many cases where pulmonary neoplasia is suspected.

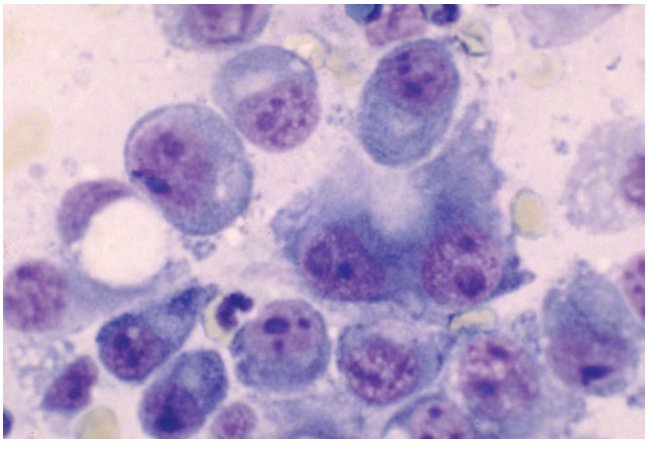


Figure 5.17 Pulmonary carcinoma (dog). The cells present in this BAL are showing obvious morphological features of malignancy (anisocytosis, anisokaryosis, variation in nuclear to cytoplasmic ratio and also variation in the size, shape and number of nucleoli within each nucleus). Source: Dunn (2010). Reproduced with permission of BMJ Publishing Group Ltd.

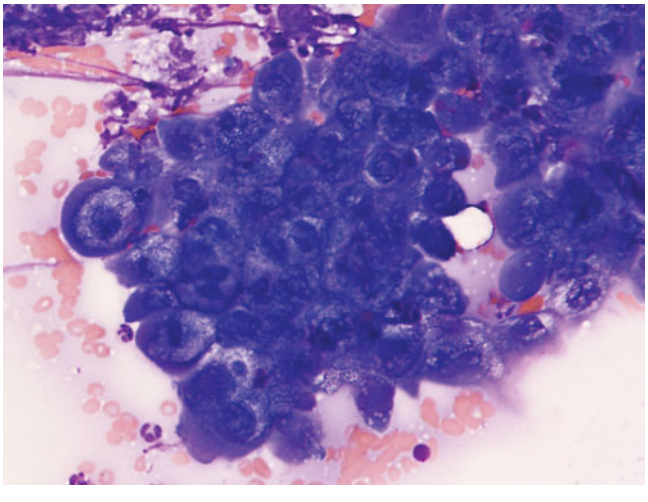


Figure 5.18 Fine-needle aspirate of a pulmonary carcinoma (dog). These epithelial cells are showing marked anisocytosis, anisokaryosis and variation in nuclear to cytoplasmic ratio. Note the perinuclear vacuolation. Source: Niki Skeldon/Axiom Veterinary Laboratories Ltd. Reproduced with permission.

6 Biochemical and Cytological Evaluation of Body Cavity Effusions

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Introduction

The visceral and parietal surfaces of body cavities are lined with mesothelium. This is bathed in serous fluid which in healthy cats and dogs is minimal, but increased amounts occur in various pathological processes. Analysis of effusions can be useful to gain information about the mechanism of formation.

Sample collection and processing

Readers are referred to standard texts for techniques for sampling abdominal, pleural and pericardial fluid (Bexfield and Lee, 2010). Processing of fluid samples is covered in Chapter 1.

Classification of effusions

Traditional system

Traditionally, effusions have been classified as **transudates, modified transudates** or **exudates** on the basis of total nucleated cell count (TNCC) and protein content (Table 6.1) (Rizzi *et al.*, 2008). This system is controversial: it is not used in human medicine and does not address aetiology, and the term 'modified transudate' is a misnomer because many fluids so classified do not form as transudates with subsequent modification of TNCC and protein.

Manual of Diagnostic Cytology of the Dog and Cat, First Edition. Edited by John Dunn.
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Table 6.1 Traditional classification of effusions based on TNCC and protein content[a]

	TNCC (× 10^9/L)	Protein (g/L)
Transudate	<1.5	<25
Modified transudate	1.0–7.0	25–75
Exudate	>7.0	>30

[a]As a general rule, protein levels are more important when distinguishing transudates and modified transudates, while cell counts are more relevant when differentiating modified transudates from exudates.
Source: Rizzi *et al*. (2008). © 2008 Elsevier. Reproduced with permission.

Cell counts

Reference laboratories provide automated cell counts. Some in-house haematology analysers provide a reasonably accurate TNCC (Giannasi *et al.*, 2013; Welles *et al.*, 2011).

Total protein

In practice, refractometry can be used to measure total protein. Many refractometers have a scale calibrated for total protein. Where samples are opaque, refractometry is performed on the supernatant after centrifugation to avoid false elevations.

Aetiological system

Recent publications classify effusions according to aetiology (Dempsey and Ewing, 2011; Stockham and Scott, 2008; Zoia *et al.*, 2009). The authors support this approach and have adapted it for the following discussion. There are six major pathogeneses of pleural and abdominal effusions:

- Transudation: altered fluid dynamics (+/– decreased plasma oncotic pressure)
- Exudation: altered vascular and/or mesothelial permeability
- Compromised lymphatic drainage or integrity
- Haemorrhage
- Ruptured viscus (+/– subsequent inflammation and exudation)
- Neoplasia: cell exfoliation and/or any of the previously mentioned mechanisms

Effusions are classified as '**transudate, protein poor**', '**transudate, protein rich**' or '**exudate**', unless fluid analysis reveals another specific mechanism, in which case they are classified as such (e.g. 'chylous', 'bilious'). Table 6.2 provides guidelines for the aetiological classification of effusions. Note that these values should be used in conjunction with cytological and other clinicopathological findings to determine the mechanism of effusion formation; they are not absolute decision limits. The causes of different types of effusion are listed in Table 6.3.

Table 6.2 Guidelines for the aetiological classification of effusions[a]

	TNCC (× 10^9/L)	Protein (g/L)[b]
Transudate, protein poor	<1.5	<20
Proteinaceous effusion[c]	<5.0[d]	>20
Exudate	>5.0[ef]	>20[g]

[a]Note that the values are guides only; they are not absolute decision limits – see text.
[b]Do not interpret in the presence of turbidity (e.g. lipid from chylous effusions).
[c]Underlying problem may be (1) primarily a low-grade or proteinaceous exudate (inflammatory) with few cells migrating to the fluid (e.g. FIP, vasculitis) or (2) primarily a protein-rich transudate with mild secondary inflammation (e.g. CHF).
[d]TNCC> 1.5 × 10^9 L with mostly neutrophils and/or macrophages supports an inflammatory component (exudation).
[e]Except exudates associated with FIP or other vasculitides, where TNCC is often <5.0 × 10^9/L.
[f]Should not be classified as an exudate if the majority of cells are not neutrophils, macrophages or eosinophils (e.g. neoplastic or chylous effusions); an effusion may be a low-grade exudate with a lower TNCC than this.
[g]Increases vary with plasma protein concentration; less protein exudation can occur with concurrent hypoproteinaemia.
Source: adapted from Stockham and Scott (2008). Reproduced with permission of John Wiley and Sons.

Transudates

Protein-poor transudates are formed when fluid dynamics are altered (see standard texts for information on Starling's Laws (Hughes and Boag, 2006)). The two main causes are severe hypoalbuminaemia (usually <12 g/L) and lymphatic vessel obstruction. Increased hydraulic pressure gradient (e.g. due to portal hypertension) can contribute and may account for protein-poor transudates where albumin is more than 12 g/L. Protein-poor transudates are more common in dogs than cats.

Protein-rich transudates form with increased hydraulic pressure in the lungs or liver, usually due to heart failure or portal venous hypertension associated with hepatic pathology, respectively. Vessels within alveolar capillaries and hepatic sinuses are relatively more permeable to plasma proteins than elsewhere.

Macroscopically, both protein-poor and protein-rich transudates are fairly nondescript; fluids can be colourless to yellow or orange in colour and clear to slightly turbid.

Cytologically, both forms of transudate consist of variable numbers of nondegenerate neutrophils, macrophages, lower numbers of small lymphocytes and mesothelial cells. Macrophages may have variable morphology, ranging from small round, basophilic cells to large round cells with pale, highly vacuolated cytoplasm (Figure 6.1). The nuclei of reactive macrophages usually appear more pleomorphic. Most chronic effusions become secondarily inflamed and as a result multinucleate macrophages, plasma cells, mast cells, eosinophils and reactive mesothelial cells may be present (Figure 6.9).

Mesothelial cell morphology depends on whether cells are shed spontaneously or harvested by traumatic puncture of the mesothelium (Figure 6.2, Figure 6.5 and Figure 6.12). It can be difficult to distinguish mesothelial cells from macrophages; the former tend to have more basophilic cytoplasm and a round

Table 6.3 Characteristics of transudates and exudates; PLN/PLE, protein-losing nephropathy/protein-losing enteropathy

Effusion	Pathogenesis	Mechanism	Conditions

Transudate, protein poor	Transudation	Altered hydraulic and oncotic pressures or decreased lymphatic drainage	• Hypoalbuminaemia[a]
			  ○ Cirrhosis (dogs)
			  ○ PLN
			  ○ PLE
			• Lymphatic obstruction
			  ○ External occlusion by neoplasia, abscess, other
			  ○ Vessel blockage by thrombosis, inflammatory or neoplastic cells
			  ○ Lymph node pathology
			  ○ Lymphangiectasia
			• Portal hypertension (pre-sinusoidal and sinusoidal)
			  ○ Cirrhosis
			  ○ Idiopathic portal hypertension

Transudate, protein rich			• Congestive heart failure (CHF) • Portal hypertension (post-sinusoidal)

Exudate	Exudation	Increased vascular/ mesothelial permeability	• Infectious
			  ○ Bacteria
			  ○ Fungi (e.g. *Blastomyces, Histoplasma, Candida*)
			  ○ Viruses (e.g. FIP)
			  ○ Protozoa (*Leishmania, Toxoplasma*)
			  ○ Parasites (*Mesocestoides*)
			• Non-infectious
			  ○ Neoplasia
			  ○ Foreign body
			  ○ Bile or urine peritonitis
			  ○ Pancreatitis
			  ○ Steatitis
			  ○ Ischaemic lesions

Haemorrhagic	Haemorrhage	Loss of endothelial integrity	• Trauma • Neoplasia • Coagulopathies

Table 6.3 (Cont'd)

Effusion	Pathogenesis	Mechanism	Conditions

Chylous	'Lymphorrhage'	Leakage of lymph from lymphatics	• Physical lymphatic obstruction
			  ○ See under 'transudate, protein poor'
			  ○ Mediastinal pathology
			  ○ Lung lobe torsion
			• Functional lymphatic obstruction
			  ○ Cardiovascular disease leading to passive venous congestion
			• Loss of lymphatic integrity
			  ○ Trauma
			  ○ Surgery
			• Congenital anomalies
			  ○ Lymphangiectasia
			• Miscellaneous
			  ○ Coughing
			  ○ Vomiting
			  ○ Diaphragmatic herniation
			  ○ Idiopathic

Bilious	Ruptured viscus and exudation	Leakage of bile from biliary tract, secondary inflammation	• Trauma • Cholelithiasis • Mucocoele • Cholangitis +/– biliary tract infection • Neoplasia • Complication of surgery or hepatic biopsy
Uroperitoneum	Ruptured viscus and exudation	Leakage of urine from urinary tract, secondary inflammation	• Trauma • Urolithiasis • Neoplasia • Iatrogenic

Neoplastic	Any of the above	Varied	• Epithelial (primary or metastatic)
			  ○ Carcinoma
			  ○ Adenocarcinoma
			  ○ Mesothelioma[b]
			• Round cell
			  ○ Lymphoproliferative disease

(*Continued*)

Table 6.3 (Cont'd)

Effusion	Pathogenesis	Mechanism	Conditions
			○ Mast cell tumours ○ Histiocytic malignancies ● Mesenchymal ○ Haemangiosarcoma
Pericardial	Varied	Varied	● Idiopathic ● Neoplasia ○ Haemangiosarcoma ○ Chemodectoma ○ Lymphoma ● Trauma ● Coagulopathies ● Cardiac disease ● FIP

[a]Increased blood volume and hydraulic pressure may also contribute in cirrhosis and PLN.
[b]Mesothelial cells are cells of mesodermal origin which display features of epithelial differentiation.
Source: adapted from Stockham and Scott (2008). Reproduced with permission of John Wiley and Sons.

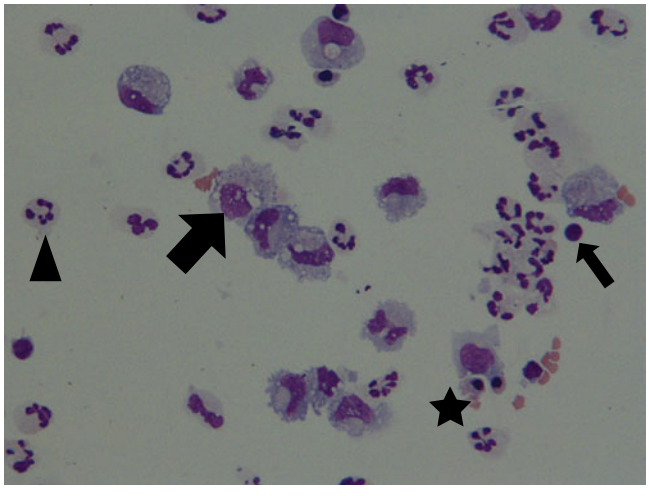


Figure 6.1 Protein-rich transudate. Abdominal effusion. Cat. Large arrow, macrophages; arrowhead, neutrophil; small arrow, lymphocyte; star, pyknotic cell.

nucleus, with stippled chromatin and sometimes prominent nucleoli, whereas macrophages tend to have a paler, more vacuolated cytoplasm and a more pleomorphic nucleus with coarse chromatin. As mesothelial cells become reactive, cytoplasmic basophilia and nucleoli become more pronounced, and multinucleation is common. Care must be taken not to over-diagnose malignancy, especially in the presence of inflammation.

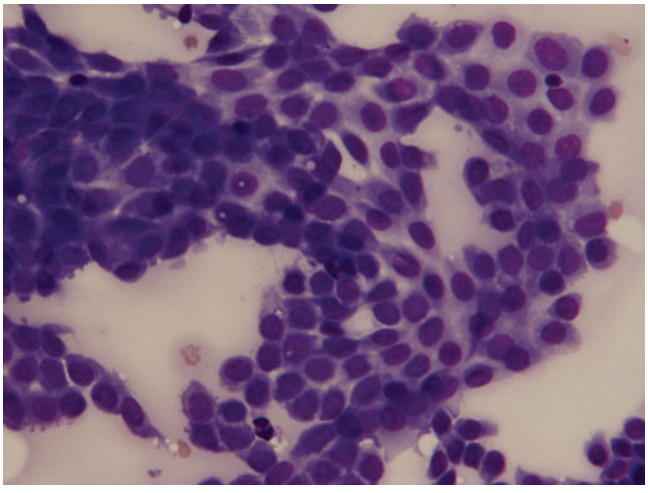


Figure 6.2 Abdominal effusion with mesothelial cells. Dog. Note flat, 'fish-scale' appearance of the mesothelial cells suggesting harvest by traumatic puncture of mesothelium.

Exudates

Exudates form due to increased vascular and/or mesothelial permeability secondary to inflammation. Inflammation may be the primary process, or the inflammatory changes occur secondary to a pre-existing effusion. Neoplastic and chylous effusions often have a TNCC in the exudate category; these will be addressed separately as they are not formed by exudation.

Macroscopically, exudates are usually turbid; simplistically, increased turbidity equates to increased TNCC. Neutrophils predominate and usually appear degenerate in septic exudates due to bacterial toxins; the nuclei appear swollen with indistinct nuclear lobes and pale-staining chromatin (Figure 6.3). Some bacteria (notably *Nocardia* and *Actinomyces* spp.) produce weaker toxins and the degenerative changes may not be as pronounced. The presence of intracellular bacteria helps distinguish infection from sample contamination (though be aware that bacterial phagocytosis by leukocytes can occur occasionally *in vitro*) (Figure 6.3). Macrophages may contain phagocytosed neutrophils (leukophagia).

Specific types of effusion

See Tables 6.3 and 6.4 for causes and confirmatory tests for these different types of effusion.

Haemorrhagic effusions

Haemorrhagic effusions usually have a PCV of at least 10%, although anything over 3% implies haemorrhage is contributing (Stockham and Scott, 2008). Haemorrhagic effusions due to ruptured vasculature or viscera must be distinguished from iatrogenic haemorrhage incurred during sampling. The presence of

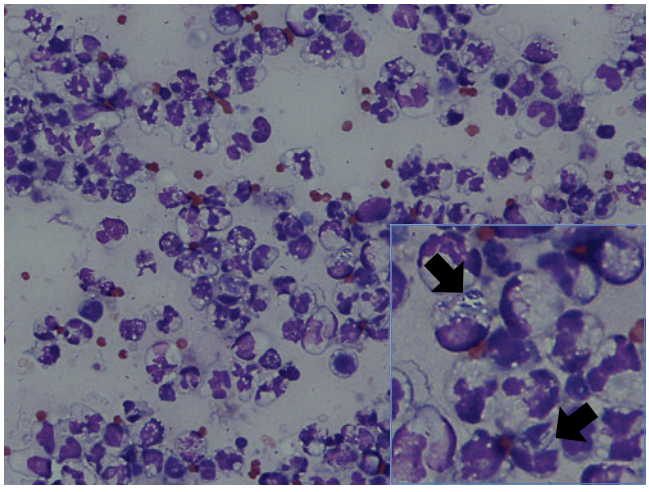


Figure 6.3 Exudate. Pleural effusion. Cat. Degenerate neutrophils. Inset: Note intracellular bacteria (arrows), some of which are filamentous (this suggests *Actinomyces/Nocardia* spp.).

erythrocyte breakdown product, e.g. haemosiderin (confirmed by Prussian blue stain) or haematoidin crystals, implies chronicity and confirms previous haemorrhage (Figure 6.4 and Figure 6.5).

The presence of macrophages containing phagocytosed erythrocytes (erythrophagia) is significant if seen on slides prepared at time of sampling; however, any delay in sample processing confounds interpretation because phagocytosis of erythrocytes released iatrogenically at sampling can occur after sample collection. Platelets are removed rapidly from body cavities; their presence implies very recent or iatrogenic haemorrhage.

Chylous effusions

Chylous effusions are usually pleural but can form in the abdomen. They occur due to leakage of chylomicron-/triglyceride-rich lymph and indicate compromise of lymphatic vessels situated somewhere between the small intestine and the thoracic vena cava (see Table 6.3 for possible causes).

Classic chylous effusions have a characteristic appearance (Figure 6.6), but not all are readily identified macroscopically, especially in anorexic animals. 'Pseudochylous' is a medical term describing homogeneous white fluids which are not chyle; these being rare in animals, the term is seldom appropriate.

Cytologically, small lymphocytes usually predominate and macrophages are highly vacuolated due to lipophagia (Figure 6.7). There may be a characteristic microvacuolated background (this is a useful feature highlighting the possibility of chyle; Figure 6.8). Chyle can induce inflammation, so with chronicity neutrophils and lower numbers of mast cells, eosinophils and plasma cells may also be present (Figure 6.9). Bacteria are rarely observed as chyle is bacteriostatic (organisms may be present if repeated drainage induces infection).

Table 6.4 Ancillary tests relevant to effusions; AGP, acid glycoprotein; RT-PCR, reverse transcription polymerase chain reaction; PARR, PCR for antigen receptor rearrangement; FeCoV, feline coronavirus

Effusion	Test	Indication	Comments/expected findings
Exudate	Culture and sensitivity	Septic exudate Any unexplained inflammation	
	Glucose[a]	Suspected infection	Serum glucose 1.1 mmol/L > effusion glucose supports sepsis
	Lactate[a]		Effusion lactate at least 1.5 mmol/L > serum lactate supports sepsis
	Lipase	Suspected pancreatitis	Ratio of effusion lipase to serum lipase is >2 (dog) Effusion lipase concentration is four times > upper serum reference limit
Haemorrhagic	PCV	Macroscopically bloody fluid	Usually >10%
	Prussian blue staining of slides	Suspected haemosiderin in macrophages	Iron stains blue/green
Chylous	Triglycerides	Macroscopically creamy effusion, predominance of small lymphocytes	Concentration in effusion > 1.14 mmol/L Concentration in effusion > concentration in serum
	Cholesterol	As for triglycerides	Concentration in effusion < serum concentration (and vice versa if non-chylous) Cholesterol: triglyceride ratio <1
Bilious	Bilirubin	Suspected biliary tract rupture, greenish fluid	Ratio of effusion bilirubin to serum bilirubin is at least 2
Uroperitoneum	Creatinine/ potassium	Suspected urinary tract rupture	Ratio of effusion creatinine concentration to serum concentration is >1[b] Effusion creatinine concentration is > 4× upper serum reference limit (dogs)
FIP	Albumin and globulins	Suspected FIP	A to G ratio (effusion) <0.4 is highly suggestive; >0.9 FIP unlikely (http://www.dr-addie.com/WhatIsFIP.htm#DiagnosisofFIP)

(*Continued*)

Table 6.4 (Cont'd)

Effusion	Test	Indication	Comments/expected findings
	Rivalta's test		See Box 6.1 and http://www.dr-addie.com/WhatIsFIP.htm#DiagnosisofFIP Specificity poor (Fischer *et al*., 2012)
	FeCoV serology		Confirms exposure only. Titres >1:1600 are supportive (Hartmann *et al*., 2003); negative titre does not rule out FIP
	Alpha 1 AGP		>1500 μg/ml supportive of FIP (Bence *et al*., 2005)
	Immunofluorescent staining of FeCoV antigen in macrophages		An *antemortem* confirmatory test, moderate sensitivity
	RT-PCR for FeCoV		Not widely available
Neoplastic	Flow cytometry for specific antigen markers	Lineage identification of atypical round cells	Sample must be fresh
	PARR	To help distinguish neoplastic from non-neoplastic lymphoid cells	

[a]Caution with interpretation: neoplastic and ischaemic conditions and aged samples can give similar results to septic samples.
[b]Note that with time the effusion and serum creatinine will equilibrate as it diffuses from the effusion to lymph and blood.

Bilious effusions

Macroscopically, the effusion typically has a yellow-brown to greenish hue and may be turbid (Figure 6.10).

Neutrophils usually predominate as free bile induces peritonitis. A characteristic feature is the presence of yellow-brown amorphous material (bile) in extracellular clumps and within macrophages; rarely, macrophages contain bilirubin crystals (Figure 6.11 and Figure 6.12).

Urinary tract rupture

Urinary tract rupture usually occurs due to trauma. The dilutional effect of urine lowers the protein concentration; an effusion with moderate TNCC but disproportionately low protein raises suspicion of uroperitoneum. Initially, the effusion resembles a protein-poor transudate, but with time inflammation develops. Spermatozoa and urine crystals may be noted.

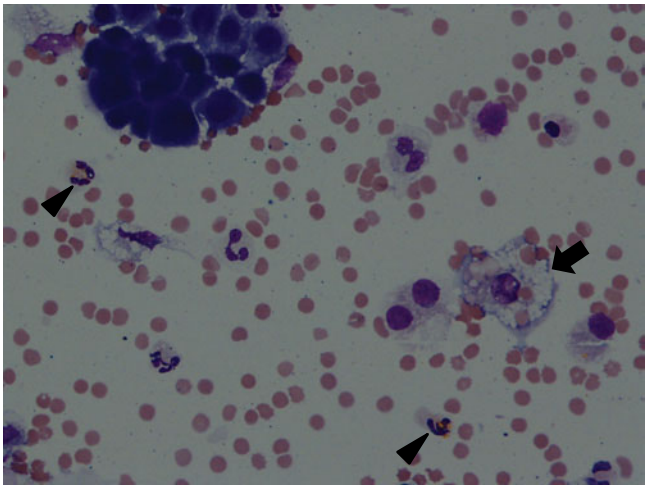


Figure 6.4 Haemorrhagic effusion (pericardial). Dog. Note the macrophage with phagocytosed erythrocytes (arrow) and haematoidin crystals in the neutrophils (arrowheads). A large cluster of mesothelial cells and numerous erythrocytes are also present.

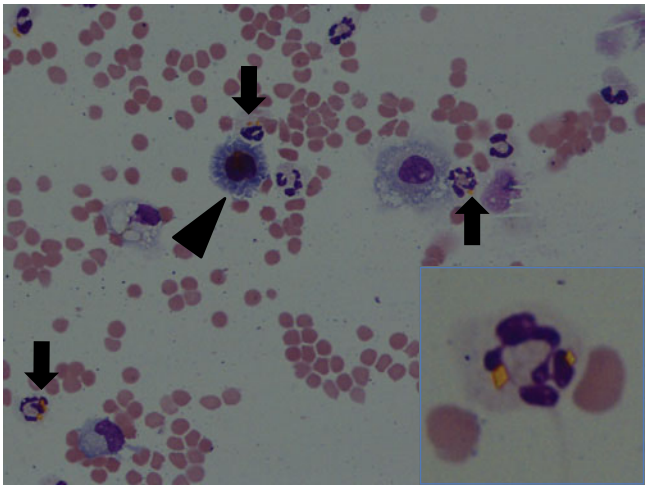


Figure 6.5 Same case as Figure 6.4. Haematoidin crystals (arrows) within three neutrophils and a reactive mesothelial cell showing cytoplasmic 'ruffling' (arrowhead). Two macrophages are also present. Inset: haematoidin crystals within a neutrophil.

Feline infectious peritonitis (FIP)

Vasculitis induced by FIP causes exudation of protein-rich fluid into body cavities. Cell counts are disproportionately low, with inflammation being in the vessel walls not the body cavity. Effusions in FIP usually have protein of >40 g/L and TNCC of 2.0–6.0 × 10^9/L (Rizzi *et al.*, 2008).

Macroscopically, the fluid is pale to deep yellow, may contain fibrin strands and may froth when shaken. Cytologically, the effusion is characterised by a



Figure 6.6 Chylous effusion. Cat. Macroscopic appearance.

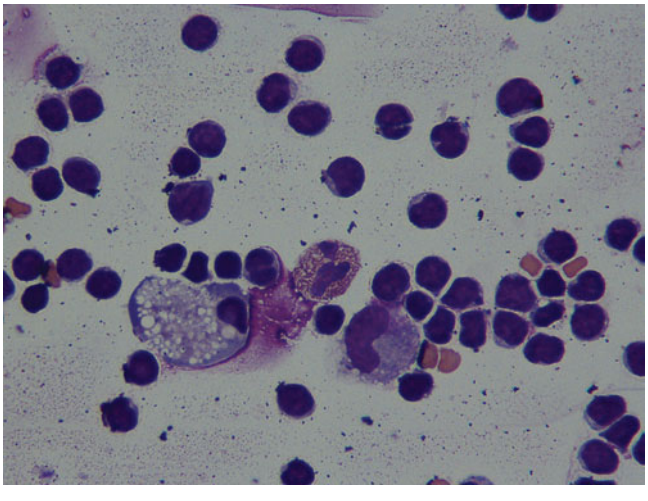


Figure 6.7 Chylous effusion. Cat. Small lymphocytes predominate. Two macrophages (one not intact), an eosinophil, erythrocytes and a non-classifiable ruptured cell are also present.

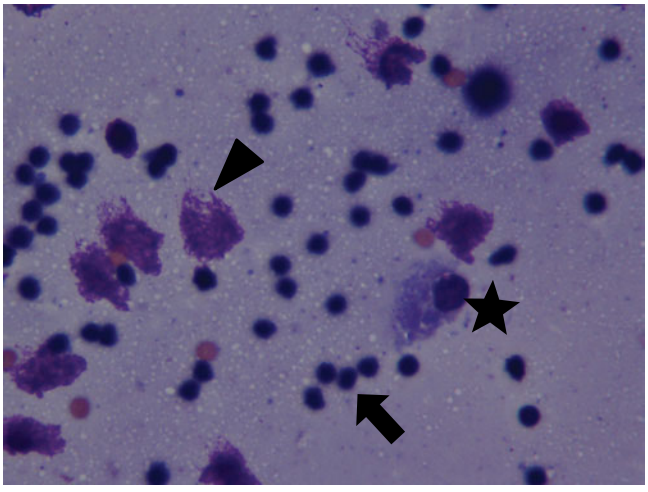


Figure 6.8 Same case as Figure 6.7. Focused to highlight the microvacuolated background containing lipid. Small lymphocytes predominate (arrow). Some cells have ruptured and are non-classifiable (arrowhead and star).

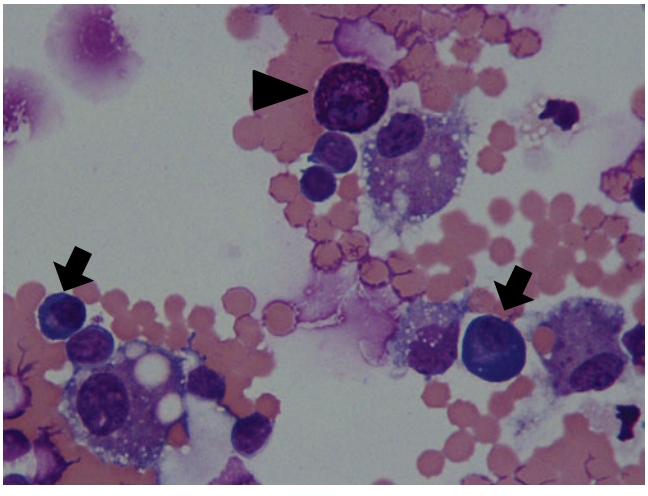


Figure 6.9 Chylous effusion with inflammation. Cat. In addition to small lymphocytes and macrophages, plasma cells (arrows) and a mast cell (arrowhead) are present. The pale pink material originates from ruptured cells.

heterogeneous leukocyte population with neutrophils usually predominating. High numbers of macrophages may also be present. The cells are often located in a stippled background fluid deposit containing protein 'crescents', a drying artefact which suggests high protein content (Figure 6.13). Histopathology remains the mainstay of diagnosis, with immunohistochemistry to demonstrate viral antigen within lesions being the gold standard (see Table 6.4 and Box 6.1 for ancillary tests which may support a diagnosis of FIP).



Figure 6.10 Bilious effusion. Dog. Macroscopic appearance.

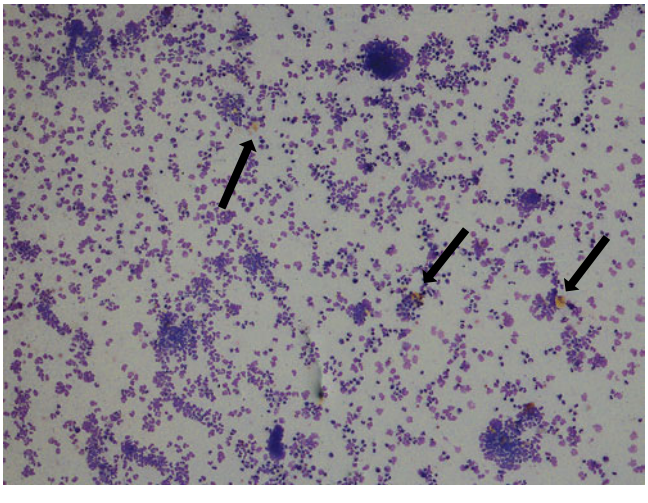


Figure 6.11 Same case as Figure 6.10. Note high cellularity of this direct smear and presence of yellow-staining extracellular material (bile) (arrows).

Eosinophilic effusions

Eosinophils have distinct rod-shaped (cats) or round (dogs) pink/orange cytoplasmic granules and a lobed nucleus. Often their presence in effusions is secondary to peripheral blood. They may be present in low numbers in inflammation. Their

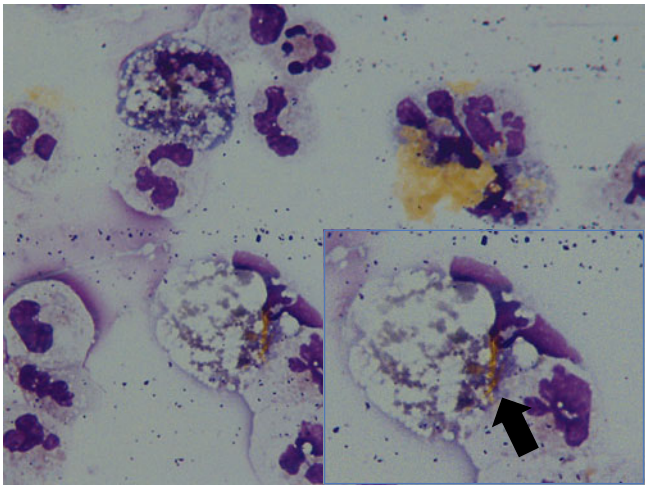


Figure 6.12 Same case as Figure 6.10. Deteriorated neutrophils, macrophages, reactive mesothelial cell and yellow-staining extracellular material (bile). Inset: One macrophage contains a needle-like bilirubin crystal (arrow).

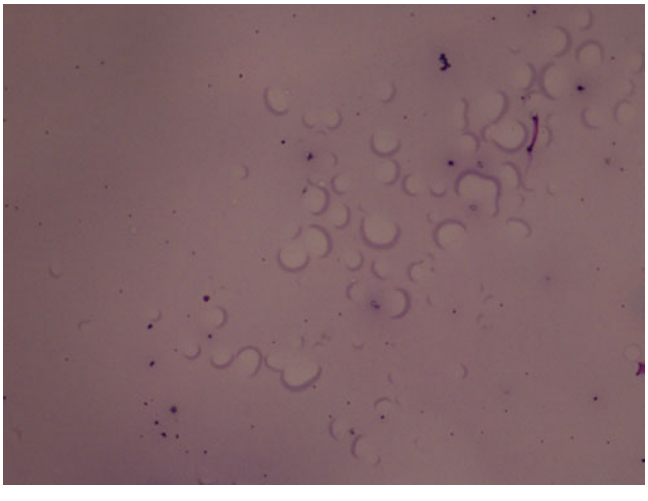


Figure 6.13 Exudate. Abdominal effusion from a cat with suspected FIP. Note the protein crescents.

Box 6.1 Rivalta's test methodology

- Mix one drop of 98% acetic acid with 5 ml of distilled water.
- Gently place one drop of the effusion on the surface of the mixture.
- Positive: layer of gel forms at surface or drop slowly floats to bottom.
- Negative: drop disperses.

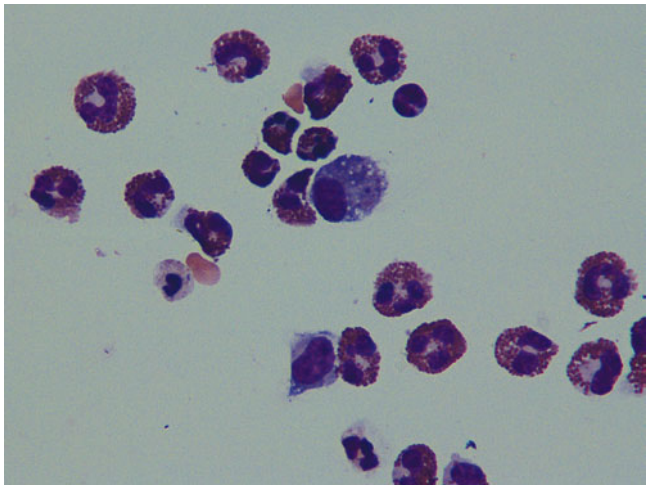


Figure 6.14 Eosinophilic inflammation. Abdominal effusion. Dog. Two macrophages, one neutrophil and one pyknotic cell are present. Eosinophilic inflammation may be associated with parasitism (e.g. *D. immitis*), allergy/hypersensitivity and neoplasia (e.g. with lymphoma or mast cell tumours).

presence in high numbers suggests parasitism (e.g. *Dirofilaria immitis* infection), allergic/hypersensitivity reactions or paraneoplastic syndrome (most commonly associated with mast cell tumours or lymphoma) (Figure 6.14).

Neoplastic effusions

Neoplastic cells from solid tumours or leukaemias may be present in effusions; absence does not rule out neoplasia. Cytocentrifugation may be necessary to detect rare neoplastic cells. The most common neoplasms diagnosed by effusion cytology are carcinomas (primary or metastatic) and round cell tumours, e.g. mast cell tumours, lymphoproliferative disease and histiocytic sarcoma (Figure 6.15, Figure 6.16, Figure 6.17, Figure 6.18, Figure 6.19 and Figure 6.20). Rarely, cells from haemangiosarcomas exfoliate into effusions.

Cells from carcinomas and adenocarcinomas can be difficult to distinguish from reactive mesothelial cells displaying features which may resemble those associated with malignancy. Mesotheliomas are rare neoplastic proliferations of mesothelial cells, almost impossible to diagnose cytologically due to the difficulty distinguishing them from their reactive counterparts and from carcinomas; histopathology is essential.

Specialist ancillary tests may be necessary to determine the lineage of atypical round cells (see Table 6.4). Low numbers of mast cells can be seen with inflammation, but high numbers imply visceral mast cell neoplasia (more common in cats than dogs). Neoplastic mast cells may have finer, less obvious cytoplasmic granulation than normal cells, especially in cats and/or when rapid stains are used.

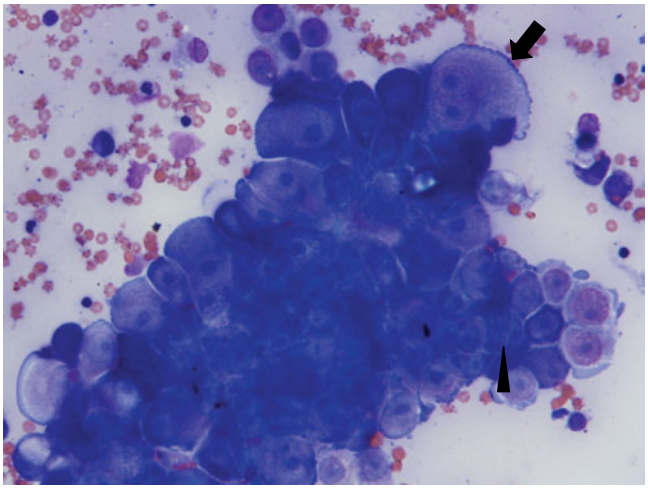


Figure 6.15 Pleural effusion. Dog. Carcinoma. Note anisokaryosis, prominent nucleoli of variable size, binucleation (arrow) and nuclear moulding (arrowhead).

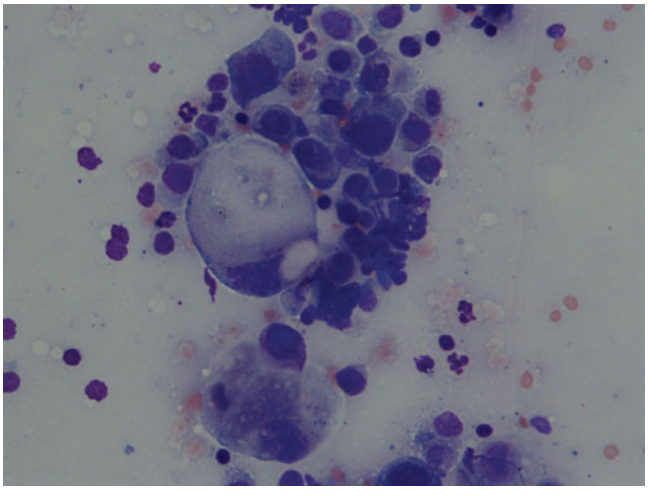


Figure 6.16 Same case as Figure 6.15, demonstrating cytoplasmic 'ballooning' of a carcinoma cell (these are often referred to as 'signet ring' cells). Note inflammation, which is common with carcinomas.

Pericardial effusions

In dogs, approximately 50% are idiopathic, with most of the remainder being neoplastic. In cats, pericardial effusions are most commonly associated with CHF and FIP. The usual criteria are applied to differentiate chronic from recent/iatrogenic haemorrhage (see section 'Haemorrhagic effusions'). Exfoliated mesothelial cells are often particularly reactive in pericardial effusions and can be difficult to distinguish from neoplastic cells (Figure 6.21).

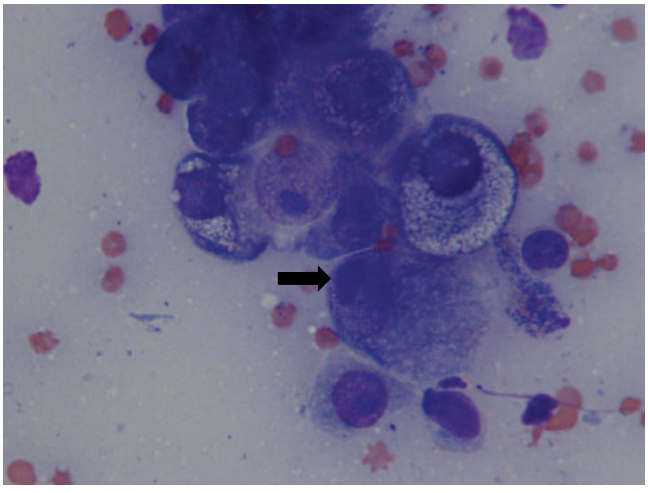


Figure 6.17 Same case as Figure 6.15, demonstrating bizarre nucleolar morphology (arrow) and cytoplasmic vacuolation suggesting secretory product.

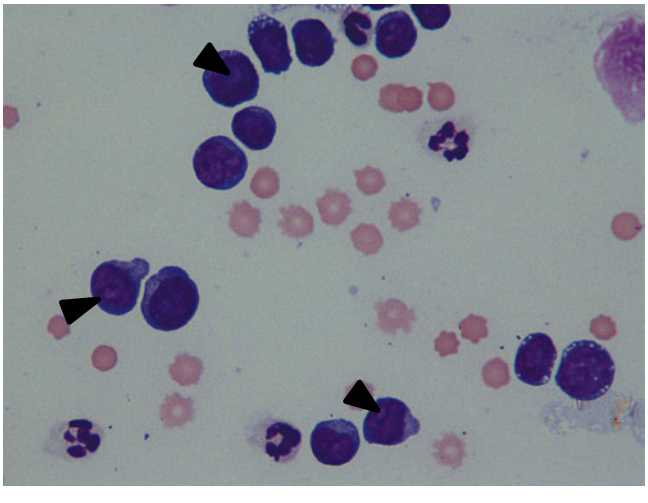


Figure 6.18 Pleural effusion. Cat. Lymphoma. Note the atypical large lymphoid cells with scant, sometimes vacuolated cytoplasm, coarse chromatin, and often a single large nucleolus (arrowheads).

Artefacts

- Common:
  - Starch granules from glove powder (see Figure 1.20).
  - Ultrasound gel (see Figure 1.19).
  - Lysed cells (especially in septic and chylous effusions due to increased cell fragility) (Figure 6.8).

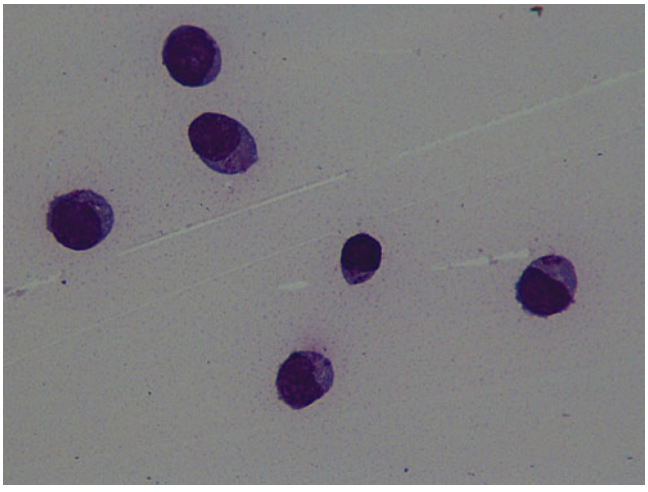


Figure 6.19 Abdominal effusion. Cat. Large granular cell lymphoma.

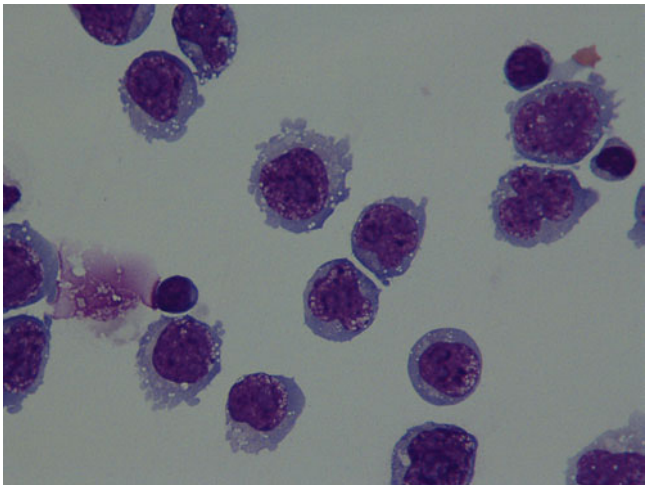


Figure 6.20 Pleural effusion. Cat. Round cell neoplasm. Large atypical round cells with relatively abundant, vacuolated cytoplasm, variable nuclear shape, coarse chromatin and prominent nucleoli. Cytomorphology suggests lymphoid or histiocytic origin, but ancillary tests are required for lineage determination. Three small lymphocytes also present.

- Infrequent:
  - *Dirofilaria* microfilariae due to peripheral blood contamination (endemic areas).
  - Copious bacteria without inflammation suggests accidental enterocentesis or sample contamination.

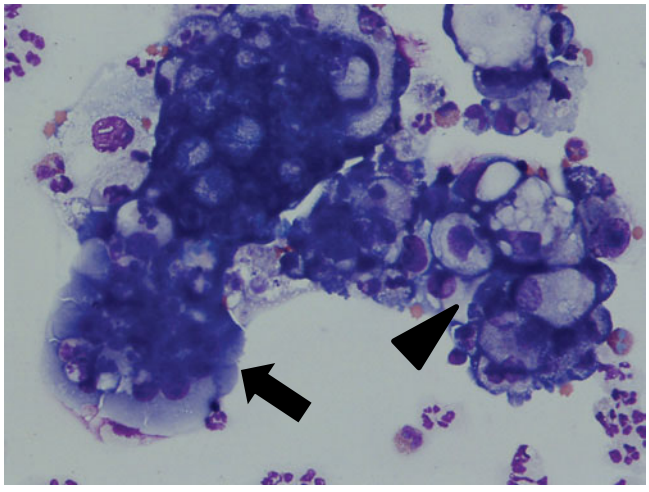


Figure 6.21 Pericardial effusion. Probably malignant. Cat. Note high cellularity. In some areas cytomorphology suggests reactive mesothelial cells (arrow). It is unclear if the highly vacuolated cells (arrowhead) belong to the same population; they could be macrophages, reactive or neoplastic mesothelial cells or carcinoma cells. Inflammatory cells are also present. Cat had an extensive thoracic mass involving pericardium.

Conclusion

Effusion cytology is useful in a practice setting and in many cases provides a definitive diagnosis. It is best performed on fresh samples. Reference laboratories are essential when examination by a specialist cytologist or ancillary tests are required.

Acknowledgment

The authors thank Dr. Mike Scott for review of this chapter and, specifically, for provision of footnotes to Table 6.2. His insightful comments were invaluable.

References

Bence, L.M., Addie, D.A. and Eckersall, P.D. (2005) An immunoturbidimetric assay for rapid quantitative measurement of feline alpha-1-acid glycoprotein in serum and peritoneal fluid. *Veterinary Clinical Pathology*, **34** (4), 335–340.

Bexfield, N. and Lee, K. (2010) *BSAVA Guide to Procedures in Small Animal Practice*, British Small Animal Veterinary Association, Quedgeley Gloucester.

Dempsey, S.M. and Ewing, P.J. (2011) A review of the pathophysiology, classification and analysis of canine and feline cavitary effusions. *Journal of the American Animal Hospital Association*, **47**, 1–11.

Fischer, Y., Sauter-Louis, C. and Hartmann K. (2012) Diagnostic accuracy of the Rivalta test for feline infectious peritonitis. *Veterinary Clinical Pathology*, **41** (4), 558–567.

Giannasi, C., Brown, A. and Skeldon, N. (2013) *Evaluation of HemoCue WBC as a bedside analyser in characterising abdominal effusions*. BSAVA Congress Scientific

Proceedings Abstracts. British Small Animal Veterinary Association, Quedgeley, Gloucester, p. 558.

Hartmann, K., Binder, C., Hirschberger, J. *et al.* (2003) Comparison of different tests to diagnose feline infectious peritonitis. *Journal of Veterinary Internal Medicine*, **17** (6), 781–790.

http://www.dr-addie.com/WhatIsFIP.htm#DiagnosisofFIP (accessed 23 August 2013).

Hughes, D.H. and Boag, A. (2006) Fluid therapy with macromolecular plasma volume expanders, in *Fluid, Electrolyte and Acid–base Disorders in Small Animal Practice*, 3rd edn (ed. S.P. DiBartola), Saunders Elsevier, St. Louis, MO, pp. 627–634.

Rizzi, T.E., Cowell, R.L., Tyler, R.D. *et al.* (2008) Effusions: abdominal, thoracic and pericardial, in *Diagnostic Cytology and Haematology of the Dog and Cat*, 3rd edn (eds R.D. Tyler, J.H. Meinkoth, D.B. DeNicola *et al.*), Mosby Elsevier, St. Louis, MO, p. 244.

Stockham, S.L. and Scott, M.A. (2008) Cavitary effusions, in *Fundamentals of Veterinary Clinical Pathology*, 2nd edn (eds S.L. Stockham and M.A. Scott), Blackwell Publishing, Ames, IA, pp. 831–867.

Welles, E.G., Oller, E., Spangler, E.A. *et al.* (2011) *Validation of an in-office automated haematology instrument, the Heska CBC-Diff, for total nucleated cell counts in body cavity effusions and comparison of differential cell counts with manual observations from prepared smears*. ASVCP Annual Meeting Abstracts, December 3–7, 2011, Nashville, TN, p. 597.

Zoia, A., Slater, L.A., Heller, J. *et al.* (2009) A new approach to pleural effusions in cats: markers for distinguishing transudates from exudates. *Journal of Feline Medicine and Surgery*, **11**, 847–855.

7 Cytology of Synovial Fluid

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Introduction

Synovial fluid is a plasma dialysate formed from the ultrafiltration of fluid through fenestrated capillaries lying just beneath a subintima (thereby excluding larger proteins such as fibrinogen) which is then modified by constituents secreted by the joint tissues. The two major cell populations of the intima are type A synoviocytes (resident tissue macrophage derived) and type B synoviocytes (secretory fibroblast related). The type B cells secrete fluid components such as collagens, fibronectin, hyaluronan and lubricin. The normal viscosity of synovial fluid is due to the hyaluronic acid content. Synovial fluid has two main functions: to aid in the nutrition of articular cartilage by acting as a transport medium for nutritional substances, such as glucose, and to aid in the mechanical function of joints by lubrication of the articulating surfaces.

Synovial fluid analysis can aid in the investigation of joint disease. Indications for analysis can include stiffness, weakness, joint pain, pyrexia, swelling of the limbs and joints (Figure 7.1) or joint deformity. Importantly, synovial fluid analysis is only a component of the work-up of joint disease, and findings require correlation with other clinical and laboratory findings including culture, serology, radiography and other imaging modalities. In some circumstances, synoviocentesis may be contraindicated, e.g. where there is overlying cellulitis or dermatitis or if the animal is bacteraemic or has a coagulopathy.

Collection

The following aspects merit consideration and preparation prior to sampling:

Manual of Diagnostic Cytology of the Dog and Cat, First Edition. Edited by John Dunn.
© 2014 John Wiley & Sons, Ltd. Published 2014 by John Wiley & Sons, Ltd.



Figure 7.1 Joint distension due to synovial effusion. Source: John Dunn, Axiom Veterinary Laboratories Ltd. Reproduced with permission.

Restraint

This relies on clinical judgement of the veterinarian to establish whether physical immobilisation, sedation or anaesthesia is necessary.

Asepsis

Routine aseptic technique is essential as normal joint spaces are sterile.

Equipment

- Clippers, sterile gloves, sterile scrub solution and alcohol.
- Sterile disposable 3ml syringes.
- 1.5 inch 22 G needles (large dogs) or 1 inch 25 G needles (small dogs and cats). Longer needles may be required for elbow, shoulder or hip joints.
- Microscope slides with frosted ends.
- Plain and EDTA tubes: ideally pre-labelled tubes and slides with patient name and joint sampled.

Approach

Synovial fluid sampling is usually performed with the animal in lateral recumbency and the joint in question uppermost. Palpation of the joint in flexion and extension should help to identify the joint space (Figure 7.2). Gentle advancement of the sampling needle is necessary to avoid any damage to the articular cartilage. The volume obtained will vary between joints, e.g. fluid is usually readily obtained from the stifle but is more difficult to obtain from carpal and tarsal joints. Swollen joints will typically yield more fluid. The plunger of the syringe should be released before the needle is withdrawn from the joint space to minimise blood contamination.

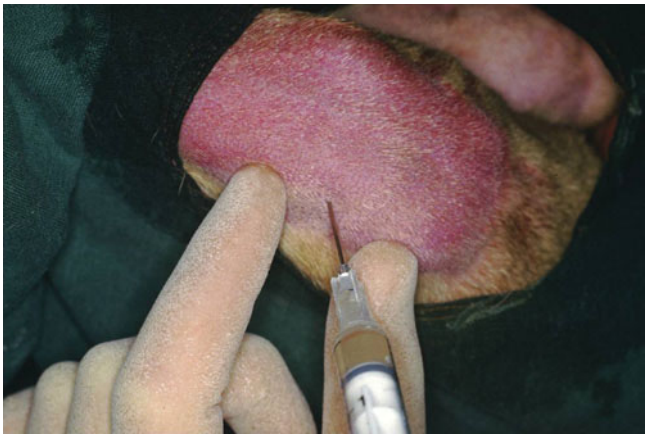


Figure 7.2 Approach to carpal arthrocentesis. Source: John Dunn, Axiom Veterinary Laboratories Ltd. Reproduced with permission.

Readers are referred to suitable orthopaedic textbooks for detailed approaches to specific joints.

Sample handling

The amount of synovial fluid collected will vary, so it is important to prioritise tests accordingly. While the sample is in the syringe volume, colour and turbidity should be noted, and as the fluid is expelled onto a glass slide, viscosity should be assessed. Any apparent blood contamination during sampling should also be noted. Direct smears should be made immediately and air-dried for subsequent cytological examination which includes a subjective assessment of cellularity and nucleated cell differential count (smear preparation is discussed in Chapter 1). Depending on the volume remaining, further tests may include total nucleated cell count and protein concentration (EDTA or plain tube; protein cannot be measured from a sample in a plain tube if sample has clotted due to blood contamination or inflammation) and culture (plain tube or sample in culture medium). Regardless of the amount collected, it is usually advantageous to prepare direct smears immediately to best preserve cell morphology. These slides should not be refrigerated prior to staining.

Synovial fluid properties

Volume

An approximate or subjective estimation of volume should be made. Volumes depend on patient size, the joint sampled and the amount of effusion present. The volume of joint fluid obtained in normal animals may range from 1 drop to 1.0 ml in dogs and 1 drop to 0.25 ml in cats.



Figure 7.3 Synovial fluid from an inflamed joint. Note the increased volume obtained and the moderate turbidity of the sample. A plain or culture media are preferred for bacterial culture. Source: John Dunn, Axiom Veterinary Laboratories Ltd. Reproduced with permission.

Colour and turbidity

Normal synovial fluid is transparent and colourless or very light yellow or straw coloured. When a fluid is blood tinged, haemarthrosis should be distinguished from iatrogenic blood contamination. With haemarthrosis fluid is uniformly bloody throughout collection. If the fluid was initially free of blood but at some point blood is seen to enter the sample in a trailing wisp, this suggests contamination. Dark yellow or xanthochromic fluids may indicate chronic haemorrhage and erythrocyte breakdown and the formation of haemoglobin breakdown products. As a general guideline more turbid fluids may have a higher nucleated cellularity (Figure 7.3).

Viscosity

Normal synovial fluid is very viscous because of high hyaluronanic acid content. Although viscosity can be measured on a viscometer, in practice this is usually a visual assessment best performed at the time of collection. When slowly ejected from a needle, normal fluid forms a long strand that is at least 2.5 cm before separating from the needle. When a drop is placed between thumb and forefinger, the strand bridges the two digits as they are moved apart.

Normal synovial fluid does not clot but may exhibit thixotropy (this is the property of certain gels to become fluid when shaken). On standing at room temperature, normal synovial fluid may assume a gelatin-like appearance but when shaken it becomes fluid again.

Mucin quality

If there is enough fluid, this may be assessed with a mucin clot test. If not performed immediately, a heparin sample is preferable to an EDTA sample since

EDTA degrades hyaluronic acid. One part synovial fluid is added to four parts 2.5% glacial acetic acid which causes the mucin to precipitate and sometimes clot. It can be performed in a test tube or a glass slide. With inflammatory conditions the proteases from the neutrophils degrade the hyaluronic acid and viscosity decreases.

Total nucleated cell count

This can be quantified using either a haemocytometer or an electronic particle counter. EDTA samples are preferable for nucleated cell concentrations and cytology due to greater cell preservation. Hyaluronidase can be added to minimise cell clumping.

These vary from joint to joint but generally normal joints have total nucleated cell counts <3000/μL and most cats <1000/μL.

Total protein

This is ideally measured by a quantitative biochemical assay because refractometry measures other solutes and protein. Joint fluid can form a thixolabile gel if left undisturbed for several hours; clots can be distinguished in normal fluid by gently shaking the sample to restore fluidity. If a specimen forms a clot after collection, this indicates intra-articular haemorrhage or inflammation with increased vascular permeability and protein exudation (e.g. fibrinogen) into the joint space. Normal synovial fluid has a low protein concentration <2.5 g/dL. Protein concentration will increase with inflammatory disease.

Cytology

Normal synovial fluid is composed predominantly of mononuclear cells and contains very few red blood cells (RBCs). The term 'mononuclear cells' includes monocytes, macrophages, lymphocytes and synovial lining cells which may not be readily distinguishable morphologically. With inflammatory processes neutrophils or even eosinophils may be identified. Cellular morphology must be interpreted cautiously in samples which are not fresh since exposure to EDTA or prolonged storage can result in altered morphology, e.g. cytoplasmic vacuolation. A subjective assessment of cellularity can be made cytologically. Generally, in the body of a normal smear, there are approximately 2 cells/field at 400× magnification (Figure 7.4). Due to the high viscosity of synovial fluid, cells tend to line up in rows (the so-called windrowing, Figure 7.5), and this characteristic pattern suggests normal viscosity although it should be noted that in hypocellular preparations it may not be possible to appreciate this feature. Cells are often located in a characteristic pink granular proteinaceous background fluid deposit (Figure 7.6 and Figure 7.7). Normal synovial fluid contains <5–12% neutrophils.

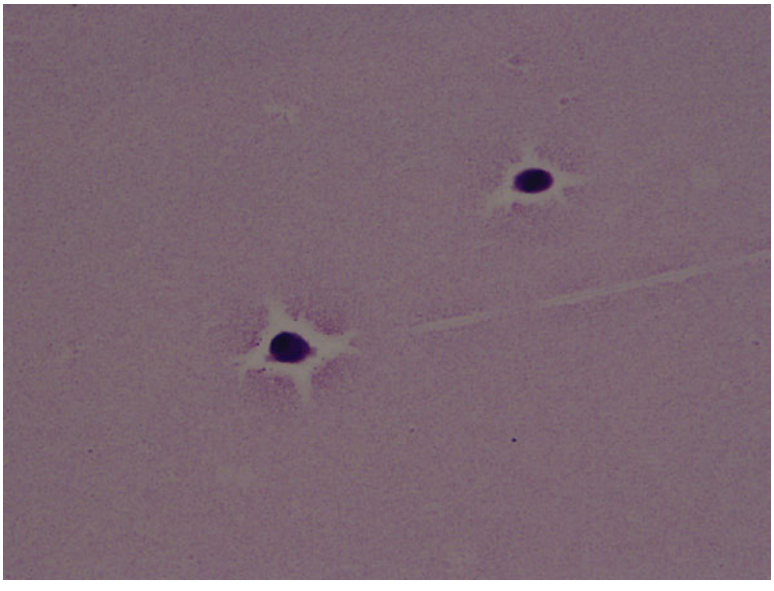


Figure 7.4 Note the low cellularity in this normal canine synovial fluid and appearance of dark-staining mononuclear cells with a moderate amount of granular basophilic cytoplasm. The cells are located in a stippled eosinophilic fluid deposit.



Figure 7.5 Cell windrowing in synovial fluid from a dog with degenerative arthropathy. Cells are seen to line up like rows of cut hay in a field. This property is due to synovial fluid viscosity. Source: John Dunn, Axiom Veterinary Laboratories Ltd. Reproduced with permission.

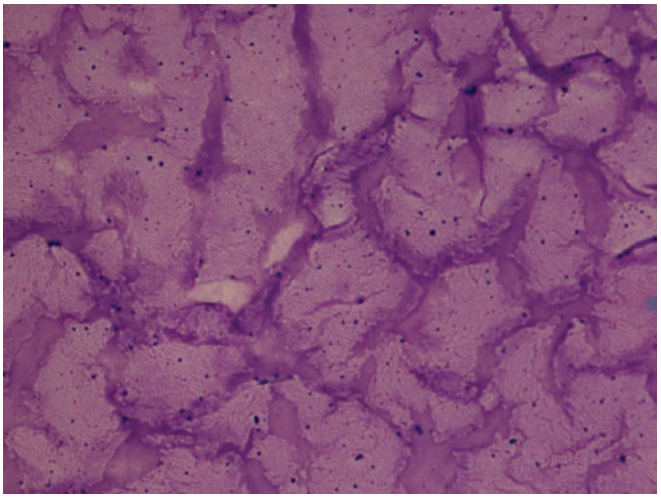


Figure 7.6 Synovial fluid from a dog. Note the thick granular to ropy background material reflecting the high mucin content.

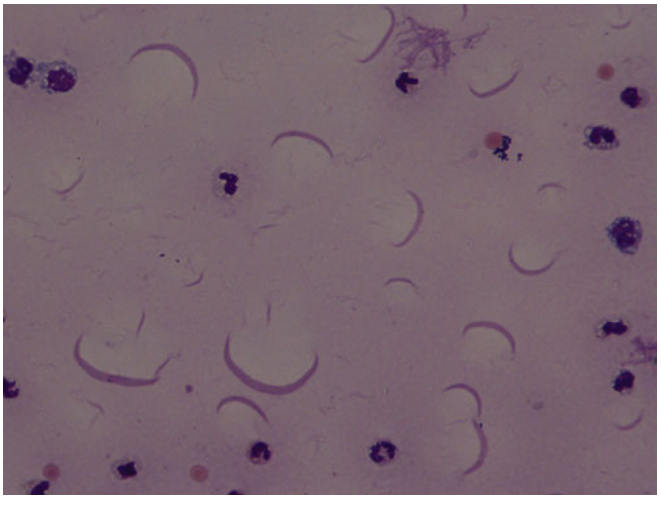


Figure 7.7 Note the increased number of neutrophils and protein 'crescents' (these are often seen with high-protein fluids and relate to inflammation here).

Culture

This can be performed on fluid samples collected into plain tubes or blood culture medium. The use of liquid blood culture media enables culture of larger volumes of joint fluid, and these media contain resins that decrease the inhibitory affects of antibiotics and intrinsic inhibitory substances in synovial fluid, as well as lytic

agents that release micro-organisms phagocytised by inflammatory cells. Blood culture tubes or bottles should be inoculated at the time of fluid collection and incubated for 24 h at 37°C then transferred to appropriate growth media. In some cases, culture of a synovial membrane biopsy may be necessary to obtain a positive culture result.

Ancillary diagnostic tests

Antinuclear antibodies (ANA)

These are key to detection of systemic lupus erythematosus (SLE), and low positive or transient ANA titres may also be seen with rheumatoid arthritis. However, low titres can also be detected with neoplastic, inflammatory or infectious disease, and sometimes, clinically normal animals and not all animals with SLE have detectable ANA.

Rheumatoid factor

This is the antibody (usually IgM) against the Fc portion of autologous IgG. This is of limited diagnostic use since positive results can be due to other causes and negative results to not rule out the disease.

Classification of joint disease

The aim of synovial fluid analysis is to distinguish inflammatory joint disease from degenerative joint disease (DJD). Haemarthrosis and neoplasia may also, less commonly, be distinguished. Further differentiation of disease processes requires correlation with other historical, laboratory and imaging findings. The classification of joint disease is summarised in Table 7.1.

Inflammatory arthropathies

These are either infectious or non-infectious (e.g. traumatic or immune mediated). Generally the greater the inflammatory reaction, the more turbid and discoloured and the less viscous the fluid becomes. They are characterised by increased numbers of neutrophils within the fluid which are typically moderately to markedly increased.

Infectious arthritis

Infectious arthritis can be caused by bacterial or fungal agents and generally presents with a very high nucleated cell count (mostly neutrophils). Neutrophil degeneration and phagocytosed organisms (Figure 7.8) are usually not features and therefore cannot be relied upon to indicate a septic aetiology. Organisms can gain access to joints

Table 7.1 Classification of joint disease

Category	Gross appearance	Viscosity	Total nucleated cell concentration	Protein	Differential	Causes
Normal	Clear to straw coloured	High	<3000/μL	<2.5 g/dL	<5% neutrophils, >95% mononuclear cells	
Degenerative arthropathy	Clear to straw coloured	Normal to decreased	1000–10,000/μL	Normal to decreased	<10% neutrophils, >90% mononuclear cells	DJD may be congenital or associated with joint instability, trauma or neoplasia
Inflammatory arthropathy	Cloudy, yellow to off white or red brown	Mildly to markedly decreased	5000–>100,000/μL	Normal to increased	10–90% neutrophils	Sepsis, immune mediated
Haemarthrosis	Red, cloudy or xanthochromic	Decreased	Increased RBCs	Increased	Number of red cells relative to leukocytes is similar to blood	Trauma, coagulopathy, neoplasia

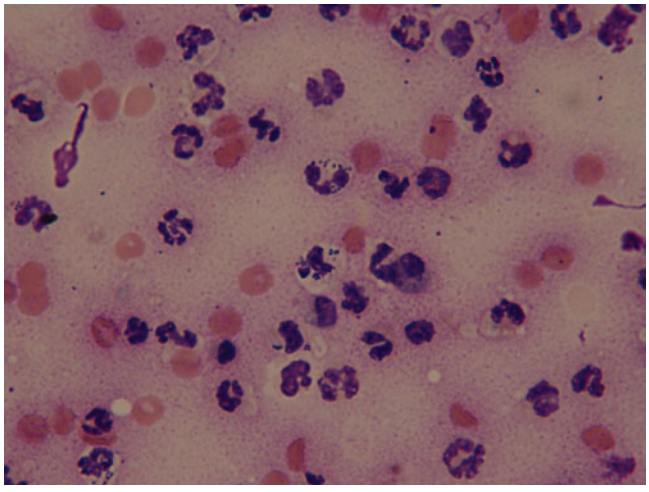


Figure 7.8 The presence of intracytoplasmic cocci bacteria from this fresh smear indicates sepsis.

either haematogenously or via direct inoculation, e.g. secondary to a penetrating wound. Infectious arthritis tends to present with solitary joint involvement and is often acute in onset. Since infectious agents and neutrophil degeneration are often not detected in septic joints, it may be advisable to always culture inflamed joints, bearing in mind that a negative culture also does to rule out infection since micro-organisms can be located in the synovial lining. Commonly cultured bacteria from joints include *Staphylococcus intermedius*, *Staphylococcus aureus* and beta-haemolytic *Streptococcus* spp. Haemolytic strains of *Escherichia coli* or *Pasteurella multocida* are most common in cats with septic arthritis. Fungal arthritides are uncommon but have been reported as a sequela to osteomyelitis or disseminated infection by *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Aspergillus* spp., *Coccidioides immitis*, *Histoplasma capsulatum* and *Sporothrix schenckii*. Other infectious organisms implicated as causative agents include mycoplasma, bacterial L-forms, spirochaetes (*Borrelia burgdorferi*), protozoa (*Leishmania donovani*), viruses (calcivirus, coronavirus) and rickettsia (*Ehrlichia canis, Rickettsia rickettsii*).

Non-infectious arthritis

With the exception of trauma, this involves an immune-mediated process, namely, a type III hypersensitivity response. Clinical signs are the result of immune complex deposition within the synovial capsule. Multiple joints are involved either concurrently or consecutively; however, occasionally, only a solitary affected joint is affected. In addition to joint pain/lameness, other signals of systemic disease include fever, generalised stiffness or ill-defined pain, peripheral blood cytopenias, neck or back pain, lymphadenopathy or proteinuria. Since affected animals may present with a 'shifting' lameness, aspiration of multiple joints is advocated.

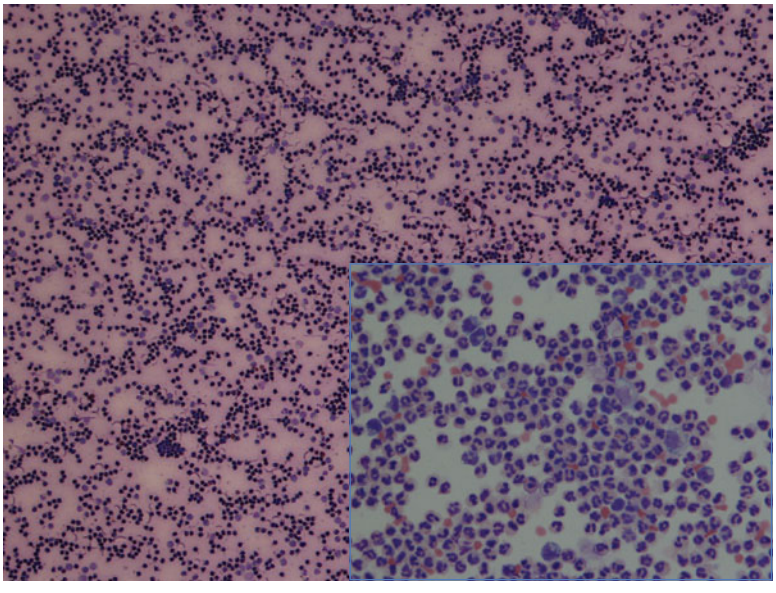


Figure 7.9 Direct smear from a dog with idiopathic immune-mediated polyarthritis. Note the protein crescents and marked increase in cellularity. Inset at higher power.

Non-erosive arthropathies

The majority of these immune-mediated arthritides are non-erosive. Immune-mediated polyarthritis may be idiopathic and drug induced, or it may occur secondary to infection or neoplasia. Other causes include borreliosis (Lyme's disease), ehrlichiosis, breed-specific polyarthritis (e.g. polyarthritis–meningitis syndrome in Bernese mountain dog, Boxer, Corgi, German Shorthaired Pointer, Newfoundland or Weimaraner; juvenile-onset polyarthritis of Akitas; and synovitis–amyloidosis of Shar-Pei dogs) and SLE. Although some cases of SLE will be seropositive for ANA, this is not necessarily the case, and a positive result may also be seen in some cases of idiopathic polyarthritis. In rare cases of SLE, lupus erythematosus cells (LE cells) may be seen. Crystal-induced arthritis (e.g. gout) has rarely been described in dogs and cats.

Cytologically, increased numbers of non-degenerate neutrophils (Figure 7.9) are seen although in rare cases increased numbers of lymphocytes and plasma cells may also be present (typically in stifle joints and associated with ACL rupture). Diagnosis of immune-mediated disease is dependent not only on demonstrating joint inflammation but also on ruling out infection via culture, serology and/or empirical therapy.

Erosive arthropathies

Types of erosive arthritis described in cats and dogs include rheumatoid arthritis, polyarthritis of greyhounds and feline progressive polyarthritis. Erosive arthritis is suggested when there are lucent cyst-like areas in the subchondral bone due to

progressive erosion of cartilage and subchondral bone in the area of synovial attachments, which results in loss of articular cartilage and collapse of the joint space with narrowing or widening of the joint spaces on radiography. There is a progressive loss of subchondral bone with deformation and destruction of affected joints. Deformities are most frequent in the carpal, tarsal and phalangeal joints and less frequent in the elbow and stifle. Other clinical features include morning stiffness, swelling of more than one joint within a 3-month period, symmetrical swelling of joints, mononuclear infiltrates observed microscopically in a synovial membrane biopsy and a positive rheumatoid factor (RF) titre. However, some idiopathic cases will also be seropositive for RF, and this is not a specific finding. Infection or neoplasia may also cause erosive joint disease.

Degenerative joint disease

DJD is characterised by degeneration of articular cartilage with secondary changes in associated joint structures. This can occur secondary to conditions such as osteochondrosis, hip dysplasia, joint instability, trauma, nutritional disorders or neoplasia. Cytological abnormalities may be detected before radiographic changes are readily apparent. The cytological changes are not as dramatic as those seen with inflammatory disease, and generally a mild increase in the number of mononuclear cells (Figure 7.10 and Figure 7.11) is the predominant finding, of which more than 10% are moderately to markedly vacuolated (Figure 7.12) or phagocytic (Figure 7.13). These cells are probably a mixture of macrophages (histiocytes) and synovial cells. Subsequent trauma to the joint may result in superimposed transient mild inflammation and haemorrhage. If damage to articular cartilage is severe, osteoclasts (Figure 7.14), osteoblasts (Figure 7.15) and chondrocytes may exfoliate into the joint fluid.

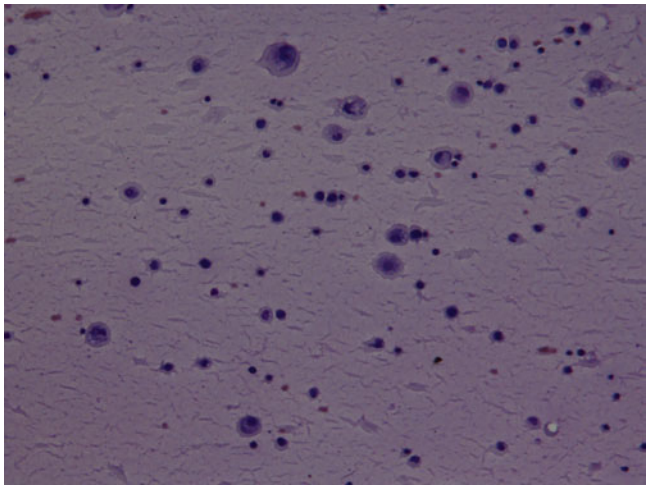


Figure 7.10 In degenerative arthropathy, large mononuclear cells can be seen within small clumps or individually.

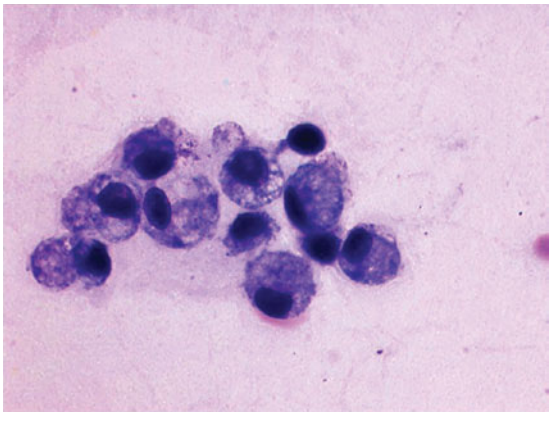


Figure 7.11 Note that the majority of these large mononuclear cells display cytoplasmic vacuolation. Source: John Dunn, Axiom Veterinary Laboratories Ltd. Reproduced with permission.

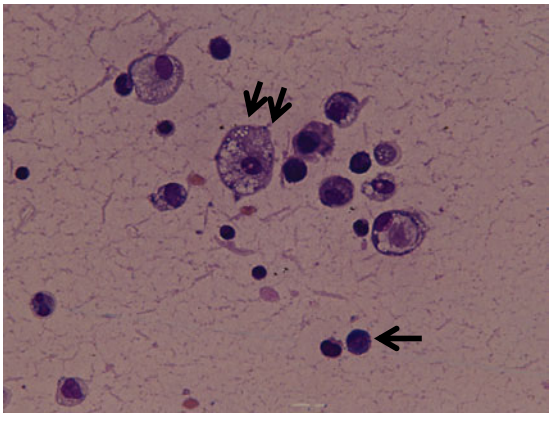


Figure 7.12 Direct smear from a dog with degenerative arthropathy showing large mononuclear cells or macrophage-type cells (double arrow) and small lymphocytes (single arrow). Reactive synoviocytes can be difficult to differentiate from reactive macrophages.

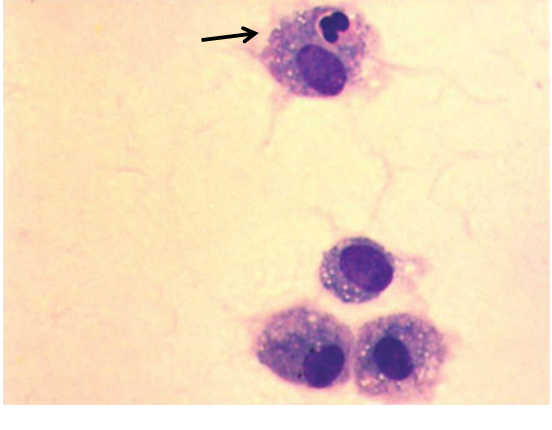


Figure 7.13 Large mononuclear cell displaying phagocytosis of a neutrophil.

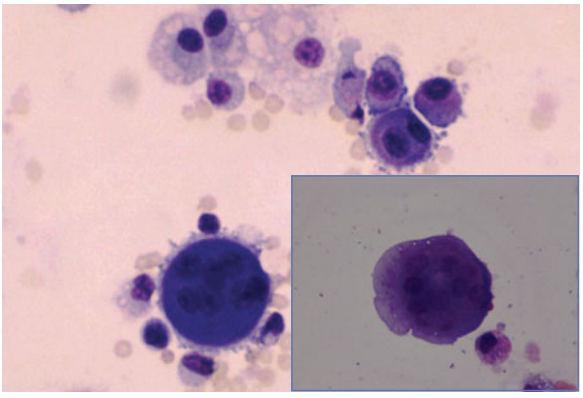


Figure 7.14 Osteoclast in synovial fluid from a dog with degenerative arthropathy. These cells indicate articular cartilage erosion and exposure of subchondral bone (500×). Inset: Osteoclast (higher power) shows a further example at the same magnification.

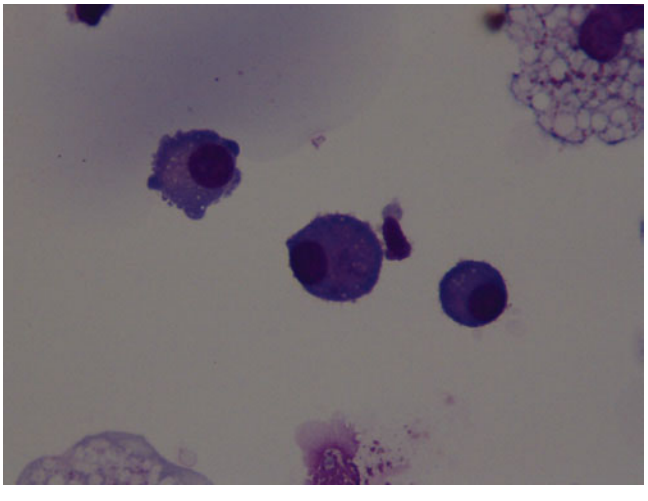


Figure 7.15 Osteoblast/chondrocytes within the same fluid as Figure 7.14.

Haemarthrosis

Recent trauma may result in joint haemorrhage. As in other instances in cytology, true haemorrhage must be distinguished from the much more common artefact of blood contamination. This is best done at the time of sample collection. If haemorrhage has occurred sometime previously, the synovial fluid may appear xanthochromic or homogenously red and cloudy. Cytologically, erythrophagocytosis and/or haem breakdown products (e.g. haemosiderin or haematoidin crystals) (Figure 7.16) may be observed within macrophages. Besides trauma other causes of haemorrhagic joint fluid include coagulation defects and neoplasia. A congenital coagulation factor deficiency should be considered in a puppy or kitten presenting with repeated episodes of haemarthrosis with or without a history of trauma.

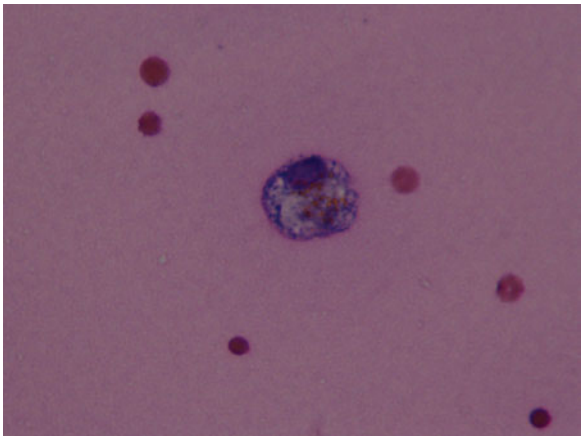


Figure 7.16 Blood contamination is common with joint aspiration. To help distinguish true haemorrhage from contamination, fresh smears should be examined for the presence of platelets, erythrophagia and haemosiderin or haematoidin crystals. The macrophage contains numerous small golden haematoidin crystals.

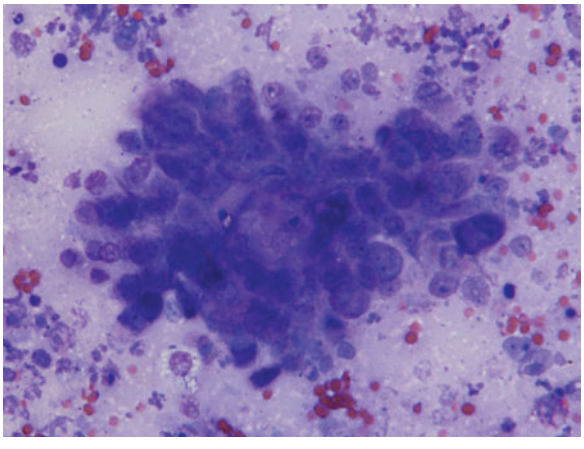


Figure 7.17 Direct smear from an aspirate from a swollen metacarpal joint in a cat. This cluster of cells displays anisocytosis, anisokaryosis and prominent nucleoli suggestive of neoplasia (suspected synovial cell or histiocytic sarcoma).

Neoplasia

Synovial neoplasia is rare in cats and dogs. Although synovial sarcomas are often cited as the most common neoplasm, studies have shown that the majority in dogs are actually of histiocytic origin. Other types include synovial myxoma, fibrosarcoma, chondrosarcoma and other undifferentiated sarcomas. Histiocytic and synovial cell sarcomas are also reported in cats. Cytological features of malignancy may be noted on aspiration of synovial fluid (Figure 7.17) although definitive characterisation of the neoplasm may require histological evaluation of a biopsy sample or, in some cases, immunohistochemical labelling to identify cell lineage.

8 Biochemical and Cytological Examination of Cerebrospinal Fluid

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Cerebrospinal fluid (CSF) collection and laboratory analysis is indicated in the investigation of disease processes involving the meninges, nerve roots or the central nervous system (CNS). CSF analysis is a sensitive test, which can help to narrow down the list of differential diagnoses, but rarely provides a definitive diagnosis. The results of CSF analysis should be interpreted in conjunction with the clinical presentation and diagnostic imaging findings.

When taking a CSF sample, the following should be taken into consideration:

- Owner consent: CSF collection is an invasive procedure requiring general anaesthesia with inherent risks for the animal. Poor collection technique may increase the risk of iatrogenic damage to the underlying CNS.
- The animal should be intubated and carbon dioxide partial pressure kept between 30 mmHg and 35 mmHg.

Contraindications

- Thrombocytopenia (platelet count < 50 x 10^9/L) or other coagulopathies, because of the increased risk of iatrogenic haemorrhage.
- Elevated intracranial pressure (ICP); the findings of diagnostic imaging and/or neurological examination findings should be considered prior to CSF collection.

CSF collection sites and technique

A CSF sample is collected routinely at the cerebellomedullary cisternal site (Figure 8.1, Figure 8.2, Figure 8.3 and Figure 8.4) or from the subarachnoid space at L5–L6 (Figure 8.5 and Figure 8.6) in dogs and cats. As a general rule, CSF

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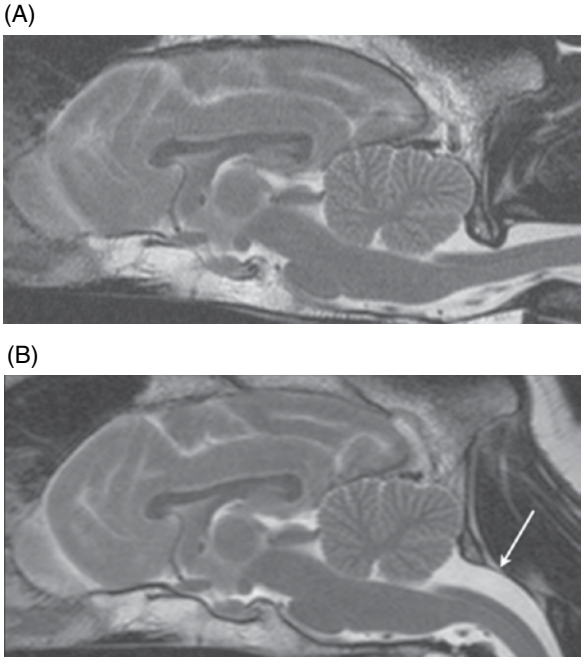


Figure 8.1 Sagittal T2-weighted magnetic resonance image of a Labrador brain with an (A) extended and (B) ventrally flexed head position. For cerebellomedullary collection, the head needs to be bent ventrally to increase the size of the subarachnoid space (arrow). Source: Holger Volk, RVC. Reproduced with permission.

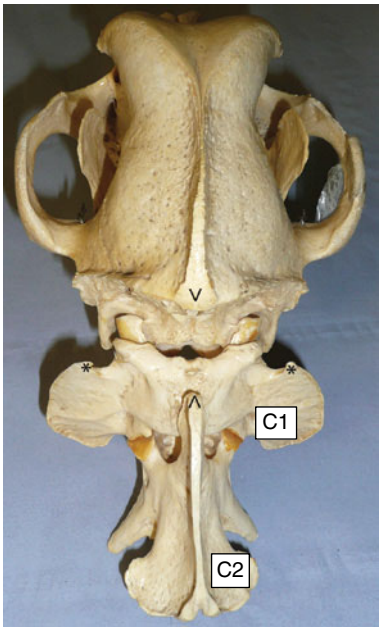


Figure 8.2 The bony landmarks for the cerebellomedullary cisternal CSF collection site. The landmarks for the midline are the external occipital protuberance ('V') and the spinous process of the axis (C2, 'Λ'). The needle with the bevelled edge pointing cranially is inserted through the midline near the cranial border of the wings of the atlas (C1), which can be found where the midline intersects with an imaginary line between the cranial borders of the wings of C1 ('*'). A slight loss of resistance is felt as the needle penetrates the dorsal atlanto-occipital membrane and enters the subarachnoid space. Source: Holger Volk, RVC. Reproduced with permission.

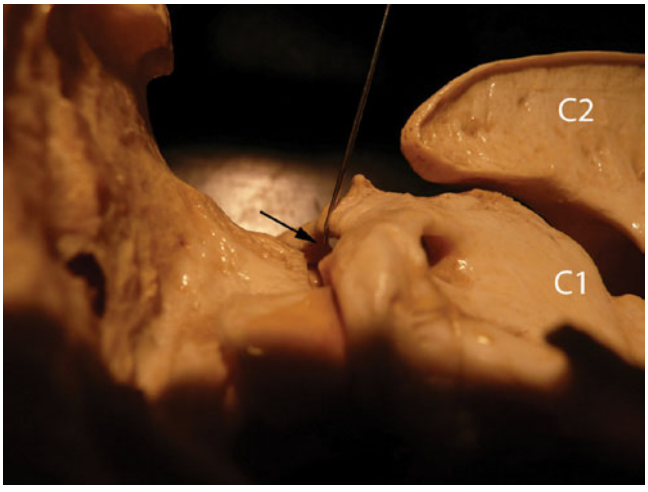


Figure 8.3 Image demonstrating the location of the tip of the spinal needle relative to the arch of C1 for CSF collection. Arrow indicates tip of the spinal needle. Source: Holger Volk, RVC. Reproduced with permission.



Figure 8.4 Aseptic collection of CSF. As soon the atlanto-occipital membrane is penetrated, the stylet is withdrawn and fluid is allowed to flow from the hub of the needle into sterile tubes. Especially for the inexperienced CSF collector, it can be easier to remove the stylet once the skin is punctured. The needle can then be advanced until CSF is retrieved. The CSF sample should be divided into at least two aliquots collected in separate tubes. The initial collection may be contaminated with blood. Source: Holger Volk, RVC. Reproduced with permission.

is obtained from the site which is both caudal and closest to the lesion. However, lumbar CSF collection is more commonly associated with iatrogenic blood contamination of the sample. A cerebellomedullary cisternal CSF sample is therefore often required even if the lesion is caudal to the cervical spinal cord.

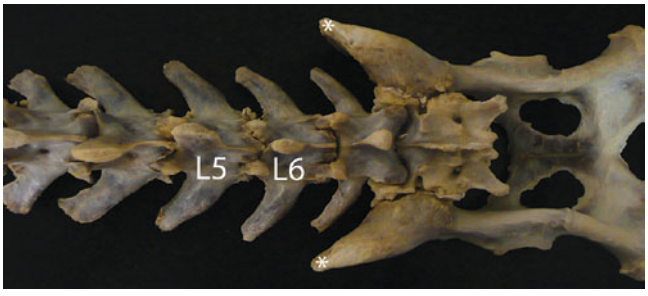


Figure 8.5 The bony landmarks for the L5–L6 CSF collection site. The landmarks for the midline are the dorsal spinal processes of L5 and L6. The dorsal spinal process of L6 can be found cranial to an imaginary line between the cranial aspect of the ilium ('∗'). The needle is inserted on the midline near the cranial border of L6 spinal dorsal process and then advanced ventrally. Source: Holger Volk, RVC. Reproduced with permission.



Figure 8.6 Image demonstrating the location of the spinal needle relative to the dorsal spinal process of L6. A slight loss of resistance is felt as the needle penetrates the dorsal interarcuate ligament. There can be a gentle twitch of the pelvic limbs. The stylet is removed and fluid is allowed to flow from the needle hub. The arrow indicates needle location in relation to the dorsal spinal process of L6. Source: Holger Volk, RVC. Reproduced with permission.

The dog or cat is placed in right/left lateral recumbency for a right-/left-handed person. The haircoat is clipped and the skin is aseptically prepared. The shortest possible (1.5–3.5 in) 22 G spinal needle with stylet can be used for most small animals. Occasionally, a 20 G spinal needle needs to be used for larger animals.

Processing of CSF

Ideally, once the CSF is obtained, it should be processed within 20 min–1 h. Chapter 1 demonstrates how to prepare a sediment smear to concentrate the cells if a cytocentrifuge is not available. Concentration of the cells is required even when there is a significant pleocytosis (increased cellularity) in the CSF, as cell numbers will still be relatively low compared to fluid samples from other sites. Although recent articles have suggested that total nucleated cell counts will not be significantly affected with 24–48-h storage, cell percentages can be significantly affected, and this will influence interpretation. If delayed processing is unavoidable, then the addition of 10% autologous serum may help preserve cell morphology but will obviously have a dilutional effect on cell numbers and will increase the measured protein concentration. If there is sufficient fluid, CSF may be split into aliquots, to which autologous serum can be added to one for cytology, and the second may be left untreated for the cell count and protein measurement.

Gross appearance

Normal CSF is clear, colourless and non-turbid. If the sample is contaminated with blood at the time of collection, it may appear haemorrhagic. Generally, if there has been bleeding into the CSF prior to collection, the sample will appear xanthochromic (yellow, similar to icterus). If the sample appears turbid, then a pleocytosis is likely, although cytological assessment will be needed to determine which cell types are present.

CSF protein concentration

The concentration of protein in normal CSF is very low (generally <0.25 g/L for cisternal samples and <0.45 g/L for lumbar samples, although actual reference intervals will depend on the laboratory used). Protein concentrations of this magnitude cannot be detected by refractometry or measured with routine protein assays on in-house chemistry analysers. A specific method for measurement of total CSF protein is required. Increased protein may be seen with any of the differentials which result in a pleocytosis as described in the following texts. Increased protein may also be seen without an increased cell count. This is referred to as cytoalbuminaemic dissociation. This may be seen with differentials such as intravertebral disc disease (IVDD), neoplasia, degenerative myelopathies or occasionally infectious agents. Atypical cells, such as macrophages, may or may not be present.

Cell counts

Cell counts are generally performed using a manual haemocytometer (Figure 8.7). A cover slip is placed on top ensuring Neubauer rings are formed on either side, and then the CSF is introduced to cover the surface. The haemocytometer is allowed to stand for 5 min before a count of nucleated cells and erythrocytes is undertaken.

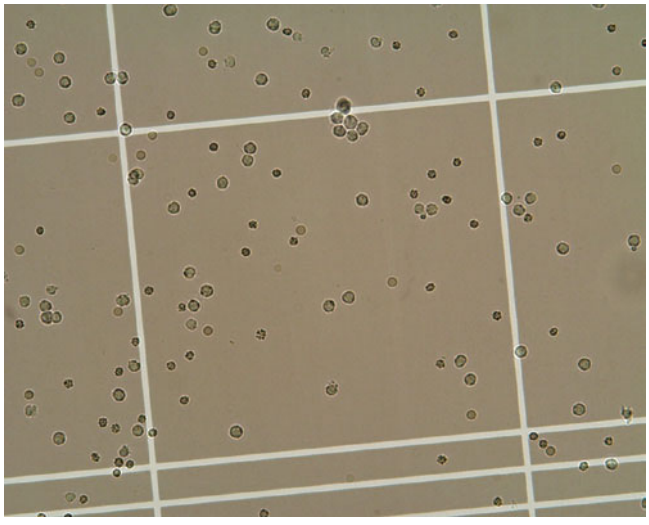


Figure 8.7 Haemocytometer. This shows one of the small squares at the edge of the larger square. The three lines at the bottom of the picture represent the outline of the larger square. This is a sample from a dog with Steroid Responsive Meningitis Arteritis (SRMA). Note the pleocytosis. Source: Kate English, RVC. Reproduced with permission.

Usually, the count is performed in the four large corner squares and the central square, and then the numbers are multiplied by a factor which depends on the depth of the haemocytometer chamber. It is considered optimal to perform a count on both sides of the haemocytometer and average the results. It can be challenging to separate nucleated cells and erythrocytes in some samples. Generally, the nucleated cells appear granular at higher magnifications, and if the condenser is moved away from the stage, the unstained cells can be seen more easily, and also the biconcave shape of the erythrocytes may become more apparent (Figure 8.8).

Nucleated cell counts are usually less than 5 cells/μL although some feline studies have cell counts of up to 8 cells/μL (mm^3). Erythrocytes are expected to be scarce. If iatrogenic haemorrhage has occurred, then moderate numbers of erythrocytes may be seen. The presence of very high numbers of erythrocytes (>500/μL) may increase the nucleated cell count. Some previous studies have attempted to use formulae to estimate the increase in the number of nucleated cells resulting from severe blood contamination; however, these are generally considered to be unreliable.

CSF cytology

Cell numbers can be semi-quantitatively assessed on cytology even if there is insufficient sample to perform other tests. Cytological examination of a stained sample of CSF is useful since atypical cells may be identified even if the cell count is low. Low numbers of large and small mononuclear cells can be seen in normal CSF (Figure 8.9). Small mononuclear cells are often small mature lymphocytes. The presence of macrophages, and large, reactive or granular lymphocytes, is unusual and may suggest a prior inflammatory stimulus.

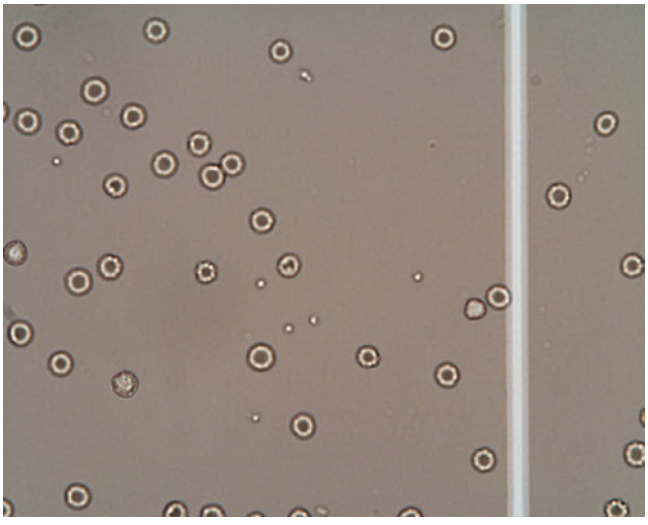


Figure 8.8 Haemocytometer. The biconcave shape of the erythrocytes can be clearly seen following lowering of the condenser to provide more contrast. Source: Kate English, RVC. Reproduced with permission.

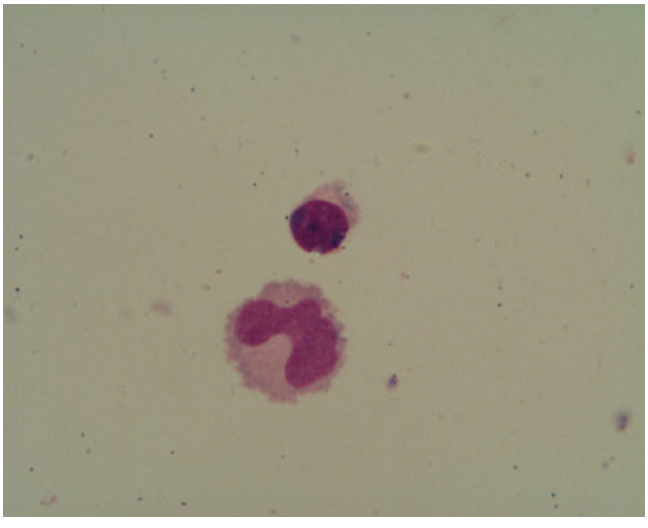


Figure 8.9 A large mononuclear cell (bottom) and a small lymphocyte (top) from an unremarkable canine CSF tap. Source: Kate English, RVC. Reproduced with permission.

Haemorrhage

The presence of erythrophagia or cells containing haem pigment suggests previous haemorrhage in a freshly processed CSF sample. Erythrophagia can occur *in vitro* if there is significant delay in processing the sample before cytology is performed (Figure 8.10 and Figure 8.11).

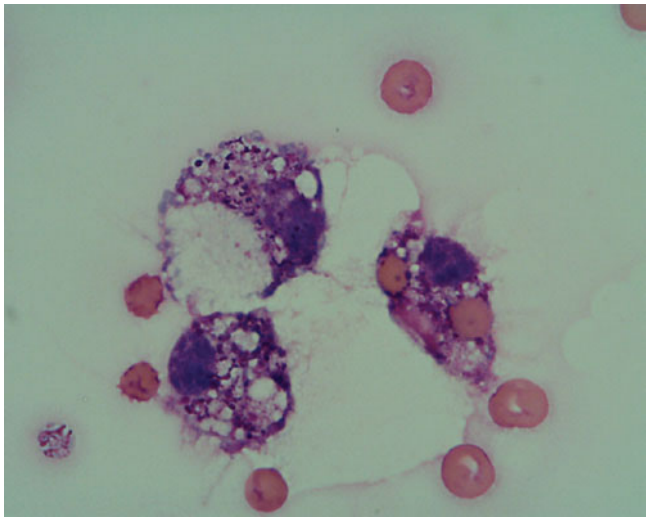


Figure 8.10 Macrophages exhibiting erythrophagia. Macrophages are present. One exhibits erythrophagia in this sample where there had been haemorrhage into the CSF prior to sampling. Source: Kate English, RVC. Reproduced with permission.

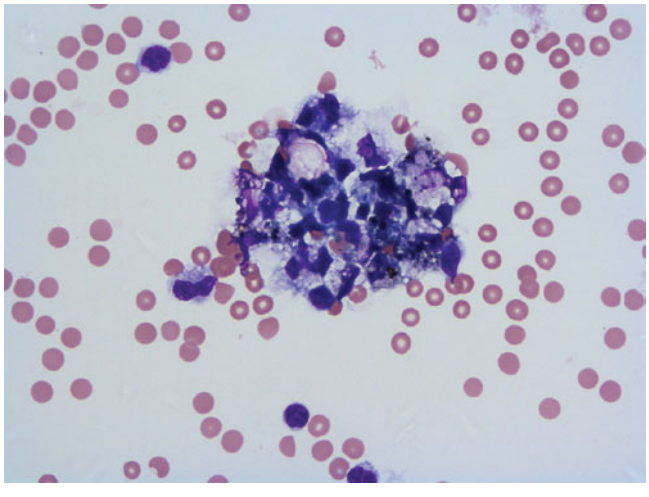


Figure 8.11 Macrophages with haemoglobin breakdown pigment. The macrophages contain blue-black pigment in this CSF sample taken from a dog following spinal surgery. Source: Kate English, RVC. Reproduced with permission.

Non-inflammatory cells

Meningeal lining cells are occasionally noted either discretely or in small clusters. These can be present due to sampling artefact and may not indicate significant pathology. However, occasionally large clumps may be present in an animal with meningioma (Figure 8.12)

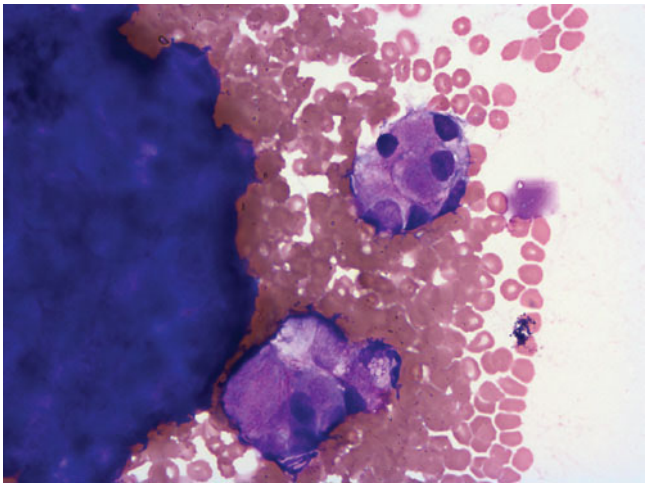


Figure 8.12 Meningeal cells. Note the abundant eosinophilic cytoplasm and small dense eccentrically located oval nucleus in the clustered cells. Source: Kate English, RVC. Reproduced with permission.

Neutrophilic pleocytosis

Neutrophils may be seen in low numbers (usually <7% of the total nucleated cell count) particularly if there is iatrogenic blood contamination. *Neutrophilic pleocytosis* exists when the number of neutrophils is increased and constitutes greater than 50% of the total nucleated cell population (Figure 8.13). In dogs, neutrophilic pleocytosis is commonly associated with steroid-responsive meningitis–arteritis (SRMA) which often presents with neck pain and joint effusions. In cats, the differential diagnosis includes the dry form of feline infectious peritonitis (FIP). Other causes of neutrophilic pleocytosis include trauma and acute IVDD. It may also occur occasionally as a response to neoplasia, e.g. meningioma. Bacterial meningitis is very rare in dogs and cats (Figure 8.14). Other infectious agents and rarely granulomatous meningoencephalomyelitis (GME) may also present with a neutrophilic predominance. Other rare differentials including a few breed-specific conditions may also be considered.

Mononuclear pleocytosis

Mononuclear pleocytosis is characterised by increased numbers of large mononuclear cells and/or small lymphocytes. In the dog, GME is a common cause of mononuclear pleocytosis. FIP (cat) and other infectious agents such as *Toxoplasma gondii* (cat and dog), *Neospora caninum* (dog) and fungal aetiologies should also be considered as differentials. These disorders may also be associated with a *mixed pleocytosis* where the distribution of mononuclear cells is similar to that described earlier, but the CSF also contains a variable number of neutrophils (Figure 8.15).

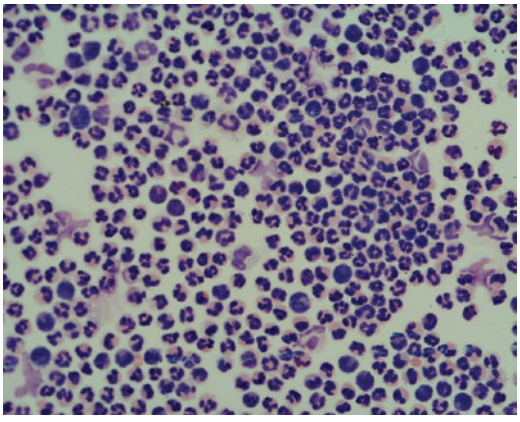


Figure 8.13 Neutrophilic pleocytosis. This sample is from a dog with SRMA. Note the high numbers of non-degenerate neutrophils. Source: Kate English, RVC. Reproduced with permission.

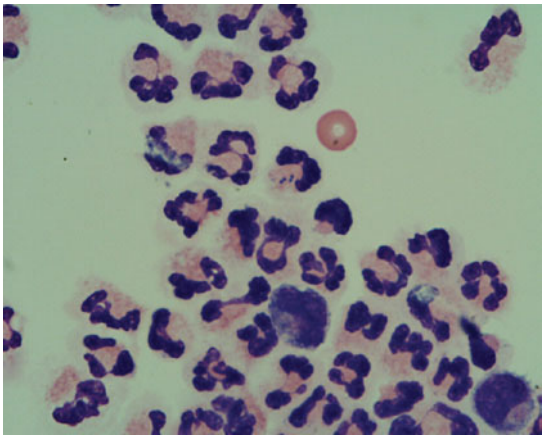


Figure 8.14 Bacterial meningitis. Note the intracellular cocci in the neutrophil next to the erythrocyte. Source: Kate English, RVC. Reproduced with permission.

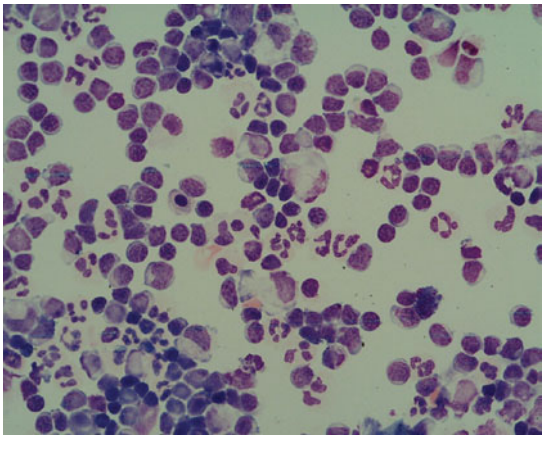


Figure 8.15 Mixed pleocytosis. This CSF sample is from a dog with GME. Note the mixed population of non-degenerate neutrophils, lymphocytes and large mononuclear cells resembling macrophages. Source: Kate English, RVC. Reproduced with permission.

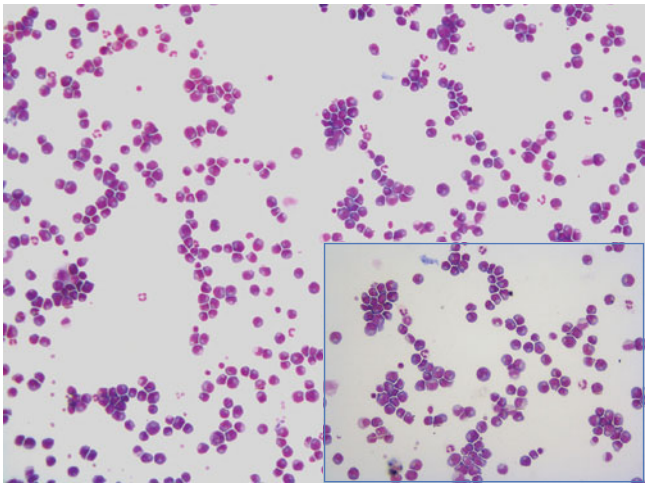


Figure 8.16 CNS lymphoma (dog). Note the high numbers of atypical lymphocytes in this CSF sample. Inset: The same cells under higher magnification. These round lymphoid cells have scant to moderate cytoplasm and an eccentrically located irregularly shaped nucleus. Note the perinuclear 'clearing' of the cytoplasm. Source: Kate English, RVC. Reproduced with permission.

Necrotising meningoencephalitis of small breed dogs and chronic canine distemper may present with a mononuclear pleocytosis where lymphocytes predominate. Other rare differentials including a few breed-specific conditions may also be considered. Resolving bacterial meningitis (following antibacterial therapy) may also have a lymphocyte predominant pleocytosis. Occasionally, a population of atypical lymphocytes may be seen in animals with lymphoma (Figure 8.16). Lymphomas involving the CNS do not always exfoliate cells into the CSF (so an absence of atypical lymphocytes in CSF does not preclude this diagnosis). CNS lymphoma is rarely primary and there is commonly involvement at other sites.

Eosinophilic pleocytosis

Eosinophilic pleocytosis is uncommon (Figure 8.17). A steroid-responsive eosinophilic meningitis occasionally occurs in dogs and cats. Other potential causes include protozoal and fungal infections and migrant parasites. It has also been reported with IVDD.

Myelin

Myelin can quite often be seen in CSF samples (Figure 8.18). It can appear as eosinophilic foamy material or as fibrillar strands or ribbons (this material stains positively for myelin with Luxol fast blue). Myelin is more often seen in lumbar samples. It may reflect sample artefact and therefore may not be indicative of CNS pathology.

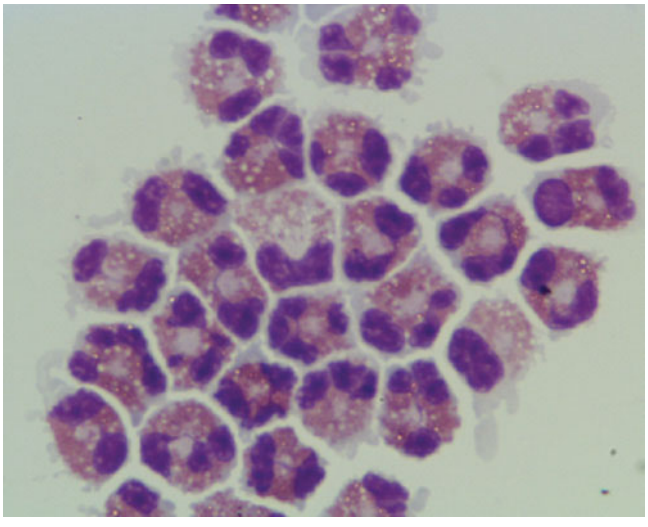


Figure 8.17 Eosinophilic pleocytosis CSF from a dog which was diagnosed with systemic candidiasis. Source: Kate English, RVC. Reproduced with permission.

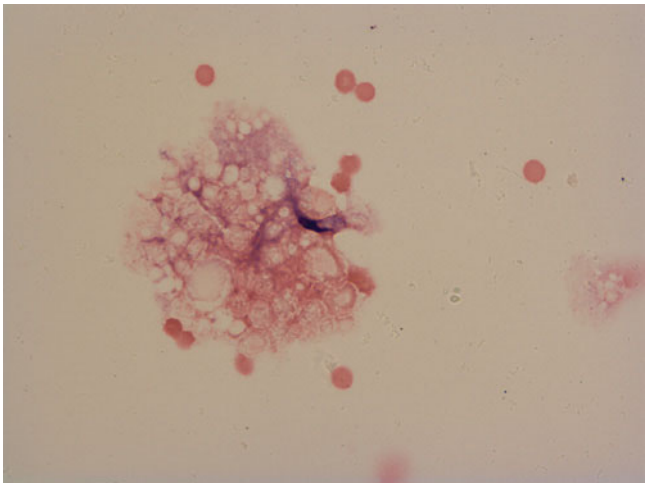


Figure 8.18 Myelin. The myelin stains as foamy eosinophilic material. Source: Kate English, RVC. Reproduced with permission.

Additional testing

Currently, biochemical or enzyme markers are not considered routinely useful. In inflammatory diseases, measurements of immunoglobulin fractions have been used to try and better characterise the underlying disease. Antibody or antigen tests or PCR testing may also be performed on CSF to check for certain infectious agents.

9 Cytology of the Eye and Adnexal Structures

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Eyelids

Indications for cytological investigation

Eyelid conditions that may benefit from cytological investigation include infectious, immune-mediated and neoplastic causes of blepharitis (Table 9.1). Not all of these conditions can be definitively diagnosed by cytological investigation, but the procedure may allow diagnosis of some conditions (e.g. demodicosis, dermatophytosis, lymphoma, mast cell tumour) or aid in the exclusion of others.

Collection techniques

With the exception of eyelid biopsy, most collection techniques can be performed in the conscious patient.

Hair pluck

A number of hair samples should be taken from different areas of the eyelid, including hairs close to the eyelid margin. Squeeze the skin and then gently pluck the hairs with 'padded' forceps in the direction of the hair growth. Low-field microscopic examination under oil, ideally after applying a cover slip, should be performed, examining the hair shaft for evidence of mites and spores.

Eyelid skin scrape

Apply a drop of oil to the skin or a small scalpel blade. Gently squeeze the skin to be sampled between finger and thumb to express follicular and possibly glandular material. Scrape with the blade in the direction of the hair growth until capillary ooze is seen to ensure sufficient depth for deeper parasites such as *Sarcoptes* spp.

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Table 9.1 Causes of blepharitis

Aetiology		Examples
Infectious	Viral	Canine papillomavirus (Figure 9.1), feline poxvirus, feline herpesvirus-1
	Bacterial	*Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp. (Figure 9.2), others

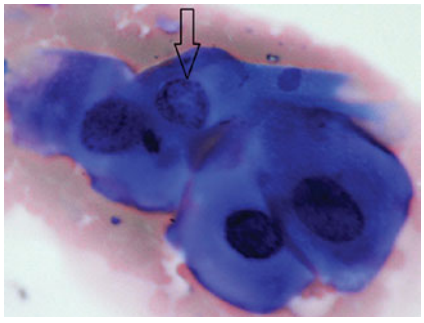


Figure 9.1 Four nucleated squames with intranuclear papilloma virus inclusions – indistinct hyalinised lightly basophilic ovoid structures (arrow). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

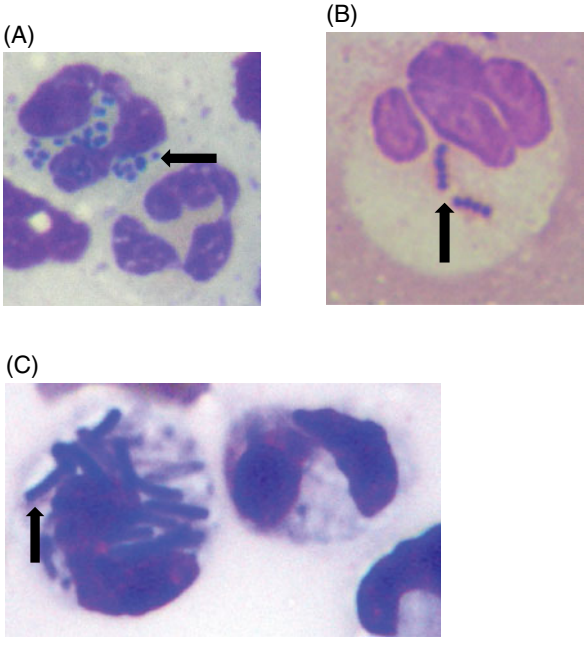
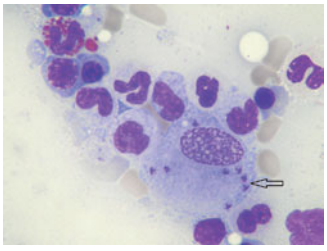
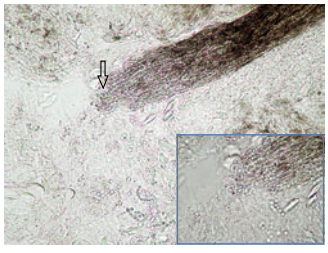
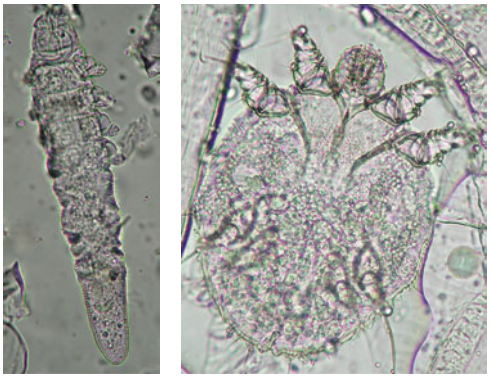


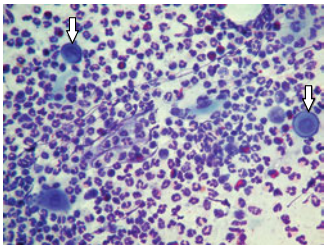
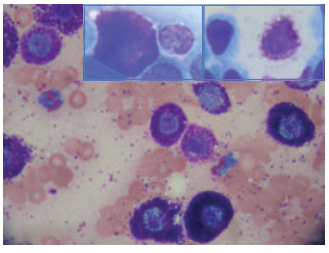
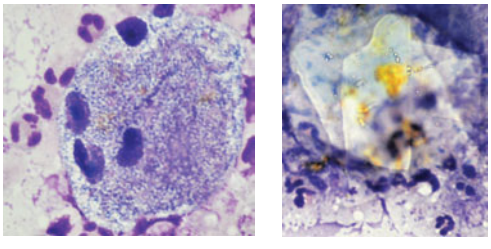
Figure 9.2 Karyolytic neutrophils with (A) diplococci (staphylococci) (B) two chains of cocci (streptococci); (C) phagocytosed large bacterial rods (*Pseudomonas* sp.). Source: A and B, Roger Powell, PTDS Ltd; C, David Gould, Davies Veterinary Specialists. Reproduced with permission.

Table 9.1 (Cont'd)

Aetiology	Examples
Protozoal	*Leishmania* spp. (Figure 9.3)
	
	Figure 9.3 Macrophage (largest cell) with eight phagocytosed *Leishmania* amastigotes (arrow). Source: Roger Powell, PTDS Ltd. Reproduced with permission.
Fungal	Dermatophytes (Figure 9.4)
	
	Figure 9.4 Hair shaft showing chains of refractile circular structures (arrow) indicating arthrospores (dermatophytosis – unstained KOH scrape). Source: Roger Powell, PTDS Ltd. Reproduced with permission.
	(*Microsporum canis, Trichophyton mentagrophytes*, *Microsporum gypseum*)
Parasitic	*Demodex* spp., *Sarcoptes* spp. (Figure 9.5)
	(A) (B)
	
	Figure 9.5 (A) Single *Demodex canis* and (B) *Sarcoptes scabiei* (unstained KOH scrapes). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

(*Continued*)

Table 9.1 (*Cont'd*)

Aetiology	Examples
Immune mediated	Pemphigus (Figure 9.6), systemic lupus erythematosus, uveodermatological syndrome, atopic/allergic disease
	
	Figure 9.6 Inflammatory cells with two deeply basophilic larger acantholytic nucleated squames (arrows), consistent with pemphigus foliaceus. Source: Roger Powell, PTDS Ltd. Reproduced with permission.
Neoplastic	Sebaceous adenoma (trichoblastoma), histiocytoma (see Figure 4.23), lymphoma (see Chapter 2 and Figure 4.26), squamous cell carcinoma (see Figure 4.13), mast cell tumour (Figure 9.7), adenocarcinoma, melanoma, papilloma, feline apocrine hidrocystoma, others
	
	Figure 9.7 Fresh blood with several discrete well-granulated mast cells and two eosinophils. Left inset: Well-granulated mast cell. Right inset: Same cell rapid stained ('Diff-Quik'), the granules not being 'seen'. Source: Roger Powell, PTDS Ltd. Reproduced with permission.
Miscellaneous	Meibomian gland impaction or granuloma (chalazion; Figure 9.8)
	(A) (B) 
	Figure 9.8 (A) Multinucleated giant macrophage with densely vacuolated cytoplasm (fat and yellow bilirubin) surrounded by mature neutrophils. (B) Several overlapping notched rectangular crystalline 'clefts' indicating cell degeneration and cholesterol deposition in cystic structures such as chalazion. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

Aetiology	Examples
	Histiocytosis Nodular fasciitis Feline eosinophilic blepharitis Foreign body (Figure 9.9) Secondary to entropion or ocular surface disease

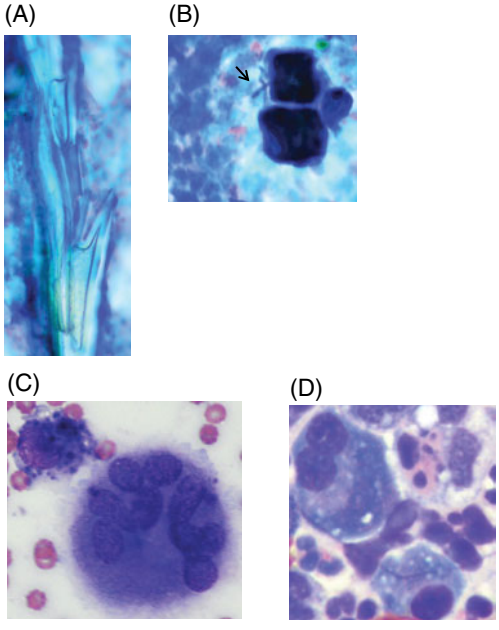


Figure 9.9 (A) Barbed plant material in a foreign body reaction. (B) Three square fragments of plant material, alongside blebbing basophilic linear fungal/yeast strands (arrow). (C) Multinucleated giant macrophage with a vacuolated phagocytic macrophage. (D) Two plasma cells, one binucleate (top left). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

Gently smear the material as thinly and evenly as possible on a labelled glass slide.

The material is covered with a cover slip to aid examination. Oil can be used for this, allowing the mites to be spotted as they move around; however, if the preparation is too thick, the squames can hide the mites. The use of potassium hydroxide (KOH 1–10%) instead of oil will 'clear' the keratinised material, making it transparent so mites are more easily seen. The KOH should be carefully applied to the material under the cover slip and left for at least 30 min to allow 'clearing' before examination. Note, however, that the 10% solution of KOH kills the mites so they no longer move. Look between and along the margins of the hair shafts for parasites (Figure 9.5). Examine the base of the hair for arthrospores (Figure 9.4).

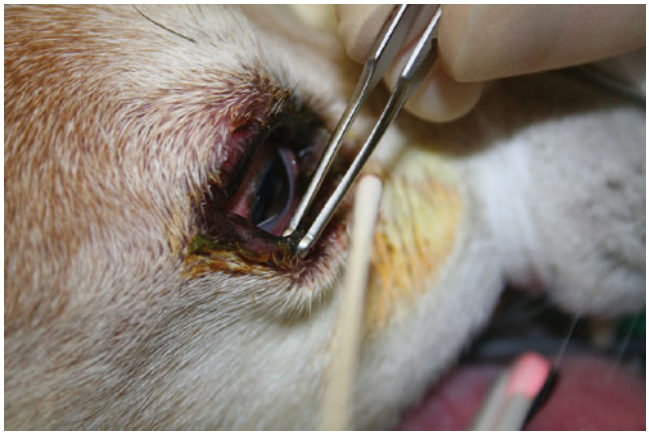


Figure 9.10 Technique for meibomian squeeze using thumb forceps. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

Meibomian gland squeeze

- Application of topical anaesthetic to the palpebral conjunctiva prior to performing this technique may aid patient compliance.
- Samples collected from the meibomian glands (Figure 9.10) should be submitted for bacterial culture and sensitivity testing.
- They may also be examined under low-field magnification for evidence of Demodex mites (Figure 9.5). If air-dried, they can also be examined for bacteria (Figure 9.2).

Eyelid biopsy

- This should be performed under general anaesthesia.
- Eyelid biopsy (Figure 9.11) samples should be fixed in preservative and submitted for histopathological examination. However, cytological examination of an impression smear of the biopsy sample immediately prior to fixation may allow early diagnostic interpretation. Ensure the smear is not exposed to formalin fumes as this distorts the cells and prevents detailed examination.
- If an infectious aetiology is suspected, eyelid biopsy samples should also be submitted for bacterial and fungal culture.

Normal cell types

- *Keratinised squames* (Figure 9.12) found on the outer haired surface of the eyelid.
- *Sebaceous epithelial cells* have clear finely vacuolated cytoplasm and a central dense staining nucleus (Figure 4.12). These are located in the epidermal layers between the outer and inner surfaces of the eyelid.
- *Columnar epithelial cells* are found on the inner surface. These are longer thin cells with blue vacuolar cytoplasm that may contain distinct irregular eosinophilic globules. They have a polar/basal circular nucleus and a small nucleolus (Figure 9.13).

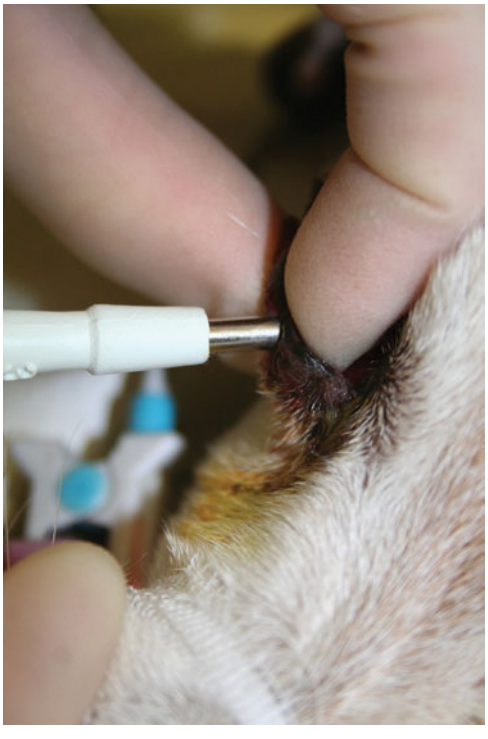


Figure 9.11 Technique for eyelid punch biopsy. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

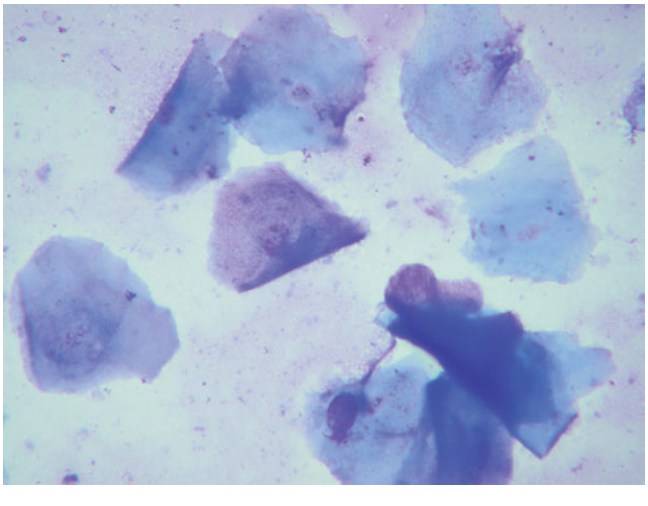


Figure 9.12 Individual anucleate angular polygonal keratinised squames with light blue-turquoise cytoplasm. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

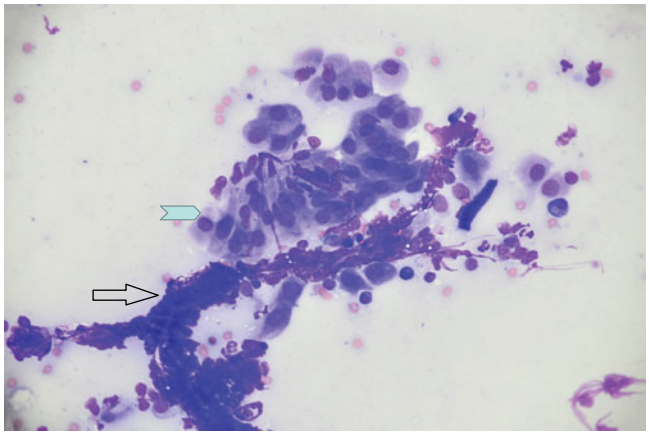


Figure 9.13 Small palisading cohesive strand of monomorphic columnar epithelial cells (arrowhead) alongside lysed cellular material (arrow) and scattered mixed inflammatory cells. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

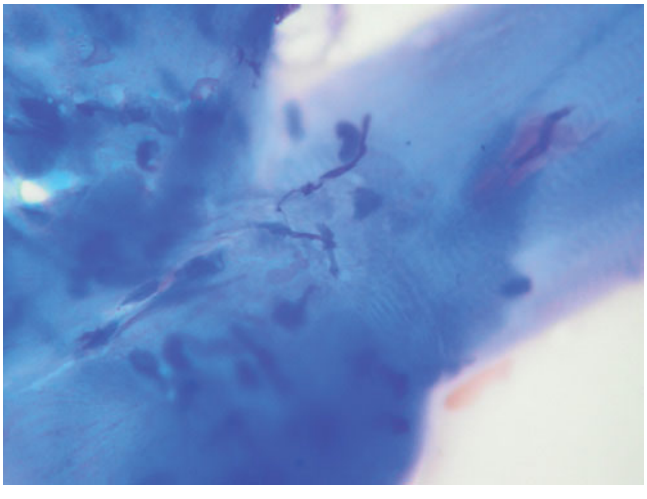


Figure 9.14 Skeletal striated muscle fragments. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

- Skeletal muscle fibres may be aspirated from the dermis and subcutis. These are dark turquoise-coloured cells with fine striations and dense strap-like nuclei (Figure 9.14).

Abnormal cytology

Inflammation

- Bacteria, most commonly cocci or rods, typically elicit a purulent neutrophilic inflammatory response. The neutrophils usually appear karyolytic (Figure 9.2). Foreign bodies and higher-order agents such as mycobacteria

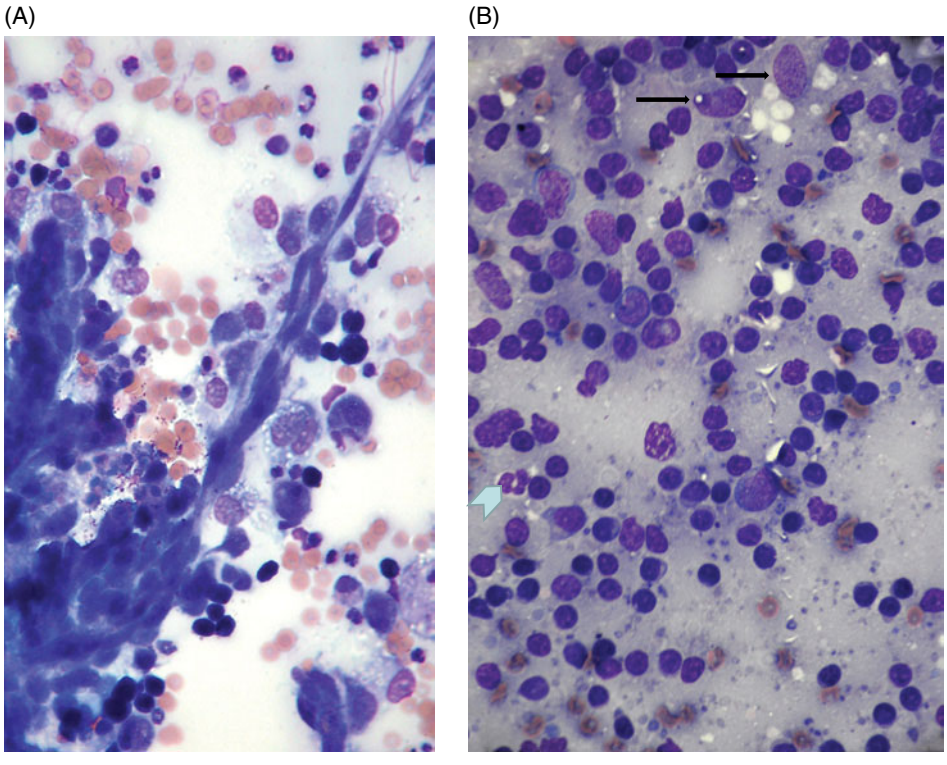


Figure 9.15 (A) Dense fibrovascular ball with neo-capillary extending up – right. (B) Mixed lymphocytes, primarily small with a few intermediate and nucleolated larger ones alongside occasional neutrophil (arrowhead) and dendritic cells (arrows) in a lymphoid follicle forming in chronic inflammation. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

may elicit a more mixed pyogranulomatous to granulomatous response characterised by increased numbers of mononuclear cells (e.g. lymphocytes, plasma cells and macrophages). Some macrophages may be multinucleated (Figure 9.9). *Mycobacteria* spp. can be seen as clear non-staining, linear or curvilinear structures within macrophages (see Figure 2.5, Figure 5.11 and Figure 15.2E), but the organisms can also be extracellular. Foreign bodies may introduce environmental opportunistic fungi or yeasts (Figure 9.9B). More chronic lesions may induce angiogenesis and nodular lymphoid follicular hyperplasia (Figure 9.15). Immune-mediated lesions are occasionally sampled (Figure 9.6).

Other infectious agents

- *Viral inclusions* are typically intra-nuclear, appearing as variably distinct circular to more oval or curved eosinophilic or basophilic smooth 'clumps' inside the nucleus, displacing the chromatin into thick black grainy strands (Figure 9.1).

- *Leishmania amastigotes* consist of a central circular to oval dense nucleus and a linear bar-shaped kinetoplast, positioned at right angles to the nucleus (Figure 9.3).These may intracellular (within macrophages) or extracellular.
- *Dermatophytosis*. Arthrospores can be seen as chains of circular refractile structures at the base of the hair shaft (Figure 9.4). When stained, they appear as dark blue circular structures inside macrophages or hair shafts.

Neoplasia

The following neoplasms may more commonly involve the eyelids:

- *Sebaceous adenoma*
- *Apocrine (basal cell) adenoma or trichoblastoma*
- *Squamous cell carcinoma*
- *Histiocytoma*
- *Lymphoma*
- *Mast cell tumour*

Please refer to Chapter 4 for images and cellular descriptions of these tumours.

Conjunctiva and cornea

Indications for cytological investigation

Causes of conjunctival and corneal disease in dogs and cats are listed in Table 9.2. Diagnostic cytology should be employed in cases of severe, progressive or recurrent conjunctivitis and keratitis and in those cases that are resistant to empirical treatment.

Collection techniques

The appropriate collection techniques for conjunctival and corneal investigations are listed in the following texts.

Conjunctival or corneal swabs

- Swabs should be submitted for bacterial culture and sensitivity testing. Since topical anaesthetics can inhibit bacterial culture, samples for bacterial culture should be taken prior to application of a topical anaesthetic. To reduce the risk of contamination, care should be taken to avoid contact of the swab with the lid margin or facial skin.
- If *Mycoplasma* infection is suspected, specific transport medium should be used.
- Alternatively plain swabs can be used for PCR testing for certain agents such as herpesvirus, *Chlamydophila* and *Mycoplasma* spp.

Table 9.2 Causes of conjunctival or corneal disease

Aetiology		Examples
Infectious	Viral	Feline herpesvirus-1, canine distemper virus, canine adenovirus, canine herpesvirus-1
	Bacterial	*C. felis* (Figure 9.16), *P.* spp. (Figure 9.2), *Streptococcus* spp. (Figure 9.2), *Mycoplasma* spp., others
	Protozoal	*Leishmania* spp. (Figure 9.3)
	Fungal	Fungal keratitis (*Aspergillus* spp., others) (Figure 9.17)
Immune mediated		Chronic superficial keratitis (pannus; Figure 9.18), keratoconjunctivitis sicca, eosinophilic keratoconjunctivitis (Figure 9.19A and B), nodular granulomatous episclerokeratitis, atopic/allergic disease
Neoplastic		Lymphoma, mast cell tumour, melanoma, papilloma, squamous cell carcinoma, others (see Chapter 4)
Miscellaneous		Feline corneal sequestrum, corneal epithelial cyst, foreign body, subconjunctival orbital fat prolapse
		Secondary to eyelid, lacrimal, intraocular or orbital disease
		Secondary to external irritants

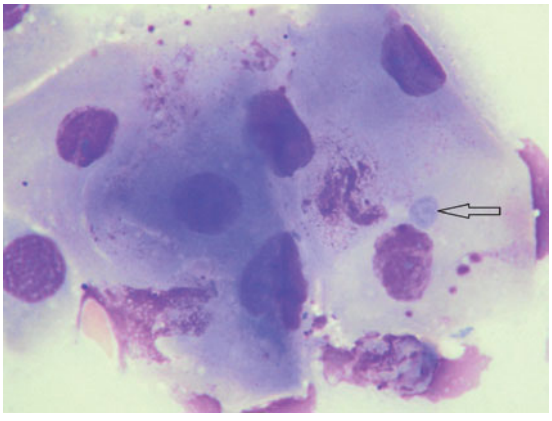


Figure 9.16 Several nucleated squames, one with a basophilic *Chlamydophila* initial body (arrow). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

Exfoliative cytology

- Samples for cytological examination ideally should be collected with a cytobrush (Figure 9.20), which results in superior cell quality and yield in comparison to collection with a swab. A topical anaesthetic should be applied prior to sampling to improve patient compliance.

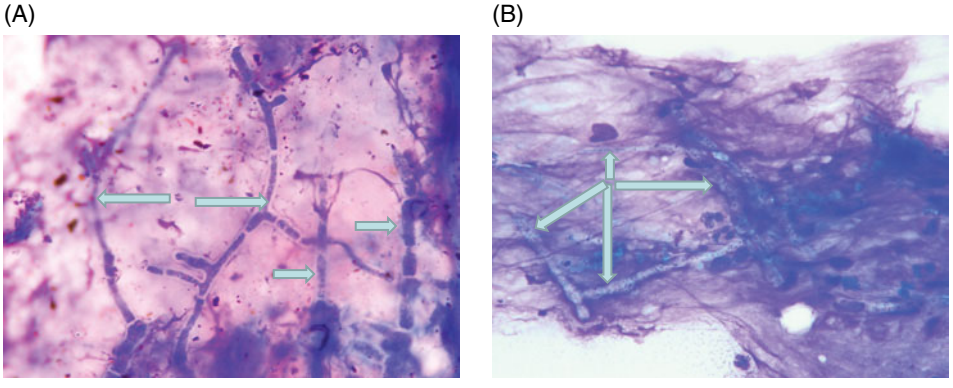


Figure 9.17 (A) Fungal right-angle branching basophilic hyphae (arrows). (B) Less staining and more irregular grainy branching fungal hyphae (arrows), genetic sequencing confirming *Scedosporium apiospermum*. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

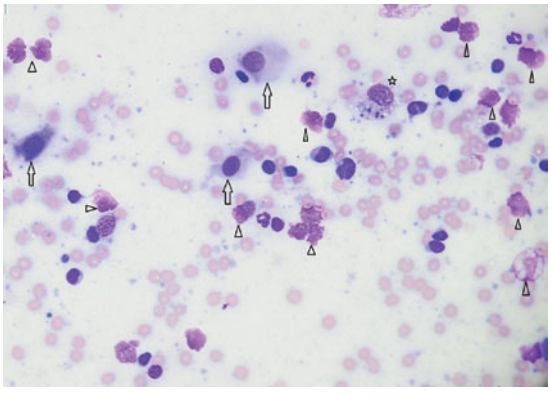


Figure 9.18 Epithelial cells (arrows) with bare lysed nuclear remnants (arrowheads) and mixed inflammatory cells, mainly small lymphocytes, scattered mature neutrophils and a macrophage (*). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

- Conjunctival snip biopsies can be taken under topical anaesthesia, light sedation or general anaesthetic (Figure 9.21). An impression smear of the sample may allow early diagnostic interpretation prior to fixation and submission of the sample for histopathological examination, ensuring no formalin fume exposure.

Normal cell types

- *Nucleated squamous epithelial cells* are round to cuboidal in appearance (Figure 9.22A). *Goblet cells and columnar epithelial cells* may also be present (Figure 9.22B).
- When sampling, if previous ultrasound examination has been performed, ensure that gel does not contaminate the slides and obscure morphological detail (see Figure 1.19).

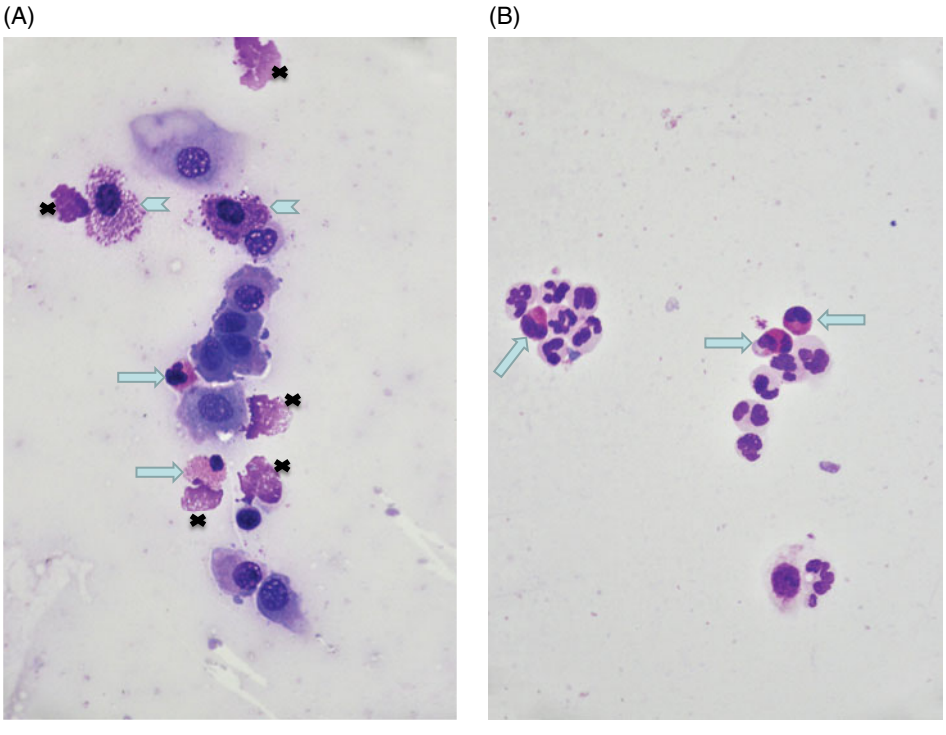


Figure 9.19 (A) Nucleated squames, lysed cells (x), two eosinophils (arrows) and two mast cells (arrowheads). (B) Groups of neutrophils with three eosinophils (arrows), the latter not always dominating in cases of eosinophilic keratitis as they are variably present at various depths and also multifocally. Source: Roger Powell, PTDS Ltd. Reproduced with permission.



Figure 9.20 Cytobrush for collection of conjunctival material. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

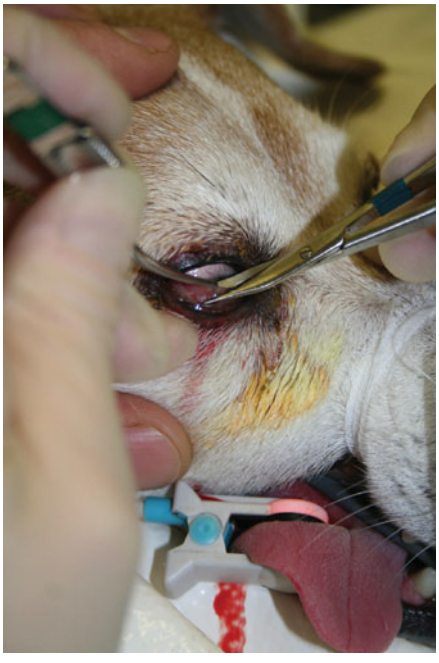
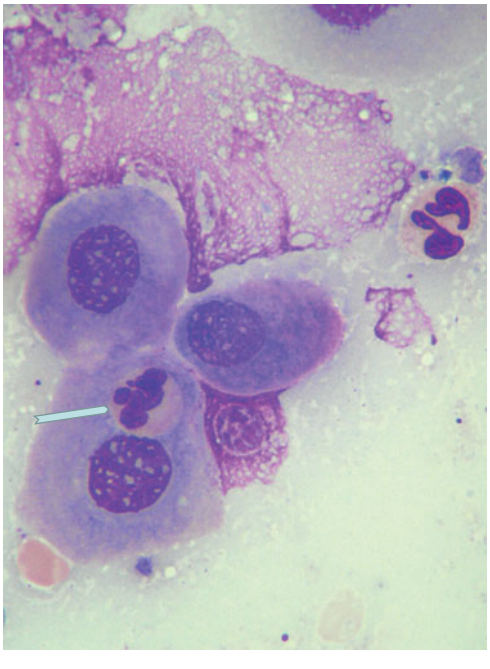


Figure 9.21 Technique for conjunctival snip biopsy. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

(A)



(B)

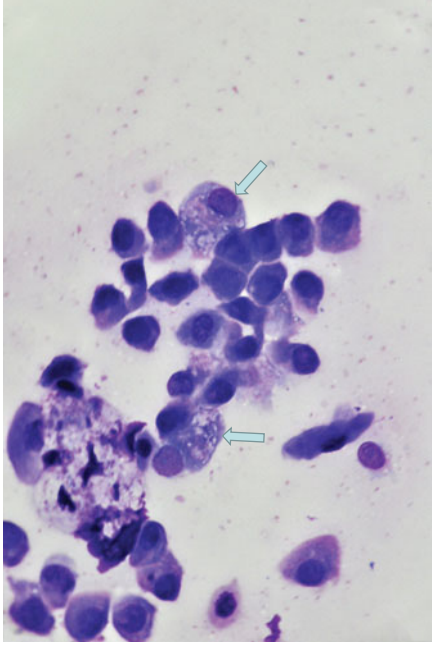


Figure 9.22 (A) Nucleated squames with a trafficking cytoplasmic neutrophil (arrowhead) – emperipolesis – seen in inflammation. (B) Goblets cells (arrows), which look similar to columnar cells but more rounded with vacuolar cytoplasm that can be globular and eosinophilic, especially with more chronic lesions. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

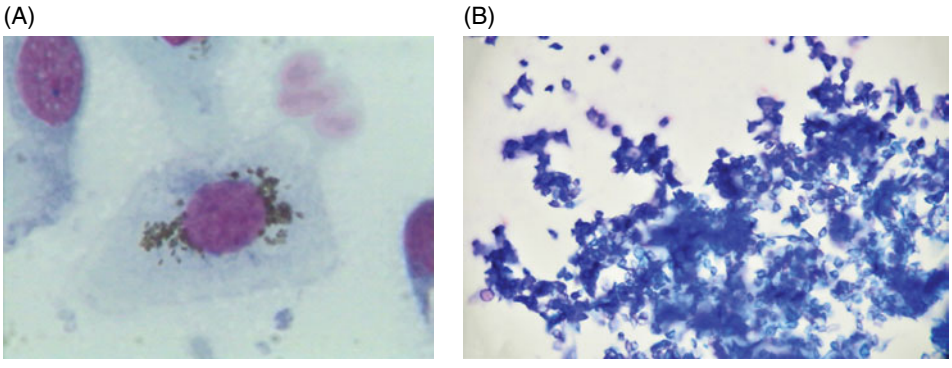


Figure 9.23 (A) Superficial nucleated squame with perinuclear grains of melanin seen in chronic inflammation. (B) Irregular refractile crystalline clear material with a basophilic 'halo' consistent with calcium salts seen in chronic keratitis. Source: Roger Powell, PTDS Ltd. Reproduced with permission.

Abnormal cytology

Inflammation

- Neutrophilic inflammation may be due to acute bacterial or viral infection but consider also immune-mediated disease or traumatic ulceration especially if culture (and/or PCR) results are negative. *Pannus* is characterised by a more chronic mixed or pyogranulomatous inflammatory response (Figure 9.18). Chronic inflammation may result in epithelial dysplasia and the presence of perinuclear melanin granules in squamous epithelial cells (Figure 9.23A). It can also be associated with dystrophic calcification if long standing (Figure 9.23B).

Eosinophilic keratitis is often associated with herpesvirus infection in cats, or it may be immune mediated. The inflammatory response consists of variable numbers of mast cells and eosinophils with lower numbers of neutrophils and small lymphocytes (Figure 9.19).

Other infectious agents

- *Chlamydophila felis* appears as indistinct basophilic to slightly purple initial and/or elementary intracytoplasmic bodies in squamous epithelial cells (Figure 9.16).
- *Mycoplasma* spp. appear as small indistinct basophilic 'dots' on squamous epithelial cells (see Figure 15.2H).
- *Fungal keratitis*. Fungal hyphae stain as linear septate structures with parallel walls. Hyphae branch at various angles. The presence of fruiting bodies (conidiophores) may allow speciation, or it can be done by culturing or genetic sequencing (Figure 9.17).

Neoplasia

- The tumour types are similar to those that can involve the eyelids.

Anterior chamber

The normal anterior chamber is essentially acellular but in anterior uveitis inflammatory cells traverse the blood–aqueous barrier and enter the aqueous humour where they cause aqueous flare. Causes of anterior uveitis are listed in Table 9.3.

Most cases of anterior uveitis do *not* warrant cytological investigation of the anterior chamber. If aqueous flare is not clinically visible via ophthalmoscopic or slit-lamp examination, then aqueocentesis is not indicated.

Collection technique

- Aqueocentesis should be performed with the patient under general anaesthesia or heavy sedation. Immediately prior to aqueocentesis, topical anaesthetic should be applied and the ocular surface should be aseptically prepared using dilute povidone–iodine solution at 1:50 dilution.
- A 25–30 G needle attached to a syringe is passed into the anterior chamber via the limbus. Forceps are required to stabilise the globe during passage of the needle (Figure 9.24). It is important to closely visualise the needle tip during passage to ensure that it enters the anterior chamber and does not damage the iris or lens. Approximately 0.1–0.2 mls of aqueous humour is slowly drawn into the syringe before the needle is briskly withdrawn from the anterior chamber. If volumes greater than 0.2 mls are withdrawn, the anterior chamber should be re-inflated with balanced salt solution or sterile normal saline.

Table 9.3 Causes of uveitis in dogs and cats

Infectious	Viral (adenovirus and herpesvirus in dogs; feline infectious peritonitis, feline immunodeficiency virus, feline leukaemia virus, feline herpesvirus-1 in cats) Bacterial (e.g. *Bartonella* spp., *Leptospira* spp., *Borrelia burgdorferi*, *Ehrlichia canis*, penetrating injury or septicaemic spread) Protozoal (toxoplasmosis, neosporosis, leishmaniasis) Mycotic/algal Parasitic (e.g. *Angiostrongylus vasorum*)
Non-infectious	Traumatic Lens induced Systemic disease (e.g. toxaemia, bleeding disorders, diabetes mellitus, hyperlipidaemia, systemic hypertension, granulomatous meningoencephalomyelitis, systemic histiocytosis) Neoplasia (most commonly lymphoma) Autoimmune/immune-mediated disease Idiopathic

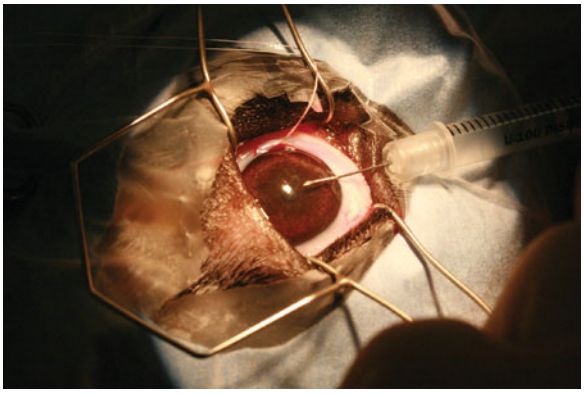


Figure 9.24 Technique for aqueocentesis sampling. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

Indications for cytological investigation

Aqueocentesis sampling may be indicated if lymphoma is suspected in an animal with active aqueous flare. It is not generally useful in the diagnosis of other types of intraocular tumours since these do not tend to shed cells into the aqueous humour in sufficient numbers. Less commonly, aqueocentesis is performed to collect samples for culture, PCR or antibody testing in selected cases of infectious uveitis.

Sampling of the anterior chamber carries some risk, including bacterial contamination, iatrogenic damage to the intraocular structures (specifically the iris and lens) and retinal detachment. Aqueocentesis should therefore only be performed by veterinary surgeons experienced in the technique.

Normal cell types

As mentioned previously, aqueous humour is essentially acellular (only the occasional rare small lymphoid cell may be present). The presence of increased numbers of any cell type is therefore considered abnormal.

Abnormal cytology

Inflammation

- Many cases of anterior uveitis are idiopathic. Infectious causes are listed in Table 9.3. The inflammatory response may vary from neutrophilic to lymphocytic, mixed or pyogranulomatous (Figures 9.25). These different inflammatory categories may reflect the same disease process over time. Neutrophils may be hypersegmented and pyknotic due to hydropic degeneration or centrifugation of the sample (Figure 9.25C). Macrophages often contain phagocytosed melanin

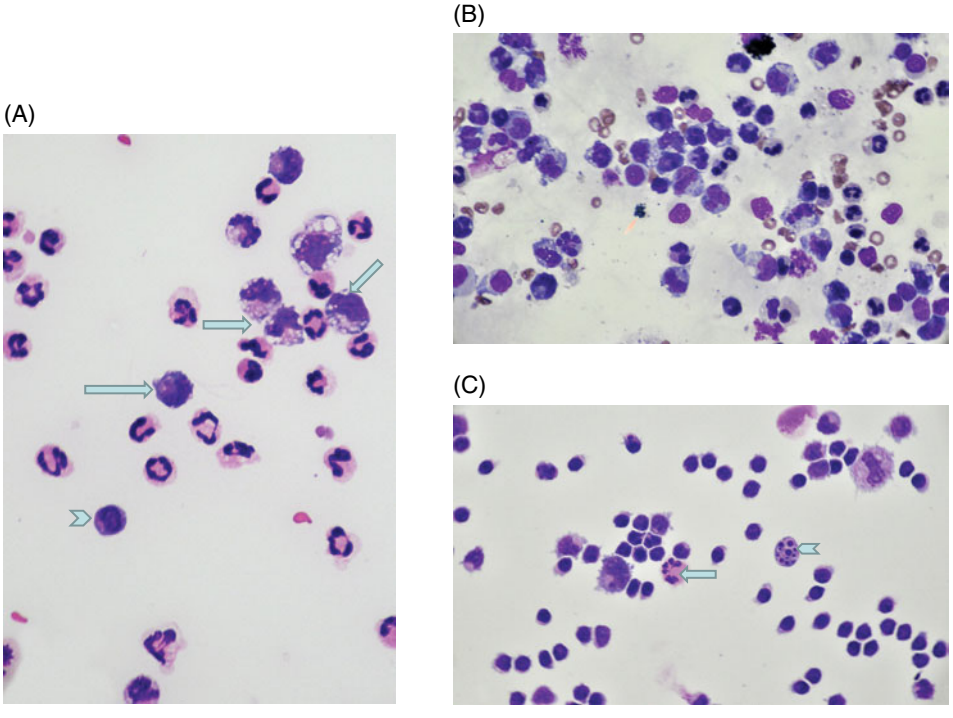


Figure 9.25 (A) Neutrophilic mixed uveitis with macrophages (arrows) and a small lymphocyte (arrowhead). (B) Pyogranulomatous inflammation with admixed small lymphocytes, macrophages and mature neutrophils. (C) Mixed inflammatory, primarily lymphocytic, infiltrate showing a hypersegmented neutrophil (arrow) and a karyorrhectic pyknotic neutrophil (arrowhead). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

granules (Figure 9.26A and B). They may also be leukophagic (Figure 9.26C), or if hyphaema is present, they may contain phagocytosed red blood cells or degraded haemoglobin product, e.g. haemosiderin (Figure 9.26D). Lymphocytes may also be larger and reactive (Figure 9.27).

Neoplasia

- Tumour types are similar to those that may involve the eyelids and conjunctiva, lymphoma being the most commonly diagnosed. Neoplasia is often associated with neutrophilic or (pyo)granulomatous inflammation.

Other intraocular tumours such as melanoma or adenoma/adenocarcinoma rarely shed cells into the anterior chamber, although fine-needle aspiration (FNA) may be useful for diagnosing some of these cases. Prior to sampling, the advice of an experienced veterinary ophthalmologist should be sought since FNA of intraocular tumours carries significant risk, including haemorrhage and damage to intraocular structures.

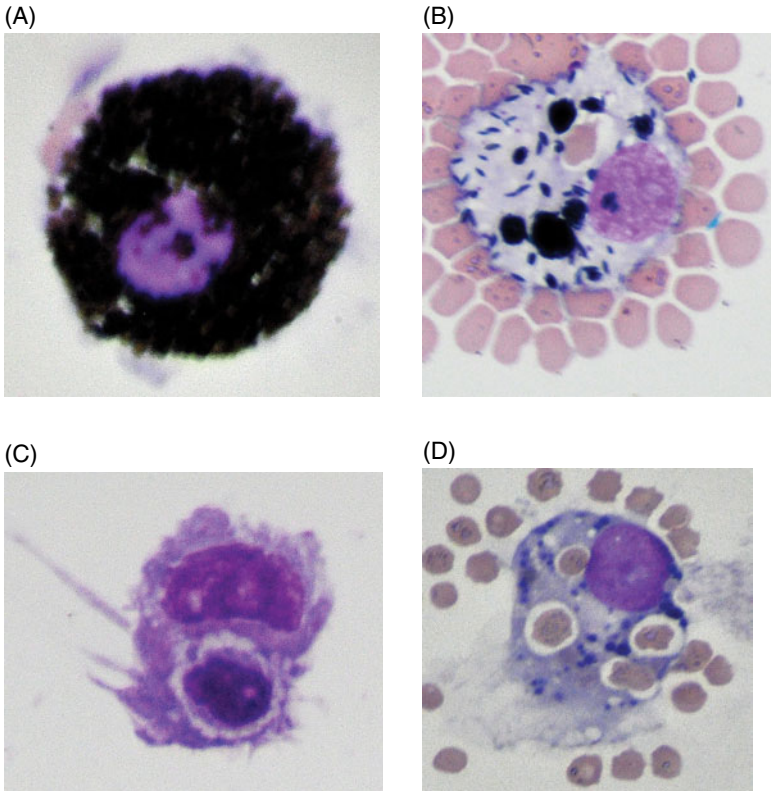


Figure 9.26 (A) 'Classic' macrophage with irregular very dense melanin phagosomes obscuring the light purple nucleus. (B) Macrophage with melanin can have more linear phagosomes resembling bacteria but are black, non-dividing and variable in size. (C) Macrophage with a phagocytosed neutrophil seen in chronic inflammation and also in the reparative phase. (D) Macrophage with erythrophagocytosis and also phagosomes with incompletely degraded haemoglobin, often blue-black and irregular in appearance (haemosiderin). Source: Roger Powell, PTDS Ltd. Reproduced with permission.

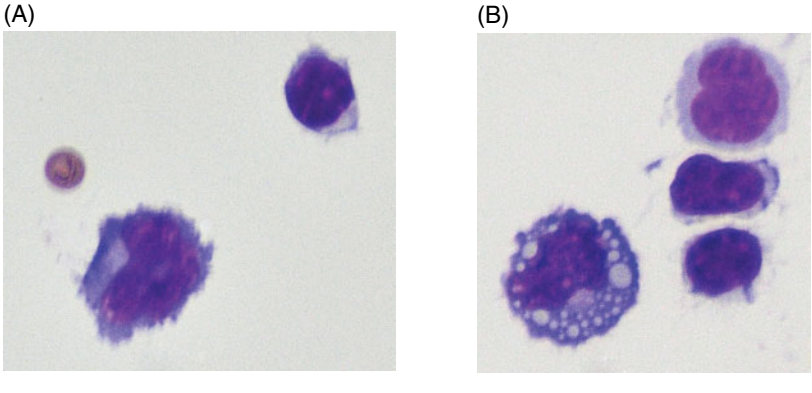


Figure 9.27 (A) Reactive 'transformed' lymphocyte (left) that is now activated by the inflammatory process and circulating cytokines. (B) Activated reactive lymphocyte (left) producing antibody in light blue vacuoles called Russell bodies. Source: Roger Powell, PTDS Ltd. Reproduced with permission.



Figure 9.28 Technique for vitreocentesis sampling. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

Vitreous

Indications for cytological investigation

Cytological sampling of the vitreous is very rarely indicated. Because of the serious risk of iatrogenic damage to the retina and lens, the technique should only be performed by an experienced veterinary ophthalmologist.

The main indication for vitreocentesis is infection of the intraocular structures (endophthalmitis). Less commonly it may be performed in cases where neoplastic infiltration of the vitreous is suspected.

Collection technique

Vitreocentesis is performed with the patient under general anaesthesia. Immediately prior to sampling, topical anaesthetic should be applied and the ocular surface should be aseptically prepared using dilute povidone–iodine solution at 1:50 dilution.

Using forceps to stabilise the limbus, a 23–27 G needle attached to a syringe is passed transclerally into the vitreous (Figure 9.28). The needle entry point should be 7 mm behind the dorsolateral limbus in the dog. Great care must be taken to avoid iatrogenic damage to the lens, ciliary body and retina. After sample collection, the needle is briskly withdrawn from the vitreous.



Figure 9.29 Ultrasound-guided FNA of the orbit. Source: David Gould, Davies Veterinary Specialists. Reproduced with permission.

Normal cell types

Since the vitreous is essentially acellular, the presence of more than a few cells (especially non-lymphoid) within a vitreous sample is considered abnormal. The types of cells seen are similar to those aspirated from aqueous humour.

Orbit

Indications for cytological investigation

Major causes of orbital disease in dogs and cats include:

- Abscess/cellulitis/foreign body
- Neoplasia
- Cyst (e.g. zygomatic salivary gland cyst, lacrimal cyst)
- Inflammatory disease (e.g. myositis)

Collection techniques

Orbital FNA is performed with the patient under general anaesthesia. It may be performed trans-orally or periocularly. Ultrasound guidance during sampling greatly improves collection rates and reduces risk of iatrogenic damage to the eye (Figure 9.29).

Normal cell types

The cell type varies depending on the area sampled. Aspirates often include epithelial cells (keratinised squames, nucleated squames, sebaceous or salivary elements and, potentially, skeletal muscle fibres (Figure 9.14)).

Abnormal cytology

Inflammation

Retrobulbar abscess. Aspirates consist of a large number of karyolytic neutrophils containing ingested bacteria. Free plaques or rafts of bacteria may also be seen.

Neoplasia

Carcinomas are more common although the tissue of origin may not be determined either cytologically or histopathologically. The cells of salivary gland carcinomas can show more obvious malignant features, and they may form acinar or tubular structures similar to carcinomas located at other sites.

10 Cytology of the Urinary Tract

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Three of the most common conditions affecting the urinary system are infections, kidney disease and neoplasia. An indication of a disorder involving the urinary tract (urethra, bladder, ureters and kidneys) can be obtained from microscopic observation of urine sediment, along with the urine specific gravity (SG). This, coupled with a recent history and physical examination, can determine further investigations and/or treatments. It is important to bear in mind, however, that only around 30% of neoplasms which involve the urinary tract can be detected by urine sediment analysis. Likewise, not all infections can be detected by sediment analysis alone.

Collection techniques

Urine

Urine can be obtained by 'free catch', catheterisation or cystocentesis. The latter method is advisable if bacterial culture is required.

Bladder and urethra

Samples for cytology can be obtained by flushing the bladder with warm saline via a urinary catheter and recovering the flush fluid. If a mass is visible on ultrasound examination and is accessible, a suction biopsy of the mass can be performed under sedation or light anaesthesia to collect small fragments of tissue via the catheter tip (this technique is henceforth referred to as a catheter tip suction biopsy). If the mass is not accessible through the urethra, an ultrasound-guided fine-needle aspirate (FNA) or 'Tru-Cut' biopsy can be performed under sedation or anaesthesia.

Manual of Diagnostic Cytology of the Dog and Cat, First Edition. Edited by John Dunn.
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Kidneys

Ultrasound-guided FNA or 'Tru-Cut' biopsies are usually performed under analgesia or anaesthesia. If a 'Tru-Cut' or wedge biopsy is required for histological analysis in addition to cytology, a key hole laparoscopic or laparotomic access procedure may be used. The kidney is a very vascular organ and great care with haemostasis is required, as haemorrhage post-biopsy is not uncommon.

If the biopsy sample is obtained by ultrasound guidance, the potential for transcoelomic metastasis should be considered if a malignant tumour is diagnosed; this has occasionally been reported to occur with transitional cell carcinomas (TCC) in dogs.

Sample preparation

Urine

The urine is mixed well and centrifuged at 1000 rpm for 5 min. A set volume of 5 ml or 3 ml is routinely used. After centrifugation the supernatant is used for dipstick chemistry or discarded. The sediment pellet is gently resuspended in the remaining urine, and a single drop, with or without the addition of a drop of stain, e.g. modified Sternheimer–Malbin stain (Sedi-Stain, Becton Dickinson Co. Ltd), is placed on a glass slide and covered with a cover slip. Special disposable plastic counting chamber devices can also be used.

Washes

The fluid can be prepared as described for a prostatic wash or bronchoalveolar lavage or a small volume (100–200 μL) can be spun in a special cytocentrifuge to produce a concentrated preparation which can be air-dried and stained with one of the Romanowsky-type stains for cytology.

Fine-needle aspirates

A drop of the tissue or fluid aspirate is expelled onto a glass slide and a thin film is prepared. This is air-dried and stained with a Romanowsky-type stain in the usual way.

Biopsies (a catheter tip suction biopsy, 'Tru-Cut' or wedge biopsy)

Impression smears can be prepared by gently pressing the surface of the tissue onto a clean glass slide (the cut surface should be gently 'blotted' free of excess blood beforehand). This smear can be air-dried and stained for cytology as described in Chapter 1. The remaining tissue can then be fixed in formal saline and processed for histological examination.

Cytological evaluation

Urine sediment cytology

Urine should be evaluated within 30 min of collection or refrigerated and brought to room temperature before the sample is processed. Knowledge of the SG and method of collection is useful.

Normal urine produces very little sediment (Figure 10.1A and B). This may include:

- Less than five white blood cells (WBC) per high-power field (hpf)
- Less than five red blood cells (RBC) per hpf
- Less than five epithelial cells of the superficial bladder/urethral squamous epithelial cells per hpf
- Small numbers of crystals (see following text)
- Other materials can include fat droplets, sperm and amorphous fine granular material.

'Free catch' urine may contain epithelial cells from the lower urinary tract and contaminating organisms, bacteria, yeasts, fungi and, rarely, intestinal parasites. In unstained preparations, fat droplets may be easily confused with RBC (Figure 10.2A, B and C).

In stained preparations, stain precipitate or ultrasound gel may be mistaken for bacterial organisms (cocci; Figure 10.9B). Crystals, most commonly struvite or oxalate crystals, may also be present (Figure 10.1A and Figure 10.3).

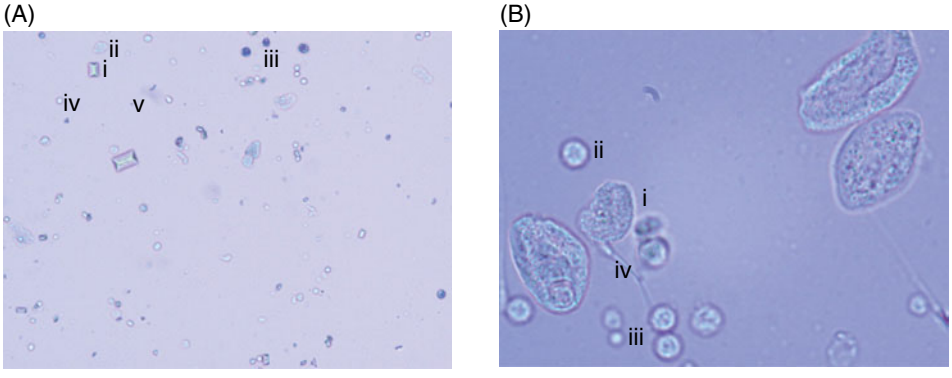


Figure 10.1 (A) Unstained urine sediment (microscope condenser lowered to view cells using a 40 × objective lens). (i) Struvite crystals may be seen in normal concentrated urine; (ii) the large cells are epithelial cells exfoliated from the bladder mucosa; (iii) the smaller cells with internal structure are neutrophils; (iv) the small round cells with no internal structure are red cells; (v) the very small round objects are fine particulate matter which may exhibit Brownian motion and may be confused with cocci. (B) Unstained urine sediment (50× oil objective lens). (i) Four large epithelial cells, (ii) intermediate-sized WBC, (iii) small round RBC and (iv) a few spermatozoa can be seen.

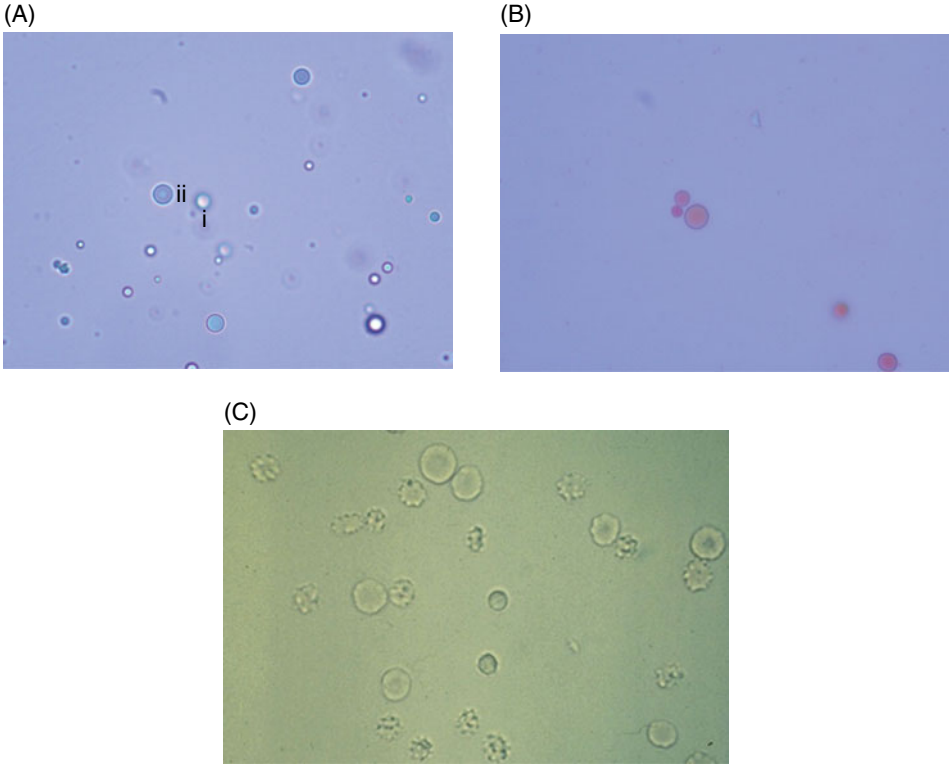


Figure 10.2 (A) Urine sediment from a cat. Large numbers of refractile fat droplets (i) of variable size are present. These are common in cat urine and can be confused with RBC (ii) in unstained preparations; (B) sample of the same urine stained with Oil Red-O (ScyTek Laboratories Inc.) which stains fat orange/red; (C) urine sediment (unstained sample). The WBC are the larger cells; RBC are smaller; and many appear crenated. Two smaller refractile fat droplets are also present.

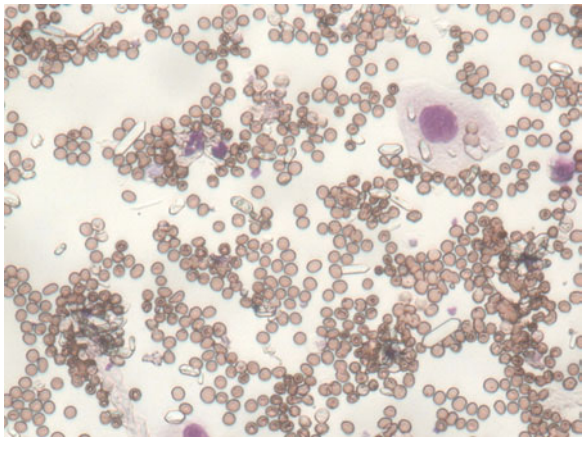
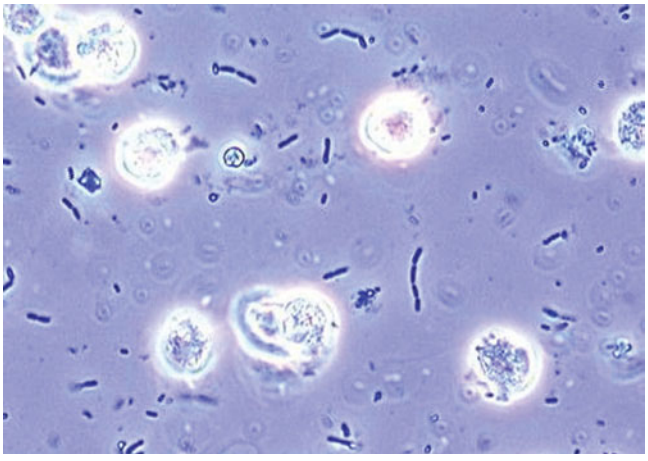


Figure 10.3 Urine sediment from a cat (stained sample). A large number of RBC, calcium oxalate monohydrate crystals and a single large epithelial cell are present. These crystals can be seen in healthy cats but also following ingestion of ethylene glycol (antifreeze) or, more commonly, toxic plants, e.g. lilies.

(A)



(B)

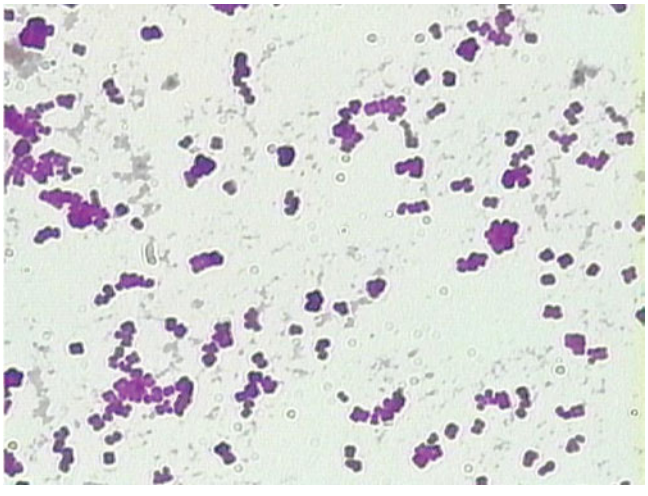


Figure 10.4 (A) Urine sediment (unstained sample) viewed with a phase contrast microscope. WBC and dark chains of rods and small round paler cocci can be seen in the background; (B) urine stained with Sedi-Stain (Becton Dickinson Co. Ltd). A large number of RBC and clusters/clumps of stained cocci are present.

Urinary tract infection (UTI)

The lower urinary tract is often infected by ascending bacteria; infections involving the kidney may also be due to ascending bacteria or may be haematogenous in origin. If a UTI is suspected, a cystocentesis sample in a sterile container is preferred for bacteriology (culture and sensitivity). A UTI is characterised by the presence of large numbers of neutrophils which often contain intracytoplasmic microorganisms (most commonly rods and cocci). These include gram-negative *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp. and less frequently *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. (Figure 10.4A, B and Figure 10.5).

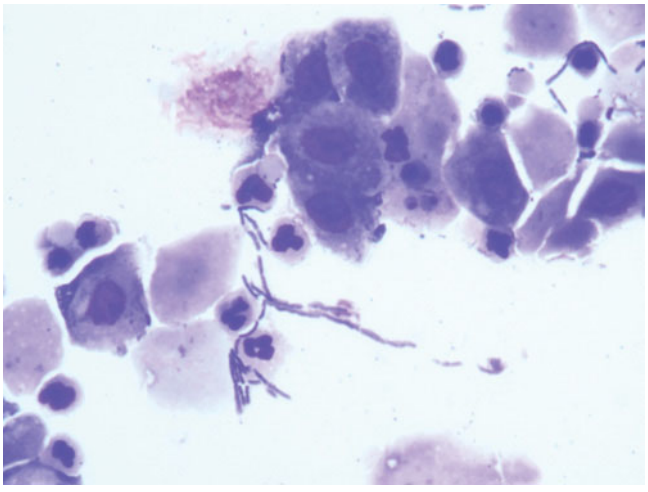


Figure 10.5 Urinary tract infection. Urine sediment showing large epithelial cells, neutrophils and bacterial rods (*E. coli*).



Figure 10.6 Unstained sediment. Large numbers of growing yeasts (*Candida* spp.) These are common contaminants in urine. They can be seen as a rare cause of UTI in immunocompromised animals.

Occasionally, yeast organisms are seen particularly in immunocompromised animals or animals on long-term antibiotic therapy (Figure 10.6). In order to visualise bacteria in urine sediment, there needs to be 10,000 rods/ml or 100,000 cocci/ml; however, only 1–10 bacterial organisms per ml need to be present for a positive bacterial culture. Therefore, an inactive or a negative urine sediment on microscopy of a dilute urine sample does not preclude the presence of a UTI. Brownian movement of particles in sediment can also be confused with cocci.

If the UTI is severe or prolonged, there may also be inflammation of the bladder/urethral mucosa, resulting in the presence of large sheets of superficial epithelial cells in the urine sediment along with large numbers of RBC. If the UTI is long standing, these epithelial cells may become dysplastic or may exhibit characteristics of squamous metaplasia (Figure 10.7).

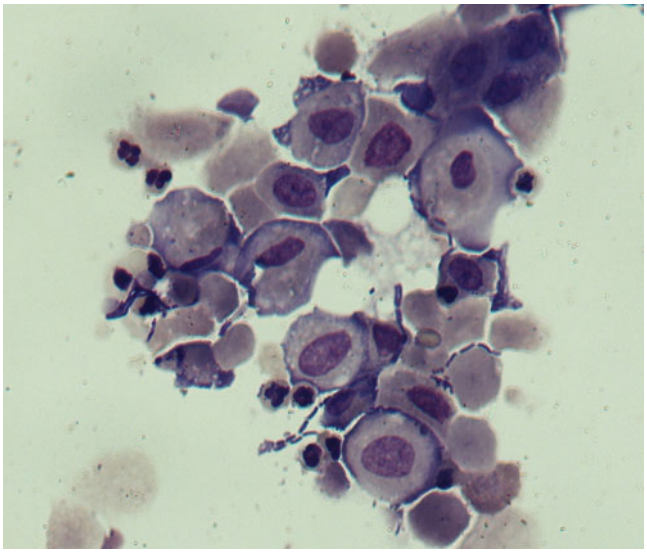


Figure 10.7 Urine sediment. Note the large epithelial cells demonstrating dysplastic changes. Neutrophils and bacteria (rods) can also be seen.

Neoplasia

If there is haematuria and/or the urine sample appears cloudy and there are few neutrophils or bacteria in the sediment, then neoplasia should be suspected. A large number of epithelial cells, particularly if there are changes in morphology, should raise suspicion of neoplasia (benign polyps should be expected to exfoliate epithelial cell types with more typical morphology). However, it is worth bearing in mind that urine is an inhospitable environment for epithelial cells, and many of the morphological changes may reflect degeneration or ageing.

Cytology of bladder washes, fine-needle aspirates and impression smears

The cytological characteristics of inflammation and infection are similar to those described earlier. Neoplasia is more likely to be detected from an FNA or an impression smear of a discrete mass in the bladder or urethra rather than by microscopic examination of urine sediment. In dogs and cats, the most common tumour is the Transitional Cell Carcinoma (TCC) (Figure 10.8A, B, C, D, and E). In cats, 15% of tumours involving the bladder or urethra are squamous cell carcinomas. Polyps and benign masses are much less common and can be differentiated from carcinomas on impression smears (Figure 10.9A and B). Other rare tumours include leiomyomas, leiomyosarcomas and botryoid tumours.

Because most samples are obtained under ultrasound guidance, they may easily be contaminated with ultrasound gel which can obscure morphological detail unless care is taken with needle placement (Figure 10.9B and Figure 1.19).

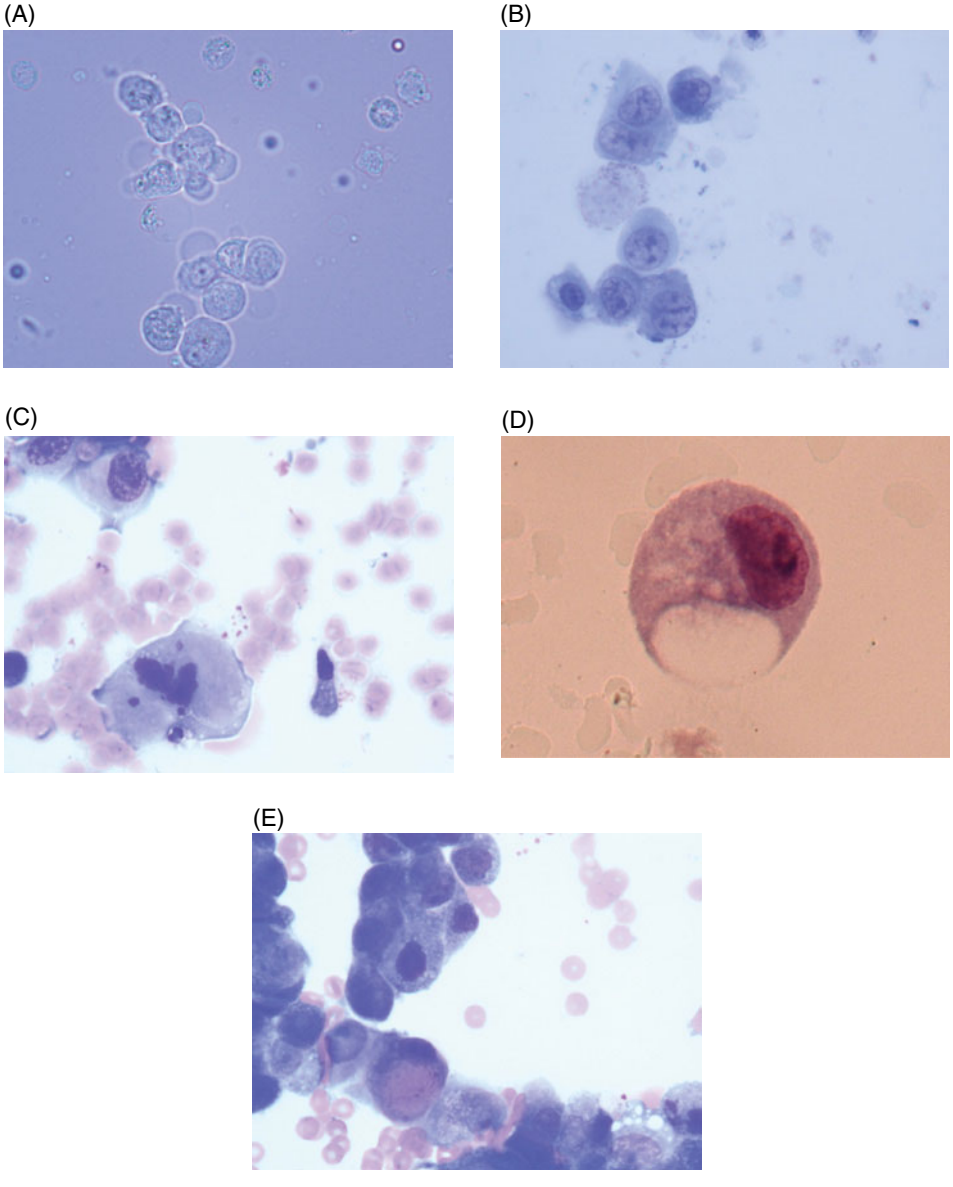
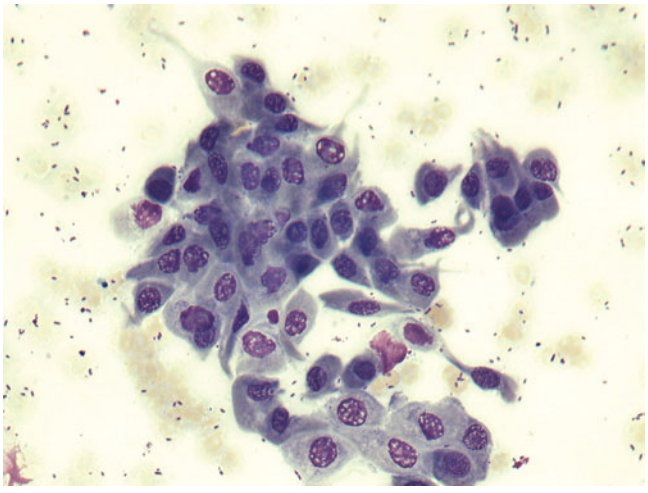


Figure 10.8 (A) Unstained sediment from a bladder wash. Rafts of epithelial cells, many with multiple nucleoli. Some cells are binucleated. The cytological appearance is suspicious for a TCC of the bladder; (B) sediment from a bladder wash. The epithelial cells possess large nuclei with multiple prominent nucleoli; (C) cytospin preparation of a bladder wash. There are numerous RBC in the background. Note the large epithelial cell in mitosis; (D) TCC. Cytospin preparation of a bladder wash. This epithelial cell has assumed a 'signet ring' appearance; (E) cytospin preparation of a bladder wash. Rafts of atypical cells consistent with a TCC are present.

(A)



(B)

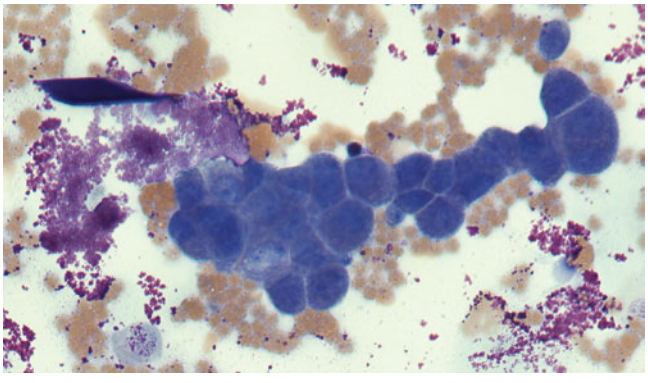


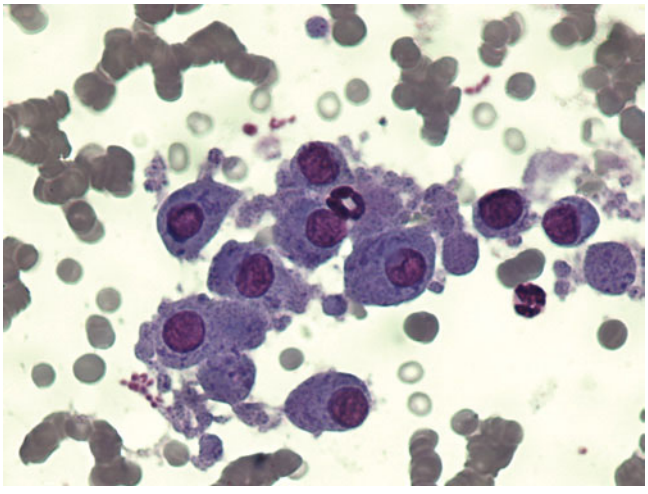
Figure 10.9 (A) Impression smear of catheter tip suction biopsy of a polypoid mass on the bladder wall which protruded into the lumen. The squamous epithelial cells show mild anisocytosis and anisokaryosis. There are RBC and bacteria but no WBC in the background. This was confirmed as a benign polyp on histology; (B) impression smear of a catheter tip suction biopsy of a mass in the bladder trigone area. The epithelial cells show criteria of malignancy including anisocytosis, anisokaryosis, binucleation and increased nuclear to cytoplasmic ratio. Numerous RBC can be seen in the background which is a common finding with tumours. The magenta-coloured material is an ultrasound gel. A TCC was confirmed on histology.

Renal cytology

Normal cell types

Individual renal tubular epithelial cells may be seen in urine and in FNA samples (Figure 10.10A and B). In dogs, the cells are caudate shaped with pale basophilic cytoplasm, while in cats they frequently contain numerous punctuate fat vacuoles. Sometimes large rafts of normal tubular epithelial cells may be aspirated (Figure 10.11).

(A)



(B)

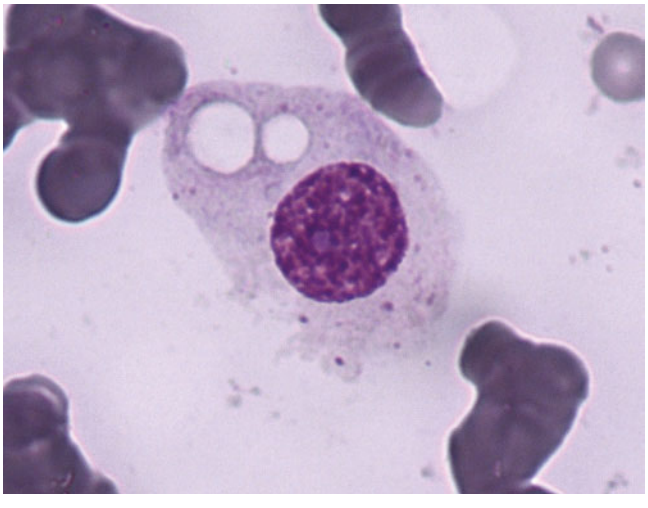


Figure 10.10 (A) FNA kidney. Clusters of normal renal tubular epithelial cells. These exhibit mild anisocytosis and anisokaryosis. Note the dark intracytoplasmic granules. Renal aspirates frequently contain a large amount of blood; (B) FNA feline kidney. A single normal renal tubular epithelial cell with a caudate shape and discrete cytoplasmic vacuoles (cytoplasmic vacuolation is more frequently observed in feline tubular epithelial cells).

Abnormal cytology

Inflammation

Aspirates may contain a few normal renal epithelial cells and a large number of inflammatory cells (neutrophils, small lymphocytes and macrophages). The latter are particularly prominent in granulomatous conditions, e.g. feline infectious peritonitis (FIP) and mycobacterial infections in cats.

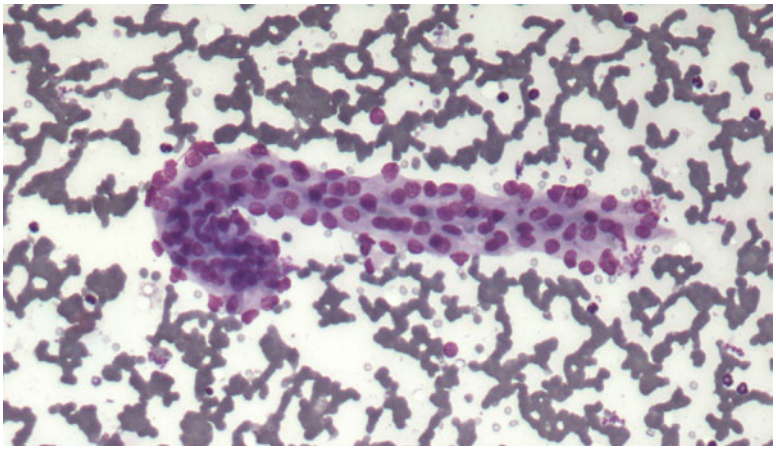


Figure 10.11 Intact renal tubule consisting of a raft of normal renal tubular epithelial cells in a background of blood.

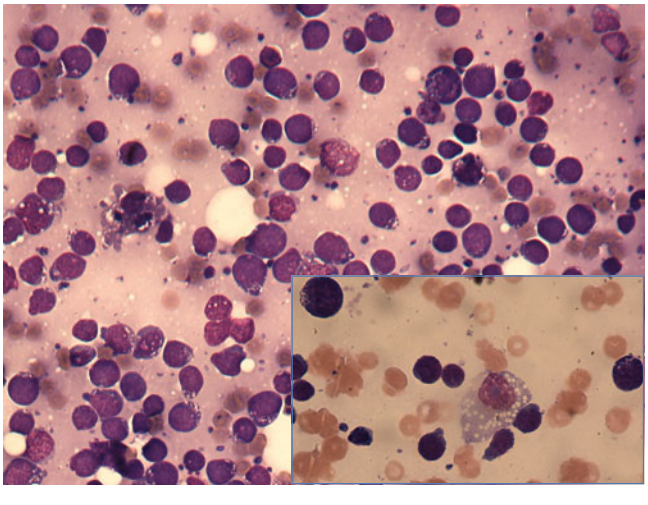


Figure 10.12 FNA kidney (cat) Lymphoma. Large numbers of lymphocytes with a few epithelial cells in a background of blood are seen. Inset: higher magnification of the FNA showing a background of blood with single renal epithelial cell and numerous lymphocytes with multiple prominent nucleoli.

Neoplasia

Primary neoplasms of the kidney are relatively uncommon in dogs and cats; however, secondary metastatic spread of some tumours to the kidney can occur.

Lymphoma

Renal lymphoma can be primary or result from secondary metastatic spread. It can occur as a single mass in one pole of the kidney or diffusely involve the renal parenchyma (Figure 10.12A and B). It is reported to be the most common tumour in cats.

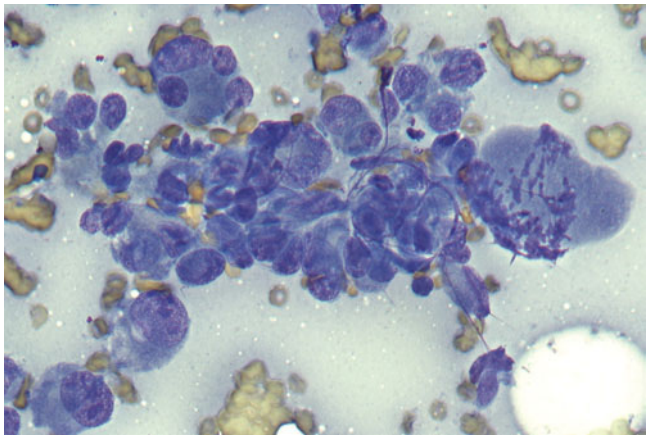


Figure 10.13 FNA kidney (dog). The atypical renal epithelial cells demonstrate many criteria of malignancy. Note the abnormal mitotic figure to the right. This was confirmed as a primary renal carcinoma on histology.

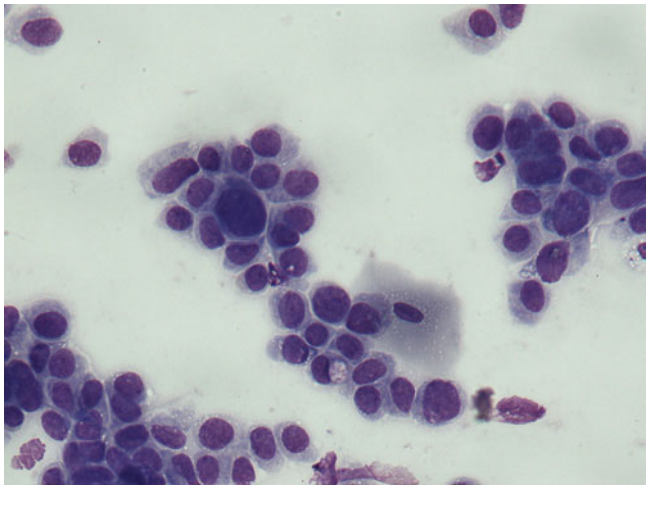


Figure 10.14 FNA kidney (dog). A raft of epithelial cells and one normal squamous epithelial cell with a small nucleus and abundant pale basophilic cytoplasm are present. Many of the epithelial cells are showing obvious criteria of malignancy (anisocytosis, anisokaryosis and variation in nuclear to cytoplasmic ratio). This was confirmed as a TCC on histology.

Carcinomas

Primary renal carcinomas can arise from renal tubular epithelial cells. Although only 1% of all tumours in dogs are located in the kidney, 85% of these are reported to be carcinomas. They are frequently unilateral (Figure 10.13). TCC occasionally arise from urothelial cells in the pelvis or ureters but are much less common than carcinomas of tubular epithelial origin or TCC involving the bladder and urethra (Figure 10.14).

Nephroblastoma

This is a rare renal tumour that usually occurs in young dogs and cats.

Mesenchymal tumours

These are uncommon in dogs and cats and constitute only 1% of all renal tumours. They can be renal sarcomas, haemangiosarcomas or fibrosarcomas. Diagnosis of such tumours is difficult from FNA preparations as they are poorly exfoliative and the cells may be difficult to differentiate from hyperplastic fibrocytes as fibrosis is common in chronic renal disease.

11 Cytology of the Liver, Exocrine Pancreas and Gastrointestinal Tract

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Cytological examination of samples collected from the liver, pancreas and gastrointestinal (GI) tract may provide useful diagnostic information and in many cases a definitive diagnosis. Limitations of this investigation include the absence of tissue architecture and suboptimal sample quality in terms of cellularity and preservation.

Liver

The presence of hepatobiliary disease can be established from the animal's history, physical examination, laboratory testing and imaging; however, a specific pathological process is rarely identified. This usually requires morphological examination of the hepatic parenchyma via biopsy (fine-needle aspirate, core, punch or wedge biopsy). Indications for cytological examination of hepatic samples include:

- The presence of abnormal laboratory test results indicative of hepatocellular damage, cholestasis and/or hepatic dysfunction
- Identification of focal or multifocal hepatic masses
- Hepatomegaly of unknown cause
- Altered hepatic echotexture on ultrasound examination
- Presence of neoplastic disease in other organs
- Assessment of therapy or progression of disease
- Suspicion of gall bladder pathology (aspiration of the gall bladder to collect bile)

Collection of hepatic samples for cytological examination should be ideally preceded by assessment of the animal's coagulation status, although complications associated with fine-needle aspiration of the liver are few.

Manual of Diagnostic Cytology of the Dog and Cat, First Edition. Edited by John Dunn.
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Correlation between cytological and histopathological findings has been evaluated in several studies and is reported to range from 28% to 83% with the highest correlation found with the cytological assessment of biopsy imprints (Fondacaro *et al*., 1999; Kristensen *et al*., 1990; Roth, 2001; Wang *et al*., 2004; Weiss and Moritz, 2002; Weiss *et al*., 2001). Diffuse diseases like lymphoma, hepatic lipidosis and neutrophilic (suppurative) hepatitis are readily diagnosed cytologically. Focal or multifocal hepatic masses may be more difficult to assess cytologically due to failure of collecting representative material. Histopathology is required for the definitive diagnosis of hepatocellular adenomas, well-differentiated hepatocellular carcinomas, nodular hyperplasia, fibrosis, chronic (e.g. lymphocytic periportal) inflammation, portovascular anomalies, amyloidosis and poorly exfoliative sarcomas.

Normal cytological findings

As in any vascular organ, peripheral blood contamination is expected. In addition to peripheral blood-derived leukocytes, occasional macrophages (i.e. Kupffer cells), mast cells and rare precursors of haemopoietic cells may also be present.

Hepatocytes are usually aspirated in sheets and clusters. Rare nuclei may contain rectangular crystalloid inclusions (Figure 11.1). Binucleated cells and presence of multiple nucleoli are infrequent findings. In addition, dense clumps of biliary epithelial cells are occasionally found in normal liver aspirates (Figure 11.2), as are sheets of mesothelial cells (see Figure 6.2). Bile can also be identified as a granular amorphous basophilic to greenish-grey material.

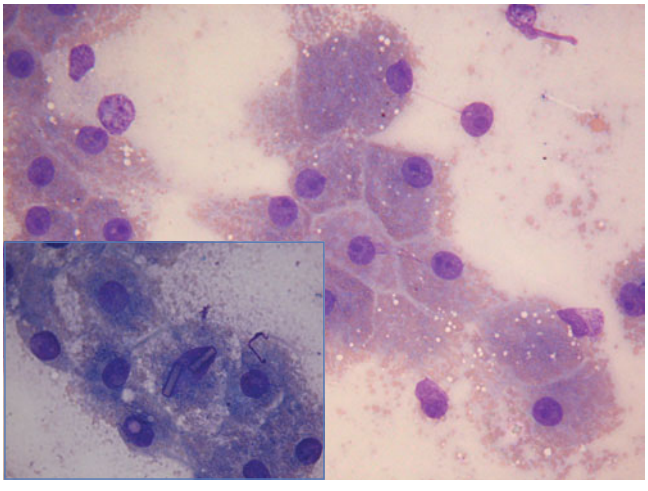


Figure 11.1 Normal hepatocytes, cat. Hepatocytes are large round to polygonal cells (i.e. three to four times the diameter of a red blood cell), with a centrally located round to slightly oval-shaped nucleus. Nuclear chromatin is coarse and most nuclei possess a single prominent nucleolus. The cytoplasm is granular and lightly basophilic with pinkish eosinophilic granules (due to the amphophilic tinctorial properties). Inset: Normal hepatocytes in an aspirate from a dog exhibiting rectangular crystalloid nuclear inclusions.

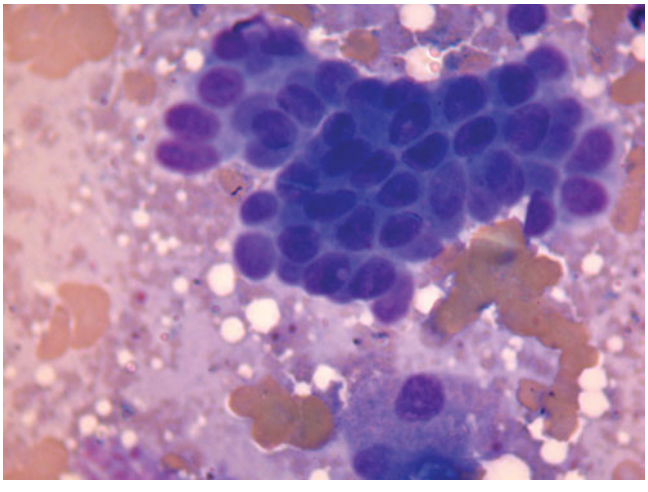


Figure 11.2 Normal biliary epithelium, cat. Biliary epithelial cells are cuboidal to columnar in shape and have a round nucleus with dark-blue clumped chromatin and a small amount of pale to mildly basophilic cytoplasm.

Non-neoplastic disorders

Nodular regenerative hyperplasia

Cytological characteristics of hepatocytes from hyperplastic and regenerative nodules are similar to those of normal hepatocytes and to those aspirated from hepatocellular adenomas, although a higher percentage of binucleated hepatocytes may be reported. Histopathology may be required for a definitive diagnosis in these cases.

Inflammation

Hepatobiliary inflammation may be difficult to detect cytologically. *Neutrophilic (suppurative) inflammation* is more commonly identified (Figure 11.3), but care must be taken to confirm that the neutrophils are hepatic in origin and not derived from peripheral blood. The detection of neutrophils in close proximity to hepatocytes provides a high degree of confidence for a diagnosis of neutrophilic inflammation. Neutrophilic inflammatory infiltrates are most frequently associated with bacterial infection, although bacteria are rarely seen.

Mixed cell inflammation consists of variable numbers of neutrophils, macrophages, lymphocytes and plasma cells. In cases where macrophages and giant cells predominate (Figure 11.4), the inflammation can be characterised as granulomatous or pyogranulomatous although the authors believe these terms are more appropriate for a histopathological rather cytological diagnosis. Causes of mixed cell inflammation include fungal infections, mycobacterial infections (Figure 11.4), feline infectious peritonitis (FIP), hepatozoonosis, leishmaniasis

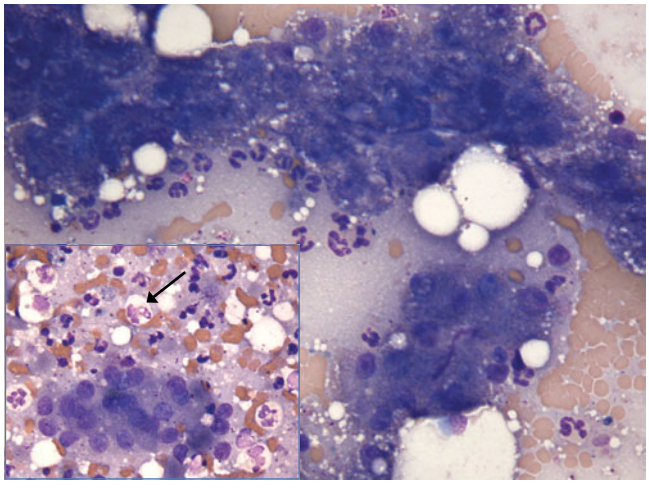


Figure 11.3 Neutrophilic inflammation (aspirate from a cat with cholangiohepatitis). Many non-degenerate neutrophils are present among the sheets of hepatocytes. Inset: Aspirate from a dog with a hepatic abscess. Many neutrophils, some showing signs of degeneration (arrow), and a cluster of poorly preserved hepatocytes are present in a background containing amorphous necrotic material.

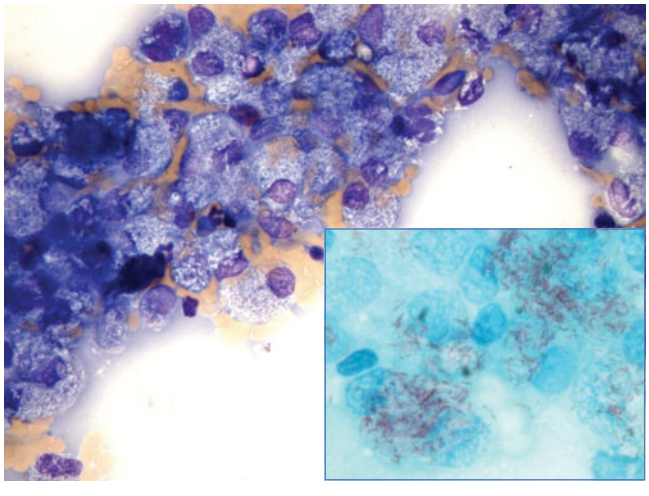


Figure 11.4 Mycobacteriosis. Liver aspirate from a dog with systemic mycobacterial infection. The macrophages contain intracellular negative staining rod-shaped bacteria. Inset: Same case stained with Ziehl–Neelsen demonstrating the acid-fast (red) bacilli inside the macrophages.

and toxoplasmosis. Mixed cell inflammation can also be seen with nodular regenerative hyperplasia.

Lymphocytic inflammation is characterised by a predominance of small lymphocytes, with or without plasma cells. This type of inflammation is usually confined to periportal areas and therefore may be missed. Differentials for lymphocytic

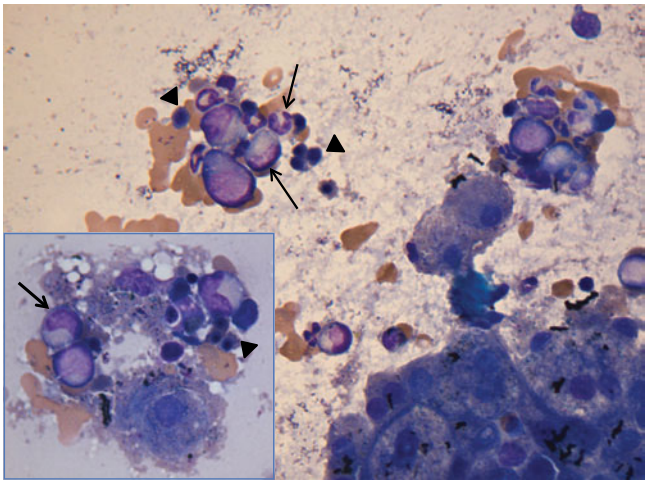


Figure 11.5 Extramedullary haemopoiesis. Late red blood cell precursors (arrowheads) and myeloid precursors (arrows) are present. Some of the hepatocytes (bottom right) contain small biliary casts. Inset: Same case at higher magnification. Late red blood cell precursors (arrowheads) and myeloid precursors (arrows) are present.

inflammation should always include small cell lymphoma and chronic lymphocytic leukaemia especially if the infiltration is extensive.

Extramedullary haemopoiesis

Increased haemopoiesis may occur as an appropriate response to anaemia or systemic inflammatory disease. Extramedullary haemopoiesis is identified by the presence of numerous haemopoietic cells at various stages of maturation, with the later stages predominating (Figure 11.5).

Fibrosis

Fibrosis is characterised by collagen deposition and presence of mesenchymal cells (Figure 11.6). It occurs as a result of chronic inflammation or tissue damage. Collagen appears as an extracellular eosinophilic matrix material which should be differentiated from amyloid A protein deposits. The latter tend to be darker in colour and can be identified under polarised light in smears stained with Congo red.

Hepatic lipidosis

Hepatic lipidosis is a common disease of the cat characterised by lipid accumulation in the hepatocytes (Figure 11.7). It can be a primary (idiopathic) disorder or secondary to a systemic disease (e.g. nutritional, metabolic, toxic).

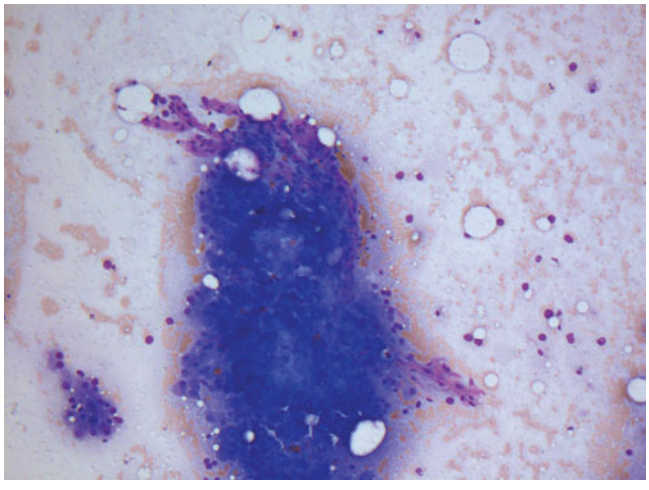


Figure 11.6 Liver aspirate, dog. Strands of pink extracellular material associated with a cluster/sheet of hepatocytes suggest fibrosis.

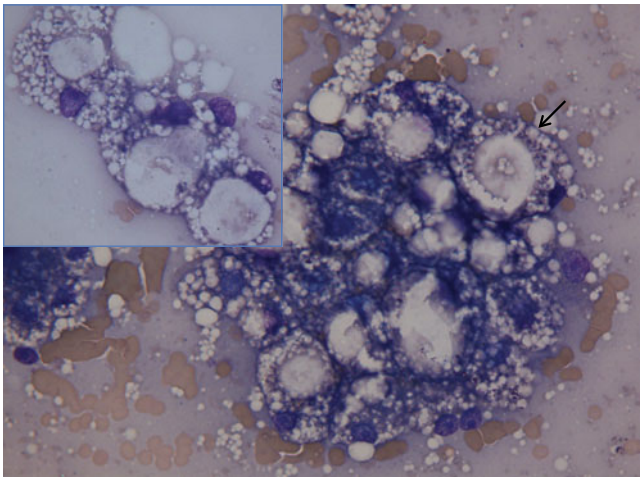


Figure 11.7 Hepatic lipidosis, cat. Accumulation of lipid in hepatocytes appears as distinct, sharply delineated and variably sized cytoplasmic vacuoles (arrow). Some hepatocytes appear distended with the nucleus pushed to the periphery. Hepatocellular degeneration and necrosis may be present in severe cases. Inset: Same case at higher magnification.

Steroid-induced hepatopathy

Steroid-induced hepatopathy is characterised cytologically by cytoplasmic rarefaction (vacuolation) of the hepatocytes (Figure 11.8). Cytoplasmic rarefaction is caused by glycogen accumulation or is a result of hydropic cell degeneration in response to a toxic insult or hypoxia.

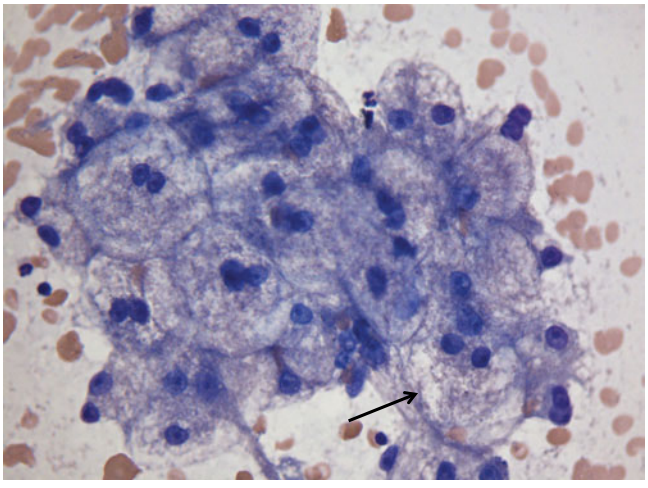


Figure 11.8 Vacuolar hepatopathy, dog. Distended hepatocytes show cytoplasmic rarefaction with indistinct vacuoles (arrow). In contrast to hepatic lipidosis where the nucleus is often displaced by the lipid vacuoles, in steroid-induced vacuolar hepatopathy, the nucleus of the hepatocyte remains central.

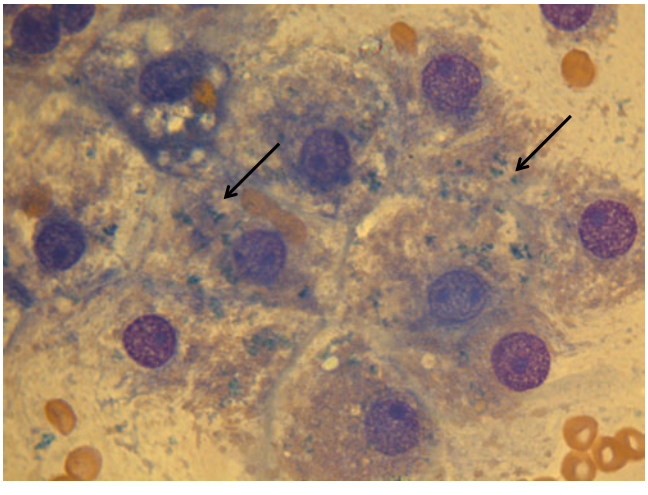


Figure 11.9 Normal hepatocytes with intracellular lipofuscin, dog. Hepatocytes exhibit a fine granular blue-green intracytoplasmic pigment (arrows).

Pigment accumulation

Four main types of pigment may be identified during cytological examination of hepatic preparations: bile, haemosiderin, copper and lipofuscin. Lipofuscin is the most commonly seen pigment. This appears as green-black granules in the cytoplasm of hepatocytes (Figure 11.9), particularly of older animals, and can be present in normal aspirates. This pigment accumulation must not be confused with the deposition of bile. Cholestasis is recognised in cytological specimens by the presence of biliary

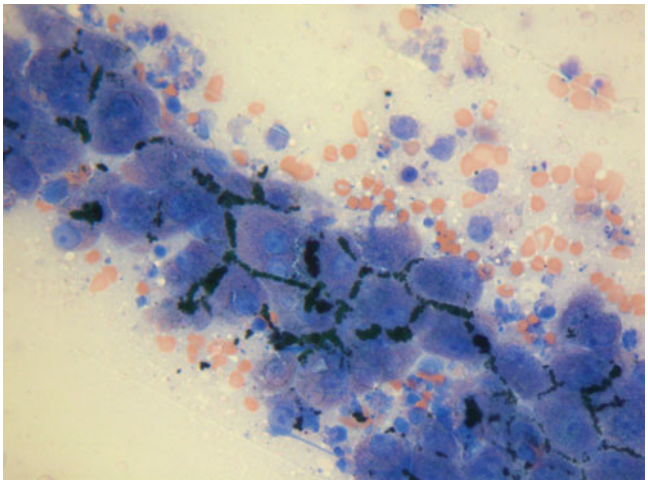


Figure 11.10 Cholestasis, dog. Hepatocyte margins are delineated by thick plugs of green-black bilirubin pigment (biliary casts). Final diagnosis was hepatic carcinoma.

casts (Figure 11.5 and Figure 11.10). Haemosiderin appears as a golden-brown to bluish-black granular pigment that may be present within hepatocytes and/or macrophages. The presence of haemosiderin can be confirmed with a Prussian blue stain and is most commonly indicative of haemolytic disease or prior blood transfusion.

A marked increase in the copper content of hepatocytes occurs in dogs with inherited copper-associated hepatopathy (e.g. in Bedlington terriers) and in some dogs with chronic hepatitis. Cytologically, hepatocytes may contain refractile coarse pale blue-green granules which are often difficult to identify. Staining the smears with rubeanic acid stain confirms the presence of copper.

Neoplasia

In dogs, most primary hepatic tumours are hepatocellular in origin, whereas bile duct carcinomas are the most common primary hepatic neoplasm in cats. Other frequently diagnosed neoplasms in the dog include lymphoma, sarcoma (mostly metastatic with haemangiosarcoma being the most common) and undifferentiated carcinoma. In the cat, bile duct carcinoma and lymphoma account for the majority of all hepatic neoplasms.

Hepatocellular neoplasia

Hepatocellular neoplasms range from a benign well-differentiated adenoma to the poorly differentiated malignant carcinoma (Figure 11.11). A recent article reviewed the cytological features of well-differentiated hepatocellular carcinomas in dogs, which included dissociation of hepatocytes, acinar and palisading arrangements, multinuclearity, increased nuclear to cytoplasmic ratio and presence of 'naked' nuclei and capillaries (Masserdotti and Drigo, 2012). However, some of these

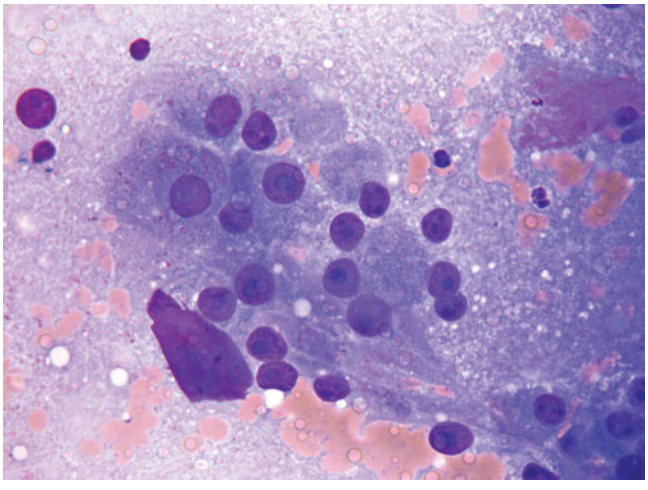


Figure 11.11 Hepatocellular carcinoma, dog. Relatively well-differentiated hepatocytes exhibiting nuclear characteristics of malignancy (anisokaryosis, anisonucleolosis, macronucleoli, variation in the number of nucleoli).

features have been described in cytological reports from hyperplastic nodules, and no comparison has been made between these two groups. In addition, cytology alone cannot differentiate a hepatoma from a well-differentiated carcinoma or a primary from a metastatic carcinoma (e.g. pancreatic carcinoma).

Biliary neoplasia

Biliary adenomas and carcinomas cannot be differentiated cytologically as in both neoplasms the epithelial cells can appear morphologically normal (Figure 11.12). However, in cats carcinomas occur more frequently than adenomas.

Anaplastic carcinomas and hepatobiliary carcinoids

In poorly differentiated anaplastic hepatocellular and bile duct carcinomas, the aspirated cells appear less frequently hepatoid in origin. Cytologically, these tumours cannot be differentiated from metastatic carcinomas and are termed anaplastic carcinomas.

Hepatic carcinoids are primary neuroendocrine tumours that originate from enterochromaffin cells. They cannot be distinguished cytologically from metastatic neuroendocrine tumours (e.g. adrenal, endocrine pancreatic tumours; see Chapter 12).

Lymphoma

Lymphoma is the most common hepatic malignancy of dogs and cats. Infiltration of the hepatic tissue with large lymphoid cells in animals with multicentric lymphoma is readily recognisable in cytological specimens as the cells readily exfoliate (Figure 11.13). Because lymphocytes are fragile cells, many bare nuclei

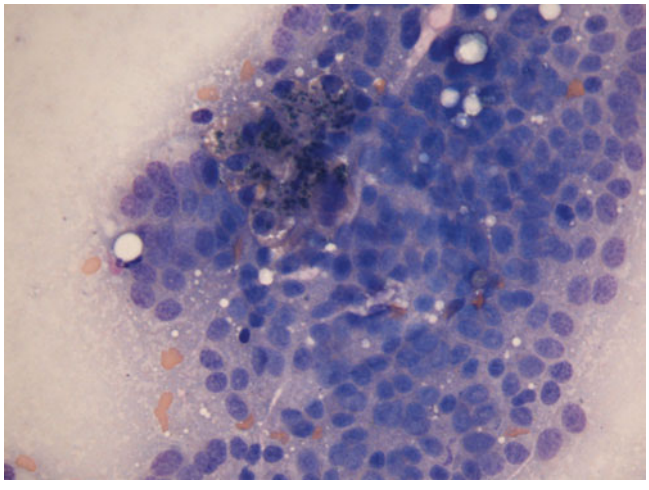


Figure 11.12 Biliary carcinoma, cat. A large number of biliary epithelial cells with round to oval deeply stained nuclei, finely reticulated chromatin and scant basophilic cytoplasm surround a small cluster of hepatocytes with intracellular pigment (lipofuscin).

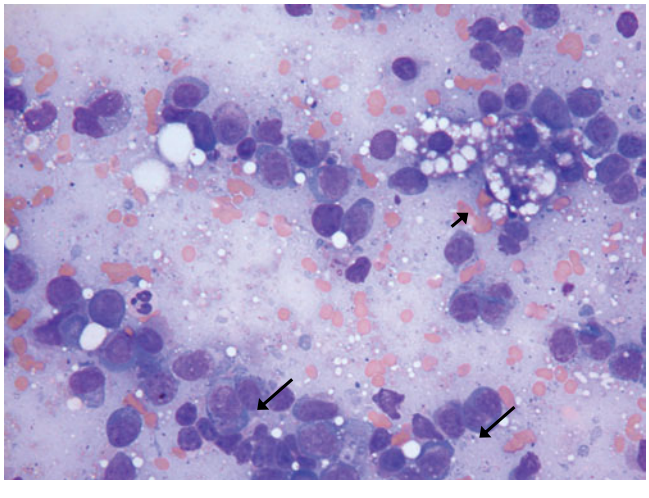


Figure 11.13 Lymphoma, cat. Three hepatocytes with vacuolated cytoplasm (short arrow) and many large atypical lymphoid cells (long arrows) can be seen.

may be present. Cellular morphology varies and immunochemistry may be used to further classify the lymphoma. Typically, the cells are large (often greater than three times RBC diameter) and round with a round or indented nucleus, coarse chromatin, distinct nucleoli and a variable amount of dark-blue cytoplasm. The lymphomatous cells may also be markedly pleomorphic, exhibiting marked

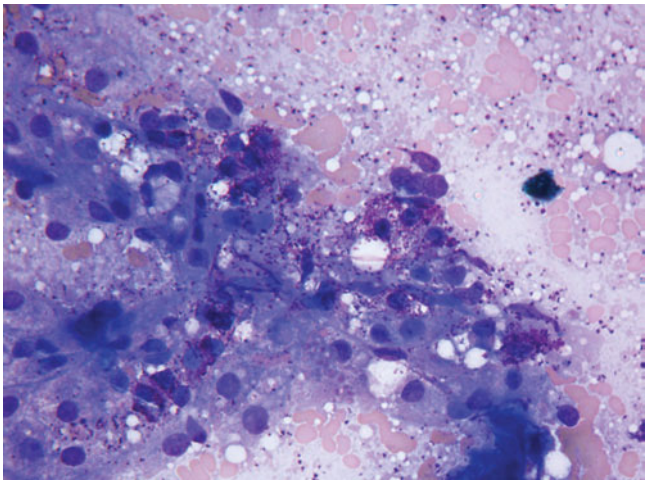


Figure 11.14 Mast cell tumour. Aspirate from a cat with mildly heterogenous liver on ultrasound and a previous history of splenic mast cell tumour. Abundant free azurophilic granules and an increased number of mast cells are seen associated with the hepatocytes.

anisocytosis, nuclear pleomorphism and multinucleation. Cells aspirated from large granular lymphomas contain variably sized azurophilic cytoplasmic granules. Less frequently lymphoid cells can be small and morphologically unremarkable and more consistent with lymphocytic inflammation. In such cases, however, small cell lymphoma or chronic lymphocytic leukaemia should be considered.

Mast cell tumour

A few mast cells can be seen in hepatic samples collected from clinically healthy animals and animals with mixed cell inflammation of the liver. The presence of increased numbers or clusters of mast cells associated with hepatocytes is highly suggestive a hepatic mast cell tumour (Figure 11.14). Other cytological features suggestive of mast cell tumour are the presence of atypical (pleomorphic and/or poorly granulated) mast cells (Book *et al*., 2011; Stefanello *et al*., 2009).

Histiocytic sarcoma

Disseminated histiocytic sarcoma is another tumour that can involve the liver. When markedly atypical cells are present (Figure 11.15), malignant neoplasia is easily diagnosed, but determining these cells as histiocytic may require immunophenotyping.

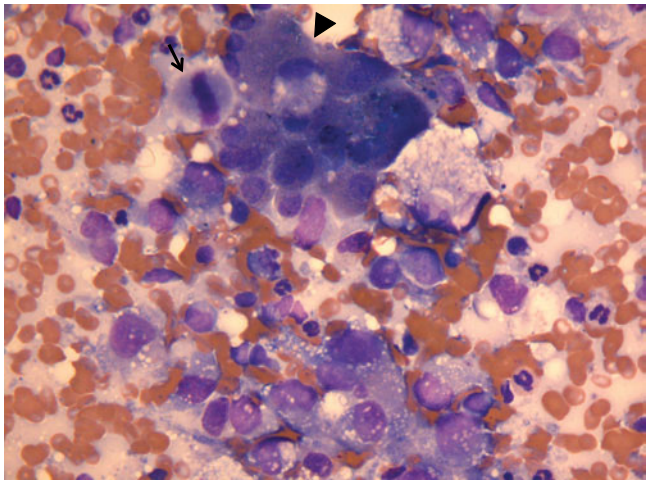


Figure 11.15 Histiocytic sarcoma. Aspirate from a Bernese Mountain dog with hepatosplenomegaly. A cluster of hepatocytes (arrow head) and a large number of pleomorphic round cells are present. Some of the latter resemble macrophages, others resemble mesenchymal cells. A mitotic figure (arrow) can also be seen.

The exocrine pancreas

The pancreas is rarely sampled unless a defined abnormal area such as a mass or a cystic structure is detected on imaging.

Normal cytological findings

The exocrine pancreas represents the majority of the pancreatic tissue, and acinar epithelial cells comprise most of the cells present in smears from normal pancreatic tissue (Figure 11.16). Pancreatic nodules may represent hyperplasia, neoplasia or inflammation. Hyperplastic nodules contain epithelial cells which morphologically appear similar to normal epithelial cells although they may exhibit a slightly higher nuclear to cytoplasmic ratio, a prominent nucleolus and more noticeable binucleation may be evident.

Non-neoplastic disorders

Inflammation of the pancreas can range from neutrophilic to lymphocytic. As most cases of pancreatitis are diagnosed based on clinicopathological findings and imaging, pancreatic aspiration and cytology are rarely performed.

The smears prepared following aspiration of an acutely inflamed pancreas are highly cellular. Neutrophils with or without evidence of degeneration predominate, and variable numbers of acinar epithelial cells, which may exhibit dysplastic changes,

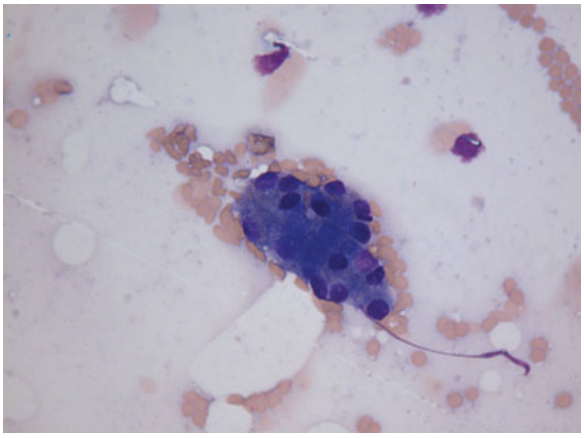


Figure 11.16 Pancreas, cat. Normal pancreatic epithelial cells are polygonal in shape and exfoliate in small sheets and clusters. They have a low nuclear to cytoplasmic ratio, an eccentrically located round nucleus with reticulated chromatin and occasionally a single small nucleolus. The granular cytoplasm is basophilic with mild amphophilic characteristics due to the abundant small pink apical granules (thought to be zymogen granules).

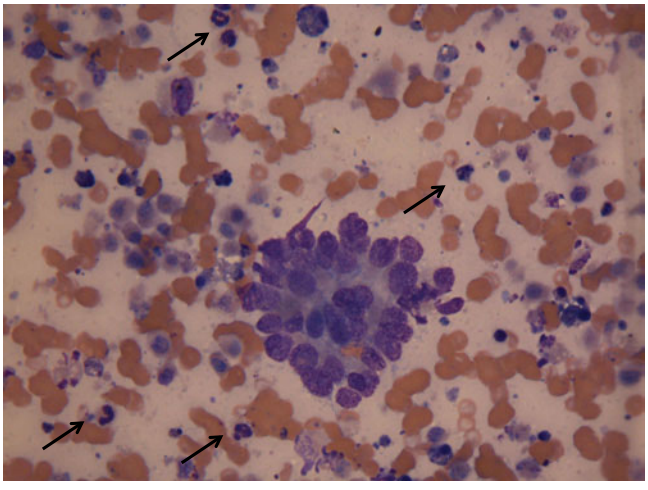


Figure 11.17 Pancreatitis, dog. An ultrasound-guided FNA of an enlarged pancreas. Neutrophils (arrows) and poorly preserved pancreatic cells are present in a background consisting of amorphous basophilic debris which is the result of the activity of the proteolytic enzymes causing rapid deterioration of the cells.

are also present (Figure 11.17). Aspirates from a chronically inflamed pancreas commonly contain low numbers of cells due to fibrosis. Cytological examination may reveal a background of red blood cells and small amounts of calcium-rich cellular debris with rare epithelial cells, the occasional reactive fibroblast and foci of lymphocytes and neutrophils.

Aspiration of pancreatic cysts and pseudocysts yields a hypocellular fluid with a total protein concentration similar to that of a modified transudate. Cytology of the fluid reveals the presence of low numbers of neutrophils, reactive macrophages and rare reactive fibroblasts.

Neoplasia

Both benign and malignant pancreatic tumours can cause obstruction of pancreatic ducts, resulting in the release of pancreatic enzymes and subsequent development of pancreatitis. If the inflamed parenchyma is aspirated rather than the neoplasm, a diagnosis of neoplasia may be missed. The main difficulty in the diagnosis of pancreatic carcinoma is distinguishing well-differentiated carcinoma cells with minimal characteristics of malignancy from the benign or dysplastic epithelial cells seen in adenomas or with pancreatitis, respectively. Pancreatic adenocarcinoma is an uncommon neoplasm but is the most frequently diagnosed neoplasia of the exocrine pancreas. It is readily recognisable on cytology as the cells exhibit characteristics similar to those seen in cells from adenocarcinomas of other organs (e.g. acinar arrangements, cellular pleomorphism, high nuclear to cytoplasmic ratio, moderate to marked anisocytosis and anisokaryosis, prominent nucleoli, cytoplasmic basophilia and fine vacuolation; Figure 11.18). Endocrine pancreatic neoplasms are discussed in Chapter 12.

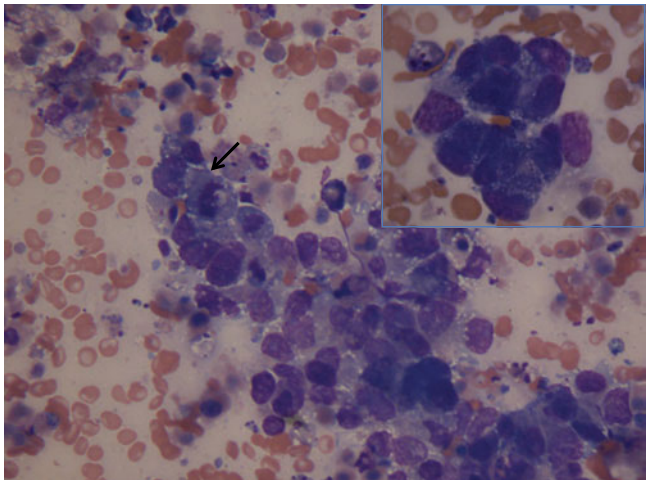


Figure 11.18 Carcinoma, pancreas, dog. Ultrasound-guided aspirate of a single pancreatic mass. A cluster of round to polygonal epithelial cells with round to oval nucleus and basophilic cytoplasm is present. Some (not depicted) were arranged in tubular or acinar formations. Criteria for malignancy included moderate anisocytosis and anisokaryosis, anisonucleolosis, nuclear moulding and atypical mitotic figures (arrow). Evidence of necrosis, inflammation and previous haemorrhage was also noted in this aspirate. Inset: Same case under higher magnification.

Rarely, lymphoma and mesenchymal tumours arising from the supporting tissue such as haemangiosarcoma, fibrosarcoma and liposarcoma can also involve the pancreas. Apart from lymphoma, the mesenchymal neoplasms exfoliate poorly and therefore are only occasionally diagnosed on cytological smears.

Gastrointestinal tract

The GI tract is most commonly evaluated by cytological examination of aspirates collected ultrasonographically, impression smears prepared from biopsy samples or endoscopically collected brushings.

The most common indications for cytological examination of the GI tract are the presence of a focal mass and/or increased intestinal wall thickness identified during abdominal ultrasonography. Histopathology is almost always required for a final diagnosis, but cytology can often provide a strong indication of neoplastic or inflammatory disease.

Stomach

Normal cytological findings

Impression smears of normal gastric samples normally consist of mucus and a variable number of mucus-secreting columnar epithelial cells arranged in sheets and clusters. Gastric epithelial cells are columnar in shape with a single round nucleus and finely granular cytoplasm. They are often arranged in a 'honeycomb' pattern (Figure 11.19). Fragments of ingesta, spiral bacteria and

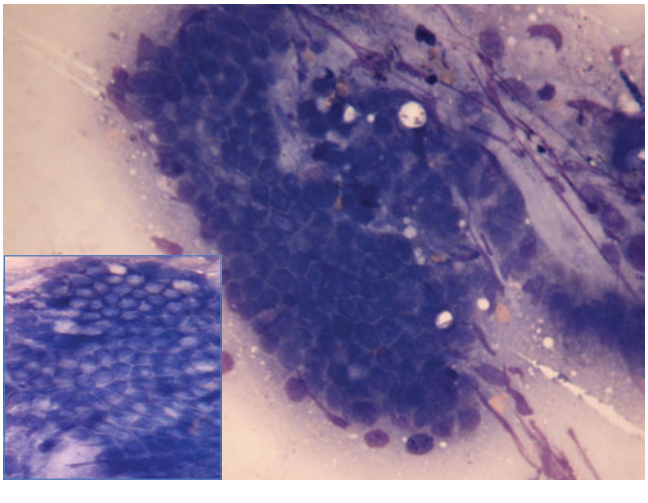


Figure 11.19 Normal gastric epithelium. Sheets of regular cuboidal to polygonal epithelial cells. Inset: Gastric epithelial cells arranged in a 'honeycomb' pattern.

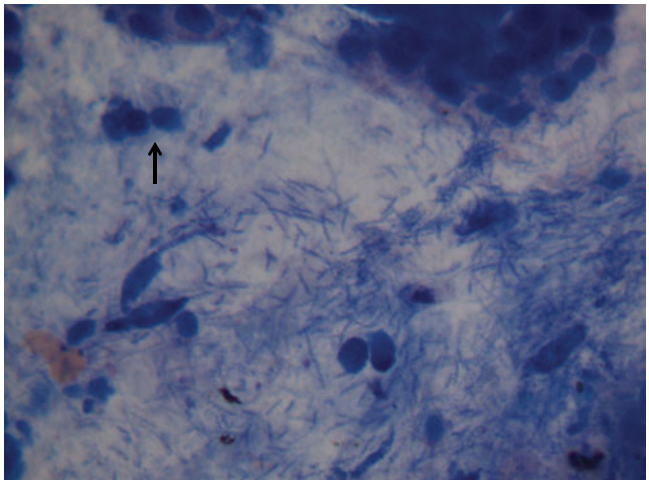


Figure 11.20 *Helicobacter* organisms. Spiral-shaped bacteria embedded in a mucus background with low numbers of condensed neutrophils (arrow).

squamous epithelial cells covered with oropharyngeal flora (including *Simonsiella* organisms) are also frequently seen.

Inflammation/infection

Lymphocytic–plasmacytic inflammation is the most common type of gastric inflammation and may be associated with chronic hyperplastic and superficial gastritis, atrophic gastritis and *Helicobacter* infection. Neutrophilic inflammation is uncommon and can also be associated with *Helicobacter* infection (Figure 11.20). Although the presence of inflammation and spiral bacteria suggests *Helicobacter* infection, other laboratory tests (e.g. PCR) are required to confirm this diagnosis.

Neoplasia

Gastric neoplasia is rare, carcinoma in the dog and lymphoma in the cat being the most commonly diagnosed neoplasms.

Carcinoma

Neoplastic cells exhibit morphological criteria of malignancy typical of carcinomas in other locations (Figure 11.21), but sometimes their appearance may resemble that of atypical lymphoid cells.

Lymphoma

Large cell lymphoma is easily diagnosed on cytology (Figure 11.24), but histopathology is required to differentiate lymphocytic inflammation from a small cell lymphoma.

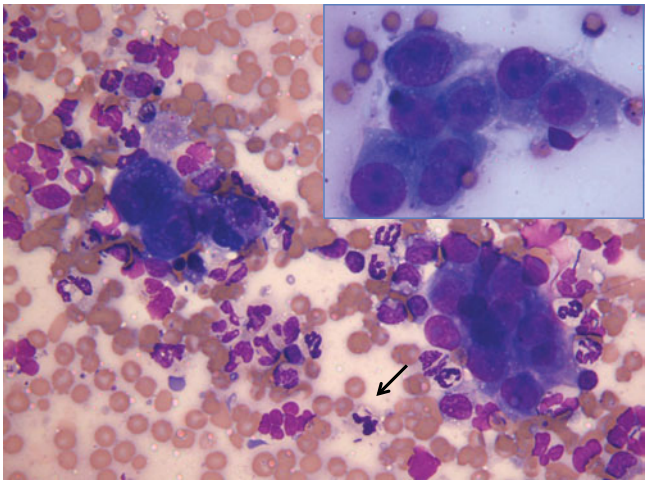


Figure 11.21 Gastric carcinoma with secondary septic neutrophilic inflammation, dog. Clumps of epithelial cells with deeply basophilic vacuolated cytoplasm exhibiting anisokaryosis, open coarse chromatin, distinct nucleoli, anisonucleolosis and nuclear moulding are present. Note also the neutrophils and extra- and intracellular rods (arrow). The septic neutrophilic inflammation was likely secondary to ulceration in this case. Inset: Higher magnification demonstrates the nuclear characteristics of malignancy.

Smooth muscle neoplasms

Leiomyoma and leiomyosarcoma are uncommon gastric mesenchymal neoplasms, mostly seen in old dogs. The cells resemble those seen in intestinal leiomyomas/leiomyosarcomas (Figure 11.25).

Intestines

Normal cytological findings

Intestinal epithelial cells are normally quite uniform, with round nuclei and basophilic cytoplasm that may appear confluent (Figure 11.22). Mucus-producing goblet cells with large cytoplasmic vacuoles and/or magenta-coloured granules may also be identified. Well-differentiated small lymphocytes or a mixed lymphoid population, including granular lymphocytes, may also be aspirated from gut-associated lymphoid follicles.

Inflammation/infection

Cytology of inflammatory lesions reveals variable numbers of neutrophils, macrophages, mast cells, lymphocytes and eosinophils. Lymphocytic–plasmacytic inflammation is the most common type (Figure 11.23), but this diagnosis cannot be always made cytologically as sampling of gut-associated lymphoid tissue will result in similar findings (see preceding text).

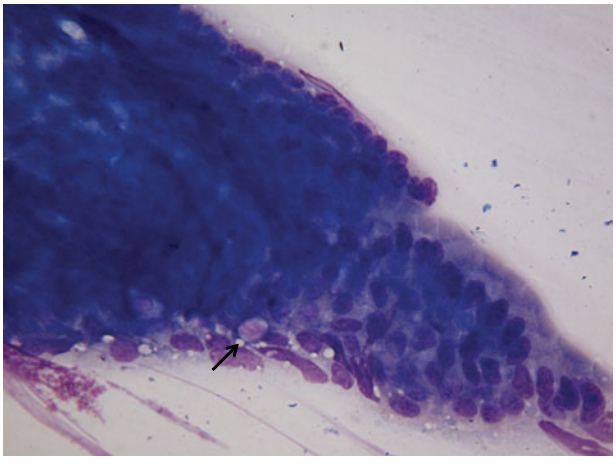


Figure 11.22 Normal duodenum. Impression smear from a biopsy sample. Sheet of regular columnar epithelial cells with a round to oval nucleus and pale basophilic cytoplasm. A single lymphocyte is also depicted (arrow).

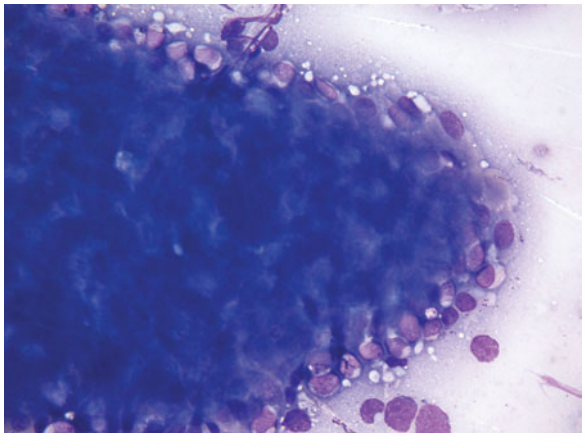


Figure 11.23 Lymphocytic inflammation. Impression smear from a duodenum biopsy sample. Small lymphocytes and large granular lymphocytes (with small intracytoplasmic azurophilic granules) surround a cluster of epithelial cells. These lymphoid cells may constitute a normal lymphoid population, or they may indicate lymphocytic inflammation or a lymphoma. Histopathological examination of a biopsy specimen is often necessary to obtain a definitive diagnosis.

Neoplasia

Lymphoma is usually characterised by high numbers of large lymphocytes (Figure 11.24). Intestinal carcinomas/adenocarcinomas share cytological findings similar to carcinomas located in other organs. Mesenchymal neoplasms, such as gastrointestinal stromal tumour (GIST), leiomyoma and leiomyosarcoma (Figure 11.25), are rare and cannot be diagnosed by cytology alone.

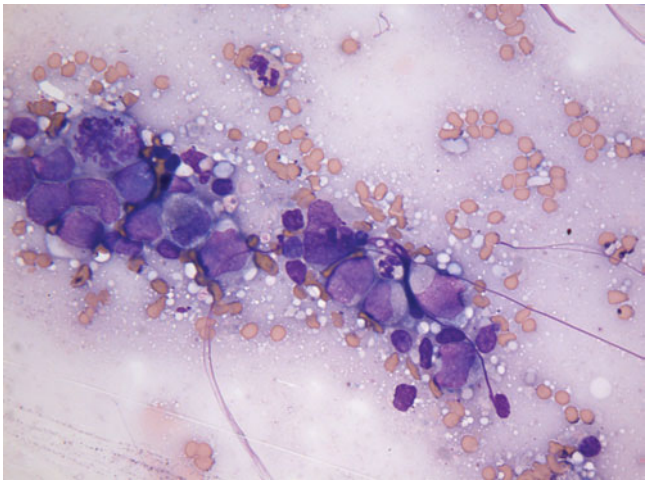


Figure 11.24 Large cell lymphoma, cat. Impression smear from a duodenum biopsy sample. Atypical large lymphoid cells. The nuclei are greater than three times red blood cells in diameter. A mitotic figure is also shown (top left).

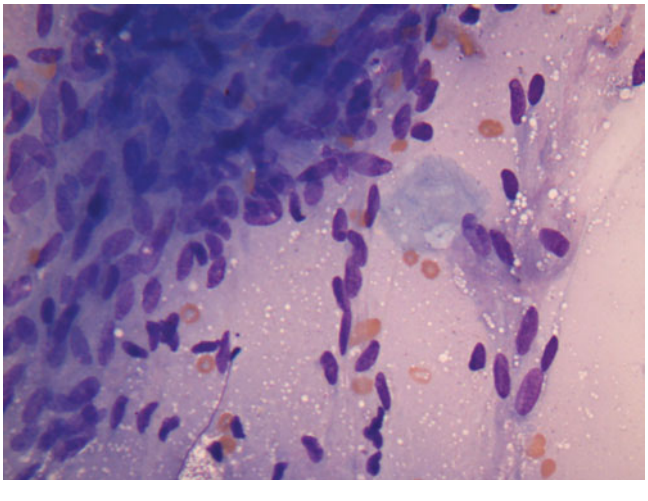


Figure 11.25 Mesenchymal neoplasm. Impression smear from a duodenum biopsy sample. A cluster of spindle-shaped cells showing mild anisocytosis and anisokaryosis is present. The cells have an oval nucleus with finely granular chromatin and no distinct nucleolus. Some cells have small clear cytoplasmic vacuoles. Cytoplasmic margins are poorly defined. Histopathological diagnosis was uncertain (differential diagnosis included GIST and leiomyosarcoma; immunohistochemistry was not performed).

References

Book, A.P., Fidel, J., Wills, T. *et al*. (2011) Correlation of ultrasound findings, liver and spleen cytology, and prognosis in the clinical staging of high metastatic risk canine mast cell tumors. *Veterinary Radiology & Ultrasound*, **52** (5), 548–554.

Fondacaro, J.V., Fuilpin, V.O., Powers, B.E. *et al*. (1999) Diagnostic correlation of liver aspiration cytology with histopathology in dogs and cats with liver disease. *Journal of Veterinary Internal Medicine*, **13**, 254.

Kristensen, A.T., Weiss, D.J., Klausner, J.S. *et al*. (1990) Liver cytology in cases of canine and feline hepatic disease. *Compendium on Continuing Education for the Practicing Veterinarian*, **12**, 797–806.

Masserdotti, C. and Drigo, M. (2012) Retrospective study of cytologic features of well-differentiated hepatocellular carcinoma in dogs. *Veterinary Clinical Pathology*, **41** (3), 382–390.

Roth, L. (2001) Comparison of liver cytology and biopsy diagnoses in dogs and cats: 56 cases. *Veterinary Clinical Pathology*, **30**, 35–38.

Stefanello, D., Valenti, P., Faverzani, S. *et al*. (2009) Ultrasound-guided cytology of spleen and livera prognostic tool in canine cutaneous mast cell tumor. *J Vet Journal of Veterinary Internal Medicine*, **23** (5), 1051–1057.

Wang, K.Y., Panciera, D.L., Al-Rukibat, R.K. *et al*. (2004) Accuracy of ultrasound-guided fine-needle aspiration of the liver and cytologic findings in dogs and cats: 97 cases (1990–2000). *Journal of the American Veterinary Medical Association*, **224**, 75–78.

Weiss, D.J. and Moritz, A. (2002) Liver cytology. *Veterinary Clinics of North America: Small Animal Practice*, **32** (6), 1267–1291.

Weiss, D.J., Blauvelt, M. and Aird, B. (2001) Cytologic evaluation of inflammation in canine liver aspirates. *Veterinary Clinical Pathology*, **30**, 193–196.

12 Cytological Examination of the Endocrine Glands

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Introduction

Cytological examination of endocrine tissues in companion animal practice is limited to some neoplastic disorders of the thyroid gland, adrenals, endocrine pancreas and other uncommon neoplasms (chemoreceptor tumours, carcinoids, extramedullary paragangliomas). Many other disorders (e.g. atrophy, inflammation, degenerative disorders) cannot be adequately investigated by cytology and require different diagnostic approaches (histopathology, serum biochemistry, hormonal testing, etc.) to achieve a definitive diagnosis. Moreover, some endocrine tumours are not easily investigated by cytology since they are often too small to be sampled (e.g. parathyroid hyperplasia/adenomas) or are difficult to access (e.g. pituitary tumours).

Cytology of the thyroid gland

The normal thyroid gland is composed of two lobes located laterally to the upper trachea; the two lobes are sometimes connected by a thin isthmus. Normal thyroid tissue is composed of follicular structures lined by a monolayered glandular epithelium of cubical follicular cells. These cells produce thyroid hormones, which are stored as thyroglobulin into the follicular cavity. This proteinaceous material is also called colloid since it appears as amorphous eosinophilic extracellular material in cytological and histological samples. Calcitonin-secreting neuroendocrine cells (C-cells) are also present between thyroid follicles. Since some ectopic thyroid tissue may be present anywhere from the base of the tongue to the heart base, some thyroid tumours may arise in unusual anatomic sites (e.g. cranial mediastinum and heart base). Normal, hyperplastic or benign neoplastic thyroid tissues have very similar cytological features. These different conditions cannot be distinguished by cytology alone, and histopathological examination of a biopsy

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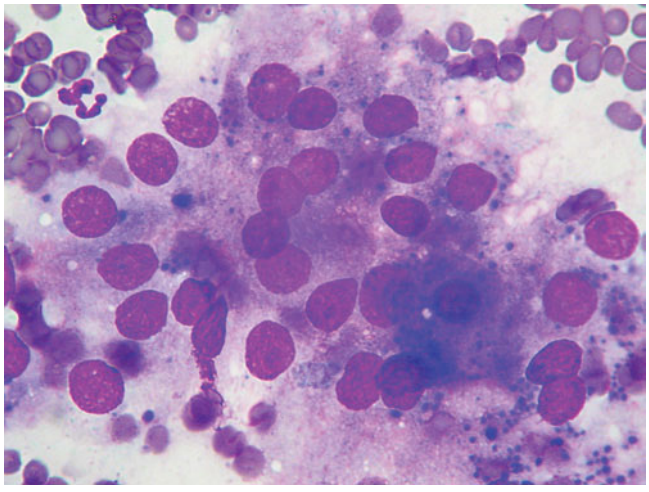


Figure 12.1 FNA from a thyroid nodule in a cat with clinical hyperthyroidism. The cells are arranged in an acinar pattern (centre), have poorly defined cytoplasmic margins, abundant basophilic cytoplasm with deep blue granules, round uniform nuclei with fine chromatin and indistinct small nucleoli.

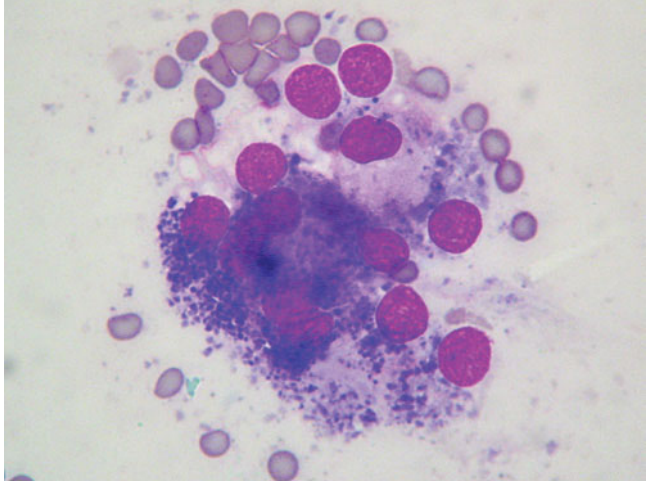


Figure 12.2 FNA from a thyroid nodule in a cat with clinical hyperthyroidism. Note the thyroid follicular cells in this field contain basophilic cytoplasmic granules.

specimen may be required to obtain a definitive diagnosis. In fine-needle aspirates (FNA) from normal, hyperplastic and benign neoplastic thyroid tissue, follicular cells are often organised in an acinar arrangement which is reminiscent of the follicular pattern seen on histology (Figure 12.1). These cells have a round to polygonal shape often with indistinct cellular borders, a moderate to abundant basophilic cytoplasm which sometimes contains deeply basophilic granules (considered to be thyroxine by some authors although this has not been substantiated) (Figure 12.2).

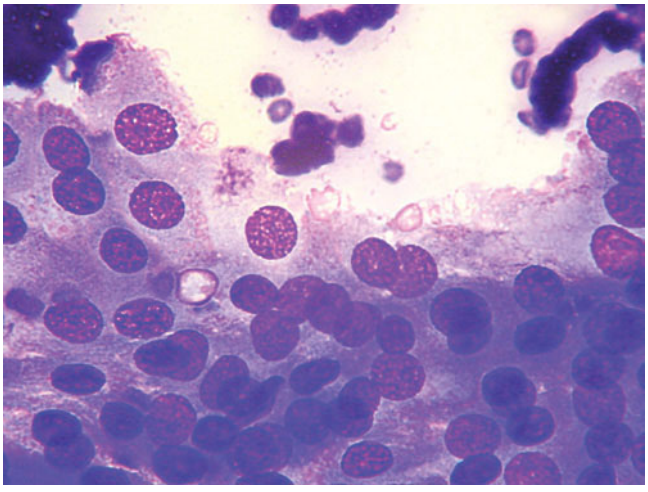


Figure 12.3 FNA from a thyroid nodule in a cat with clinical hyperthyroidism. Note the eosinophilic amorphous material which is probably colloid.

Eosinophilic intercellular or intracellular amorphous material (colloid) may also be seen (Figure 12.3). Due to their paucity, C-cells are not usually identified in cytological specimens of normal thyroid tissue. Since normal as well as neoplastic thyroid tissue is extremely vascular, blood contamination is common in FNA.

Thyroid tumours arising from follicular cells may be benign (adenomas are very common in cats) or malignant (adenocarcinomas are more common in dogs). Tumours that arise from C-cells are mainly described in dogs and are called C-cell adenomas or carcinomas (or medullary thyroid carcinomas). The clinical behaviour of a C-cell carcinoma seems to be less aggressive than that of a true adenocarcinoma in the dog (Carver *et al.*, 1995). Unless a clear follicular pattern is evident on histology, the histological appearance of follicular and C-cell tumours may be very similar. Therefore, a definitive distinction between these tumours must be based on specific immunohistochemical markers (thyroglobulin and cytokeratin 20 for follicular cells; calcitonin and other generic neuroendocrine markers, such as neuron-specific enolase (NSE), chromogranin A and synaptophysin, for C-cells) (Doss *et al.*, 1998; Espinosa de los Monteros *et al.*, 1999; Leblanc *et al.*, 1991; Patnaik and Lieberman, 1991).

In cats, an increase in thyroid gland mass is almost always due to hyperplasia or adenoma and is often associated with clinically overt hyperthyroidism. Cytology in these cases has limited utility since diagnosis is based on history, clinical examination and hormonal testing. Thyroid gland enlargement is often found during cervical palpation. Cytological examination of benign feline thyroid adenomas usually shows the microscopic features described earlier (Figure 12.1, Figure 12.2 and Figure 12.3). Cats are rarely affected by thyroid carcinomas (less than 5% of hyperthyroid cats). These tumours usually have an aggressive local behaviour and high metastatic tendency but are highly responsive to radioiodine therapy. A fixed thyroid mass that does not progress down the cervical area as it

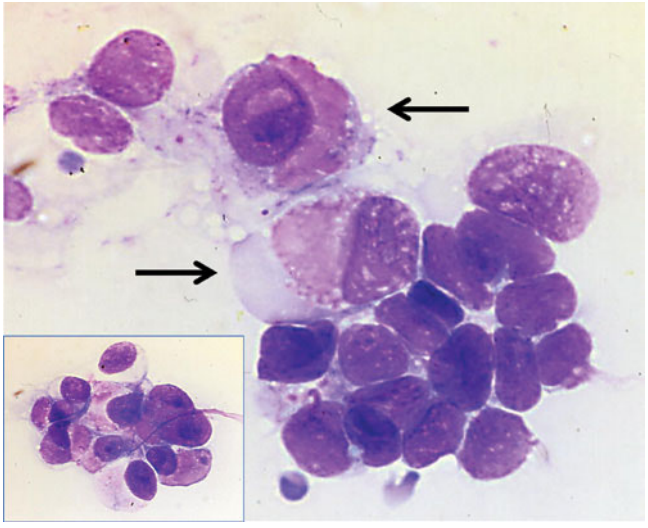


Figure 12.4 FNA from an upper paratracheal mass in a cat. A group of cohesive cells with variable nuclear to cytoplasmic ratio is evident. Two larger cells (arrows) have a larger nucleus with a prominent nucleolus and intracytoplasmic pink material. Lower left inset: Malignant thyroid follicular cells are showing marked anisocytosis and anisokaryosis, with large prominent nucleoli. This cat was submitted for surgical removal of the mass, and thyroid carcinoma was confirmed by histology (see Figure 12.5). The cat died from metastatic dissemination within 3 months (radioiodine therapy was not available at that time).

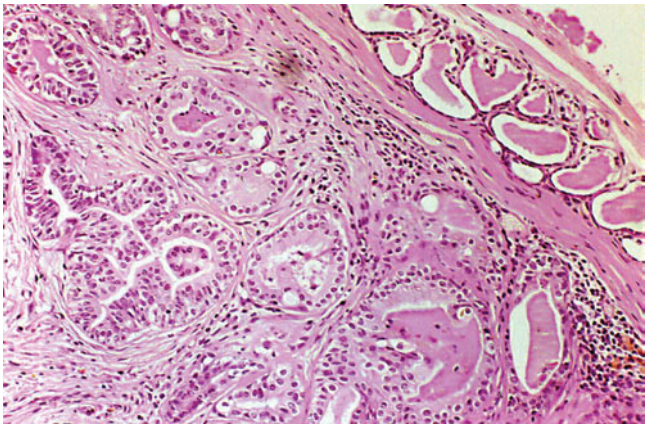


Figure 12.5 Histopathology from the same case of Figure 12.4. A neoplastic malignant proliferation (follicular thyroid carcinoma) on the left is compressing residual normal thyroid tissue (upper right) (haematoxylin and eosin 200×).

grows must be considered potentially malignant, whereas large benign thyroid tumours remain mobile and, as they become heavier, they may move to the lower third of the neck or even into the cranial mediastinum. Malignant thyroid tumours in cats may show more marked cytological atypia than benign tumours (Figure 12.4 and Figure 12.5). However, since low-grade well-differentiated thyroid carcinomas

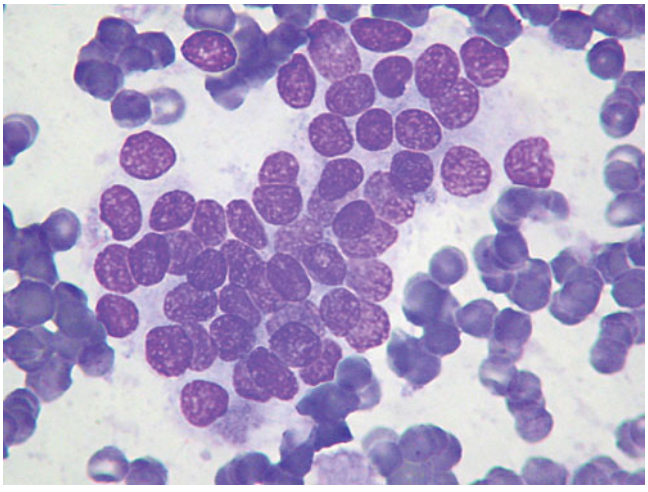


Figure 12.6 FNA from a thyroid nodule in a dog with overt hyperthyroidism and hypertrophic cardiomyopathy. A group of cohesive cells with indistinct cellular borders, uniform oval nuclei with fine chromatin and indistinct nucleoli. The clinical course of this case was more consistent with a benign thyroid tumour (the tumour was stable in size 1 year after the diagnosis), but since there was evidence of only very mild cellular atypia, differentiation between adenoma and carcinoma on a cytological basis was not possible.

are cytologically very similar to true adenomas in cats, it may be not possible to distinguish these tumours by cytology alone.

Malignant tumours of follicular origin (adenocarcinoma) are more common in dogs and show aggressive clinical behaviour despite having relatively mild malignant criteria on cytology (Figure 12.6). Adenomas are reported to comprise about 30–50% of canine thyroid tumours confirmed at necropsy. However, since they are usually small and often do not cause hyperthyroidism, they may be underdiagnosed by clinicians (Leav *et al*., 1976). On the contrary, adenocarcinomas usually present as large, invasive, fixed and poorly circumscribed cervical paratracheal masses, which may cause dyspnoea, coughing and dysphagia. Aspirates from canine thyroid tumours are usually bloody and often poorly cellular since neoplastic thyroid tissue has a higher vascular density than normal thyroid parenchyma (Harari *et al*., 1986). Therefore, several FNA samples are often necessary to achieve a definitive diagnosis. Aggregates of follicular cells from canine thyroid carcinomas are often arranged in an acinar or palisading pattern on cytology (Figure 12.7A and B). Colloid may be seen between cells, or much less commonly, intracellularly (Figure 12.8), and may appear pink to lightly blue on cytology. These cells closely resemble those observed in normal thyroid tissue and only rarely do they show moderate to severe atypia (Figure 12.9 and Figure 12.10). Naked nuclei of uniform size and shape are often seen, and these may retain the acinar organisation described earlier. Despite this 'low-grade' appearance, every canine cervical mass of thyroid origin must be considered potentially malignant, unless otherwise proven (Figure 12.11). Since cytology in most cases cannot be used to predict the clinical behaviour of the tumour, other criteria must be adopted to differentiate adenomas

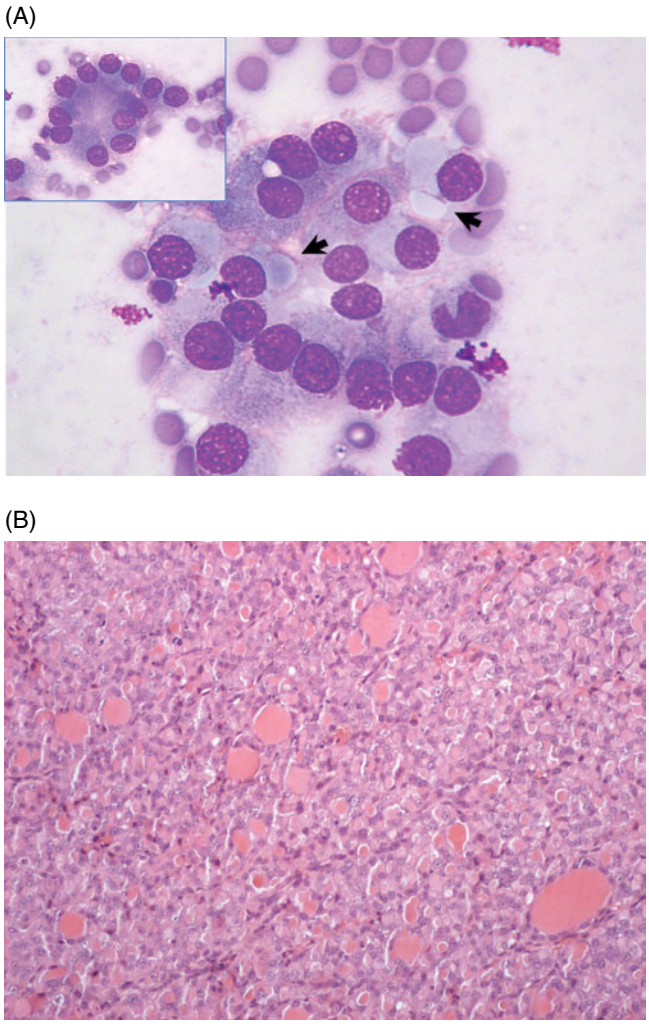


Figure 12.7 (A) FNA from an infiltrating cervical mass in a dog. A group of neoplastic thyroid follicular cells showing very mild cytological atypia, organised in a palisading or acinar (upper left inset) pattern. Some cells contain an eosinophilic intracytoplasmic globule of secretory material (arrows) consistent with colloid. (B) Histological appearance of the same mass. Note the microfollicular to solid growth pattern and the very mild cellular atypia (haematoxylin and eosin 200×).

from adenocarcinomas (size, degree of invasiveness, mobility, presence/absence of metastases, histological findings, etc.).

In one study, about one-third of canine thyroid carcinomas were proven to be of C-cell origin, based on immunohistochemistry (Carver *et al*., 1995). The cytological picture of medullary thyroid carcinomas shares common features with other neuroendocrine tumours (pheochromocytoma, islet cell tumours, chemoreceptor tumours, carcinoids), but differentiation from thyroid adenocarcinoma cannot be

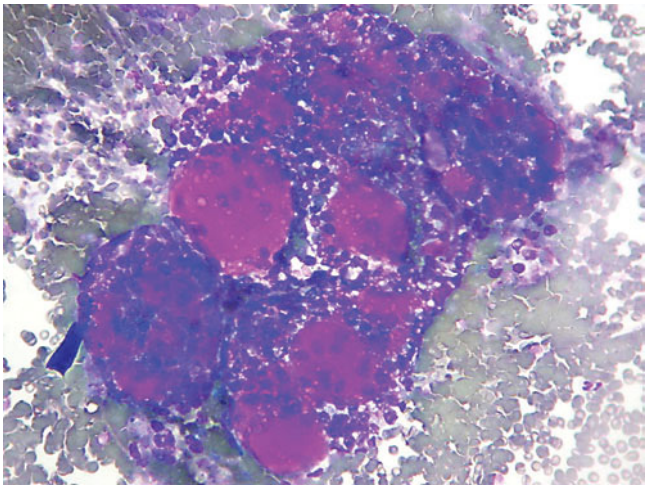


Figure 12.8 FNA from an infiltrating cervical mass in a dog. A large cluster of thyroid follicular cells is admixed with extracellular pink material which is colloid.

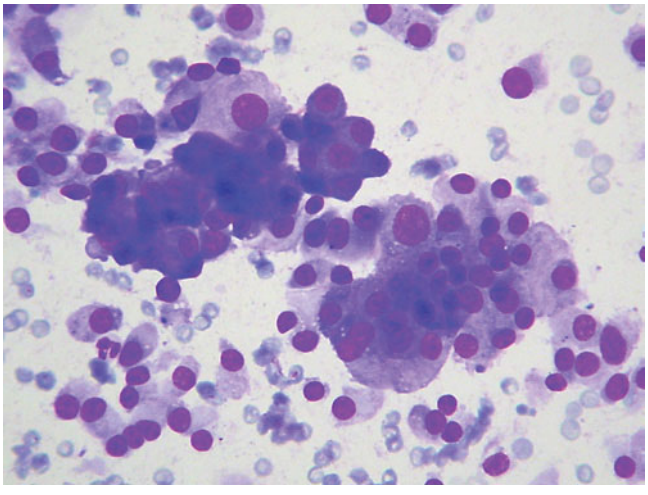


Figure 12.9 FNA from thyroid carcinoma in a dog. Clusters of malignant thyroid follicular cells showing moderate anisocytosis, anisokaryosis, variable nuclear to cytoplasmic ratio and multiple nucleoli. These malignant features are relatively uncommon in canine thyroid carcinomas.

achieved by cytology alone. Although a true acinar pattern is more typical of adenocarcinomas, cells from neuroendocrine tumours may also arrange in a rosette-like pattern. Aspirates from medullary carcinomas often consist of a large number of naked nuclei. When intact cells are present, they show a plasmacytoid appearance and may be observed alone or in loosely cohesive clusters (Figure 12.12). These cells have moderate to abundant, slightly basophilic and finely granular cytoplasm. The nuclei are usually markedly monomorphic with fine chromatin and inconsistent

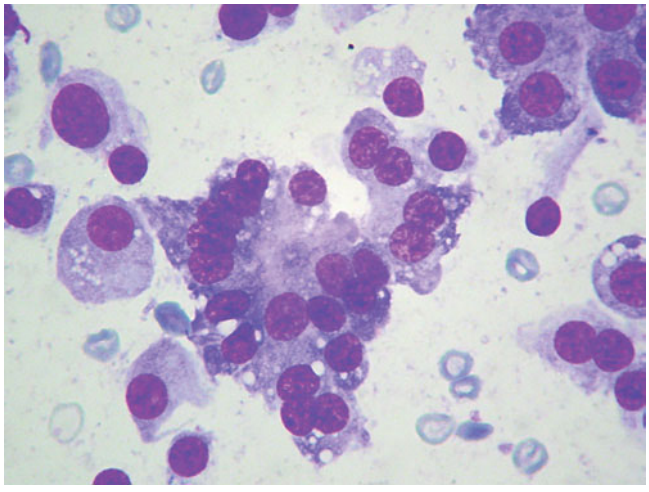


Figure 12.10 FNA from thyroid carcinoma in a dog. Clusters of malignant thyroid follicular cells showing moderate anisocytosis, anisokaryosis, variable nuclear to cytoplasmic ratio and multiple nucleoli. These malignant features are relatively uncommon in canine thyroid carcinomas.

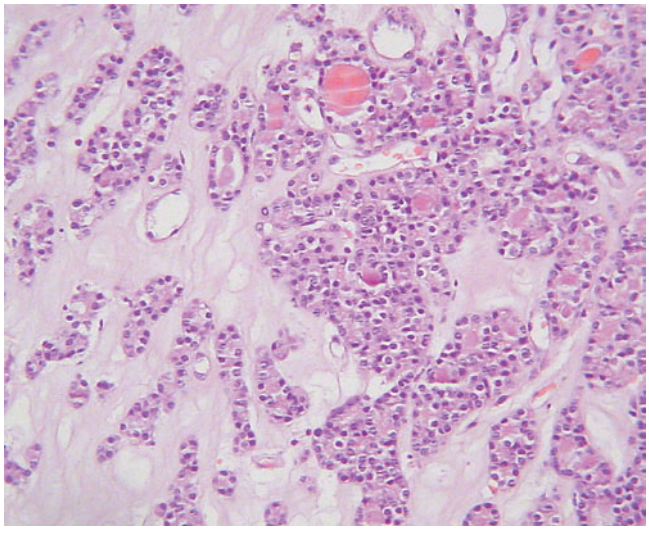


Figure 12.11 Histopathology from a thyroid carcinoma in a dog. Despite the low-grade appearance, this was a very aggressive tumour with invasive behaviour (haematoxylin and eosin 200×).

nucleoli. Nuclear pseudo-inclusion bodies are sometimes found and are considered an invagination of the nuclear membrane (Figure 12.13). Several patterns of C-cell carcinoma have been described on histopathology, including uncommon giant-cell and oxyphilic variants (Patnaik and Lieberman, 1991); therefore, some discrepancies from the previously described cytological pattern should be expected.

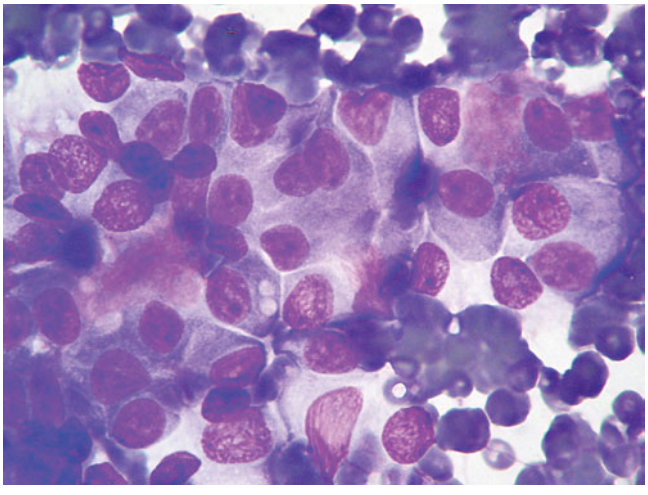


Figure 12.12 FNA from a thyroid nodule in a dog. A group of loosely cohesive cells with a plasmacytoid appearance is present. This tumour was confirmed to be a C-cell carcinoma by histology and immunohistochemistry (positivity for chromogranin A, NSE and calcitonin).

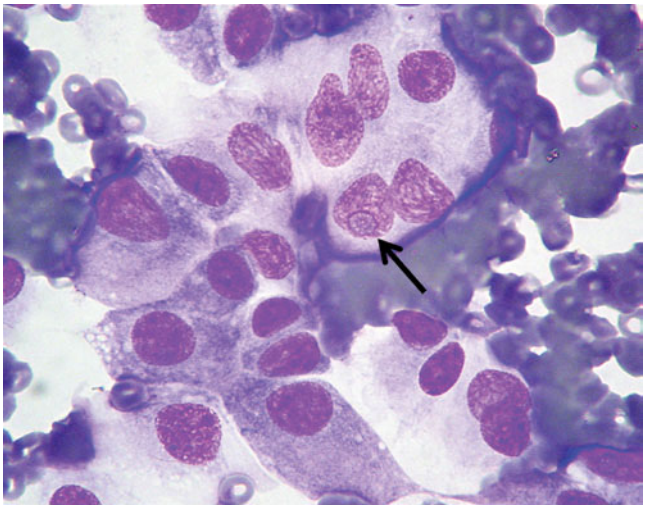


Figure 12.13 FNA from a thyroid nodule in a dog (same case of Figure 12.12). A group of cohesive pleomorphic cells from a C-cell carcinoma. A nuclear pseudo-inclusion is evident (arrow).

Cytology of the adrenal glands

Adrenal tumours are reported to be quite common in dogs and relatively uncommon in cats. Adrenal tumours may arise from different areas of the adrenal gland, which is embryologically, morphologically and functionally divided in two portions: an external cortical area (subdivided into three different

layers: glomerulosa, fasciculata and reticularis) that produces steroid hormones (aldosterone in the external glomerulosa; cortisol, other corticosteroids and sex hormones in the fasciculata and reticularis) and an internal medulla, which produces catecholamines (epinephrine and norepinephrine). The cells of the adrenal cortex are of mesodermal origin. Neoplastic proliferations of this portion are classified as adrenal adenoma and adrenal carcinoma, depending on their biological behaviour. The cells of the adrenal medulla are derived from the neuroectoderm and are classified as neuroendocrine cells. The cells in the adrenal medulla function as post-ganglionic modified neurons that release catecholamines after sympathetic stimulations. Adrenal medullary tumours are called pheochromocytomas and are therefore considered neuroendocrine tumours. Extra-adrenal pheochromocytomas may occur (usually near large thoracic and abdominal vessels) and are called paragangliomas. A third category of adrenal neoplasia can be due to metastases of tumours located at other sites, e.g. lymphoma, angiosarcoma, carcinomas, malignant melanoma and histiocytic sarcoma. Many primary adrenal tumours are functionally active and secrete an inappropriate amount of one or more hormones which cause related clinical syndromes. Primary adrenocortical tumours usually cause hyperadrenocorticism in dogs and hyperadrenocorticism or hyperaldosteronism in cats. Pheochromocytomas can cause hypersecretion of catecholamines and consequent clinical symptoms such as hypertension, tachycardia and tachypnea. Clinical findings and laboratory tests are often useful in accurately addressing the origin of many adrenal masses. However, some adrenal neoplasms are hormonally inactive, produce a minimal amount of hormones or unusual hormones and can be found incidentally during abdominal ultrasound or CT scan. In these cases, a diagnosis of adrenal 'incidentaloma' is often given.

The definitive morphological classification of adrenal tumours is based on histological features and requires a surgical approach or invasive biopsy. Cytology may offer a minimally invasive diagnostic alternative in some selected cases, although FNA is discouraged when pheochromocytoma is strongly suspected, since sudden release of catecholamines and catastrophic haemorrhage may occur with this tumour. Based on the author's experience, the distinction between adrenocortical neoplasia and pheochromocytoma is quite straightforward on cytology. Pheochromocytoma shows the usual cytological features of neuroendocrine tumours described for C-cell carcinomas of the thyroid gland (Figure 12.14). Adrenocortical tumours are instead characterised by aggregates of polygonal to rounded cells, with a low nuclear to cytoplasmic ratio, abundant basophilic and often microvacuolated cytoplasm, round eccentric nuclei with coarse chromatin and small nucleoli (Figure 12.15 and Figure 12.16). Evidence of extramedullary haematopoiesis may be seen in aspirates from adrenocortical neoplasms; therefore, megakaryocytic, erythroid and myeloid precursors may also be observed. The differential diagnostic cytological features of primary adrenal tumours are summarised in Table 12.1. Cells may be arranged perivascularly in both adrenocortical tumours and pheochromocytoma, and therefore this feature cannot be used for differential diagnosis. Unfortunately, both cytology and histology cannot be used to define the biological behaviour of adrenal neoplasms: only microscopic or macroscopic assessment of invasiveness of adjacent tissue and metastatic tendency should

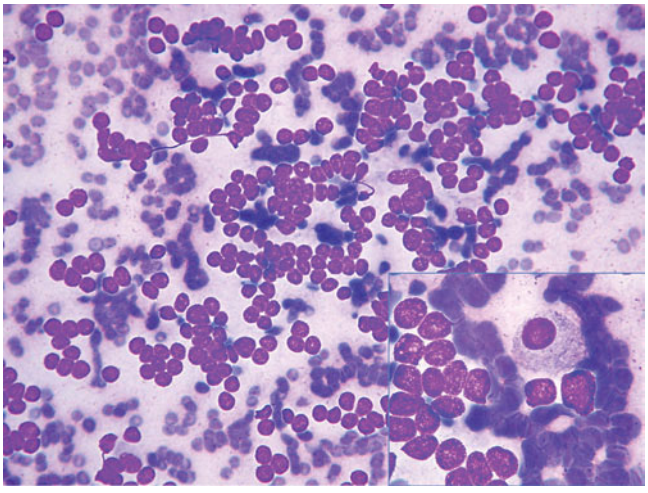


Figure 12.14 FNA from a canine pheochromocytoma. Numerous naked nuclei of uniform size and shape with fine chromatin and indistinct nucleoli can be seen in rows or rosette-like pattern. Lower right inset: A rare intact round cells with moderate amount of slightly basophilic and granular cytoplasm and an eccentric nucleus with the same features of the other naked nuclei.

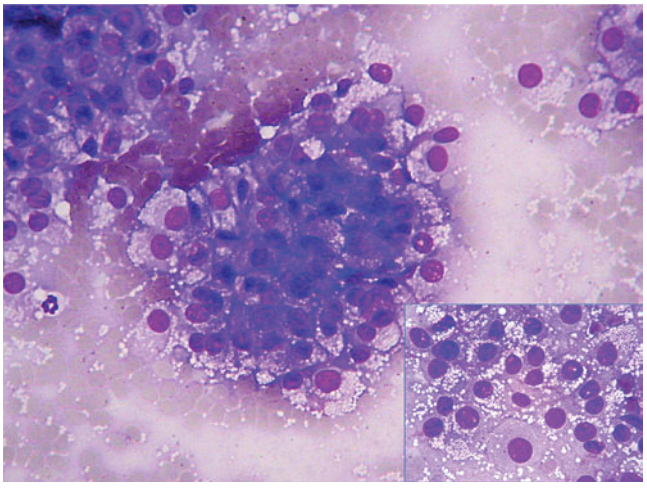


Figure 12.15 FNA from a functional adrenocortical tumour in a dog with hyperadrenocorticism. Note the clusters of cohesive cells, with moderate to abundant basophilic cytoplasm containing numerous vacuoles (inset).

be considered reliable indicators of malignancy (Labelle *et al*., 2004). A specific proliferative index of cell cycle (the nuclear protein Ki-67) has been also used as a marker of malignancy for adrenocortical tumours, but such a tool is not commonly available in diagnostic laboratory settings (Labelle *et al*., 2004).

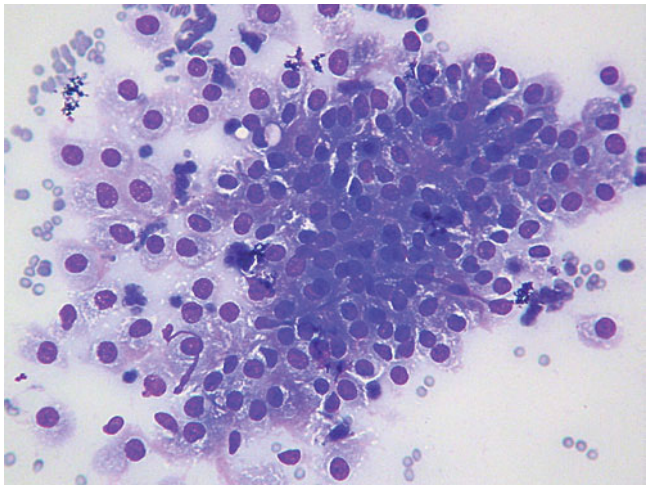


Figure 12.16 FNA from a functional adrenocortical tumour in a cat with primary hyperaldosteronism. The cytological features of this case are very similar to those found in other functional and non-functional adrenocortical tumours in dogs.

Table 12.1 Summary of cytological features of adrenocortical tumours and pheochromocytoma

	Adrenocortical tumours	Pheochromocytomas
General architecture	Many intact cells, singly or in cohesive clusters, with distinct cellular borders	Many uniform naked nuclei, often located in a finely granular and basophilic background; rare intact round and plasmacytoid cells
Nuclear to cytoplasmic ratio	Low	High
Cytoplasm	Basophilic and markedly vacuolated (small- to medium-sized lipid vacuoles)	Pale blue, finely granular
Nucleus	Round to oval, central to paracentral with coarse/ condensed chromatin	Round to oval, with fine chromatin
Nucleoli	Indistinct to prominent	Indistinct
Other features	Haematopoietic precursor cells (extramedullary haematopoiesis)	

Cytology of the endocrine pancreas

The normal endocrine pancreas consists of small islands of neuroendocrine cells scattered within the exocrine pancreatic parenchyma. These endocrine cells are morphologically identical yet functionally different based on their final hormonal

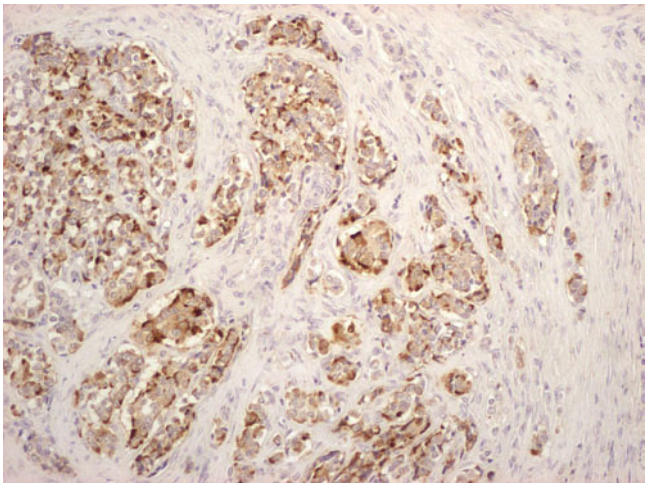


Figure 12.17 Immunohistochemistry from a gastrin-secreting tumour infiltrating the pancreas and the duodenal wall (anti-gastrin polyclonal antibodies (ABC method), haematoxylin counterstain 100×). Source: Paola Roccabianca, DVM, Dipl ECVP, University of Milan, Italy. Reproduced with permission.

product. Most of them secrete insulin, while others may produce glucagon, somatostatin and pancreatic polypeptide (and even gastrin during foetal life). Islet cell tumours are uncommon in dogs and rare in cats. They arise from the neuroendocrine islet cells and may produce and secrete one or more hormones. Based on the predominant hormonal activity, these tumours are called insulinomas, glucagonomas, somatostatinomas or gastrinomas; insulinomas are the most common in small animal practice. The definitive classification of an islet cell tumour is based on demonstration of hormonal secretion (e.g. demonstrating a high insulin activity in a dog with hypoglycaemia) or by immunohistochemistry (Figure 12.17), since they appear identical on histology and cytology. Moreover, their microscopic appearance does not predict the biological behaviour, and only the growth pattern (degree of invasion of adjacent tissue on histology) and/or the presence of metastases will distinguish between benign and malignant tumours. Although islet cell tumours are often small at the time of diagnosis, they are frequently malignant and metastatic in dogs, contrary to the situation in humans. Cytological samples from islet cell tumours share common features of other neuroendocrine neoplasms: samples are usually very cellular and composed of numerous, naked nuclei of similar size and shape located in an amorphous, slightly basophilic background (Figure 12.18). This background results from the rupture of the cellular membrane which is fragile in neuroendocrine tumours. The nuclei are oval and have finely stippled chromatin with indistinct nucleoli. Mild anisokaryosis is often evident, and the nuclei are arranged in rows or rosette-like patterns (Figure 12.18 and Figure 12.19). When intact cells are present, they show the typical plasmacytoid-like neuroendocrine appearance with a moderate amount of granular, slightly basophilic cytoplasm that may also be vacuolated in some cases (Figure 12.19). Rarely, small clusters of intact cells may be found, reminiscent of their growth pattern in small nests clearly visible in histological samples (Figure 12.20).

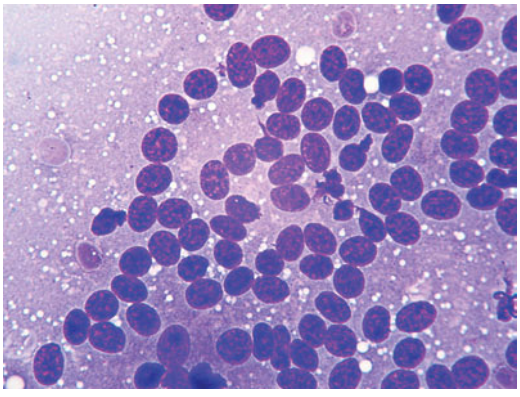


Figure 12.18 Intra-operative FNA of a pancreatic islet cell tumour in a dog. Note the naked oval nuclei arranged in rows in a basophilic background.

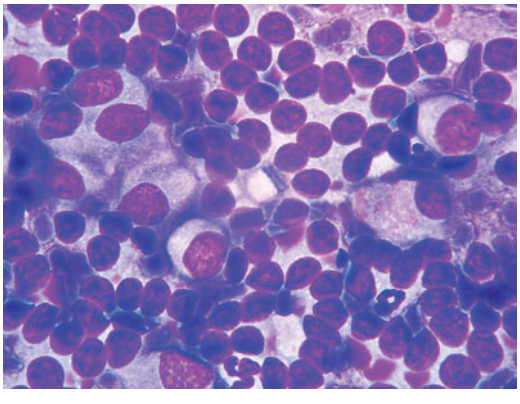


Figure 12.19 Imprint from an islet cell tumour in a dog with severe hypoglycaemia and hyperinsulinaemia. Some intact round cells with a plasmacytoid appearance can be seen among the naked nuclei.

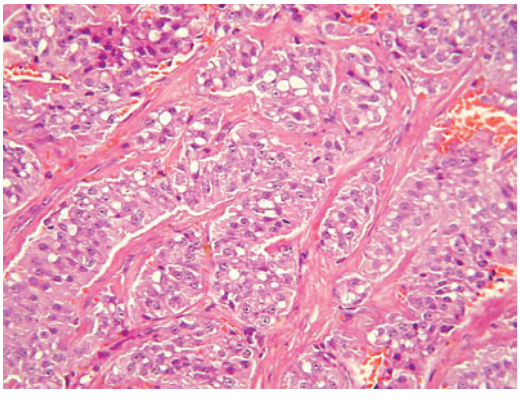


Figure 12.20 Histopathology from an insulin-secreting pancreatic islet cell tumour in a dog. Nests and cords of neoplastic neuroendocrine cells are separated by thin fibrovascular stroma (haematoxylin and eosin 200×). This histological pattern is common to many neuroendocrine tumours and histology alone often cannot be used to definitively classify a specific neoplasm.

Other neuroendocrine tumours: Chemoreceptor tumours and carcinoids

Chemoreceptor tumours (chemodectoma or non-chromaffin paraganglioma) arise from neuroendocrine cells closely associated with large vessels which are involved in the regulation of respiration, blood pressure and systemic perfusion. Although they may be found in several anatomic areas, usually near big arterial vessels, chemodectomas usually originate from the aortic body, giving rise to the typical heart-base tumour (Figure 12.21), and less commonly from the carotid body in the upper lateral cervical area. Even if most heart-base chemodectomas are biologically benign, they may cause cardiac dysfunction or they may rupture resulting in haemopericardium. Cytological sampling is usually difficult due to their anatomic location unless they are very large or they metastasise (e.g. to the lung) (Figure 12.22). The cytological appearance of chemodectomas is similar to other neuroendocrine tumours with naked nuclei in a slightly basophilic background (Figure 12.23). Anisokaryosis may be marked in chemodectomas even in benign tumours. Intact cells have the typical plasmacytoid-like appearance of neuroendocrine cells. A definitive diagnosis of a chemodectoma must be based on the typical histological pattern of a neuroendocrine tumour and immunohistochemistry (positivity for generic neuroendocrine markers such as chromogranin A, synaptophysin and NSE) (Doss *et al.*, 1998). Other differential diagnoses (C-cell carcinoma, thyroid adenoma/adenocarcinoma and parathyroid tumours) may be ruled out by histology and immunohistochemistry (positivity for calcitonin, thyroglobulin and parathyroid hormone, respectively), since all these tumours may be found in the cervical or the cranial mediastinal area.

Carcinoids are rare neuroendocrine tumours that arise from neuroendocrine cells dispersed throughout the gastrointestinal tract (gastric and intestinal wall, gall bladder and liver). They have been described mainly in dogs and rarely in cats. This group of tumours shares the same features of other neuroendocrine neoplasms (typical growth pattern on histology; histochemical positivity for Grimelius stain;

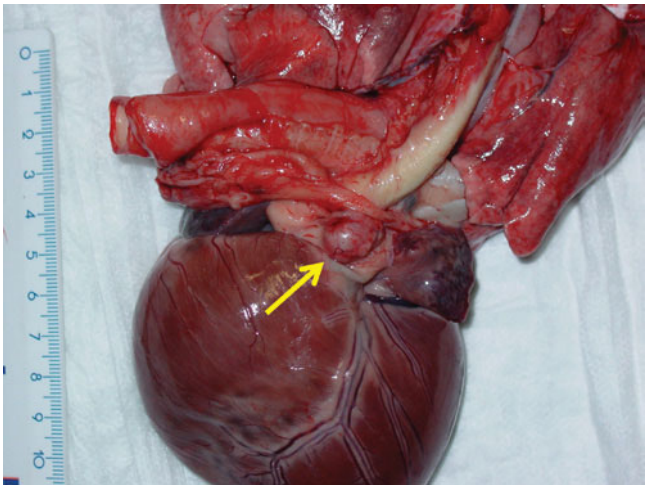


Figure 12.21 Heart-base tumour (chemodectoma) in a dog (arrow).



Figure 12.22 Lateral thoracic radiograph from a dog with metastatic chemodectoma of the heart base. Several pulmonary nodules are evident.

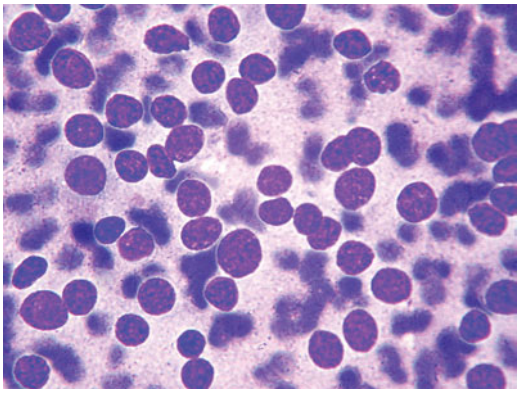


Figure 12.23 Ultrasound-guided FNA from a lung nodule in Figure 12.22 (metastatic chemodectoma in a dog). Note the similarity to other neuroendocrine tumours previously described.

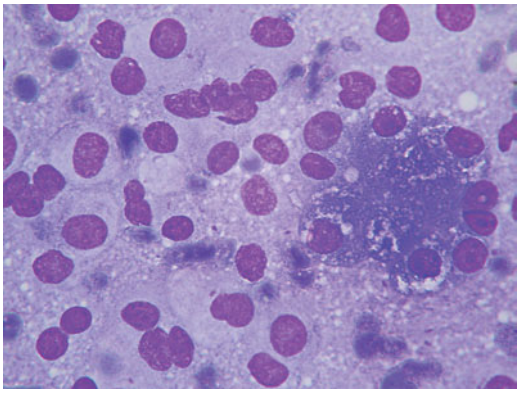


Figure 12.24 Ultrasound-guided FNA from a dog with multiple nodules in the liver (carcinoids). Naked uniform oval nuclei and the occasional round intact cell surround a small cluster of hepatocytes on the right. Note the difference between the neuroendocrine nuclei and intact hepatocytes (the latter are more rounded, with reticular to coarse chromatin and a visible nucleolus).

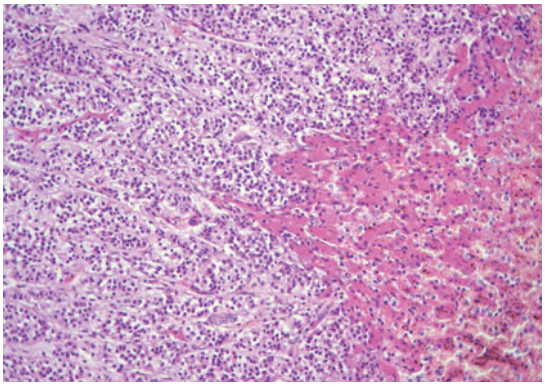


Figure 12.25 Histology from a liver nodule in a dog (carcinoid, same case as Figure 12.23). Islands of neoplastic neuroendocrine cells separated by thin fibrous stroma (on the left) are invading the liver parenchyma (on the right) (haematoxylin and eosin 100×). Source: G. Tortorella, DVM, Laboratorio La Vallonea, Italy. Reproduced with permission.

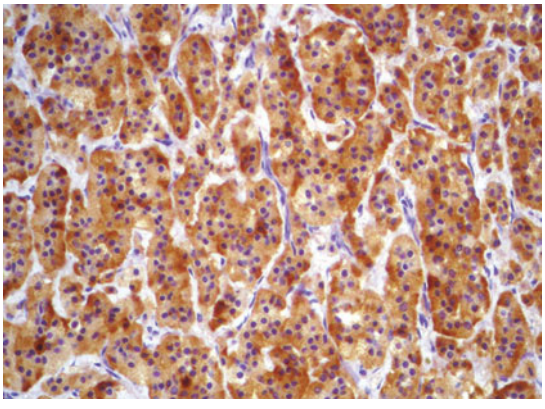


Figure 12.26 Immunohistochemistry of a liver nodule (carcinoid) in a dog (same case as Figure 12.23 and Figure 12.24). Strong cytoplasmic positivity for chromogranin A (avidin–biotin peroxidase 200×). Source: G. Tortorella, DVM, Laboratorio La Vallonea, Italy. Reproduced with permission.

immunohistochemical positivity for chromogranin A, NSE and synaptophysin; and presence of neurosecretory granules on electron microscopy). Cytology of a carcinoid is rarely performed because these tumours are often small and difficult to sample by FNA. Sometimes, however, they may reach considerable size, allowing ultrasound- or CT-guided FNA cytology. A cytological distinction between hepatic carcinoid and hepatocellular carcinoma is not easy, since aspirates from both these tumours may consist of many naked nuclei. Naked nuclei from carcinoids are however more oval with fine chromatin and inconsistent nucleoli compared with those derived from ruptured neoplastic hepatocytes, which closely resemble those of normal hepatocytes (more rounded, with coarse chromatin and a clearly visible nucleolus). The presence of intact cells with a typical neuroendocrine round-cell appearance will allow the diagnosis of carcinoid instead of hepatocellular carcinoma, although histology and immunohistochemistry are necessary for a definitive diagnosis (Figure 12.24, Figure 12.25 and Figure 12.26).

References

Carver, J.R., Kapatkin, A. and Patnaik, A.K. (1995) A comparison of medullary thyroid carcinoma and thyroid adenocarcinoma in dogs a retrospective study of 38 cases. *Veterinary Surgery*, **24**, 315–319.

Doss, J.C., Gröne, A., Capen, C.C. *et al.* (1998) Immunohistochemical localization of chromogranin A in endocrine tissues and endocrine tumors of dogs. *Veterinary Pathology*, **35**, 312–315.

Espinosa de los Monteros, A., Fernandez, A., Millan, M.Y. et al. (1999) Coordinate expression of cytokeratins 7 and 20 in canine and feline carcinomas. *Veterinary Pathology*, **36**, 179–190.

Harari, J., Petterson, M.E. and Rosenthal, R.C. (1986) Clinical and pathologic features of thyroid tumors in 26 dogs. *Journal of the American Veterinary Medical Association* , **188**, 1160–1164.

Labelle, P., Kyles, A.E., Farver, T.B. *et al.* (2004) Indicators of malignancy of canine adrenocortical tumors: histopathology and proliferation index. *Veterinary Pathology*, **41**, 490–497.

Leav, I., Schiller, A.L., Rijnberk, A. *et al.* (1976) Adenomas and carcinomas of the canine and feline thyroid. *American Journal of Pathology*, **83**, 61–93.

Leblanc, B., Parodi, A.L., Lagadic, M. *et al.* (1991) Immunocytochemistry of canine thyroid tumors. *Veterinary Pathology*, **28**, 370–380.

Patnaik, A.K. and Lieberman, P.H. (1991) Gross, histologic, cytochemical, and immunohistochemical study of medullary thyroid carcinoma in sixteen dogs. *Veterinary Pathology*, **28**, 223–233.

13 Cytology of the Male and Female Genital Tracts

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Introduction

Cytological evaluation is an important part of the clinician's armoury for diagnosis of common reproductive tract diseases. The purpose of this chapter is to provide an overview of the application of cytological investigation to common reproductive tract diseases in the dog and cat. The reader is encouraged to consult with veterinary specialists or refer to veterinary textbooks for additional information regarding the specific diagnosis and treatment of disorders of the reproductive system.

Cytology of the male genital tract

Cytological evaluation is useful for determining the cause of abnormal discharges, swellings and masses associated with the testes, scrotum, prostate, penis and prepuce as well as to evaluate semen for breeding soundness and in cases of reproductive failure. Some disorders are unique to the intact male dog (e.g. prostatitis), whereas others affect intact and neutered male dogs equally (e.g. prostatic tumours).

Collection of specimens

Preputial discharge collected on saline-moistened cotton-tipped swabs can be gently rolled onto a glass slide, and an additional sample transferred to bacterial transport medium in case culture is indicated. Fine-needle aspirates (FNA) of masses or swellings associated with the testes, prostate or external genitalia can be collected with a 22 G or 25 G needle. Complications from FNA are uncommon but may include dissemination of infection, seeding of tumour cells or haemorrhage. Ultrasonographic guidance for FNA of the testes and prostate is recommended to target the intended

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lesion and provide a measure of safety. Collection of semen and prostatic fluid through ejaculation for reproductive evaluation is described later.

Prostatic wash can be used to collect cytological specimens from dogs with prostatomegaly. The bladder first should be emptied and rinsed with sterile saline, and the last wash fluid saved for possible culture (pre-massage). The length of urinary catheter required to reach the prostate should be estimated, and the penis retracted from the prepuce and cleaned. The catheter is then inserted into the penis and advanced to the prostate. The appropriate location of the catheter tip is confirmed by digital rectal palpation, and the prostate is gently massaged before 5–10 ml sterile saline is infused with a syringe and then re-aspirated. Squash smears can be made from tissue particles retrieved from the wash fluid or from the cellular pellet after centrifugation, and an aliquot saved for possible culture (post-massage). Culture of pre- and post-prostatic massage samples as well as a urine sample aids in localisation of the infection to the prostate, urinary tract or both.

Disorders of the testicle and scrotum

Changes in the size or consistency of the scrotum may be unilateral or bilateral and acute or chronic in presentation. In addition to a complete medical work-up, examination of semen and FNA of the testes can be useful in establishing the aetiology and likely prognosis.

Infectious orchitis and epididymitis

In dogs and cats with acute orchitis/epididymitis, substantial swelling, heat and pain are typically present in the testes. In countries, where *Brucella canis* is present (rare in the United Kingdom), this organism must be considered for any dog with this presentation. Cytological examination of FNA of the testes reveals neutrophilic or pyogranulomatous inflammation in cases associated with bacterial or fungal infection, respectively. Frequently there will be inflammatory cells in the ejaculate if the male is able to ejaculate.

Testicular tumours

In many cases of testicular neoplasms, there is enlargement of one testis with a normal-sized or atrophied contralateral testis. Sertoli cell tumours and seminomas are more common in cryptorchid testes (these may be diagnosed by an FNA of the abdominal or inguinal mass). Common testicular tumours including Sertoli cell tumours (Figure 13.1), interstitial (Leydig) cell tumours (Figure 13.2 and Figure 13.3) and seminomas (Figure 13.4) often can be differentiated cytologically. Sertoli cell tumours may produce oestrogen, which can result in feminisation syndrome (gynaecomastia and attraction of male dogs), symmetrical hair loss, prostatic squamous metaplasia and irreversible bone marrow hypoplasia.

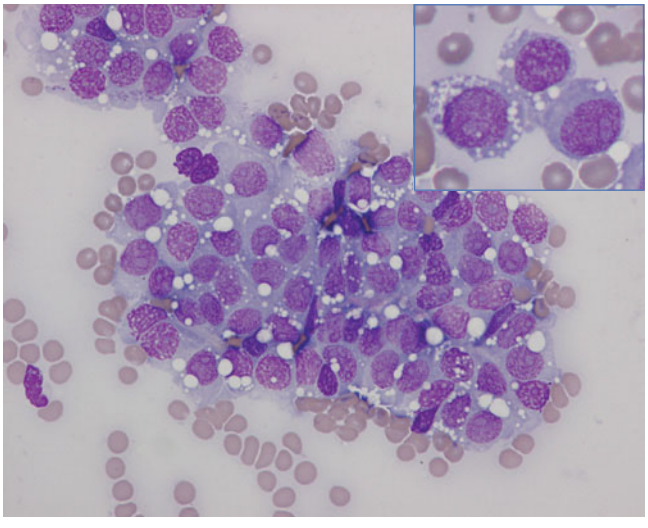


Figure 13.1 Sertoli cell tumour, dog (FNA). Tumour cells are round with moderately high nuclear to cytoplasmic (N:C) ratios and are arranged individually (inset) or in aggregates. The basophilic cytoplasm often contains moderate numbers of clear vacuoles. Nuclei have finely stippled chromatin.

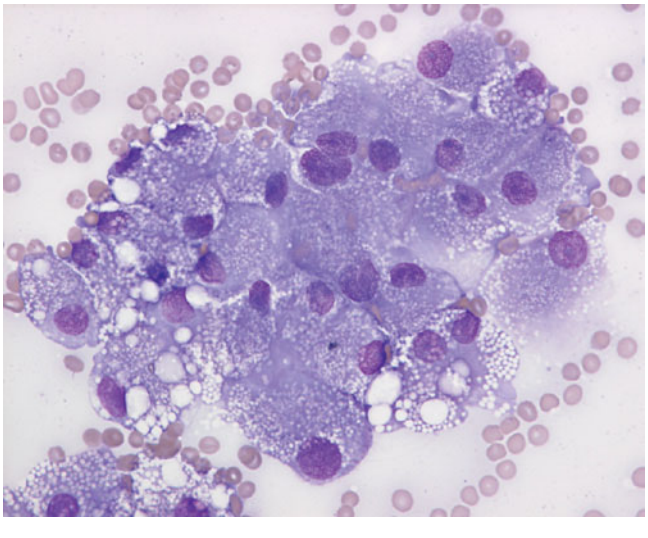


Figure 13.2 Interstitial cell tumour, dog (FNA). Tumour cells are arranged in clusters and have abundant basophilic cytoplasm containing numerous punctate vacuoles or a few larger vacuoles. Nuclei are eccentrically located.

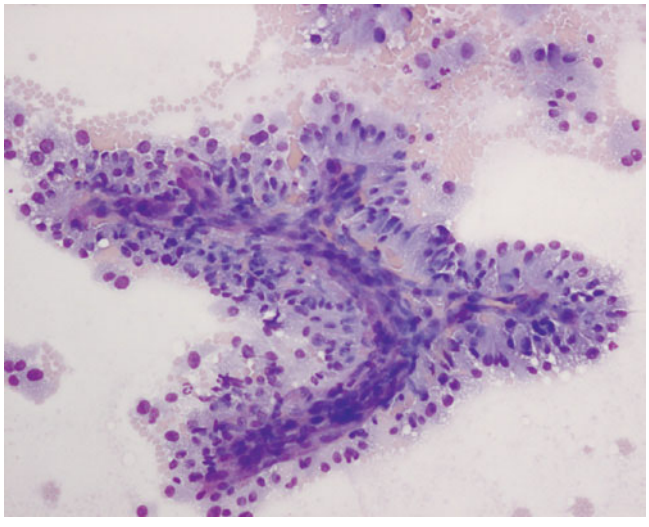


Figure 13.3 Interstitial cell tumour, dog (FNA). Palisading of tumour cells along the long axis of a capillary is observed in this low power view.

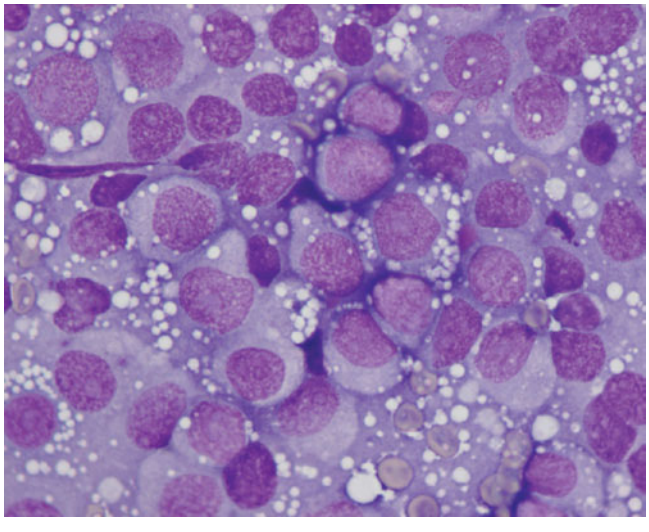


Figure 13.4 Seminoma, dog (imprint). Tumour cells are round and individualised with high N:C ratios. The nuclei have finely stippled chromatin, often with a prominent nucleolus. The small amount of lightly basophilic cytoplasm may contain a few discrete vacuoles.

Disorders of the prostate

Normal cell types

Cytology of the normal prostate consists of sheets or rows of uniform cuboidal epithelial cells with moderate amounts of basophilic cytoplasm and small, round basally located nuclei with condensed chromatin.

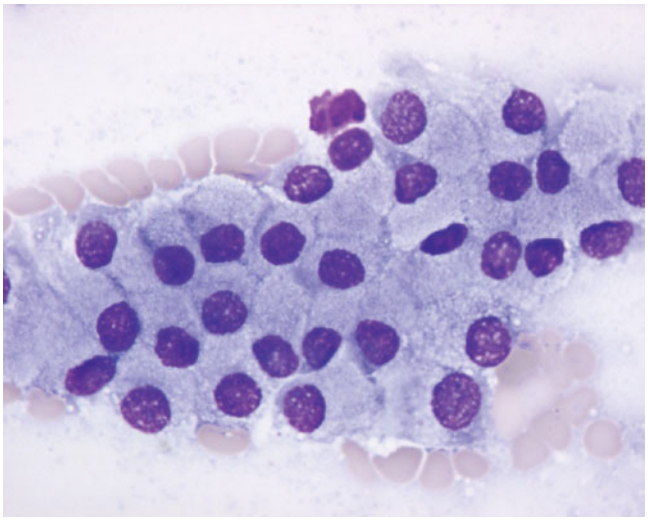


Figure 13.5 Benign prostatic, hyperplasia, dog. Hyperplastic prostatic epithelium with increased N:C ratio and cytoplasmic basophilia.

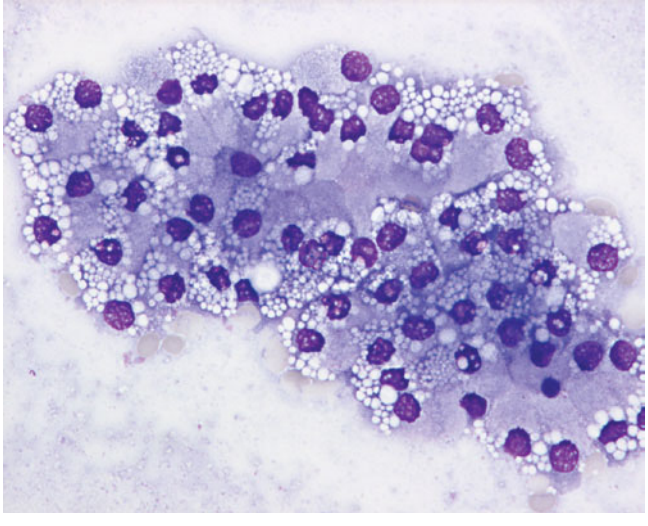


Figure 13.6 Benign prostatic hyperplasia, dog. These prostatic epithelial cells have prominent cytoplasmic vacuolisation.

Benign prostatic hyperplasia

Benign prostatic hyperplasia (BPH) is a common aging change seen in intact dogs. Glandular enlargement is symmetrical and non-painful but can lead to partial or complete urethral obstruction. In early stages, haemospermia is noted within the ejaculate without an increase in the number of white blood cells. Cells collected by aspiration or prostatic wash resemble those of normal prostatic

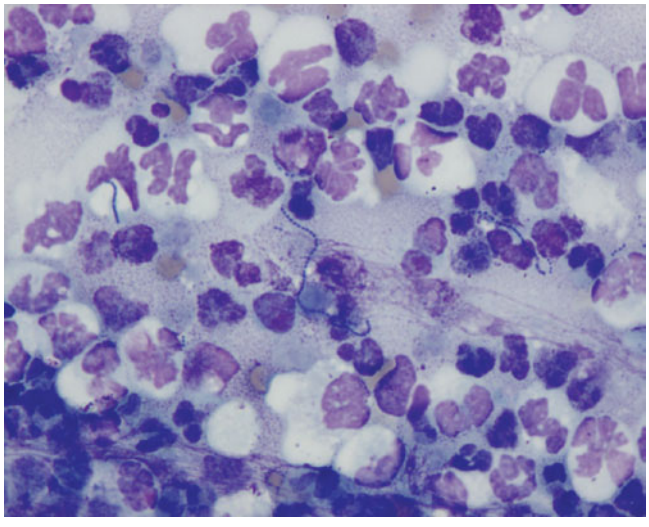


Figure 13.7 Infected prostatic cyst, dog (FNA). Numerous degenerate neutrophils with pale, swollen nuclei are present. Chains of cocci are seen within neutrophils and in the background.

epithelium and are often arranged in large 'honeycomb' clusters. Additional cytological features of hyperplasia are present (higher nuclear to cytoplasmic (N:C) ratio, increased cytoplasmic basophilia and vacuolisation, finely stippled chromatin and a small nucleolus; Figure 13.5 and Figure 13.6). Prostatitis may be a sequela to BPH.

Prostatic and paraprostatic cysts

Prostatic and paraprostatic cysts are primarily seen in intact dogs and may occur in combination with BPH. Large cysts may cause tenesmus, constipation, stranguria and abdominal distension. Aspirated fluid is often amber, blood tinged or green brown in colour. Frequently the fluid is acellular or may contain a few red blood cells, neutrophils, normal prostatic epithelial cells and macrophages. Prostatic cysts that become secondarily infected contain higher numbers of neutrophils containing intracellular bacteria (Figure 13.7).

Prostatitis and prostatic abscess

Prostatic infections are generally limited to intact dogs. Dogs with prostatitis may present with fever, caudal abdominal pain, penile discharge and stranguria. The prostate may be enlarged and painful upon rectal palpation. Ultrasonographic imaging of the prostate may detect the presence of cavitations that indicate infected cysts or an abscess. Fine-needle aspiration is not recommended if

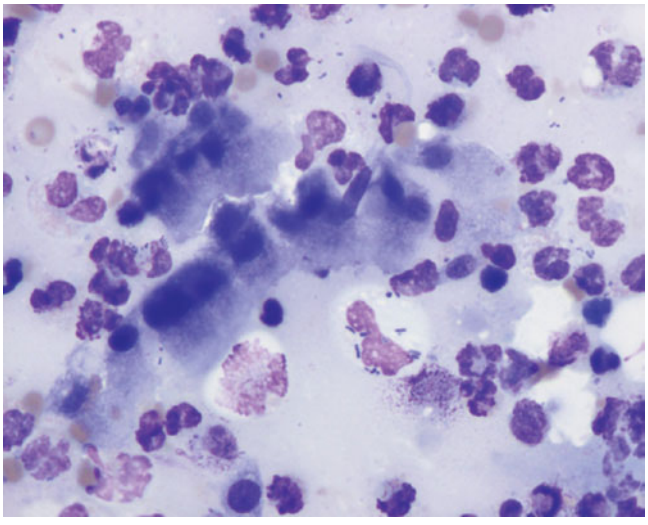


Figure 13.8 Prostatitis, dog (FNA). Necrotic prostatic epithelial cells are surrounded by degenerate neutrophils. Bacterial rods are seen within neutrophils and in the background. *Escherichia coli* was cultured from this sample.

prostatic abscessation is suspected because of the risk of rupture and subsequent peritonitis (a safer option is to collect the sample by prostatic wash or by ejaculation if the dog is not in too much pain). Smears prepared from a prostatic wash contain numerous degenerate and non-degenerate neutrophils, some of which may contain bacterial organisms (Figure 13.8). Intact or necrotic prostatic epithelial cells may be present. Samples of wash fluid or aspirated material should be submitted for culture and sensitivity, and castration should be considered in non-breeding dogs.

Prostatic neoplasms

Both intact and neutered male dogs may develop prostatic tumours. Common presenting signs include stranguria or difficulty defecating due to prostatomegaly. Metastasis to regional lymph nodes and vertebrae is common with wider dissemination possible. Transitional cell carcinoma (TCC) and primary prostatic adenocarcinoma occur most commonly and generally are cytologically indistinguishable. In addition, TCC of the bladder and urethra may extend into the prostate. Aspirates of prostatic tumours consist of moderately to highly pleomorphic epithelial cells arranged in sheets, in clusters and occasionally as individual round cells (Figure 13.9). Multinucleation and cytoplasmic vacuolisation are common, and some cells may contain a round pink cytoplasmic inclusion which may represent glycosylated protein (Figure 13.10). Necrosis may accompany large tumours. In washes of the prostate, tumour cells are less well preserved than in FNA, but clusters of pleomorphic epithelial cells often are readily identified (Figure 13.11).

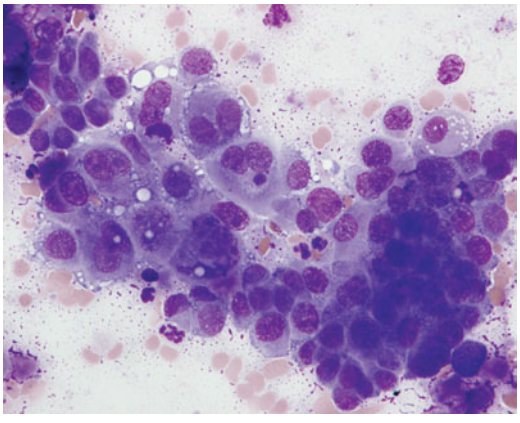


Figure 13.9 Prostatic carcinoma, dog (FNA). Moderately pleomorphic tumour cells are arranged in sheets and clusters. Several binucleate and multinucleate cells are present.

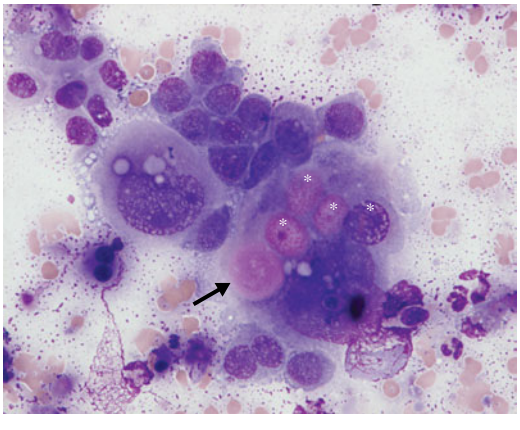


Figure 13.10 Prostatic carcinoma, dog (FNA). Cell and nuclear size and N:C ratio vary markedly. The large multinucleate cell contains one large (lower) and four smaller nuclei (asterisks) and a smooth magenta cytoplasmic inclusion (black arrow). Nuclear chromatin is clumped, and prominent nucleoli are visible.

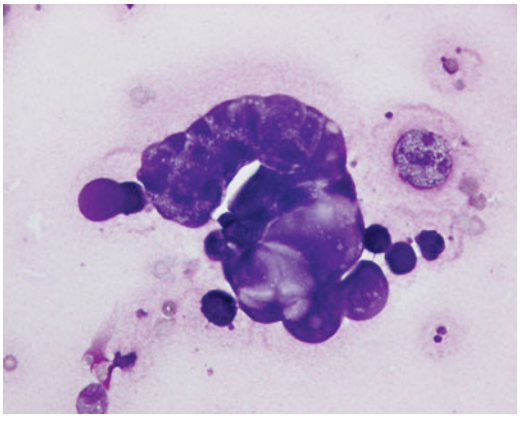


Figure 13.11 Prostatic carcinoma, dog (wash). Variation in cell size and cytoplasmic vacuolisation are apparent within this cluster of poorly preserved tumour cells.

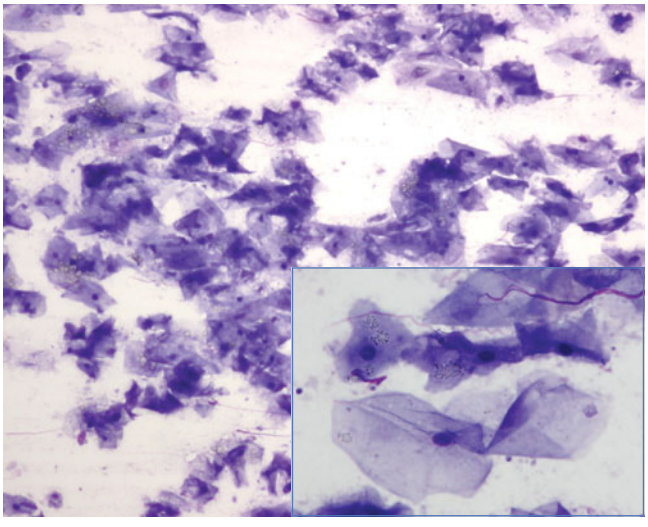


Figure 13.12 Prostatic metaplasia secondary to oestrogen exposure, dog (FNA). The specimen consists exclusively of mature, cornified squamous cells. Inset: Squamous epithelial cells (higher magnification).

Squamous metaplasia

Squamous metaplasia results from chronic exposure to oestrogen usually secondary to an oestrogen-producing Sertoli cell tumour. Detection of prostatic squamous metaplasia may be the first clue to the presence a Sertoli cell tumour in a scrotal or undescended testicle. The prostatic wash consists of numerous mature, cornified squamous epithelial cells (Figure 13.12). Concurrent neutrophilic inflammation or bacterial infection may be present.

Disorders of the penis and prepuce

Balanitis and posthitis

Bacterial infections of the external genitalia may result in purulent discharge that can be examined cytologically. Smears contain numerous neutrophils, intra- and extracellular bacteria and exfoliated mature squamous cells.

Penile and preputial neoplasms

Tumours of the penis and prepuce are rare with squamous cell carcinoma (SCC) and transmissible venereal tumour (TVT) most commonly seen. The cytological appearance of SCC is similar to that in other locations (see Figure 4.13). TVT is a contagious tumour endemic within large populations of stray dogs in some tropical and subtropical urban environments. TVT may be found on the mucosa

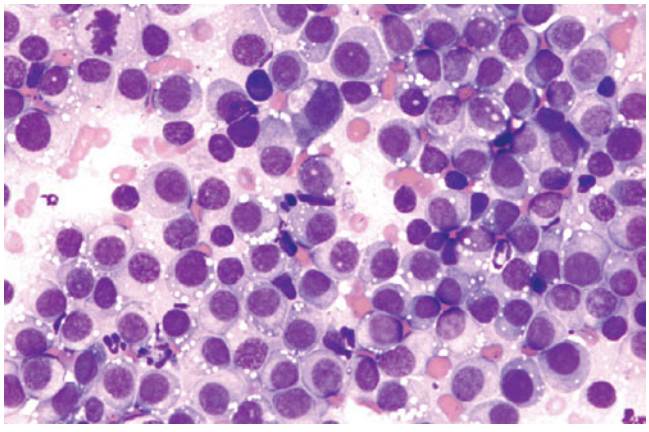


Figure 13.13 Transmissible venereal tumour. Tumour cells are round and individualised with moderately high N:C ratios. Many contain discrete cytoplasmic vacuoles. Nuclei are eccentrically located with coarsely stippled chromatin and often possess a prominent nucleolus.

of the penis or prepuce of young, sexually mature dogs and less often on the oral or nasal mucosa. The tumours are pink, friable, expansile masses that bleed easily and have a characteristic cytological appearance (Figure 13.13).

Semen collection and evaluation

Semen quality is often studied in an attempt to predict the fertilising capability of the male animal. Semen collection in the dog has been performed using an artificial vagina, although most commonly it is collected by manually stimulating the penis in the presence of a teaser bitch. Using this method, the three fractions can be collected separately into test tubes with the second (semen-rich) and third (prostatic fluid) fraction being most clinically relevant. Semen can be collected successfully from the tom cat using an artificial vagina if the tom previously has been trained. Full evaluation of semen involves assessment of sperm motility, concentration, morphology and percent live sperm. This section will focus on the cytological evaluation of stained semen specimens, and the reader is referred to other sources for information regarding a complete fertility examination in a male dog (Root Krustritz, 2007).

Common conditions affecting semen quality

Disorders of sperm

The absence of sperm in an ejaculate is called azoospermia and may result from specific testicular pathology or from incomplete ejaculation in a young or nervous dog. Abnormal sperm morphology is referred to as teratozoospermia. In gen-

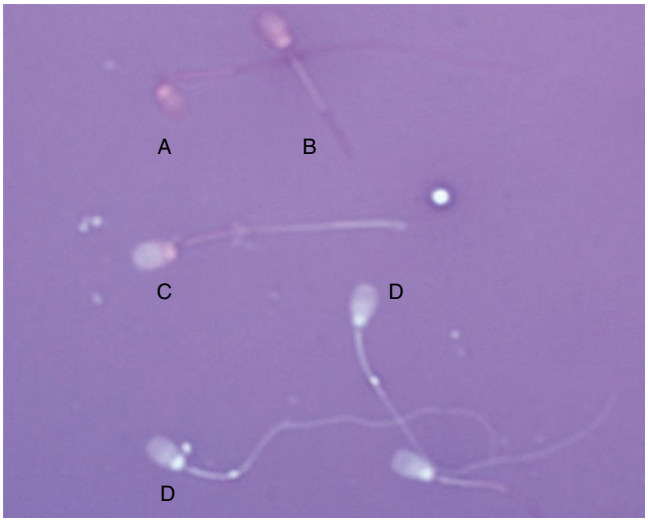


Figure 13.14 Multiple sperm defects in a case of early testicular degeneration. (A) Dead sperm with broken neck. (B) Dead sperm with bent tail. (C) Live sperm with hairpin bend to tail. (D) Live sperm with distal cytoplasmic droplet. Dog, nigrosin–eosin, 1000× oil immersion.

eral, when more than 40% of sperm are morphologically abnormal, there is a decline in the fertility. The causes of teratozoospermia are often unknown, although many cases are the result of early stages of testicular degeneration (Figure 13.14) following some (often unknown) testicular insult. Many of these animals subsequently become azoospermic. Repeated semen evaluation after two months should allow the progression of the disease process to be established. In some cases, teratozoospermia is seen in older dogs as a result of senile degenerative changes within the testes.

Dogs and cats that have severe bacterial inflammatory disease affecting the testes, epididymides, prostate or urethra are usually unable to ejaculate as a result of pain. However, in mild cases, it is possible to identify inflammatory cells including neutrophils, lymphocytes and macrophages in collected semen samples, and this often has an adverse impact upon sperm morphology and motility. Identification of these cytological findings should prompt further diagnostic investigation of the reproductive tract to identify the site of the primary problem.

Cytology of the female genital tract

Cytological evaluation is useful for determining the cause of abnormal discharges, swellings and masses associated with the ovary, uterus, vagina, vestibule and vulva. Disorders of the reproductive tract primarily occur in intact females. Cytological evaluation of vaginal smears plays an important role in the accurate timing of breeding and should be part of the assessment of bitches that fail to conceive.

Collection of specimens

Smears may be prepared from vaginal or uterine discharges by collecting material on a saline-moistened cotton swab and rolling it onto a glass slide. An additional sample can be transferred to transport medium in case culture is warranted. A vaginal speculum may be used to visualise the vestibule, urethral opening and vaginal mucosa and facilitates collection of FNA from vaginal masses. Cranial vaginal and cervical abnormalities require endoscopic inspection and collection of either a 'grab biopsy' or FNA through the instrument port of the endoscope. Ovarian or uterine masses within the retroperitoneal space and abdominal cavity can be aspirated using ultrasonographic guidance; however, tumour seeding may be a concern when aspirating ovarian tumours. Collection of vaginal cytology for breeding management is described later.

Infectious and inflammatory disorders

Vulval discharge may originate from the vagina or uterus and may vary in character from clear to mucopurulent, purulent or bloody (Table 13.1). A thorough reproductive history along with physical examination, imaging and assessment of cytological specimens is necessary to differentiate between the various causes of vulval discharge. Small numbers of neutrophils and normal bacterial flora can be expected in vaginal swabs from dogs without reproductive disease. However, large numbers

Table 13.1 Differentials for vaginal discharge

Quality of discharge	Differentials
Clear	Oestrus
Mucoid	Metoestrus Normal pregnancy
Purulent	Juvenile vaginitis Vaginitis
Purulent/haemorrhagic	Pyometra Metritis
Haemorrhagic	Pro-oestrus Oestrus Follicular cysts Vaginal ulceration Placental separation Sub-involution of placental sites Transmissible venereal tumour Cystitis Urinary tract neoplasms
Haemorrhagic (brown coloured)	Abortion
Haemorrhagic (green/brown coloured)	Parturition Dystocia, placental separation

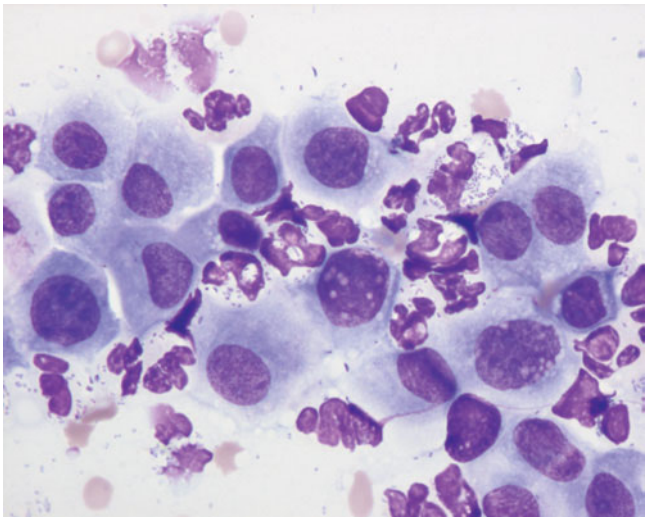


Figure 13.15 Vaginitis, bitch (cotton swab). Intermediate vaginal epithelial cells are accompanied by numerous degenerate neutrophils, some of which contain bacterial rods.

of neutrophils and bacteria, especially if the bacteria are found within neutrophils, support an infection within the reproductive tract (Figure 13.15). Diagnosis of a closed pyometra is based on a recent history of oestrus and corroborating results from physical examination, clinical pathological tests and imaging. Aspiration of a fluid-filled uterus is not recommended because of risk of leakage and subsequent peritonitis.

Neoplastic disorders

Tumours of the vulva and vagina may be found in intact and spayed females and are more common than tumours of the uterus. Leiomyomas are the most common tumour at these locations. Cytologically, these tumours consist of aggregates of elongate spindle cells with cigar-shaped nuclei. Where it is endemic, transmissible venereal tumours may be seen on the external genitalia of sexually mature female dogs that roam (Figure 13.13). A variety of other tumours may involve the skin and submucosa of the vulva and vagina including mast cell tumours (see Figure 2.23 and Figure 4.24), melanocytic tumours (see Figure 4.21) and TCC.

Ovarian tumours are uncommon in cats and dogs, and there are few reports of the cytological appearance of these tumours (Bertazzolo *et al.*, 2004). Ovarian tumours include epithelial or papillary tumours, germ cell tumours (dysgerminoma, teratoma and embryonal carcinoma) and sex-cord stromal tumours (granulosa cell tumour, thecoma and luteoma). Ovarian cysts also may be reported as a mass in the region of the ovary. Dysgerminomas and granulosa cell tumours appear cytologically similar to seminomas and Sertoli cell tumours, respectively.

Vaginal cytology for breeding management

Exfoliative vaginal cytology

Microscopic examination of exfoliated vaginal epithelial cells is a simple method for monitoring the stage of the oestrous cycle when performed serially. The increase in plasma oestrogen during pro-oestrus and oestrus causes thickening of the vaginal wall, probably as a mechanism to protect the otherwise delicate mucosa at the time of mating. Oestrogen causes thickening of the vaginal mucosa predominantly via an increase in the number of cell layers. The mucosa changes from a cuboidal epithelium (in anoestrus) through a transitional phase (during pro-oestrus) into a stratified, keratinised squamous epithelium (during the fertile period). After the end of the fertilisation period, as progesterone concentrations increase, there is rapid sloughing of the newly developed epithelium and an uncovering of a simple cuboidal epithelium similar to that observed during anoestrus.

Vaginal cells may be collected by aspiration of the vaginal cavity using a plastic catheter or by using a saline-moistened cotton swab gently rolled over the surface of the vaginal mucosa. Swabs should be introduced and removed using a small speculum or guard to avoid contact of the swab with the vestibule or skin, since collection of cells from these locations can give erroneous results. The swab should be lightly rolled onto a glass slide, or aspirated fluid can be spread as a thin film using a blood film technique (see Chapter 1). Routine Romanowsky-type stains are sufficient for evaluation and are easy to use. Trichrome stain has the advantage of identifying keratinised cells, but the procedure is technically more complicated. During pro-oestrus, as plasma oestrogen concentrations increase, the surface epithelial cells change from small circular cells with little cytoplasm ('parabasal cells') to larger, irregularly shaped flatter (squamous) nucleated cells ('intermediate cells') (Figure 13.16). During oestrus they become large inter-

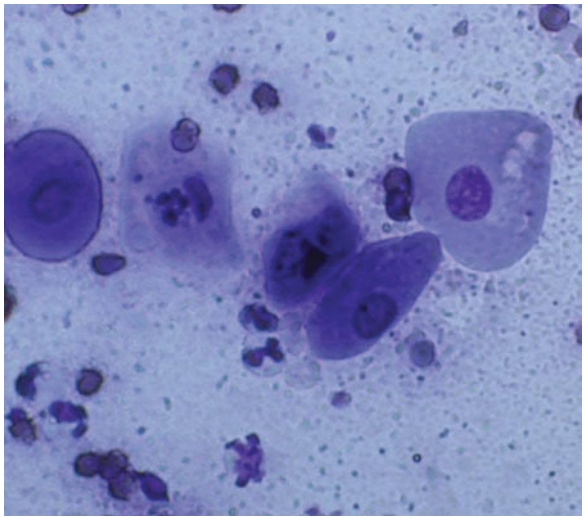


Figure 13.16 Pro-oestrus. Parabasal epithelial cells, neutrophils and a small number of erythrocytes are present.

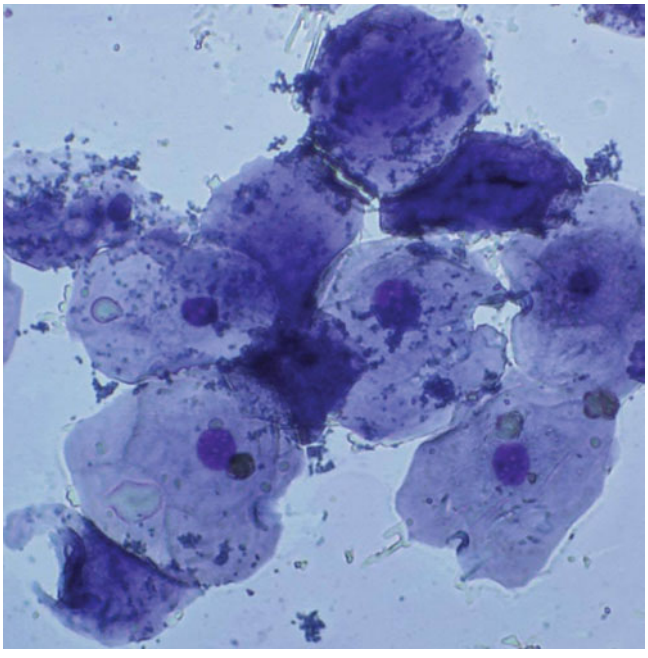


Figure 13.17 Early oestrus. Large intermediate epithelial cells, many covered in bacteria, and erythrocytes are present. Neutrophils are absent.

mediate epithelial cells (Figure 13.17) and then cornified squamous cells ('superficial cells'). The latter are anuclear or have a 'ghostlike' or small pyknotic nuclear remnant (Figure 13.18). After the end of the fertilisation period during sloughing of the epithelium, the superficial cells disappear and small parabasal and intermediate cells reappear in the vaginal smear (metoestrus) (Figure 13.19). Neutrophils are generally present in small numbers in the anoestrus vaginal smear but disappear during the fertile period because the thickened mucosa is a barrier to their migration to the mucosal surface. These neutrophils reappear, often in large numbers, during metoestrus.

Breeding should be attempted throughout the period when more than 80% of epithelial cells are anuclear as in most bitches this coincides with the fertile period (Figure 13.20). Further mating or insemination is rarely successful once metoestrus is identified.

In queens, similar changes are noted in vaginal cytology during pro-oestrus and oestrus; however, although epithelial cells are large and irregular in outline, they often remain nucleated. Furthermore, at the end of oestrus, whether ovulation occurs or not, a substantial influx of neutrophils is not apparent.

Ovarian remnant syndrome

Bitches and queens that are thought to have an ovarian remnant after ovariohysterectomy usually return to oestrus at intervals similar to those in intact females. Collection of a vaginal smear when the female is demonstrating oestrous behaviour

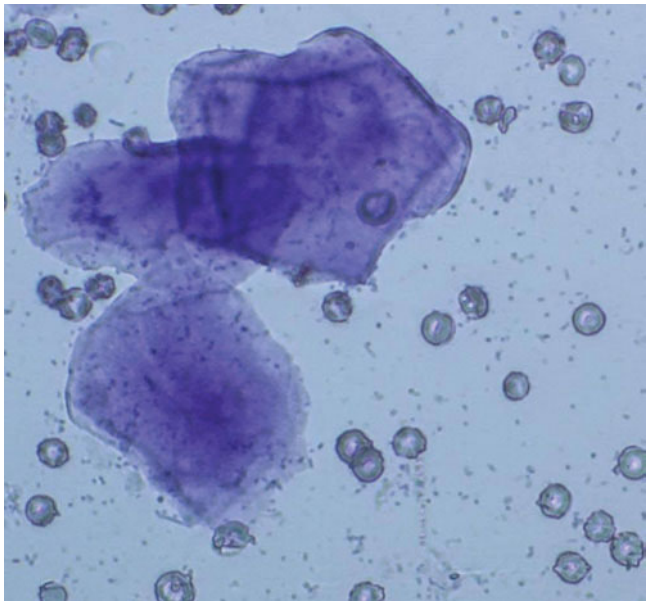


Figure 13.18 Oestrus (mating time). The majority of epithelial cells are cornified and anuclear. Cells are large and irregular and covered in bacteria. Neutrophils are absent.

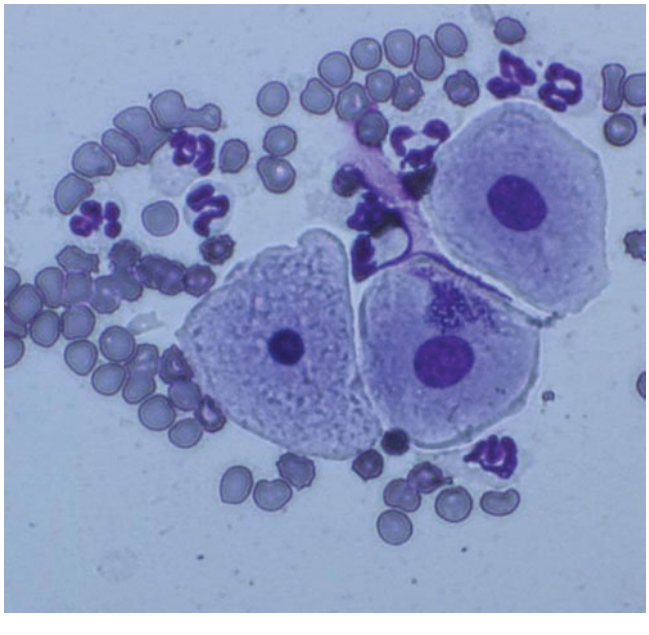


Figure 13.19 Early luteal phase. There is a substantial influx of neutrophils, and the epithelial cells are no longer cornified. Here small intermediate epithelial cells are present. Some erythrocytes remain.

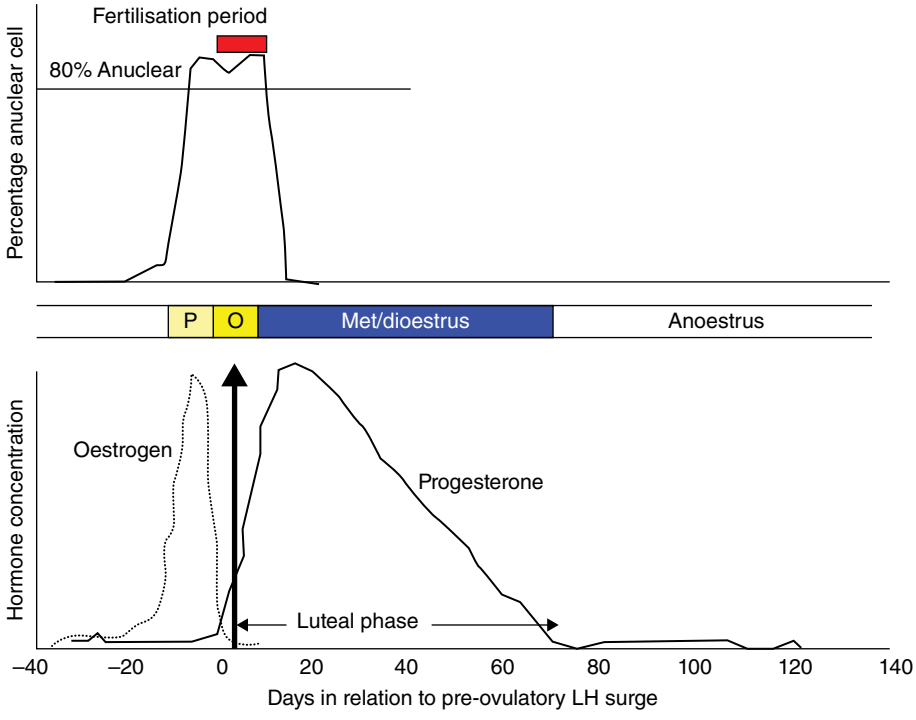


Figure 13.20 Schematic representation of the hormone concentrations and stages of the oestrous cycle in the bitch (P = pro-oestrus, O = oestrus [vertical arrow]) in relation to the fertilisation period and percentage of anuclear vaginal epithelial cells. Source: Adapted from England, G.C.W. (2013) *Dog Breeding, Whelping and Puppy Care*, Wiley-Blackwell, Oxford.

will show the presence of large intermediate and cornified epithelial cells. Care should be taken to ensure that the smear has not been contaminated with vulvar or perineal epithelial cells.

References

Bertazzolo, W., Dell'Orco, M., Bonfanti, U. *et al.* (2004) Cytological features of canine ovarian tumours: a retrospective study of 19 cases. *Journal of Small Animal Practice*, **45**, 539–545.

Root Kustritz, M.V. (2007) The value of canine semen evaluation for practitioners. *Theriogenology*, **68**, 329–337.

14 Cytology of Mammary Gland Lesions

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Optimal cytological interpretation requires availability of representative samples with adequate cellularity and access to detailed patient clinical information (i.e. good communication with the clinician) and experience regarding the cytological patterns and biology of mammary tumours in dogs and cats.

Indications for cytological investigation

The primary objective of cytological examination of mammary lesions is to differentiate primary inflammatory lesions from benign hyperplastic or neoplastic lesions and malignant tumours with respect to prognosis and treatment. The most reliable predictors of malignancy are clinical and histological evidence of tumour infiltration into surrounding tissue or vessels or metastases. Although infiltrative behaviour cannot be directly assessed by cytological examination, cytological detection of local and distant metastases in possible target organs confirms that a tumour is malignant. If a mammary lesion is suspected to be malignant, further investigations should be undertaken in order to more precisely define the prognosis before surgical excision, which is usually the treatment of choice, is attempted. These include diagnostic imaging of potential metastatic sites (e.g. three-view thoracic radiographs and abdominal ultrasound) and cytological examination of ultrasound-guided fine-needle aspirates of regional lymph nodes, suspected metastatic lesions in distant organs (e.g. liver, spleen and lung) and/or body cavity effusions. In the case of carcinoma (especially inflammatory carcinoma), cytological detection of distant metastases including bone marrow infiltration implies a poor prognosis.

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Collection techniques

Samples from mammary lesions

Cytological specimens from solid and cystic lesions can be obtained by fine-needle aspiration biopsy (FNAB) which may be ultrasound guided, whereas imprints and scrapings can be taken from ulcerated lesions, surgical biopsies or surgically removed lesions. FNAB is especially useful for the investigation of small mammary lesions during the early stages of development when it may be difficult to persuade an owner to opt for a surgical biopsy. However, early diagnosis and treatment is very important, because early surgical resection is an important prognostic factor.

With respect to the often mixed or complex composition of mammary lesions (variation of cell type and morphology within a particular lesion or different lesions within the same gland), multiple aspirates are crucial in order to increase the probability of harvesting representative cells from all relevant components of the lesion (this is especially true for mesenchymal tumours which tend to exfoliate cells in low numbers). Sample collection from different areas of the lesion also reduces the risk of non-diagnostic specimens, e.g. from inflamed, cystic or necrotic areas of a primarily malignant neoplastic lesion. When aspirating large mammary gland lesions or an enlarged lymph node for metastatic tumour cells, avoid sampling the centre of the node which may be necrotic.

Mammary secretion

Smears prepared from mammary nipple secretions are usually adequate for cytological diagnosis of diffuse inflammatory lesions, whereas diagnosis of focally inflamed or infected mammary gland lesions requires FNAB. Nipple secretions are unlikely to provide adequate information for the diagnosis of neoplastic mammary gland lesions.

Normal cell types and cytological appearance of normal lactating mammary gland

As a consequence of marked hypertrophy, aspirates from lactating mammary glands have a higher cellularity than non-lactating glands which usually contain no or only a very small number of tissue-specific cells. Mammary secretory epithelial cells which derive from secretory acini are uniform in size and shape and have a moderate amount of granular, basophilic cytoplasm and a round, dark nucleus (the nuclei are also of similar size and shape). Occasionally, small sheets and clusters of secretory cells are observed, which may be arranged in acinar formations (Figure 14.1). Epithelial cells derived from excretory ducts have basilar, ovoid nuclei with scanty basophilic cytoplasm (Figure 14.2). Clusters and small sheets or fragments of ductules with prominent intercellular borders may also be present. Because ductal and acinar epithelial cells and intra- and interlobular connective tissue undergo regular cycles of proliferation, individual hyperplastic and dysplastic

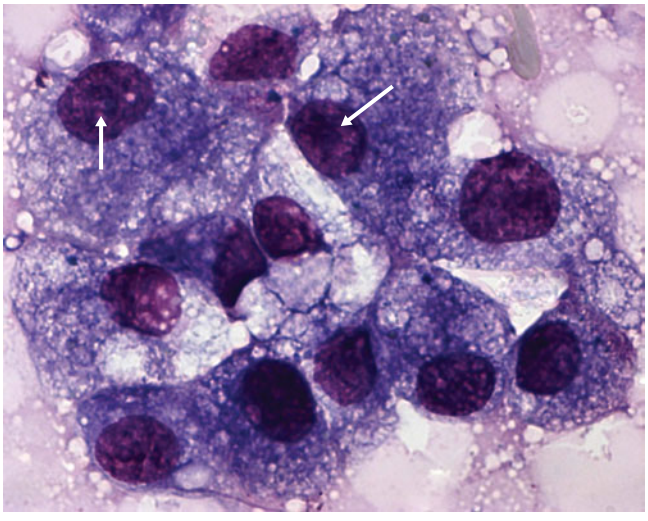


Figure 14.1 Imprint of normal canine mammary gland. Sheet of apocrine epithelial cells with abundant basophilic cytoplasm and a round nucleus, most of which contain a centrally located nucleolus (arrows).

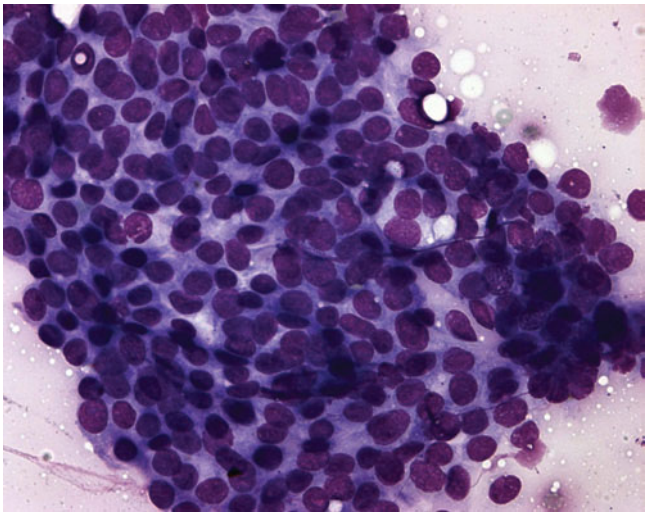


Figure 14.2 Imprint of normal canine mammary gland. A monolayered sheet of normal ductal cells characterised by scanty basophilic cytoplasm and ovoid nuclei.

cells may be present in older animals. In addition, myoepithelial cells, which line the secretory acini and teat sinuses, may be seen as spindle-shaped cells or free, darkly staining, oval nuclei (Figure 14.3). Aspirates from lactating mammary glands may also contain foam cells (Figure 14.4) and/or pigment-laden macrophages (Figure 14.5).

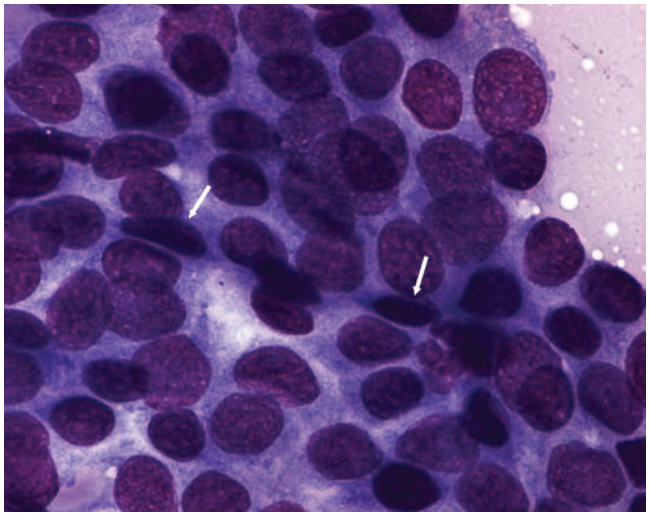


Figure 14.3 Normal myoepithelial cells are elongated darker cells with oval nuclei (arrows). They can be seen here within a sheet of ductal epithelial cells.

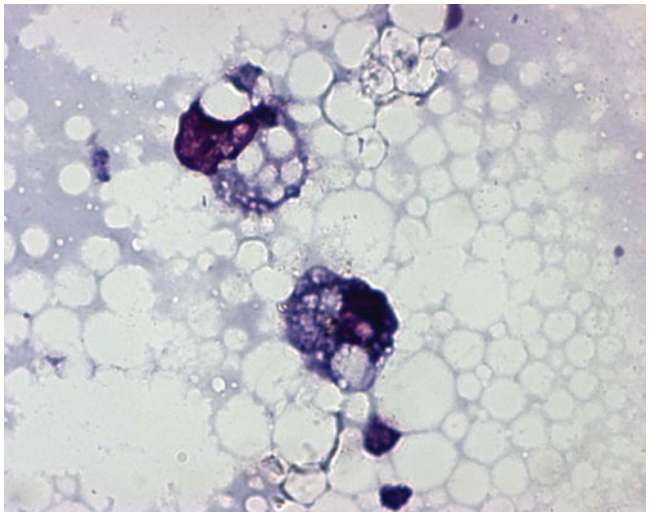


Figure 14.4 Two foam cells (lipid-laden macrophages) in normal canine mammary secretion. These are large individual cells with abundant cytoplasm containing clear cytoplasmic vacuoles and a round to oval-shaped, eccentrically located nucleus. Note the low nuclear to cytoplasmic ratio.

Foam cells originate from histiocytes, and immunophenotypic analysis suggests they may also derive from mammary epithelial cells. In addition, erythrocytes, clear lipid droplets and adipocytes may be present. The background usually contains a moderate amount of basophilic proteinaceous material.

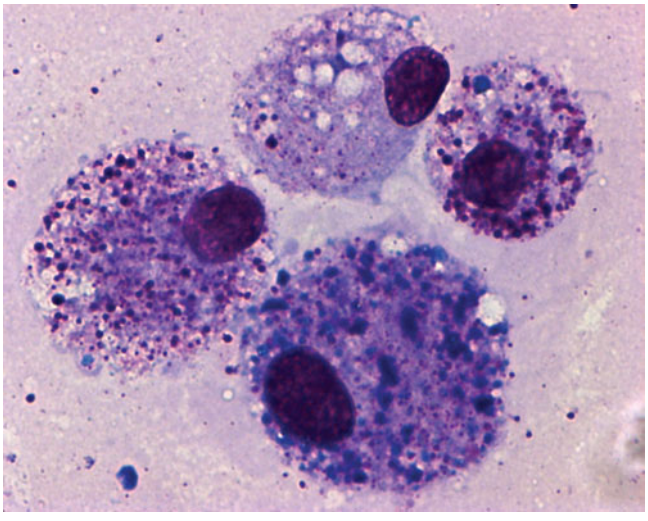


Figure 14.5 Normal canine mammary secretion. The pigment-laden macrophages contain basophilic secretory material and are situated in a lightly basophilic proteinaceous background.

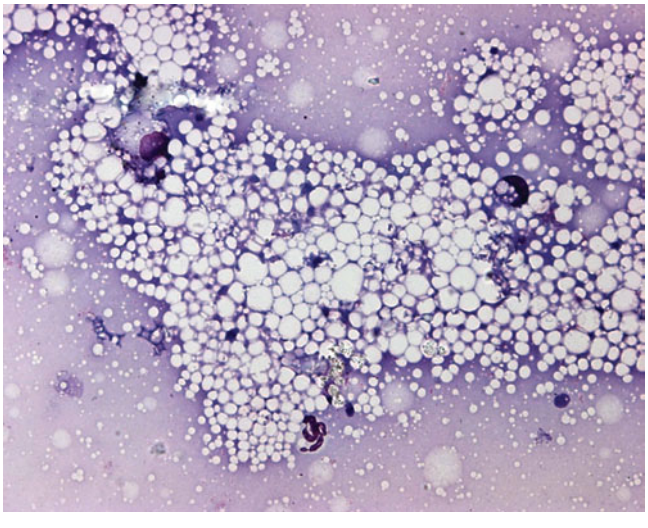


Figure 14.6 Normal canine mammary secretion with individual foam cells and a single neutrophil in a basophilic proteinaceous background containing many lipid droplets.

Normal mammary secretions are protein rich but hypocellular. Low numbers of foam cells, individual secretory and ductular epithelial cells, macrophages and the occasional neutrophil and lymphocyte are present in an eosinophilic to basophilic proteinaceous background containing large numbers of lipid droplets (Figure 14.6).

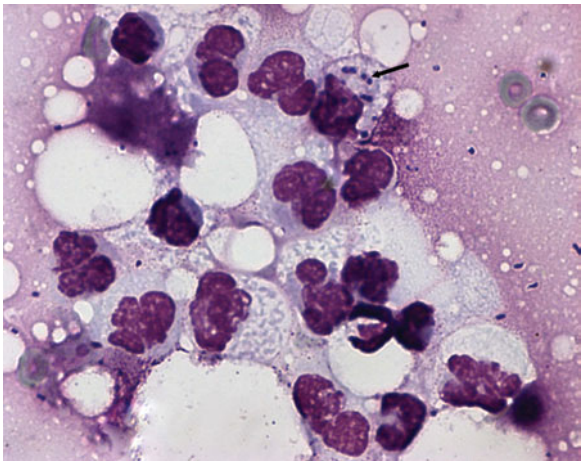


Figure 14.7 Purulent necrotising mastitis in a cat caused by *Escherichia coli*. This aspirate reveals degenerate neutrophils with swollen unstructured nuclei (note the absence of well-defined nuclear lobulation). The bacterial organisms can be seen intracellularly (arrow) and extracellularly.

Mastitis

Aspirates from focal or diffuse, inflammatory mammary lesions are usually associated with postpartum lactation or pseudopregnancy. They are cellular, and the composition of inflammatory cell types varies with the type and pathogenicity of the causative agent and the stage of disease. Bacterial infection results in purulent inflammation, and neutrophils may exhibit degenerative changes (karyolysis and/or karyorrhexis), suggesting the presence of bacterial toxins (Figure 14.7). In addition, such smears contain large amounts of necrotic cellular debris. Bacteria (especially coliforms, *Streptococcus* spp. and *Staphylococcus* spp.) can be visualised in neutrophils and, less commonly, in macrophages (Figure 14.7). Secretions of inflamed mammary glands or aspirated material should be submitted for culture and sensitivity in order to determine appropriate antibiotic therapy.

In more chronic cases and non-septic inflammatory lesions, non-degenerate neutrophils, reactive pigment-laden macrophages, lymphocytes and plasma cells are present in varying numbers (Figure 14.8). Dysplastic mammary epithelial cells may also be present in association with inflammatory changes. These cells require cautious interpretation since they may exhibit morphological changes which mimic those associated with malignancy (Figure 14.9 and Figure 14.10).

Feline mammary fibroadenomatous hyperplasia

Feline mammary fibroadenomatous hyperplasia may be suspected on the basis of the signalment, history and clinical appearance of the lesions (i.e. it is observed most frequently in young intact females or in older male and female cats; it may

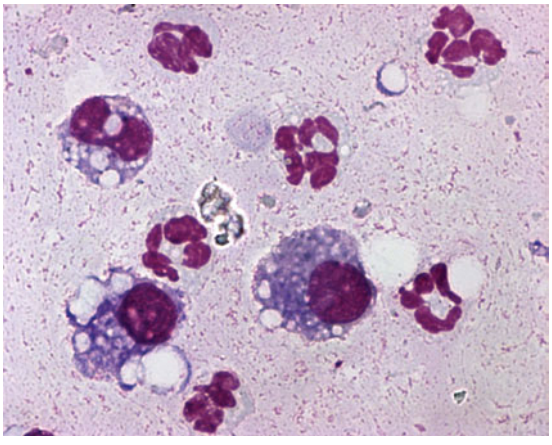


Figure 14.8 Non-degenerate neutrophils and foamy macrophages in the secretion of an inflamed mammary gland in a dog.

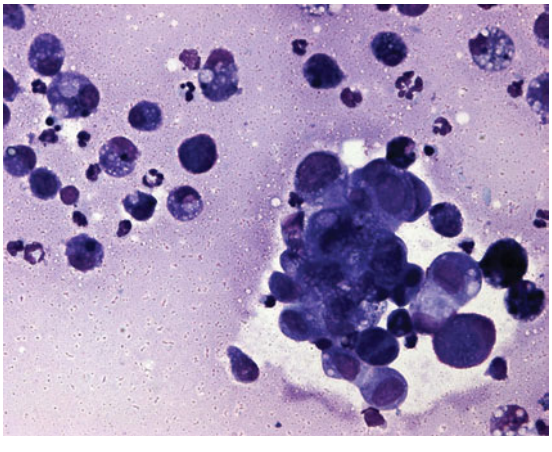


Figure 14.9 Mammary secretion of a dog with mastitis. Cluster of epithelial cells (bottom right) showing marked dysplastic changes (e.g. marked anisocytosis and anisokaryosis).

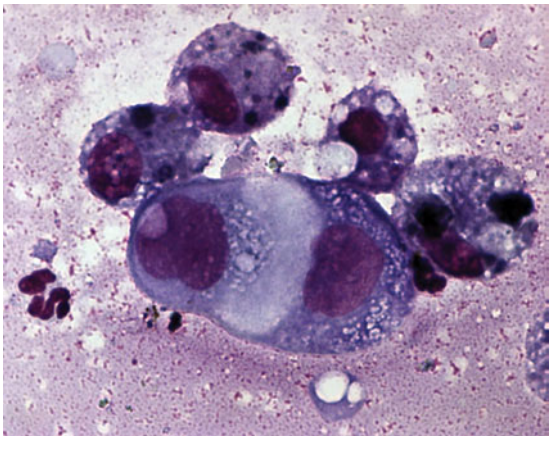


Figure 14.10 Same case as in Figure 14.9. Epithelial cells showing marked dysplastic changes (e.g. macrokaryosis) are surrounded by macrophages and a neutrophil.

be induced by the use of synthetic progesterone compounds). Aspirates may help to confirm the tentative diagnosis and rule out the main differential diagnoses which are malignant neoplasia and mastitis. The cytological appearance is characterised by small clusters of relatively uniform cuboidal ductular epithelial cells with round nuclei, small nucleoli and scant cytoplasm. A mild to moderate degree of anisocytosis and variation in nuclear to cytoplasm ratio may be evident, but the cells lack the nuclear criteria of malignancy. A reactive mesenchymal component is represented by spindle-shaped stromal cells with indistinct cytoplasmic margins, oval-shaped nuclei with prominent nucleoli and increased nuclear to cytoplasmic ratio. The stromal cells are usually embedded in a pink intercellular matrix material.

Solid benign lesions (adenoma, canine lobular hyperplasia)

Aspirates from benign neoplasms of the mammary gland such as adenomas and papillomas appear similar and cannot be differentiated with confidence on a cytological basis from lobular hyperplasia. Aspirates normally contain sheets or clusters of relatively uniform epithelial cells with small nuclei (Figure 14.11). The cells may be arranged as palisading, papillary, acinar and trabecular structures which may provide clues to the histopathological of the lesion under investigation. Pigment-laden macrophages may also be present. There is a tendency for cytology to underdiagnose malignancy, and since there is a low incidence of benign mammary tumours in cats, diagnosis of a benign lesion requires extreme caution especially

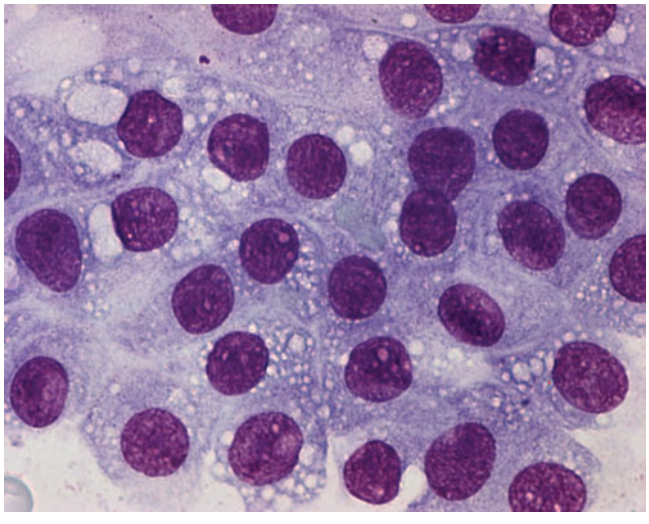


Figure 14.11 Mammary adenoma in a dog. This tissue aspirate depicts a sheet of glandular epithelial cells which are relatively uniform in appearance. These have a low nuclear to cytoplasmic ratio. The nuclei are of similar size with a fine granular chromatin pattern and small round nucleoli. Source: Simon, D., Schoenrock, D., Nolte, I. et al. (2009) Cytologic examination of fine-needle aspirates from mammary gland tumors in the dog: diagnostic accuracy with comparison to histopathology and association with postoperative outcome. *Veterinary Clinical Pathology*, **38**, 521–528. Reproduced with permission of John Wiley and Sons.

in an older cat with no history of progesterone administration. The aspirated cells from most mammary tumours are usually located in a background containing variable amounts of blood, basophilic proteinaceous material, lipid droplets and foam cells.

Benign mixed and complex mammary tumours

In dogs, benign tumours often involve epithelial and stromal elements (e.g. complex adenomas, fibroadenomas, benign mixed tumours). Benign complex adenomas or papillomas, fibroadenomas and benign mixed tumours characteristically yield specimens consisting of sheets and clusters of uniform-appearing epithelial cells in addition to individual or clumped spindle-shaped myoepithelial cells (complex tumours) or spindle or oval-shaped cells of mesenchymal origin (mixed tumours; Figure 14.12). Myoepithelial cells may also appear as free oval-shaped nuclei. The mesenchymal component may be represented by fibrous, cartilaginous or osseous elements (i.e. fibroblasts, chondroblasts or osteoblasts and osteoclasts may be present). Pink extracellular matrix material (e.g. collagen, chrondroid or osteoid) may be observed either as small discrete deposits or it may form a more diffuse background. Myoepithelial and mesenchymal cells tend to exfoliate poorly; therefore, these benign complex or mixed tumours may be difficult to diagnose on a cytological basis due to the low number of cells present.

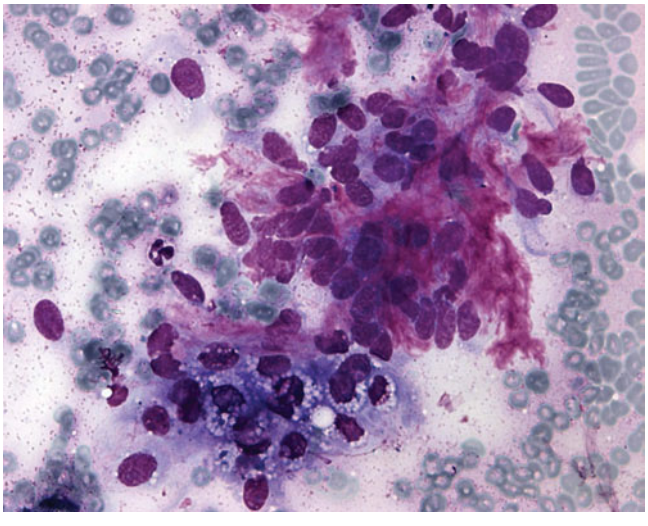


Figure 14.12 Aspirate of a mixed mammary tumour in a dog. This lesion consists of a secretory epithelial component (bottom) and mesenchymal component consisting of spindle-shaped cells associated with a large amount of an extracellular pink matrix material (top).

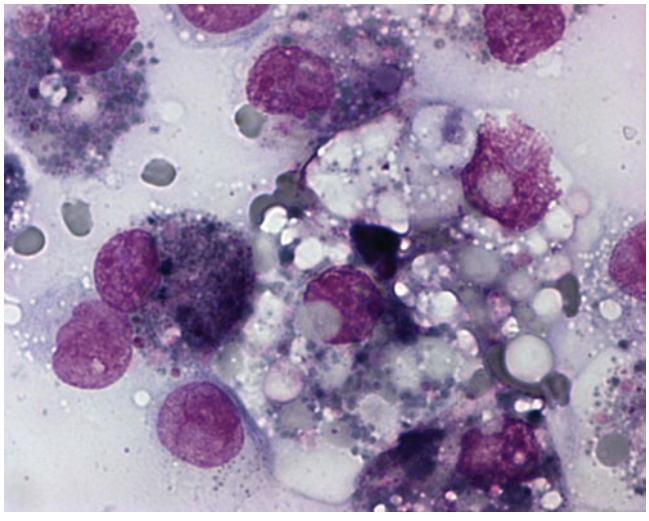


Figure 14.13 Aspirate of a mammary gland cyst (dog). These macrophages which tend to accumulate along the feathered edge of the smear contain lipid vacuoles and pigment.

Mammary cysts

Mammary gland cysts are benign lesions which occur most commonly in middle-aged or older bitches. They form as a consequence of mammary dysplasia in which dilated ducts expand to form large cavities. Mammary cysts can occur as individual or multiple lesions (polycystic mastopathy), and they may be associated with benign and malignant mammary tumours. Therefore, aspiration should also include solid areas, if present, to rule out the presence of concurrent mammary gland neoplasia. The yellow-brown or blood-tinged aspirated fluid is generally hypocellular unless there is concurrent inflammation (Figure 14.13). Vacuolated foam cells and pigment-laden macrophages predominate. Numerous neutrophils may be present in cysts which become inflamed or infected (Figure 14.14). Cholesterol crystals resulting from the breakdown of cellular membranes (Figure 14.15) and epithelial cells derived from the cystic lining may also be seen especially in aspirates from cysts lined with papillary outgrowths. These epithelial cells may be arranged in dense sheets or clusters, and mild variation in nuclear size and shape may be evident.

Malignant epithelial neoplasms

Adenocarcinomas arising from glandular epithelium are the most common malignant mammary neoplasm (and are the most common mammary tumour in cats). Less common malignant epithelial neoplasms include ductal carcinomas, anaplastic carcinomas and squamous cell carcinomas. The diagnosis of malignant epithelial mammary tumours is based on the general cytological characteristics, i.e. the presence of pleomorphic epithelial cells showing marked

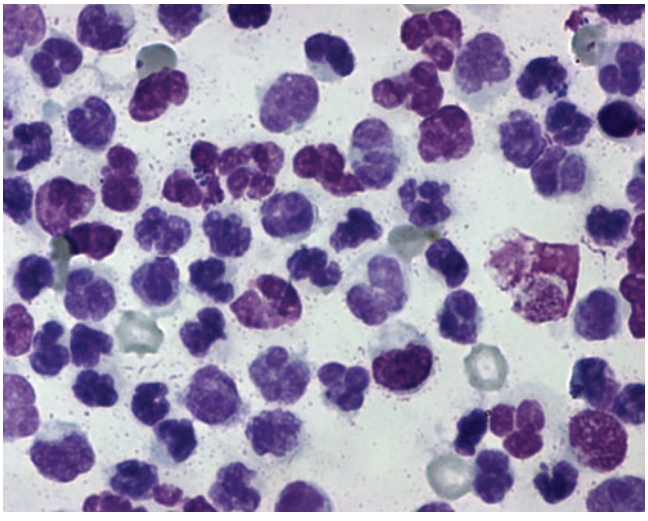


Figure 14.14 Aspirate of a mammary gland cyst (dog). There is evidence of inflammation and associated bacterial infection. Some of the degenerate neutrophils contain intracellular bacteria.

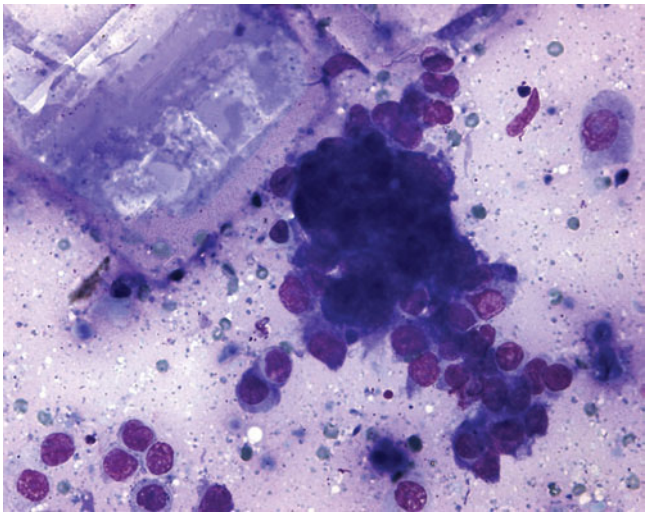


Figure 14.15 Imprint of a feline mammary carcinoma containing cystic areas. Note the cholesterol crystals (top left). These appear as large, rectangular clear crystals of varying size, often with a notched corner.

anisocytosis. The cells, which may be single or arranged in sheets and irregular clusters of variable size, demonstrate more than three nuclear criteria of malignancy in a representative percentage of cells (Figure 14.16 and Figure 14.17). Note, however, that different histological types often have similar cytological features. A characteristic feature of adenocarcinomas is the tendency for the

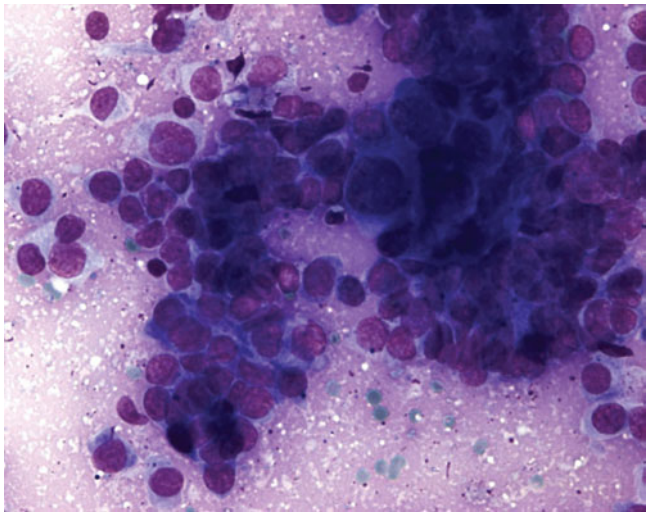


Figure 14.16 Mammary adenocarcinoma (cat). This imprint depicts a large cluster of extremely pleomorphic epithelial cells.

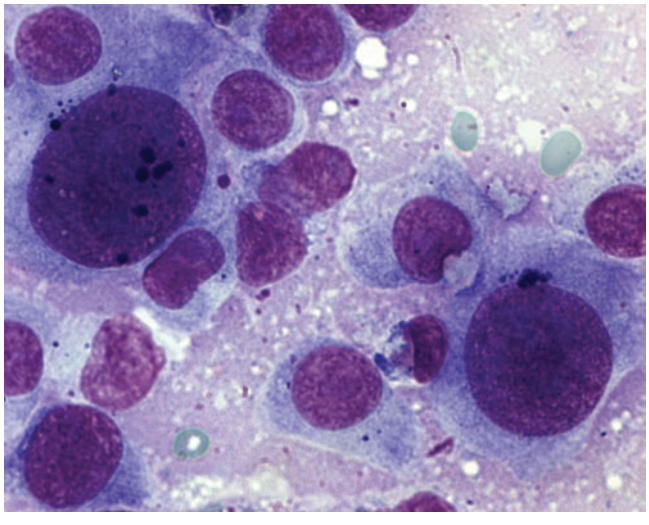


Figure 14.17 Same case as in Figure 14.16. Note the cellular pleomorphism and nuclear criteria of malignancy anisokaryosis, macrokaryosis, and prominent, large nucleoli.

cells to form acinar arrangements which can sometimes be observed especially in imprint specimens (Figure 14.18).

Epithelial cells are typically round or polygonal with distinct cell borders. They have a round to oval, eccentrically located nucleus and a moderate amount of basophilic cytoplasm which may be vacuolated. The vacuoles may be clear or may contain basophilic secretory product. Some cells may contain one large cytoplasmic vacuole which displaces the nucleus peripherally (these are referred to as 'signet ring' cells).

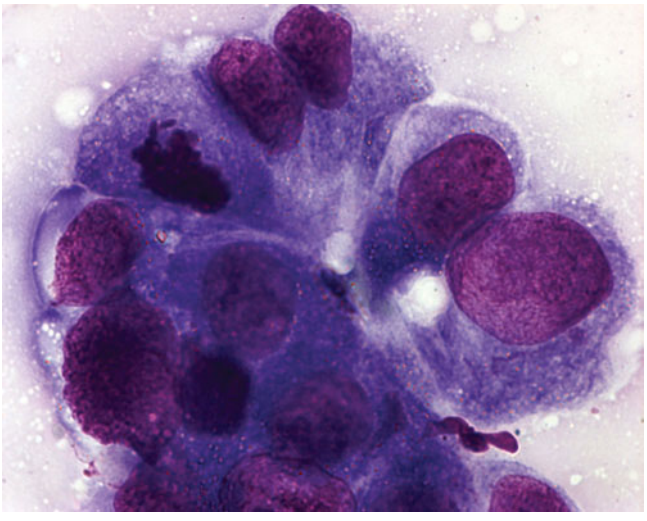


Figure 14.18 Acinar structure in an aspirate of a canine mammary adenocarcinoma. A mitotic figure can be seen in the upper left corner.

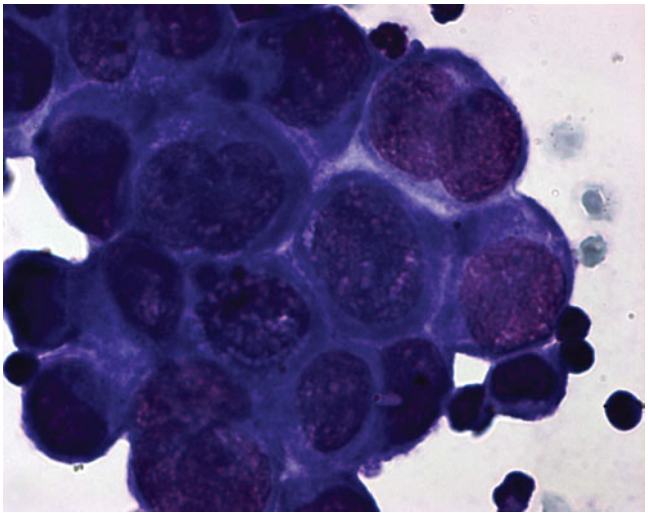


Figure 14.19 Fine-needle aspirate of the inguinal lymph node of a dog. Lymph node metastases of an anaplastic mammary carcinoma.

Nuclear criteria of malignancy are usually marked and include moderate to marked variation in nuclear size, macrokaryosis, high nuclear to cytoplasm ratio, nuclear moulding, binucleation and multinucleation, multiple prominent nucleoli, macronucleoli and nucleoli which vary in shape. Increased mitotic activity and abnormal mitotic figures may be present (Figure 14.18). Detection of metastases within local lymph nodes and distant metastases helps to confirm the biological behaviour of the tumour (Figure 14.19).

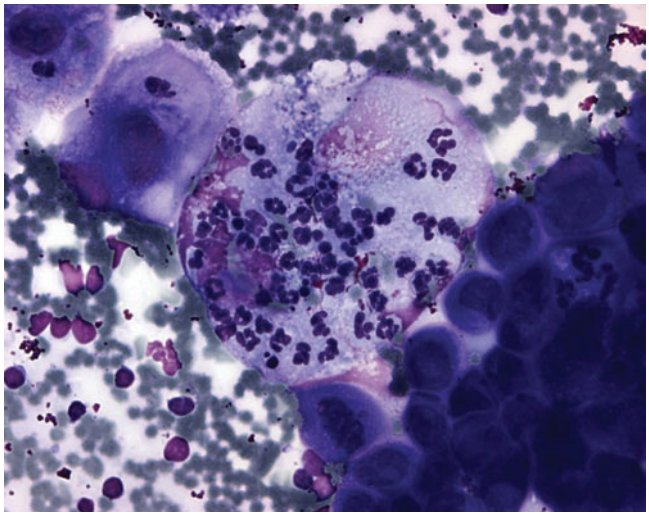


Figure 14.20 Inflammatory mammary carcinoma. Aspirate shows a cluster of highly pleomorphic epithelial cells and a large number of non-degenerate neutrophils.

Ductular carcinomas express similar cytomorphologic criteria as adenocarcinomas without the acinar structures, secretory product and cytoplasmic vacuolation; instead papillary and trabecular arrangements may be observed.

Anaplastic carcinomas have a poor prognosis. The neoplastic cells may exfoliate individually or in small clusters. They are extremely pleomorphic, have a high nuclear to cytoplasm ratio and marked anisokaryosis is evident. Bizarre nuclear and nucleolar forms, frequent multinucleated cells and abnormal mitotic figures are common. In contrast to adenocarcinomas, the hyperchromatic cytoplasm does not contain intracytoplasmic vacuoles (Figure 14.19).

Inflammatory carcinoma ('mastitis carcinomatosa') is an aggressive form of mammary carcinoma characterised by the presence of a large number of non-degenerate neutrophils, a smaller number of macrophages, and large, pleomorphic anaplastic epithelial cells exhibiting numerous criteria of malignancy (Figure 14.20). Although tumour cells are usually present in adequate numbers, aspirates from multiple sites are advisable in order to maximise the likelihood of obtaining an adequate number of cells which are indisputably malignant. History, signalment and detection of metastases may also be helpful in this respect.

The cytological appearance of **squamous cell carcinomas**, which often adhere to the overlying dermis and may be ulcerated, resembles those detected in other organs (see Figure 4.13).

Mammary sarcomas

Primary mesenchymal tumours of the mammary gland (osteosarcomas, fibrosarcomas and liposarcomas) are rare. The cytological appearance of these tumours is similar to those located at other sites (Figures 2.19, 4.17 and 4.20).

Malignant mixed mammary tumours

Malignant mixed mammary tumours are uncommon tumours in dogs. Cytological specimens contain epithelial and mesenchymal spindle-shaped cell populations, of which at least one or both (carcinosarcomas) demonstrate cellular and nuclear characteristics of malignancy. The heterogenous distribution of the cells may result in a predominance of one cell type in a particular area; therefore, examination of aspirates from multiple sites may be helpful.

15 Cytology of Selected Infectious Organisms

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Introduction

The goal of this chapter is to describe general guidelines for the cytological diagnosis of selected infectious diseases and illustrate representative examples of different classes of organisms. Types of infectious agents include common bacteria, higher bacteria, yeasts, fungal hyphae, protozoa, parasites and viruses. Descriptions of specific infectious agents will be limited. Readers are referred to specialised textbooks, literature articles or the Internet for more complete descriptions of diagnosis, biology, pathogenesis and treatment of various infectious diseases.

The photographs were taken by the author from his clinical cases and from cases in annual slide sets obtained from the American Society of Clinical Pathology Annual Meeting Case Presentation Sessions from 1975 to 2010.

Very important in cytological diagnosis is the collection, preparation and staining of samples, and for those aspects the reviewer is referred to Chapters 1 and 2 (see also Further Reading). Most organisms and certainly the inflammatory cells responding to them stain well and are best illustrated with Romanowsky-type stains including Wright's, Giemsa and the 'quick' stains that mimic the blue-red colours of those classic stains.

Identification of infectious organisms is based on their size and shape (Table 15.1). Erythrocytes and neutrophils are often present in samples and may be used to estimate the size of any organism (Figure 15.1).

Bacterial infections

Infections caused by *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp. *and E. coli* are commonly encountered infections in clinical practice. Cytology may be used to identify the presence of bacteria in a lesion and characterise the bacteria into various patterns. The pattern most suggestive of a primary infection is when

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Table 15.1 Summary of sizes, forms and location of various infectious agents

Organism	Size	Form	Location
Staphylococcus spp.	0.4–0.8 µm	Cocci	In neutrophils and background
Streptococcus spp.	0.5–1.2 µm	Cocci in chains	In neutrophils and background
Bacilli in general	0.5–1 × 1–4 µm	Rods	In neutrophils and background
E. coli	1–1.5 × 2–6 µm	Rods	In neutrophils and background
Bartonella spp.	0.5 × 1	Rods, may be slightly curved	Difficult to identify by morphology
B. bronchiseptica	0.3 × 0.5–2 µm	Rods	Attached to cilia of cells
Helicobacter spp.	2–15 µm	Thick rigid spirals (spirillum)	Gastric crypts and background
Spirochaetes	0.4 × 3–500 µm	Thin flexible spiral	Mouth, faeces, urine (*Leptospira* spp.)
Simonsiella spp.	8–12 µm	Oval, flat 'trichome' of 12–20 rods	On surface of epithelial cells
Mycoplasma spp.	0.3–1 µm	Pleomorphic 'cocci' (no cell wall)	On surface of epithelial cells
Actinomyces spp.	0.8 × 5–20 µm	Filamentous, branching plus rods	Find colonies in pus and smears
Aspergillus spp.	5–7 µm wide	Uniformly wide, septate hyphae	Look for colonies, use 4× lens
B. dermatitidis	5–20 µm	Round broad-based budding yeast	Free in exudate and debris
H. capsulatum	1–4 µm	Round to oval, budding yeast	In macrophages and neutrophils
Cryptococcus spp.	8–40 µm	Yeast with variably thick, clear capsule	Yeast without capsule 4–8 µm
Coccidioides immitis	10–100 µm	Thick walled, dark spherules	Free in exudate
C. immitis	2–5 µm	Endospores	Inside spherules
S. schenckii	2–7 µm	Pleomorphic 'cigar'-shaped	Lung, in macrophages or background
Pneumocystis carinii	5–10 µm	Round cysts with 4–6 internal bodies	Lung, free in background
P. carinii	2–7 µm	Pleomorphic trophozoites	Lung, in macrophages or background

Table 15.1 (Cont'd)

Organism	Size	Form	Location
M. pachydermatis	3–5 µm	Oval yeast with unipolar budding	On epithelial cells
C. albicans	2–10 µm	Round to oval, may have pseudohyphae	On epithelial cells
T. gondii	1–4 µm	Crescent 'banana'-shaped	In macrophages or background
N. caninum	1–5 µm	Crescent 'banana'-shaped	In macrophages and neutrophils
O. (Filaroides) osleri	232–266 µm	Larvae in a coil	Free in BAL fluid; use 4× lens
Filaroides hirthi	240–290 µm	Looks like *O. osleri*	Free in BAL fluid; use 4× lens
C. vulpis	4–16 mm	Males have a copulatory organ	Free in BAL fluid; use naked eye
Dirofilaria immitis	290–330 µm	Microfilaria in blood	In bloody background
Paragonimus kellicotti	100 × 50 µm	Yellow oval ova (1 operculum)	Lung (cat/dog), free in background; use 4× lens
A. pseudofelineus	25–35 × 12–15 µm	Operculated fluke ova	Liver, pancreas, faeces (cat/dog)
Eucoleus aerophilus	70 × 35 µm	Bipolar operculum egg (Capillaria)	BAL, sputum, faeces; use 4× lens
Aelurostrongylus	80 × 70 µm	Brown oval ova	BAL fluid (cats), free in background; use 4× lens
Erythrocytes	5–7 µm	Round	Background
Neutrophils	11–17 µm	Round	Background
See with naked eye	> 0.1 mm	1 mm = 0.001 m; 1 µm = a millionth of a meter	

Note: BAL, bronchoalveolar lavage. For full Genus species name of organisms, see text.

only one type of bacterial organism (e.g. small cocci in chains) is found (Figure 15.2A). Secondary infections more likely have a mixed population of bacteria (e.g. different sized rods and cocci) (Figure 15.2B). Bacteria which appear to be normal flora are recognised by their presence on the surface of epithelial cells and a lack of inflammatory response. Normal flora includes rods, spirochaetes and Simonsiella in the mouth and *Staphylococcus* spp. and yeasts on the skin.

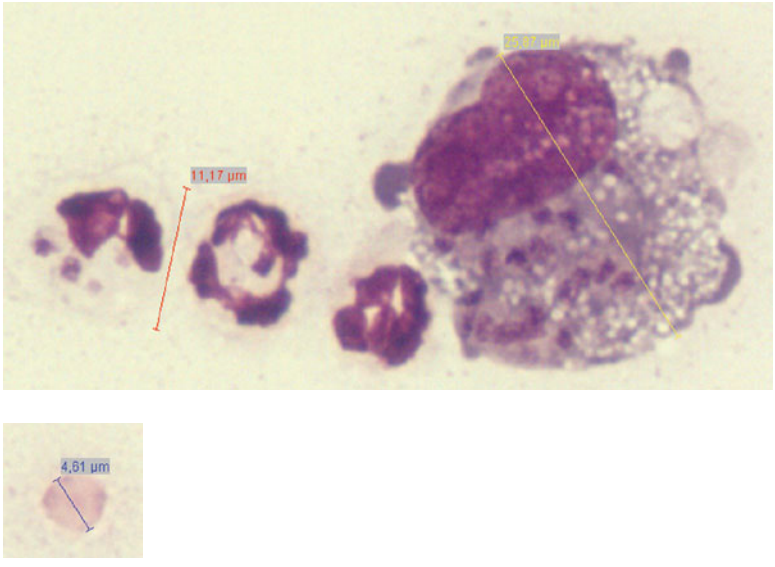


Figure 15.1 Cell sizes. Erythrocytes and neutrophils may be used to judge the size of infectious organisms and other cells in the smear. The diameter of cells will vary due to variation in thickness of the smear, so this is an approximate but practical method. Cells lay flatter and have a wider diameter in thinner areas of the smear. Erythrocytes are about 4–7 µm and neutrophils about 11–17 µm. Cells in this illustration are at the lower end of those ranges probably because they were located in a thicker area of the smear. The erythrocyte is 4.6 µm, neutrophils about 11.2 µm and the macrophage about 26 µm (the macrophage contains *T. gondii* organisms). The size of erythrocytes is affected less by the thickness of a smear than neutrophils and is thus recommended as the cell to use for estimating the size of infectious agents. Wright's-type stain about 1000×.

The cytological description of the number and types of bacteria and pattern of bacteria seen in a sample is important for interpretation of bacterial culture results. The presence of a mixed bacterial flora suggests these bacteria are secondary invaders and that culture should similarly identify a mixed population. Most bacteria cannot be specifically identified by shape and size, but the finding of one type of bacteria in significant numbers often inside neutrophils allows a fairly confident cytological diagnosis of infection. Discordance between cytology and culture results may occur if the bacterial organism is an anaerobe which does not grow on routine culture media, if the animal has recently received antibiotics or other bacteriostatic substances or if the stain has been contaminated with organisms. Consider also human error and the sensitivity of the methods (culture usually detects smaller numbers of bacteria in a sample than cytology).

Gram staining is not recommended for routine examination of cytological samples. Gram-negative bacteria (red) are difficult to see in a red background of protein and neutrophils. Bacteria are often present in small numbers and may only be identified in occasional fields of view. The intensity and consistency of Gram staining are also too variable in smears that vary greatly in thickness. Gram staining should be restricted to bacteriology laboratories looking daily at uniformly thick smears from colonies on blood agar plates.

Some bacteria are sufficiently unique to recognise on cytological smears. These include *Streptococcus* spp., *Actinomyces* spp., *Clostridium* spp., *Mycobacterium* spp. and *Simonsiella* (see Figure 15.2A–K). *Clostridium* spp. are large rods with a clear round spore in them (Figure 15.2C). *Simonsiella* spp. form large characteristic multicellular units called trichomes (Figure 15.2D). Trichomes are large, oval and flat in shape. Long, rod-shaped, bacterial cells bind together along their long sides to form subunits of perhaps 12–20 rods.

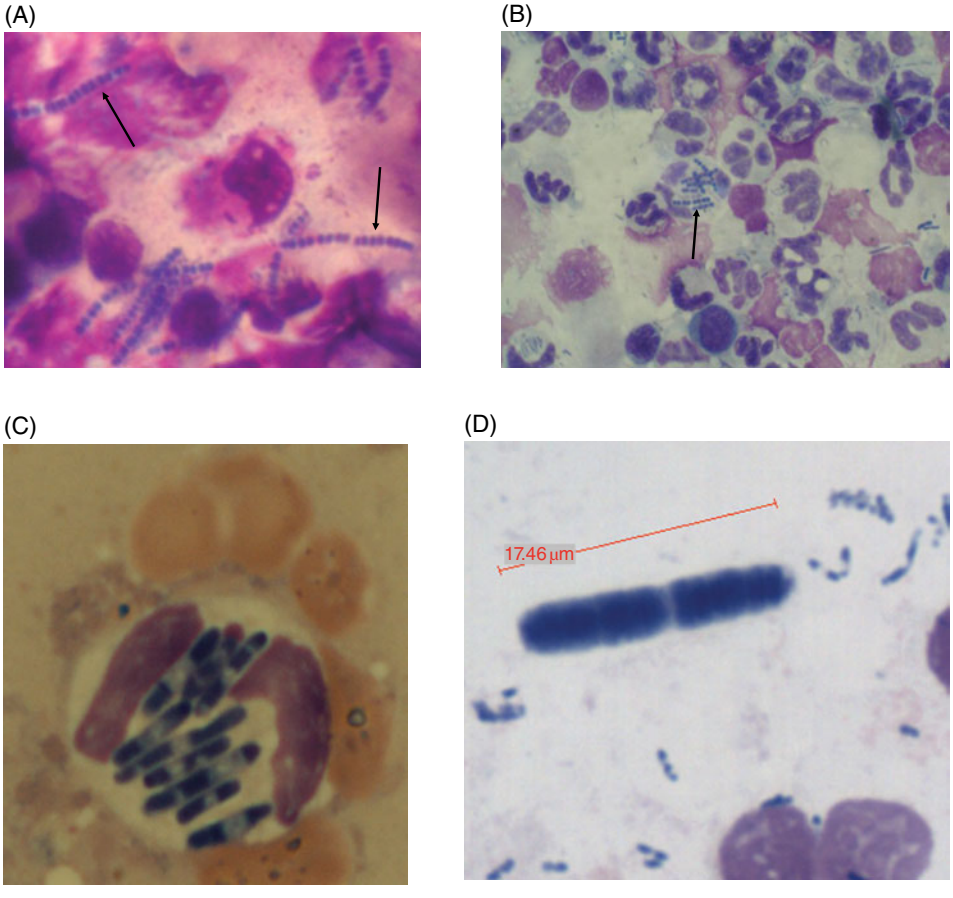
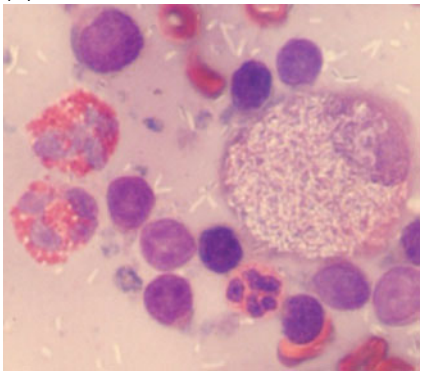
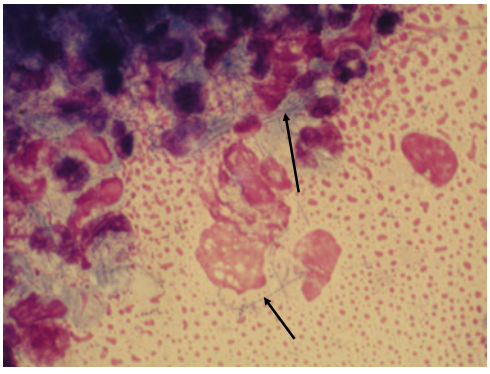


Figure 15.2 Some bacteria have sufficiently unique morphology to allow identification. (A) *Streptococcus* is a coccoid bacterium typically found in chains (arrows). (B) A mixed bacterial population is more suggestive of secondary infection. Seven chains of *streptococci* are present in a neutrophil (arrow). Different sized rods can also be seen free and within neutrophils. The rods cannot be speciated. (C) Hepatic aspirate from a dog with a neutrophil containing clostridial type rods. *Clostridium* spp. are large spore-forming bacteria. The clear round area in the bacilli is a spore. These organisms are about 7–12 µm long and 2–3 µm wide. (D) *Simonsiella* is a huge bacterium in which 15–20 rod-shaped bacterial cells fasten to each other's long axis to form characteristic multicellular subunits called trichomes. These two trichomes on an epithelial cell from a dog's mouth are 17.46 µm long. (*Continued*)

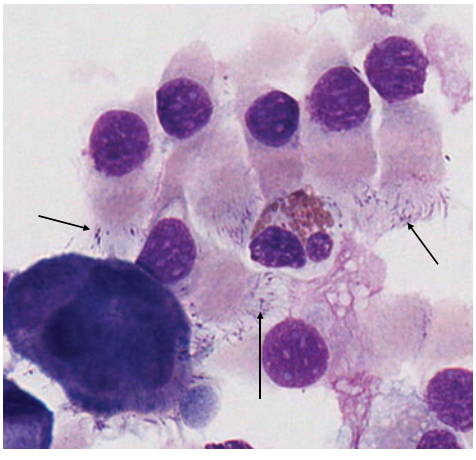
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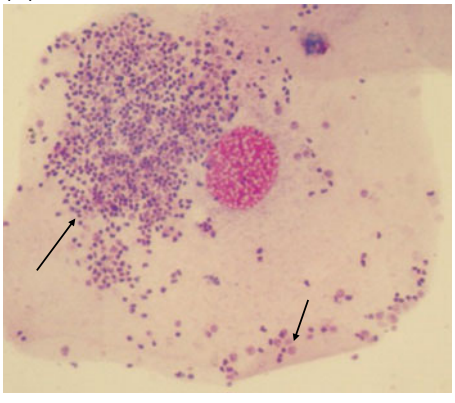


Figure 15.2 (*Continued*)
(E) Mycobacteria are rod-shaped bacteria characterised by a lack of staining (negative staining). Note the white rods in the proteinaceous background and in the macrophage. Mycobacteria have a waxy coat that prevents penetration of the stain. *Mycobacterium* spp. are intracellular organisms which proliferate in large numbers in macrophages. Note also that an eosinophilic response is evident here. (F) *Actinomyces* is a higher bacterium that is thin and branches. This filamentous bacterium (arrows) may be differentiated from branching fungal hyphae which have two distinct cell walls on each side of the organism. *Actinomyces* is much thinner. Staining along the filament is uneven and described as 'beaded'. The neutrophils in this smear are very degenerate. (G) Some bacteria attach to a surface such as *B. bronchiseptica* which are thin short blue rods (arrows) attached to ciliated respiratory epithelial cells in this BAL of a dog. (H) *Mycoplasma* spp. are bacteria which lack a cell wall and thus are very pleomorphic. Spherical forms may collapse, giving a 'ring form' appearance. *Mycoplasma* attach to the surface of cells such as this epithelial cell from the conjunctiva. (*Continued*)

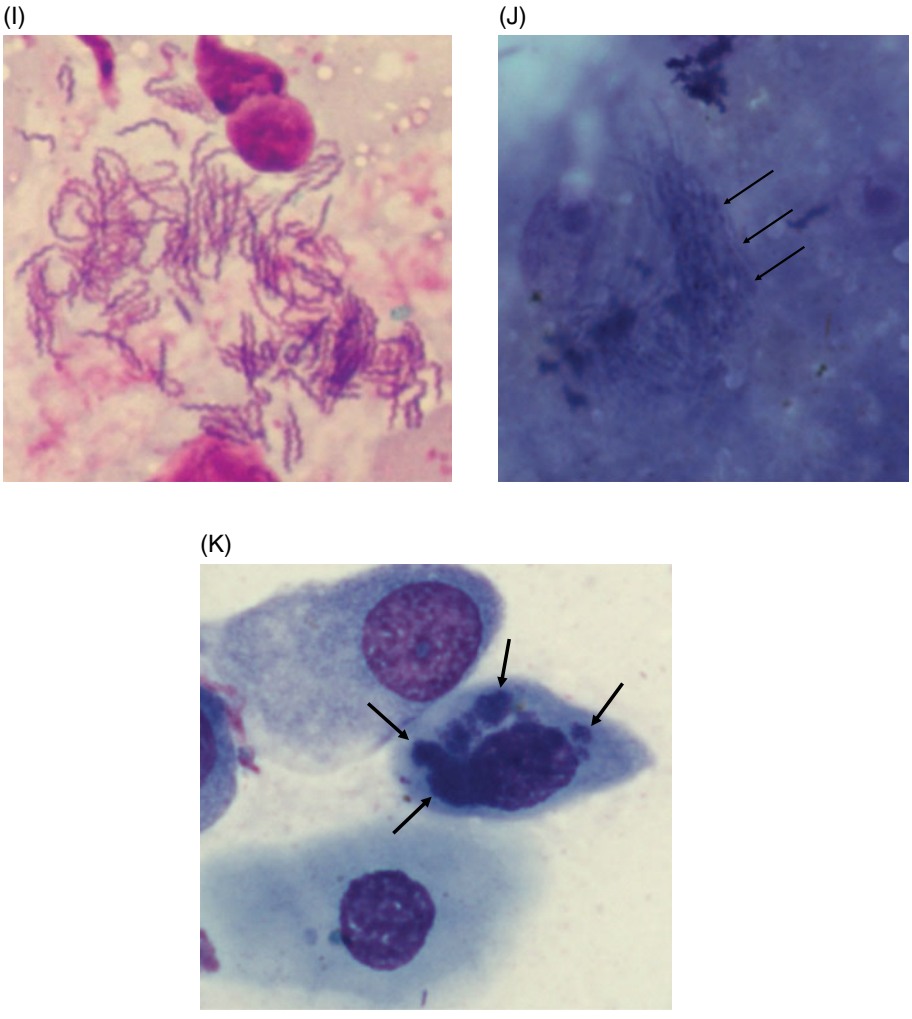


Figure 15.2 (*Continued*)
(I) *Helicobacter* is a spiral-shaped, 7–10 µm bacterium (arrows) commonly identified in gastric samples from dogs. (J) Tyzzer's disease is caused by *C. piliforme*, a long filamentous organism. Several parallel organisms can be seen within a necrotic equine hepatocyte (arrows).
(K) *C. felis* is seen in this ocular swab of a cat in the middle epithelial cell as several reticulate bodies (arrows) containing several initial bodies (0.5–1.5 µm dots). *Chlamydophila* organisms are more often found near the nuclei than *Mycoplasma* spp. which tend to be visualised nearer to the edge of the epithelial cells.

Mycobacteria are unique in that they do not take up stain so they appear as clear rods, which are negatively stained by darker material around them (Figure 15.2E).

Pleomorphic bacteria include *Actinomyces* spp. (Figure 15.2F). *Actinomyces* is a branching filamentous organism but it also has diphtheroidal forms. These smaller

forms are short, straight to curved rods that may have clubbed ends. Thus, a pure infection of *Actinomyces* may look like a mixed infection of filamentous and smaller rod-shaped or coccobacillary bacteria.

Confidence in the conclusion that the bacteria seen are causing a problem in the animal is greatly strengthened by finding them inside neutrophils (Figure 15.2B and C). Bacteria are more easily identified in the clear cytoplasm of neutrophils than in a background which may contain granular debris or in macrophages with phagocytised particulate material. This suggests the neutrophils were attracted to the bacteria and ingested them and the bacteria are less likely to be contaminants. Bacteria are usually phagocytised by neutrophils and not macrophages, although some intracellular types of bacteria, e.g. *Mycobacterium* spp., can survive and even multiply in macrophages (Figure 15.2E).

Some bacteria such as *Bordetella bronchiseptica* (Figure 15.2G) and *Mycoplasma* (Figure 15.2H) are found on the surface of epithelial cells. *Helicobacter* is found in the mucus on the gastric mucosa and in gastric crypts (Figure 15.2I). Tyzzer's disease is caused by *Clostridium piliforme* is uniquely found within hepatocytes of foals (Figure 15.2 J). *Chlamydophila felis* infection in feline conjunctivitis is often suggested by cytological examination (Figure 15.2 K).

Fungal infections

The most common fungal infections detected by cytology are overgrowth of yeasts such as *Malassezia* on warm moist surfaces in the ear or between the toes (Figure 15.3A). *Candida albicans* also can grow on the skin (e.g. between folds of skin) or in the mouth. Yeasts like *Malassezia* and *Candida* spp. have the classic oval form with a bud and cell wall (Figure 15.3A and B). *C. albicans* uniquely forms pseudohyphae (Figure 15.3B). Dermatophyte infections of skin involving *Microsporum* spp. and *Trichophyton* spp. are also common. These organisms form hyphae that break into arthrospores (Figure 15.3C). As with bacteria, culture is required to obtain a more specific classification. Dermatophyte test medium is useful for identifying ringworm-type infections.

Systemic fungal infections usually involve yeast forms of fungi, and the incidence of these infections varies greatly with the geographic region. Major systemic yeast infections include *blastomycosis*, *histoplasmosis* and *cryptococcosis* (Figure 15.3D–F). These infections are caused by budding yeasts which can be identified by the size of the yeast, size of the base of the bud and other morphological characteristics; for example, *Cryptococcus neoformans* has a clear capsule and *Histoplasma capsulatum* is present within phagocytes. *Sporothrix schenckii* is found inside macrophages and is very pleomorphic (Figure 15.3G). Organisms which are round or spherical in shape include *Prototheca*, *Rhinosporidium* and *Coccidioides*. *Prototheca* is an alga that resembles a fungus (Figure 15.3H).

Aspergillus spp. most commonly cause infections in the respiratory track. *Aspergillus* is likely when septate hyphae with uniform width are identified

(Figure 15.3I). Note also that many 'soil' (saprophytic) or atypical fungi can cause opportunist infections often secondary to penetrating wounds on the feet. These organisms are classified as pigmented and non-pigmented fungi, and they vary in shape from hyphal to spherical (even the morphology of the same organism may differ). *Pneumocystis* is now classified as a yeast-like fungus found in the lungs often in dachshunds and Cavalier King Charles spaniels (Figure 15.3J). *Alternaria* is a common mould that is found as a contaminant in many cytological samples (Figure 15.3K).

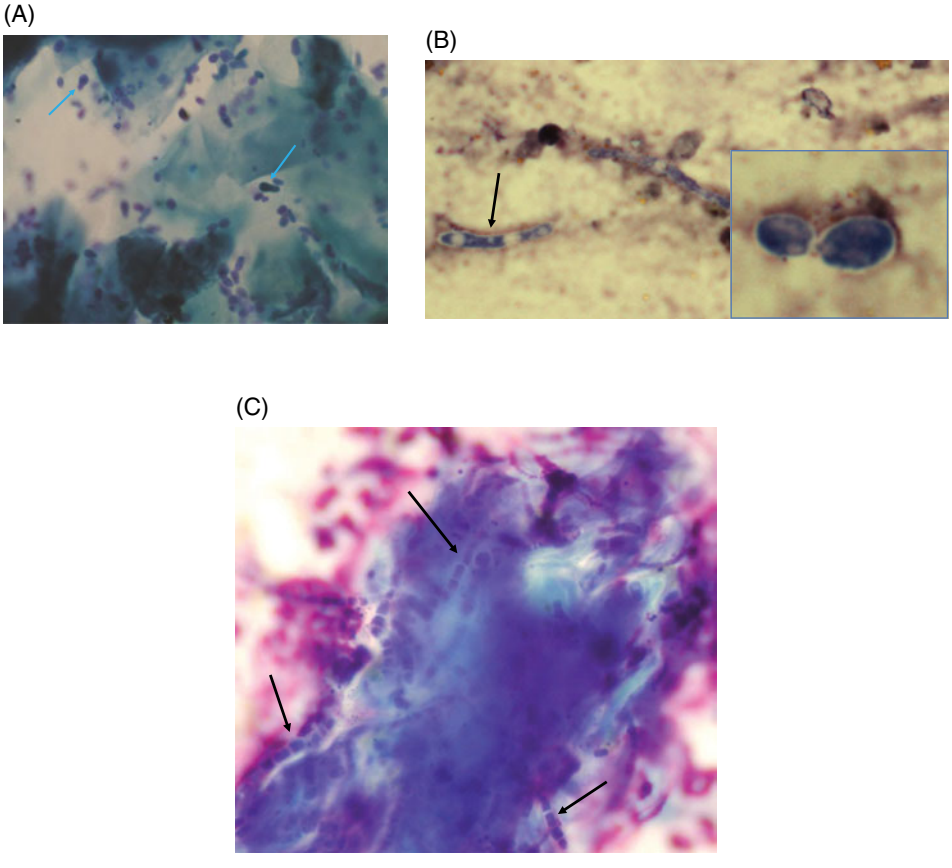
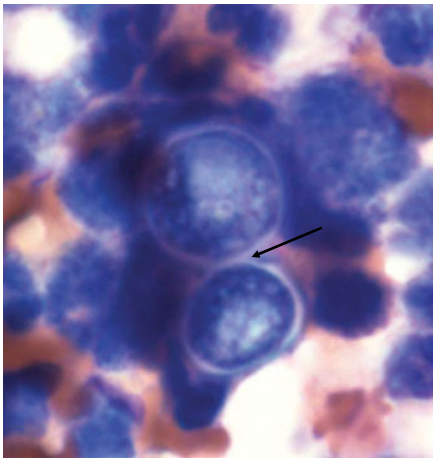
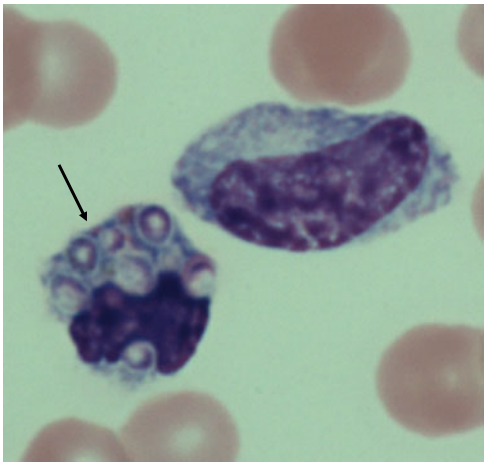


Figure 15.3 Fungal agents. (A) *Malassezia pachydermatis* infection in the ears of dogs with chronic otitis. *Malassezia* is a small oval-shaped budding yeast. *Malassezia* in cats may be smaller and even be mistaken for large *Staphylococcus* organisms. A typical pattern is shown here (arrow) with large numbers of yeast on thin squames without neutrophil exudation. (B) *C. albicans* can form pseudohyphae (arrow) or look like budding yeast (inset). This sample is a corneal scrape from a dog with keratitis. (C) Dermatophyte infection of a dog's skin. There are many septate hyphae growing on some squames. The hyphae are developing into chains of rectangular arthrospores (arrows). This was not cultured but could be *Trichophyton mentagrophytes* or *Microsporum canis*. (*Continued*)

(D)



(E)



(F)

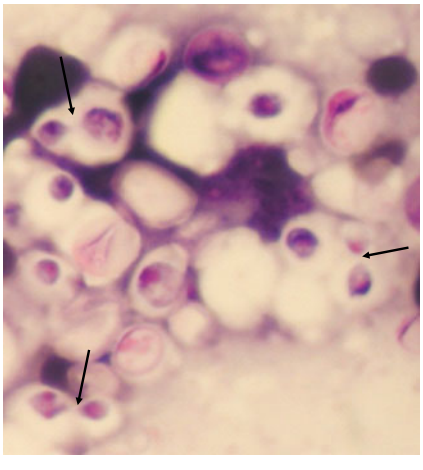
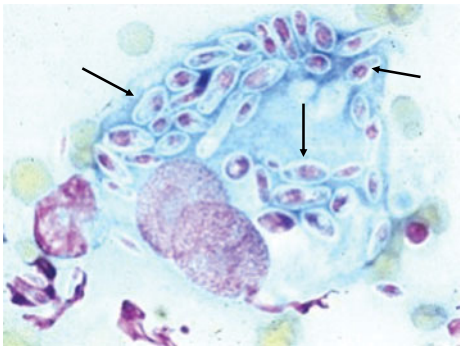
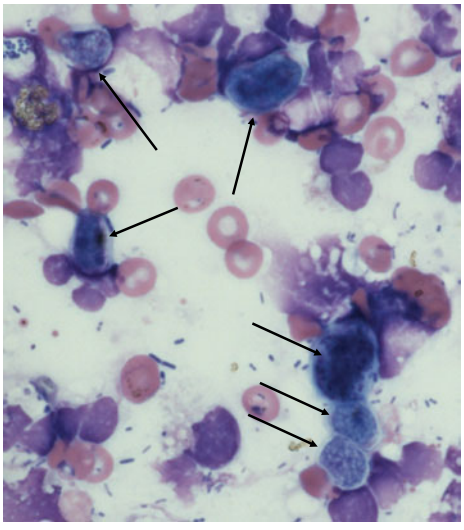


Figure 15.3 (*Continued*)
(D) Pulmonary aspirate from a dog. *Blastomycosis* (*Blastomyces dermatitidis*) is a systemic yeast infection. Note the thick cell wall and broad-based bud (arrow). Neutrophils in the background are out of focus but smaller than each of the two daughter yeasts. Each neutrophil is about 15 µm in diameter. (E) *H. capsulatum* is a small yeast causing a systemic infection. A budding yeast and other yeast with a distinct cell wall have been ingested by the neutrophil in this canine blood smear. The organism is about 1–4 µm in diameter.
(F) Aspirate from a throat abscess in a cat. *Cryptococcosis* (*C. neoformans*) is a systemic yeast infection. Note at least three budding yeasts with a narrow-based bud. *Cryptococcus* organisms have a moderately thick, clear capsule around the yeast. (*Continued*)

(G)



(H)



(I)

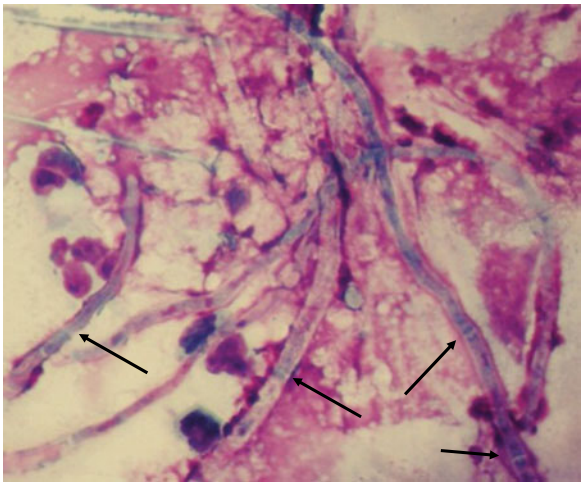
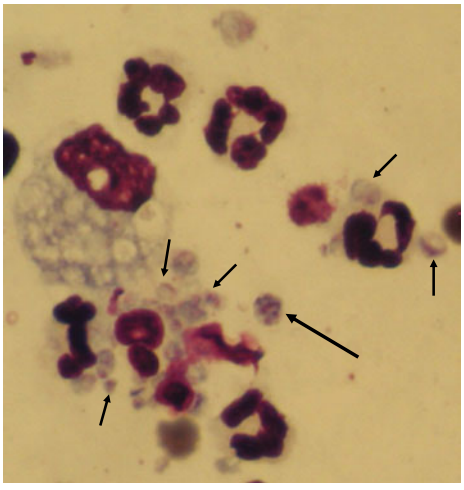


Figure 15.3 (Continued)
(G) *S. schenckii* is a pleomorphic yeast and is classically cigar-shaped. Small budding forms are in the cytoplasm of this macrophage. This was pus from an infected declaw operation site in a cat. (H) *Prototheca* spp. are spherical organisms with a cell wall that look like yeasts but are algae. Six *Prototheca* organisms from a rectal lesion in a dog can be seen here. They are about 1.5–2 times the diameter of an erythrocyte and have a granular internal texture. (I) Aspergillosis is the most common infection involving a hyphal form of fungus. It most frequently causes infection in the respiratory tract. *Aspergillus* spp. have septate hyphae of fairly uniform thickness. The younger, actively growing parts at the ends of the hyphae are thicker, have rounded ends and take up stain better. Many bacteria are also visible in the background. (*Continued*)

(J)



(K)

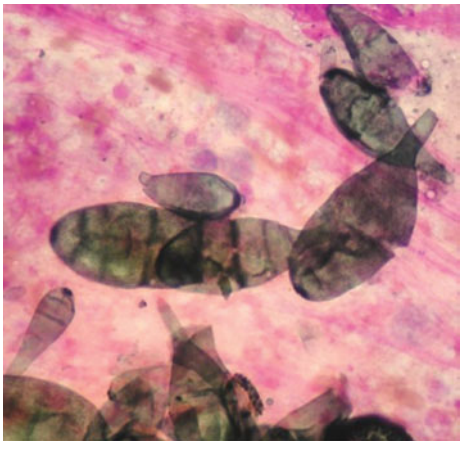


Figure 15.3 (Continued)
(J) *Pneumocystis* spp. in this BAL has free trophozoites (2–7 µm, short arrows) and cysts (long arrow) with four to eight internal bodies. (K) *Alternaria* is a mould with thick golden-coloured hyphae and characteristic club-shaped macroconidia. This photo illustrates several macroconidia. *Alternaria* is a common contaminant of cytological specimens.

Protozoal infections

Protozoa are usually seen in cytological samples as single oval- to crescent-shaped organisms with a thin cell wall and nucleus. Other stages of their life cycle are uncommonly seen. Many protozoa look very similar cytologically, e.g. *Neospora caninum* and *Toxoplasma gondii* (Figure 15.4A and B), so final diagnosis requires ancillary testing such as PCR and serology. The geographic area in which the animal lives often aids the diagnosis. For example, *Leishmania* spp. have a characteristic rod-shaped kinetoplast (Figure 15.4C), and infection is well recognised in dogs from Spain and Israel, whereas *Trypanosoma cruzi* (Figure 15.4D) looks quite similar but is more likely in a dog from Mexico or Texas. Protozoa are often found in macrophages, but morphological detail is often superior in free protozoa that have been released from ruptured macrophages.

Parasitic infections

Parasites may be suspected because of the presenting clinical or radiographic signs or if an eosinophilic inflammatory response (e.g. in a bronchoalveolar lavage (BAL)) is evident. If a parasitic infection is suspected, it is important to screen all slides with 4× and 10× lenses for ova or larvae (Figure 15.5A and B). Fluid samples collected from the animal should be examined with the naked eye for the presence of adult parasites

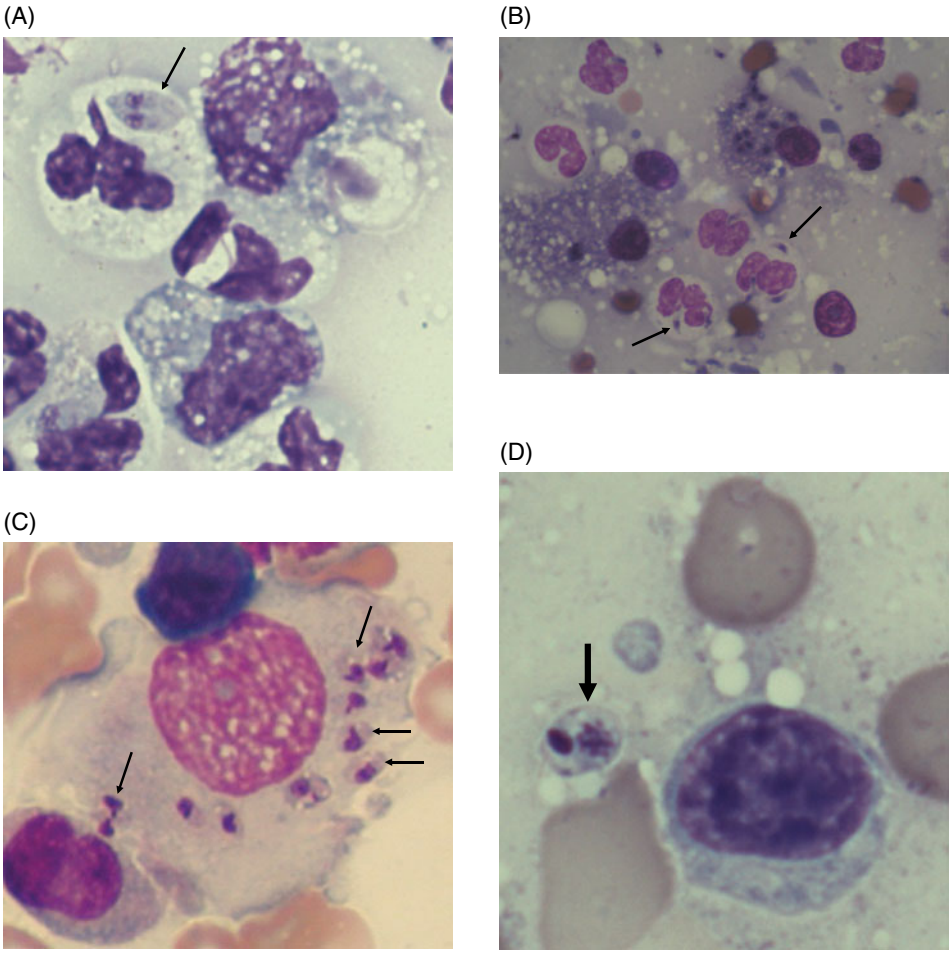


Figure 15.4 Protozoal infections. (A) *N. caninum* is a pointed oval 'banana-shaped' protozoan found in phagocytes in this aspirate from the canine lung. The upper left neutrophil contains two organisms. Morphology does not distinguish *N. caninum* from *T. gondii*. (B) *T. gondii* in the abdominal fluid of a cat appear as pointed oval-shaped protozoa with a nucleus within these neutrophils. Free organisms are also present. (C) Leishmaniasis is common in Southern Europe around the Mediterranean basin and is a common cause of lymphadenopathy in that region. This bone marrow macrophage contains about ten protozoa with a thin cell membrane, round nucleus and a rod-shaped kinetoplast. (D) *T. cruzi* is a protozoan which also has a nucleus and rod-shaped kinetoplast. It looks similar to *Leishmania* but is rounder in shape with a larger, rounder kinetoplast.

such as *Crenosoma vulpis* (Figure 15.5C). Parasites are large, may be infrequent and are easily missed if the examination is performed mainly under higher magnification (45× or 100× lenses) of stained smears. Intact adult parasites may not be seen on a smear, but crushed pieces of nematodes, flukes, tapeworm cysts or mites, etc. may be identified. The remains of crushed adults and immature forms can appear as confusing

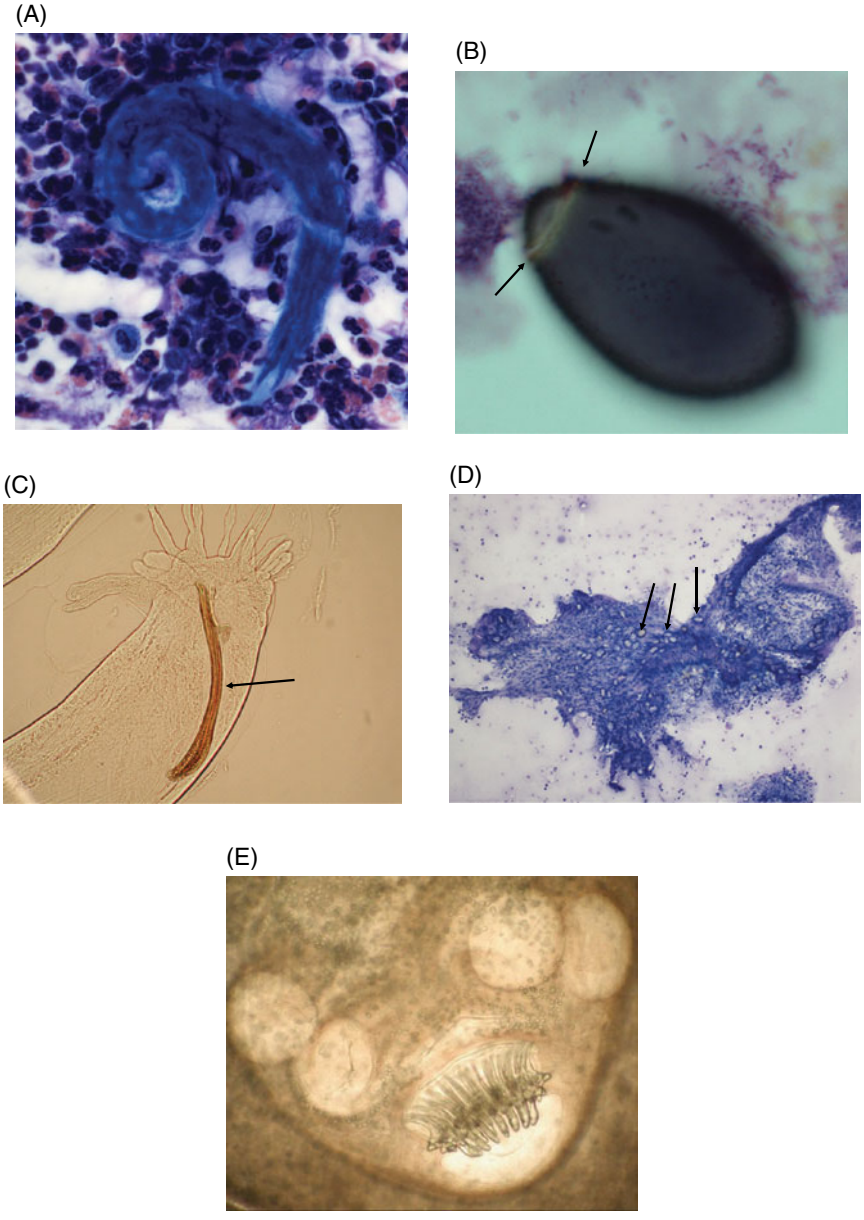


Figure 15.5 Parasitic infections. (A) Larval parasite likely *Oslerus osleri* from a BAL of a dog with tracheitis. *O. osleri* is 232–266 µm in length (approximately 2 eosinophils wide and about 30 eosinophils long). *O. osleri* is often seen in a coil, but this specimen is open, and the internal detail of the parasite can be seen. Note the abundance of eosinophils around the parasite. (B) Operculated (arrows) fluke-type ova of *Amphimerus pseudofelineus* in an aspirate of a cat's liver. Ova are 25–35 × 12–15 µm. (C) *C. vulpis* (*unstained specimen*) in a BAL from a dog. The photograph shows the genital complex of a male adult. The golden-coloured structure is the copulatory organ (gubernaculum). Adult males are 3.5–8 mm long and about 0.3 mm wide. Females are 12–16 mm long and 0.3–0.5 mm wide. (D) Crushed remains of *Mesocestoides* spp. in the abdominal fluid of a dog appear as a sheet of cells, and many calcareous corpuscles (white mineralised bodies) suggest it is a cestode (tapeworm). (E) Cysticercus is found in pericardial fluid of a dog. This has the head of a *Taenia* tapeworm with crown of hooks. The parasite was later identified as *Taenia crassiceps*.

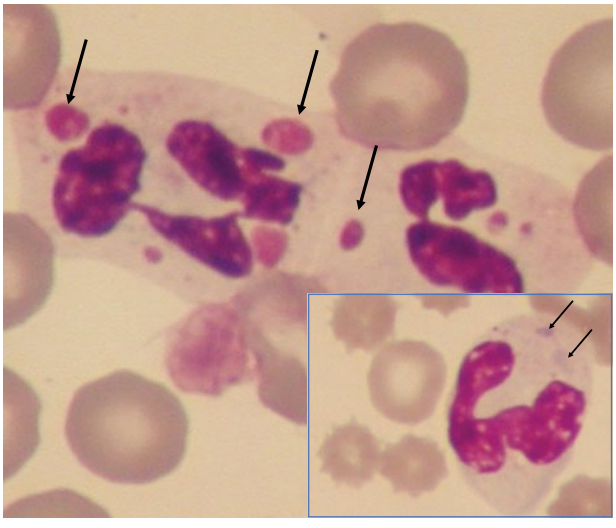


Figure 15.6 Viral infections. Distemper inclusion bodies in canine neutrophils appear reddish. They may appear grey and may be seen in erythrocytes. This was stained with Diff–Quick. Inset: The inclusions in neutrophils of the same dog are very poorly stained with Wright's stain.

large aggregates of cells (Figure 15.5D). Eggs, oocysts and larvae are small enough to identify intact on smears. Diagnosis is heavily based on in which organ of the body the parasite was found, species of animal as well as the size and shape of the organism. Cysticerci can sometimes be identified in body cavity effusions (Figure 15.5E). A dissection microscope with indirect lighting and low magnification is very helpful for the initial classification of the parasite (e.g. nematode, fluke, mite). Note the 10× ocular objective of the microscope may be used as a magnifying glass by removing it from the microscope, inverting the lens so as to look in the opposite direction and examining closely the structure (like a jeweller looking at a diamond).

Viral infections

Cytological diagnosis of viral infections in dogs and cats is rare in regions with good vaccination procedures. Distemper inclusion bodies may be seen in leukocytes and erythrocytes during the viremic phase in dogs. Figure 15.6 shows the variation in appearance of intracellular inclusions due to type of staining.

Further Reading

General

Baker, R. and Lumsden, J.H. (2000) *Color Atlas of Cytology of the Dog and Cat*, Mosby Inc., St. Louis, MO.

Cowell, R.L., Tyler, R.D., Meinkoth, J.H. *et al*. (2008) *Diagnostic Cytology of the Dog and Cat*, 3rd edn, Mosby Inc., St. Louis, MO.

Raskin R.E. and Meyer D.J. (2010) *Canine and Feline Cytology*, 2nd edn, Saunders Elsevier Inc., St. Louis, MO.

Sink, C.A. and Weinstein, N.W. (2012) *Practical Urinalysis*, Wiley-Blackwell, Ames, IA.

Villiers, E. and Blackwood, L. (2005) *Manual of Small Animal Clinical Pathology*, 2nd edn, British Small Animal Veterinary Association, Cheltenham.

Collection Techniques and Principles of Interpretation

Dunn, J. and Villiers, E. (1998a) General principles of cytological interpretation. *In Practice*, **20** (8), 429–437.

Dunn, J. and Villiers E. (1998b) Cytological and biochemical assessment of pleural and peritoneal effusions. *In Practice*, **20** (9), 501–505.

Villiers, E. and Dunn, J. (1998) Collection and preparation of smears for cytological examination. *In Practice*, **20** (7), 370–377.

Respiratory Tract Cytology

Dunn, J. (2010) Cytological examination of the lower respiratory tract in dogs and cats. *In Practice*, **32** (4), 150–155.

Gastrointestinal Tract Cytology

Bjorneby, J.M. and Kari, S. (2002) Cytology of the pancreas. *Veterinary Clinics of North America: Small Animal Practice*, **32** (6), 1293–1312.

Weiss, D.J. and Moritz, A. (2002) Liver cytology. *Veterinary Clinics of North America: Small Animal Practice*, **32** (6), 1267–1291.

Manual of Diagnostic Cytology of the Dog and Cat, First Edition. Edited by John Dunn.
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Endocrine Cytology

Bailey, D.B. and Page, R.L. (2007) Tumors of the endocrine system, in *Withrow & MacEwen's Small Animal Clinical Oncology*, 4th edn (eds S.J. Withrow and D.M. Vail), Saunders Elsevier, St. Louis, MO, pp. 583–609.

Capen, C.C. (2002) Tumors of the endocrine glands, in *Tumors of Domestic Animals*, 4th edn (ed. D.J. Meuten), Iowa State Press, Ames, IA, pp. 607–696.

Kisseberth, W.C. (2007) Neoplasia of the heart, in *Withrow & MacEwen's Small Animal Clinical Oncology*, 4th edn (eds S.J. Withrow and D.M. Vail), Saunders Elsevier, St. Louis, MO, pp. 809–814.

Maddux, J.M. and Shull, R.M. (1989) Subcutaneous glandular tissue: mammary, salivary, thyroid and parathyroid, in *Diagnostic Cytology of the Dog and Cat* (eds R.L. Cowell and R.D. Tyler), American Veterinary Publications, Goleta, CA, pp. 83–92.

Cytology of the Lymphoid System

Atwater, S.W., Powers, B.E., Park, R.D. *et al.* (1994) Thymoma in dogs: 23 cases (1980–1991). *American Veterinary Medical Association*, **205**, 1007–1013.

Bernard, A., Boumsell, L., Bayle, C. *et al.* (1978) Hand-mirror cells in T-cell lymphoma. *Lancet*, **2** (8093), 785–786.

Christopher, M.M. (2003) Cytology of the spleen. *Veterinary Clinics of North America: Small Animal Practice*, **33**, 135–152.

Cowell, R.L., Dorsey, K.E. and Meinkoth, J.H. (2003) Lymph node cytology. *Veterinary Clinics of North America: Small Animal Practice*, **33**, 47–67.

Day, M.J. (1997) Review of thymic pathology in 30 cats and 36 dogs. *Journal of Small Animal Practice*, **38**, 393–403.

van Heerde, P. (1984) Malignant lymphomas and histiocytic tumours: cytology and other diagnostic methods. Thesis, Uitg Rodophi, Amsterdam.

Lennert, K. and Mohri, N. (1978) Histopathology and diagnosis of non-Hodgkin's lymphomas, in *Malignant Lymphomas Other than Hodgkin's Disease*, Springer-Verlag, New York, pp. 111–469.

Mills, J.N. (1989) Lymph node cytology. *Veterinary Clinics of North America: Small Animal Practice*, **19**, 697–717.

Teske, E. and van Heerde, P. (1996) Diagnostic value and reproducibility of fine-needle aspiration cytology in canine malignant lymphoma. *Veterinary Quarterly*, **18**, 112–115.

Teske, E., Wisman, P., Moore, P.F. *et al.* (1994) Histological classification and immunophenotyping of canine non-Hodgkin's lymphomas. Unexpected high frequency of T-cell Lymphomas with B-cell morphology. *Experimental Hematology*, **22**, 1179–1187.

Thrall, M.A. (1987) Cytology of lymphoid tissue. *Compendium on Continuing Education for the Practising Veterinarian*, **9**, 104–111.

Zinkl, J.G. and Keeton, K.S. (1979) Lymph node cytology – I. *California Veterinarian*, **33** (1), 9–11.

Zinkl, J.G. and Keeton, K.S. (1979) Lymph node cytology – II. *California Veterinarian*, **33** (4), 20–23.

Zinkl, J.G. and Keeton, K.S. (1981) Lymph node cytology – III, Neoplasia. *California Veterinarian*, **35** (5), 20–23.

Cerebrospinal Fluid

Vernau, W., Vernau, K.A. and Bailey, C.S. (2008) Cerebrospinal fluid, in *Biochemistry of Domestic Animals*, 6th edn (eds J.J. Kaneko, J.W. Harvey and M.L. Bruss), Elsevier Inc, Ames, IA.

Genital Tract Cytology

Dahlbom, M., Mäkinen, A. and Suominen, J. (1997) Testicular fine needle aspiration cytology as a diagnostic tool in dog infertility. *Journal of Small Animal Practice*, **38**, 506–512.

Hiemstra, M., Schaefers-Okkens, A.C., Teske, E. *et al*. (2001) The reliability of vaginal cytology in determining the optimal mating time in the bitch. *Tijdschr Diergeneeskd*, **126**, 685–689.

Kraft, M., Brown, H.M. and LeRoy, B.E. (2008) Cytology of the canine prostate. *Irish Veterinary Journal*, **61**, 320–324.

Moxon, R., Copley, D. and England, G.C.W. (2010) Quality assurance of canine vaginal cytology: a preliminary study. *Theriogenology*, **74**, 479–485.

Powe, R., Canfield, P.J. and Martin, P.A. (2004) Evaluation of the cytologic diagnosis of canine prostatic disorders. *Veterinary Clinical Pathology*, **33**, 150–154.

Santos, M., Marcos, R. and Caniatti, M. (2010) Cytologic study of normal canine testis. *Theriogenology*, **73**, 208–214.

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