NOTES ON DIAGNOSTIC CYTOLOGY AND BODY FLUID ANALYSIS SESSIONS FOR VETERINARY PRACTITIONERS AND NURSES

INTRODUCTION

These notes are by no means all encompassing, but it is hoped that they will provide useful revision and present you with an approach to utilising cytopathology and body fluid analysis in veterinary practice. As I was reviewing these notes, I realised that whilst information has rapidly expanded, the fundamentals have stayed the same. As a competent veterinary practitioner or nurse, it is important for you to get the process of investigation right, as you will always be behind with the latest information. Don’t let this information deficit scare you! Good decision making should give you confidence when diagnosing disease – and you can always use Google Scholar® to follow up your decisions.

DIAGNOSTIC CYTOLOGY (EXFOLIATIVE CYTOLOGY; CYTOPATHOLOGY)

Diagnostic cytology is the examination of individual cell details for the purpose of diagnosis and prognosis. Although more information can usually be gained from the examination of tissues (histopathology) rather than individual cells, cytology does have distinct advantages over histopathology:

a) Cytological samples are cheaper and easier to obtain if superficial (eg skin and peripheral lymph nodes)
b) Cytological samples are simpler to process. Consequently, the result from a cytological preparation can usually be quickly obtained.

c) Agents of disease are sometimes easier to detect because of better resolution at 100 x magnification

d) Cell detail is usually better because of better resolution under an oil objective

Cytological preparations may be stained with blood stains, e.g. Giemsa, Diff Quik®. Commonly, a diagnosis on the basis of cytological data is limited and broad. Histopathological examination usually needs to be done to support the cytological findings (by biopsy or at necropsy), and is the only way that tissue architecture (including margins of a lesion) can be examined. Consequently, cytopathology and histopathology complement one another.

a) Solid tissue cytology

i) **Tissue imprints/scrapings** are useful for examining accessible soft tissues (in the living animal, from biopsy material, from necropsy material). The surface of the tissue is blotted free of debris and blood and a slide touched onto the exposed surface. The imprint allows examination of individual cell details, cell relationships (if the imprint is performed carefully) and examination for the presence of etiological agents. In firm tissues or in sites inaccessible to imprints (e.g. conjunctiva) tissue scrapings can be done. The tissue is cleaned as for an imprint and then material collected on the `scraper' is then gently spread over a slide (‘buttering’) or transferred to a slide and spread as for a peripheral blood film.

ii) **Fine needle cell aspirates** are principally of use in the living animal (examination of internal and external solid/fluid masses). The cellular material is aspirated with a fine gauge needle (usually 21-23) and syringe (2 ml or 5 ml commonly) and then placed on a slide and gently smeared (as a squash preparation or as a for a peripheral blood film). Some cytologists suggest that soft lesions may not need aspiration to collect a good sample and only inserting a needle will do the trick.

Solid tissue cytology is approached the same way as histopathology: **detect, describe and deduce (the three ‘Ds’ for diagnosis)**. Since cytological smears are often examined under the 100x objective, the focus for description is cellular detail, although both acellular material and living and non-living agents of disease may be detected. The interpretation is based firstly on the **five basic pathological processes (degeneration and necrosis; inflammation and repair; vascular disturbance; disorder of growth; and pigmentation/deposit)**. In most cases, the lesions are proliferative (hyperplastic or neoplastic – ie a disorder of growth), inflammatory or degenerative (the last including pigments and deposits) (see Table 1). Vascular disturbances are hard to detect with cytopathology because everything bleeds with sampling! Further differentiation of a cytological smear will depend on cytological and acellular features. Neoplastic lesions can often be differentiated into benign or malignant epithelial, mesenchymal (connective tissue) and round cell types. Solid tissue cytology is particularly useful for detecting and identifying round cell neoplasms (eg mastocytoma, lymphosarcoma, benign cutaneous histiocytoma). Inflammatory lesions may be differentiated into acute and chronic (including granulomatous) processes. Cytology is also useful for detecting agents of disease. These may be **physical** (eg foreign bodies), **living** (eg acid fast bacteria, toxoplasmal organisms, chlamydial organisms) or **chemical** (eg calcification of tissue).

An example of the use of solid tissue cytology would be to investigate lymphadenomegaly. A fine needle cell aspirate of an enlarged lymph node may be able to distinguish among benign lymphoid hyperplasia, lymphadenitis, lymphosarcoma and metastatic neoplasia.
1. Primary degenerative lesions

These include those lesions that are cystic in nature (eg epidermal cyst, renal pseudocyst or cyst), those that are due to deposits of minerals or pigments (eg calcium in calcinosis circumscripta, haemosiderin in haemorrhages, silicon or asbestos in lung macrophages), and those that are due to damage (eg physical trauma or toxic substances).

2. Inflammatory lesions

Inflammatory lesions are characterised primarily by the presence of inflammatory cells or by the presence of infectious agents (eg bacteria, viruses, fungi). Cytology is extremely useful in detecting infectious agents and may at times provide positive identification without further investigation; for example the detection of Distemper virus or chlamydial inclusions in conjunctival smears. However, in most instances, further investigation (eg culture, molecular studies) is required to positively identify the infectious agent.

Lesions caused by infectious agents often feature large numbers inflammatory cells. Occasionally, as in some cases of cryptococcosis or clostridial hepatitis, few inflammatory cells are present. These lesions are still classified as inflammatory. Not all inflammatory lesions are caused by infectious agents. Other causes include foreign material (eg sterile penetration wound; injected irritant) or endogenous irritants (eg ruptured epidermal cyst and release of keratin; bile or urine escape into the abdomen).

Inflammatory lesions, apart from being characterised by the presence of their cause, can be further classified by the types of inflammatory cells present. This can provide clues to the cause of the problem.

Neutrophils indicate acute inflammation in response to damage or to infective agents such as bacteria. Neutrophils can also form a component of ongoing ('chronic') inflammation if the chemotactic factor (such as bacteria) is persistent in the lesion (active chronic inflammation). The term purulent exudate refers to a neutrophil predominance and is often further classified as septic (bacteria are present, especially within neutrophils or non-septic (other causes such as fungi, sterile irritants or immune-mediated disease).

Based mainly on nuclear morphology, neutrophils can be described as lytic or nonlytic (degenerate and non-degenerate). The nucleus of lytic neutrophils appears faded and can have ragged or distorted edges. Lytic neutrophils develop under the influence of toxins and are often interpreted as indicating the presence of bacteria. Lytic neutrophils, however, can also be seen in some non-bacterial infections (eg fungal) or as a result of exposure to chemical toxins as in bile peritonitis or uropertioneum. Not all septic exudates feature lytic neutrophils. The term “degenerate” is often used interchangeably with “lytic”.

Eosinophils commonly accompany lesions which have a strong adaptive immune component and which usually take some time to develop (eg response to some parasites, allergies). They may also be present in lesions involving mast cells (eg mastocytoma in dogs; inflammatory lesions involving the skin, respiratory tract, urogenital tract or gastrointestinal tract) or other neoplastic cells (paraneoplastic phenomenon due to the production of chemicals that stimulate eosinopoiesis and/or attract eosinophils). Some predominantly eosinophilic inflammatory lesions are of unknown cause (eg the eosinophilic granuloma complex in cats, idiopathic pulmonary infiltrates with eosinophils in dogs).
Macrophages (also called histiocytes) are important for the phagocytosis of macromolecular material. They usually form a component of chronic inflammatory lesions and are enlisted by a demand for phagocytosis and by lymphocyte derived cytokines in the adaptive immune response. Macrophages can also be present very early on in an inflammatory reaction if the need for phagocytosis is great eg in the face of much tissue damage or foreign debris. This is especially so in certain organs or tissues such as the lower airways.

The predominance of macrophages, especially large, aggregated or multinucleate macrophages, in an inflammatory lesion may suggest a granulomatous response. This is a special form of chronic inflammation defined by a particular histopathological arrangement of macrophages that may be caused by certain agents of disease such as foreign debris, certain fungi, protozoa, multicellular parasites and bacteria (eg staphylococcal, mycobacterial, nocardial organisms). Many of these agents of disease can also cause a pyogranulomatous response in which both neutrophils and macrophages are found in significant numbers.

Lymphocytes and plasma cells are present in lesions due to adaptive immune stimulation and commonly accompany prolonged inflammation. However, in some skin lesions, such as allergies, they can accumulate quickly. While their presence suggests an immune component to the inflammation, it does not automatically mean that the immune response is the primary cause of the problem (ie autoimmunity or hypersensitivity).

Fibroblasts are normally present in chronic inflammation (therefore, also in granulomatous inflammation) and in healing wound. Beware, reactive fibroblasts can be quite pleomorphic and can overlap in appearance with neoplastic spindle cells.

3. Proliferative (hyperplastic/neoplastic) lesions

Because hyperplastic and neoplastic lesions tend to produce masses that are relatively easy to sample, this is the most common category to be diagnosed by cytology.

As mentioned previously, inflammation can accompany many tumours and can be the more prominent feature. Neoplasms can cause damage to surrounding tissue and many tumours have a high cell turnover. Both these processes produce factors that are chemotactic for inflammatory cells. Examples include the intense inflammation that commonly accompanies a squamous cell carcinoma (the irritant nature of free keratin also contributes to the inflammation); the nonseptic exudates that accompany neoplastic cells exfoliating into the pleural, pericardial and peritoneal cavities; septic inflammation due to clostridial infection of necrotic liver tumours and septic peritonitis secondary to a perforating neoplasm of the gastrointestinal tract. If tumour necrosis is extensive, the neoplastic cells can be difficult to find or identify. Experienced cytopathologists realise this and often warn the practitioner with the phrase ‘suspect underlying neoplasia as a cause’.

Much has been written about diagnosing neoplasia by cytology, especially in relation to certain tumours such as mast cell neoplasia. The suggested approach involves a logical, stepwise approach using general principles. It allows identification of the neoplastic process and often leads to diagnosis of the specific neoplasm. Questions to be asked, in order of importance, are:

1. Is the lesion primarily proliferative (disorder of growth) and, if so, is it hyperplastic, benign or malignant?

Proliferative lesions can be hyperplastic or neoplastic, benign or malignant. Differentiation can be extremely difficult and is not always straightforward based on nuclear and cytoplasmic features.
It is probably not critical or possible to distinguish between hyperplastic and benign neoplastic processes on cytology. Detection of malignancy, however, is highly desirable but, again, not always possible. The hallmarks of malignancy are anaplasia, invasion, and metastasis. The presence of invasion into surrounding tissue can only be assessed on gross and histological examination. Metastases can be detected by imaging and cytology eg aspiration of lymph nodes, organ masses. Anaplasia, which refers to the fact that the neoplastic cells are poorly differentiated and, consequently, highly variable in appearance, can be assessed cytologically. Anaplasia is a common feature of malignancy, but does not have to be present in every malignant tumour. Some malignant neoplasms can have relatively 'benign' cellular features (ie the cells look like well differentiated origin cells and show only slight nuclear and cytoplasmic variation). A common example would be some thyroid carcinomas in the dog. Even the cells from their metastases can look relatively well differentiated and be in a glandular pattern!

Despite this limitation, many tumours can be designated as malignant on cytological features. Although it is difficult to make rules for cells that break rules to become established and spread, it can generally be said that the more bizarre the cells appear the more likely are they to be malignant (exceptions – plasmacytomas, fibroblasts). Cytological features that are most likely to indicate malignancy include, numerous and abnormal mitoses, cell and nuclear pleomorphism, cytoplasmic basophilia, enlarged or bizarre nucleoli, and multinucleation (especially with variably sized nuclei).

If you suspect neoplasia from the history but your cytological sample is not supportive, don’t disregard your suspicion. Re-aspirate if the answer is not clear cut. Neoplastic cells may be difficult to detect, especially if there has been blood dilution, tissue necrosis or masking by inflammatory cells as mentioned previously.

2. Are the predominant cells round, spindle, or epithelial?

These are the three basic cell groups responsible for proliferative lesions and are classified according to cell shape and embryonic origin.

Round cells include all haematopoietic cells (including lympho-histiocytic cells) and mast cells. As suggested, the cells are round in shape. They usually show little tendency to aggregate in smears (unless the smear is thick) and are of embryonic mesoderm origin.

Spindle cells suggest a mesenchymal (embryonic mesoderm) origin (ie all connective tissues, muscle, vessels etc). Not all spindle cells are tapering, some can appear rounded or angular. While, spindle cells typically occur as individual cells in smears, some mesenchymal neoplasms exfoliate well to produce epithelial-like aggregates of cells eg haemangiopericytomas.

Epithelial tumours are derived from ectoderm or endoderm. Cells vary from square to columnar, and at least some should be found in groups in smears.

Some specific tumours derived from neuroectoderm (melanocytes and Schwann cells) can present in either spindle or epithelial forms. Malignant melanocytes may even present as round cells.

3. Can the cell types be subcategorised?

Subcategorisation depends on the detection of specific arrangements of cells or the production of a cell product. For example, if the epithelial cells are in a duct or acinus arrangement or if they are in a solid clump but have an intracellular product, then they are identified as glandular. Intracytoplasmic
melanin will suggest a melanotic tumour while intracytoplasmic, purple granules could suggest mast cell neoplasia.
Table 1. Approach to interpretation of cytological smears (based on predominant pathological process)

<table>
<thead>
<tr>
<th>Predominant pathological process</th>
<th>Main feature for characterisation</th>
<th>Usefulness of cell type</th>
<th>Basis for further characterisation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary degenerative changes</strong></td>
<td>cysts (eg renal cyst)</td>
<td>Macrophages are sparse</td>
<td></td>
</tr>
<tr>
<td></td>
<td>damage (eg burn, trauma)</td>
<td>Neutrophils will come into play as inflammation develops</td>
<td></td>
</tr>
<tr>
<td></td>
<td>deposits and pigments (eg calcinosis circumscripta)</td>
<td>May get macrophages and even giant cells against some deposits</td>
<td></td>
</tr>
<tr>
<td><strong>Primary inflammatory lesions</strong></td>
<td>On predominant cell type</td>
<td>neutrophils (purulent inflammation - septic due to bacteria; non-septic due to other causes)</td>
<td>Gram stain and culture</td>
</tr>
<tr>
<td><em>ie not secondary to primary degenerative lesions or neoplastic conditions</em></td>
<td>eosinophils (strong immune component in the inflammation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>macrophages (histiocytes) - epitheloid cells and multinucleates may indicate granulomatous inflammation</td>
<td>Gram stain, acid fast stains and culture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lymphocytes and plasma cells indicate chronic immune stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hyperplastic and neoplastic lesions</strong></td>
<td>On main cell type</td>
<td>Epithelial</td>
<td>glandular or ductular in arrangement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>squamous differentiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spindle</td>
<td>pattern and acellular deposits may allow differentiation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Round</td>
<td>mastocytoma, lymphosarcoma, haematopoietic tumours, histiocytic tumours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>variable shape (some mesodermal and neuroectodermal tumours)</td>
<td>melanomas should have cytoplasmic pigment; osteosarcomas should be producing osteoid</td>
</tr>
</tbody>
</table>
b) Diagnostic cytology as a component of body fluid analysis
Cytological preparations for fluids can be obtained by direct smearing of the fluid or by centrifuging the fluid and smearing the sediment. Fluids of an inflammatory nature should be placed in anticoagulant (e.g. EDTA) as clotting interferes with smear preparation.

Interpretation of the cell smears will depend on the site of origin of the fluid and will be discussed under body fluid analysis.

\textit{NB: Cells deteriorate rapidly in body fluids. If a delay is envisaged before the sample reaches the laboratory then smears should be made directly from the fluid prior to despatch.}

\textbf{BODY FLUID ANALYSIS}

a) Body cavity effusions
This is mainly referring to abdominal and thoracic fluids but there is applicability to pericardial and other cavities)

An effusion refers to a pouring out of any fluid into a body cavity or tissue, i.e. excess fluid. By assessing \textit{gross characteristics} and determining \textit{protein levels} (by refractometer or by the chemical Biuret method), \textit{total nucleated cell count}, and \textit{differential cell count} (on a smear). Abdominal and thoracic cavity fluid is commonly assessed when there is an effusion. In cattle and horses, abdominal cavity fluid may be assessed as part of an abdominal cavity disease investigation even when increased fluid (an effusion) is not present. Effusions can be divided into:

i) \textit{Pure transudates}
These may occur in hypoproteinemic states due to lowered plasma osmotic pressure and in some forms of chronic liver disease in the dog. They are really normal body fluid in excess (therefore low protein and low cells). These are non-inflammatory and uncommon.

ii) \textit{Modified transudates}
These are still non-inflammatory in origin. They are modified by the addition of protein or cells (but limited compared to values for exudate), e.g. ascites due to chronic passive congestion, neoplasia, chylothorax. These are very common.

\textit{NB: A process may start off as a pure transudate and, with time, progress to a modified transudate and even to a non-septic exudate (once serum protein components, neoplastic cells etc start to accumulate they may create chemotaxis for phagocytes, thereby causing secondary inflammation). Consequently, it may be difficult at times to separate some modified transudates from early non-septic exudates due to variable alterations of protein and cells.}

\textbf{Chronic heart failure in the dog} tends to cause thoracic and abdominal effusions that quickly pass from pure transudates to modified transudates (erythrocytes are common in the fluid). Occasionally they may progress to non-septic exudates. \textbf{Chronic cardiac disease in the cat} can cause thoracic effusions, which are commonly classified as \textbf{chyrous} (ie chylothorax).
iii) **Chylous effusions**

These most commonly occur in the thorax (but can be present in the abdomen) and are assumed to be due to leakage from major or minor lymphatics. Consequently, chylous effusions are characterized by many small lymphocytes and high triglycerides (higher values than in plasma). A variety of conditions can give rise to chylothorax (e.g., neoplastic interference to lymphatic flow, trauma to lymphatics, heartworm, idiopathic), but the most common cause in the cat is chronic heart disease. In contrast, chylous effusion in the thorax of the dog is less common than in the cat and is rarely associated with chronic heart disease (I think I’ve seen one case). Instead, it is most commonly due to thoracic duct compression (rarely rupture), but may occasional occur in other diseases affecting lymphatics.

Most chylous effusions are milky white but exceptions do occur. In addition, some milky white fluids may be **pseudochylous. These are extremely rare.** They are turbid fluids that can be caused by exfoliating neoplasms or tissue inflammation. With cell breakdown large amounts of cholesterol build up in the fluid. However, the milky colour of the fluid is not due to fat but due to cell debris and chemical complexes). To differentiate chylous from pseudochylous effusions, it is best to support gross and cytological findings with simultaneous analyses of **triglycerides** and **cholesterol** in both effusion and plasma (or just effusion and determine the ratio). Chylous effusions will have high triglycerides and low cholesterol effusion levels when compared to plasma levels; and **vice versa** for pseudochylous effusions.

iv) **Exudates (high protein and high total nucleated cell numbers)**

1) **non-septic exudate**

This type of effusion either develops from a modified transudate or is due to direct inflammation caused by irritants that usually produce few toxic changes in neutrophils, e.g., sterile foreign bodies, ruptured urinary bladder (this more commonly causes a modified transudate), bile peritonitis, Feline Infectious Peritonitis (FIP— it has been suggested that this condition gives rise to a modified transudate rather than a non-septic exudate as cell counts are often limited. However, since both cells and protein are increased, and it is known to be an inflammatory process, it is more appropriate to call it a non-septic exudate).

2) **septic exudate**

This type of effusion is the product of inflammation caused by a wide variety of microbes that are toxic to neutrophils (principally causing karyolysis due to interference with water balance). Values for protein and total nucleated cells are high compared to the other categories.

v) **Haemorrhagic effusion (recent or long standing)**

Recent haemorrhage will be characterised by clear supernatant on centrifugation and platelet clumps on examination of the smear. With time, the supernatant will become red-brown or yellow due to degradation of erythrocytes. A smear will show erythrophagocytosis and/or haemosiderin in macrophages.

It is important to remember that the basis for the classification of body fluid effusions depends upon **protein estimation, total nucleated cell count** and **differential cell count on a smear.** Values are
provided for abdominal fluid analysis (probably can be applied to pleural and pericardial fluid analysis with care): Refer to the following table. Values provided by texts for protein estimations by refractometer and total nucleated cell numbers for the different categories may vary from these. Consequently, the following values should be used as guides and not be regarded as absolute.
### Table of characteristics of body fluid effusions for the dog, cat and horse (mostly applies to peritoneal fluid)

<table>
<thead>
<tr>
<th></th>
<th>Pure transudate*</th>
<th>Modified transudate</th>
<th>Chylous effusion</th>
<th>Non-septic exudate</th>
<th>Septic exudate</th>
<th>Haemorrhagic effusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Gross characteristics</strong></td>
<td>clear/colourless (S); clear/pale yellow (H)</td>
<td>variable but often cloudy</td>
<td>often turbid white or pink</td>
<td>Cloudy</td>
<td>marked cloudiness</td>
<td>cloudy red or brown</td>
</tr>
<tr>
<td><strong>2. Total protein g/L (refractometer)</strong></td>
<td>&lt;25</td>
<td>25-50 (S); 25-30 (H)</td>
<td>&gt;25 (inaccurate due to presence of the fat)</td>
<td>30-70</td>
<td>30-70</td>
<td>variable but usually high</td>
</tr>
<tr>
<td><strong>3. Total nucleated cell count (x10⁶/L)</strong></td>
<td>500-1500 (S); 1500-10,000 (commonly &lt;7,500) (H)</td>
<td>300-5000 (S); 5000-12,000 (H)</td>
<td>variable but usually 500-5000</td>
<td>5000-100,000 (S); &gt;12,000 (H)</td>
<td>5000-100,000 (S); &gt;12,000 (H)</td>
<td>variable but usually high</td>
</tr>
<tr>
<td><strong>4. Differential cell count (smear)</strong></td>
<td>monocytes, lymphocytes and non-lytic (non-degenerate) neutrophils</td>
<td>as for pure transudate but increased non-lytic (non-degenerate) neutrophils and some erythrocytes and reactive mesothelial cells</td>
<td>Small lymphocytes usually predominate</td>
<td>mainly non-lytic (non-degenerate) neutrophils, some erythrocytes and mesothelial cells</td>
<td>mainly lytic (degenerate) neutrophils, microbes, some macrophages and erythrocytes</td>
<td>mixed peripheral blood cells plus macrophages and mesothelial cells</td>
</tr>
</tbody>
</table>

*also applies to values for normal abdominal fluid

H = horse; S = dog and cat; if not stated applies to all 3 species
Notes On Body Fluid Effusions:

i) **Horse abdominocentesis**: The procedure can be done even though significant effusion may not be present (e.g., in evaluation of GIT disturbances, colic). In horses, lactate levels in peritoneal fluid may be analysed in conjunction with plasma levels to assess abdominal problems. In healthy horses lactate levels in peritoneal fluid are usually lower than in plasma. With abdominal crises lactate levels in peritoneal fluid exceed those in plasma, and the wider the gap, the poorer the prognosis (lactate levels are not used for diagnosing abdominal catastrophes, just for prognosis).

ii) Pathological haemorrhage needs to be differentiated from iatrogenic haemorrhage. If blood vessel penetration has occurred at the time of abdominocentesis then the blood is often streaky. Splenic penetration will produce a high PCV and many lymphocytes. Erythrocytes can accompany modified transudates and exudates (via diapedesis), but will never reach the levels experienced in hemorrhage due to rhexis.

iii) In **cattle abdominocentesis** has been used to diagnose peritonitis. Total nucleated cells and protein levels, however, are not as useful as the differential. In healthy animals protein levels, as determined by the refractometer, are up to 30 g/L while total nucleated cells are up to 10,000 x 10^6/L (10 x 10^6/L). Eosinophils are commonly high in normal peritoneal fluid (>30%) while neutrophils are usually less than 50% (nb healthy cows, less than two weeks post-partum may have a nucleated cell count greater than 10,000 x 10^6/L, while either neutrophils or eosinophils may predominate in the differential). In peritonitis, protein levels vary from 20 g/L upwards (top of around 60) while total nucleated cells vary from 1,000 x 10^6/L upwards (top can be over 200,000). However, more importantly, the proportion of neutrophils increases while the proportion of eosinophils drops. The presence of appropriate bacteria and high fibrinogen levels support the diagnosis of peritonitis.

iv) Neoplastic effusions have an extremely variable appearance. Diagnosis is dependent on examination of the cytological smear and identification of neoplastic cells. Malignant neoplasms which commonly exfoliate into body cavities include mesotheliomas, adenocarcinomas and lymphosarcoma. If neoplastic cells have not exfoliated, then the effusion can present as a modified transudate, chylous effusion, an exudate or as a haemorrhagic effusion.

b) Synovial fluid analysis

**Introduction**

Except for septic arthritis, synovial fluid analysis rarely provides a specific diagnosis. What it does do is to provide information that allows placement of the joint problem into a general category (e.g. degenerative, inflammatory and neoplastic). When this is combined with other physical, radiological and laboratory findings a specific diagnosis may be obtained.

Synovial fluid is a dialysate of plasma to which mucus is added by synovial cells as the plasma diffuses through the synovial membrane into the joint cavity.

**Laboratory evaluation of synovial fluid**

i) **Normal gross characteristics**

1) volume: 0.01 - 1 ml for the dog (less in the cat, more in horse)
2) colour: colourless (may be light yellow in the horse)
3) transparency: clear
4) viscosity: noticeably viscous (due to hyaluronic acid content)
5) mucin content: determined by the **mucin clot test**: One ml of synovial fluid is mixed with 5 ml of 2.5% acetic acid and left for at least 1/2 hour. Normal synovial fluid,
which is high in mucin, produces a tight ropy clot in a clear solution (good mucin clot). A fair mucin clot is a soft mass in a slightly turbid solution. A poor mucin clot is a small friable mass in a turbid solution. A very poor mucin clot is a few flecks in a turbid solution.

ii)  **Normal cytological characteristics**

A total nucleated cell count is determined from synovial fluid collected in EDTA. A differential cell count is determined from a smear of synovial fluid (preferably made directly from withdrawn synovial fluid). The dog has a total nucleated cell count of less than 3000x10⁶/L (this depends on the joint sampled) while a horse usually has less than 500x10⁶/L. In health, 90% of the nucleated cells are lymphocytes and monocytes.

iii) **Other characteristics (investigated when indicated by disease)**

Protein levels are mainly of use in the horse. Although not completely accurate, the refractometer can be used to estimate total protein (in a healthy horse the value is usually 10-15 g/L). Glucose levels in health, are often equal or slightly higher than in the serum, but may be reduced in disease. Certain enzymes (e.g. AST, ALP) can be measured in the horse.

*When collecting synovial fluid, place some in an EDTA tube and some in a plain tube (sterile if you suspect septic inflammation). Make a smear immediately from withdrawn fluid. Often in the dog and cat not enough fluid is obtained to do all tests. Priority should be given to the smear (for differential cell count) and a total nucleated cell count, unless the history indicates otherwise.*

**Changes in synovial fluid characteristics with diseases:**

Refer to table on following page.

Basically, synovial fluid changes due to disease are categorised as acute degenerative/traumatic, low grade/long term degenerative or primary inflammatory (non-septic or septic). Note: lytic (degenerate) neutrophils, which are common in septic inflammation anywhere, are sometimes not obvious in septic synovial fluid due to the apparent protective nature of chemicals present. Neoplasms involving joints and causing synovial fluid changes are uncommon.
### Table for synovial fluid analysis showing changes in disease

<table>
<thead>
<tr>
<th></th>
<th>Acute degenerative/ traumatic conditions</th>
<th>Low grade/Long term degenerative conditions</th>
<th>Primary inflammatory (non-septic)</th>
<th>Primary inflammatory (septic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>Increased</td>
<td>normal to increased</td>
<td>increased</td>
<td>increased</td>
</tr>
<tr>
<td>Colour</td>
<td>normal to discoloured (red)</td>
<td>normal to discoloured (red-brown)</td>
<td>discoloured</td>
<td>discoloured</td>
</tr>
<tr>
<td>Turbidity</td>
<td>slight to marked</td>
<td>normal to slight</td>
<td>slight to marked</td>
<td>marked</td>
</tr>
<tr>
<td>Fibrin clot</td>
<td>-ve to +ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Variable</td>
<td>normal to slight</td>
<td>decreased</td>
<td>decreased</td>
</tr>
<tr>
<td>Mucin</td>
<td>fair to poor</td>
<td>normal to fair</td>
<td>fair to very poor</td>
<td>poor to very poor</td>
</tr>
<tr>
<td>Total nucleated cells</td>
<td>slight to moderate</td>
<td>normal to slight increase</td>
<td>moderate to marked increase</td>
<td></td>
</tr>
<tr>
<td>Differential cell count</td>
<td>erythrocytes, neutrophils</td>
<td>macrophages (cartilage fragments may be seen)</td>
<td>mainly neutrophils (non-lytic (non-degenerate) for non-septic, usually lytic (degenerate) for septic) plus erythrocytes (via diapedesis), macrophages and lymphocytes (microbes for septic)</td>
<td></td>
</tr>
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c) Cerebrospinal fluid evaluation

Introduction
Cerebrospinal fluid (CSF) is produced partly by diffusion from plasma and partly by active secretion from the choroid plexuses and ependymal linings. In the dog and cat, CSF is collected usually from the subarachnoid space at the atlanto-occipital articulation. In the horse and dog the subarachnoid space at the lumbosacral articulation can be used.

Laboratory evaluation of CSF

i) Physical Examination
1) Colour
In the healthy dog, cat and horse CSF is colourless. Xanthochromia (yellowing of the CSF) may occur in a jaundiced animal or in an animal that has had previous haemorrhage in the CSF. Red CSF may indicate recent haemorrhage (pink to orange supernatant) or haemorrhage at the time of collection (clear supernatant). A grey-green CSF may indicate suppuration.

2) Turbidity
Normal CSF is clear. A turbid CSF suggests excess cells (turbidity is only observed when the cell count is in excess of 500x10^6/L) or perhaps protein or bacteria.

3) Coagulation
Normal CSF doesn't clot. Clotting may be due to inflammation or haemorrhage.

ii) Total nucleated cell count
CSF is collected in an EDTA tube (in case of risk of clotting) and in a plain serum tube (for protein estimation). If infection is suspected some CSF should be collected in a sterile tube. The total nucleated cell count (usually done on the EDTA tube) has to be performed quickly as there is rapid degeneration of cells (usually within a few hours). Pleocytosis (an increase in the number of nucleated cells in CSF—in health: the healthy dog, cat and horse have counts usually 5 x 10^6/L or below; 5-8 is a grey zone for increases) may be due to trauma, primary inflammatory conditions, toxic conditions or neoplasia. Pleocytosis requires the performance of a differential cell count (if total cell count is greater than 500x10^6/L then a smear should be made directly from the CSF, if the total count is less than 500x10^6/L then ideally a smear should be made of the sediment of CSF after unit centrifugation).

iii) Differential cell counts
In practice, smears should be made at the time of collection, irrespective of total nucleated cell count. This is because cells in CSF deteriorate rapidly.

In a normal sample most cells are small lymphocytes (around 60-70%) and monocytes/macrophages (20-30%). In pyogenic infections the cells are mostly lytic (degenerate) neutrophils. In haemorrhage the cells are mostly non-lytic (non-dégénerate) neutrophils. In viral, uremic, some chronic bacterial and toxic conditions, lymphocytes and monocytes usually predominate. Neoplastic cells may be seen in meningeal tumours.

iv) Protein estimation
Normal CSF has a low level of protein (less than 0.3 g/L for dog, 0.2 g/L for cat and 0.67 g/L for horse). Most of the protein is albumin. Increases in CSF protein may occur in a variety of inflammatory conditions (primary or secondary) and principally involve increases in globulins. To detect increased levels of protein, either total protein (Ponceau S method) or
globulin (Pandy test) levels can be determined. Urine protein reagent strips will provide an indication of increased protein, but it is a crude method.

**General comments on disease affecting CSF**

The greatest alterations to CSF usually occur with meningeal disease. Nervous parenchymal disease may cause minimal changes to the CSF. CSF analysis is an aid to diagnosis and requires to be analyzed in conjunction with history, clinical signs and other diagnostic procedures performed.

Commonly, CSF changes are categorized as **degenerative** (eg disc disease), **inflammatory** (subcategories include non-suppurative, suppurtive and granulomatous depending on proportions of cells present) and **neoplastic** (primary or metastatic). Sometimes, infectious agents may be detected on the cytological smear (eg cryptococcal and toxoplasmal agents).

d) Analysis of airway and pulmonary lesions by respiratory washes (transtracheal aspirates and bronchoalveolar lavages)

Respiratory washes for airway and pulmonary disease can be performed in any animal species, but is commonly done in the dog, cat and horse

**Sampling techniques**

Collection of cells from the tracheal and bronchoaveolar surfaces can be done in a number of ways. **Bronchoaveolar lavage (BAL)**, via a bronchoscope or catheter, provides the **best cellular samples of the lower respiratory tract** (alveolar spaces and smaller airways). Occasionally, oropharyngeal contamination may occur by this method (usually related to contamination from the endotracheal tube). **Transtracheal aspiration (TTA)** will usually provide **sterile samples for microbiological investigation** but cell retrieval, especially from the alveolar spaces, may be limited.

**Oropharyngeal contamination of respiratory washes**

This will be recognised by the presence of squamous epithelium (often with associated bacteria). Specific oropharyngeal bacteria, such as *Simonsiella* sp, may be present. This will jeopardise the investigation of a possible infectious process affecting the lower respiratory tract. Moreover, any neutrophils present may be related to oropharyngeal inflammation rather than lower respiratory inflammation.

**Types of lining cells and other structures present in respiratory washings**

Cellular features will vary depending on whether the sample has been taken by TTA or by BAL. BAL: will contain a greater proportion of alveolar macrophages and lower airway epithelial cells, and will not usually have oropharyngeal contamination.

**Ciliated columnar epithelium**

The majority of tracheal and bronchial cells will be **ciliated columnar cells**. They have small round to oval nuclei situated at the end of the cell not displaying the plate of cilia. Cilia are often lost in the washing process and will be found free: they can be mistaken for filamentous bacteria.

**Mucosecretory (goblet) cells**

These are large oval to bulging, elongated cells containing numerous large deep-pink granules in their cytoplasm.
**Bronchoalveolar cells**
These refer to the small, round to square cells, often present in clusters. Their nuclei are usually round and they have moderate amounts of blue-grey cytoplasm. Some of these cells are basal cells while others may be lining cells from the lower airways. Some may be pneumocytes just becoming macrophages.

**Alveolar macrophages**
Alveolar macrophages are usually large with obvious cytoplasmic vacuoles or ingested material (eg blue granules of haemosiderin, black-brown granules of carbon). There are always some alveolar macrophages present in washes and, rather than indicate disease, may simply indicate that the sample is truly representative of alveolar spaces. Of course, increased numbers, their appearance and the presence of specific inclusions may indicate disease.

**Mucus**
This is commonly present in washes and will be pink to light blue. Excessive and prolonged secretion of mucus will occur in a variety of chronic respiratory conditions (inflammatory or neoplastic). In these circumstances the mucus often stains a darker blue. In addition there may be dark blue to purple spirals of inspissated mucus called Curschmann's spirals.

**Inflammatory cells**
Inflammatory cells (neutrophils, eosinophils, mast cells and lymphocytes) may be present in normal respiratory washes but they are usually in low numbers (eg less than 5% for neutrophils, only occasional mast cells). An exception is eosinophils in cats where levels of up to 15% can be normal.

**Commonly diagnosed airway and pulmonary conditions on the basis of respiratory washes**
Many of these are simply divided into pathological processes, the main ones being inflammatory, vascular or neoplastic.

**Acute (active) inflammation**
In the dog, cat and horse this will be characterised by high levels of neutrophils and lesser numbers of alveolar macrophages. In some cases this will be related to **bacterial infection** (with appropriate intracellular bacteria – septic inflammation). At other times there will no bacteria visible, nor will any be cultured. This **type of sterile (non-septic) inflammation** may accompany neoplastic conditions but can also be idiopathic (even though some appear to respond to antibiotics in dogs and cats!). Active inflammation can be present in chronic conditions of the respiratory tract. Septic inflammation is often a sequellae of viral pneumonia.

**Allergic or parasitic (hypersensitivity) inflammation**
This will commonly be characterised by significant numbers of eosinophils. Mast cells and neutrophils will be present in variable numbers. Often, many of the eosinophils and mast cells will degranulate, thereby producing a granular background. Parasites, such as lungworm or microfilaria, may be visible in the washes.

**Chronic Obstructive Airway Disease** in horses is regarded as a hypersensitivity. However, the presence of increased numbers of eosinophils is variable, and may just be a feature of early disease. Most features are consistent with those seen for chronic respiratory disease.

**Mycotic (fungal) inflammation**
Eosinophils may also be a prominent component of mycotic (fungal) inflammation due to similar hypersensitivity mechanisms which occur in allergies or parasitic infections. However, the inflammatory profile is more variable depending on the agent and the duration of the disease. Both purulent and pyogranulomatous responses may occur. Yeasts may be present in Cryptococcus neoformans infection, while fungal hyphae may be present in Aspergillus spp infection. Multinucleated macrophages may be present but are not specific for this type of infection. They can also occur in foreign body reactions and be present non-specifically in any form of chronic lung disease.

**Pulmonary haemorrhage**

This is not usually a specific feature and can occur in most conditions affecting the lungs. However, it can be a prominent feature in trauma, heart failure, infarction (e.g. heartworm), bleeding disorders, certain types of neoplasia and certain poisons (e.g. paraquat toxicity). Erythrophagocytosis by alveolar macrophages will be visible in acute haemorrhage, while haemosiderin-laden macrophages are more of an indication of past or chronic haemorrhage.

**Exercise-Induced Pulmonary Haemorrhage (EIPH)** occurs in horses as a consequence of strenuous exercise. TTA, and especially BAL will support the diagnosis. In the early stages erythrophagocytosis will be prominent, while haemosiderin-laden macrophages will develop and remain for weeks after the event.

**Chronic respiratory disease**

The features of chronic respiratory disease are variable and depend on the cause (i.e., these are non-specific findings that may be related to a wide variety of causes). Mucin and increased numbers of macrophages are common features but are not highly specific. Goblet cell and general epithelial hyperplasia are common if there is airway involvement. There may be squamous metaplasia with chronic irritation to lining cells.

**Primary and secondary neoplasia**

Neoplasia of the lungs cannot always be diagnosed on respiratory washes. There is more chance of diagnosing primary epithelial lung tumours (commonly adenocarcinomas) than metastatic tumours. Fine needle aspiration or biopsy of the pulmonary tissue is usually required to diagnose neoplasia. Consequently, most of the information on pulmonary neoplasia has been presented in the next part. Primary and metastatic neoplasia are rare in the horse.

e) Urinalysis

Apart from its obvious uses in detecting renal disease and lower urinary and genital tract disease, it is useful also in analysing many extra-renal problems e.g., diabetes mellitus. Therefore, urinalysis will be presented in a form that covers its uses for all possible abnormalities.

Urinalysis involves the following procedures and should be done completely in the first instance as often the results are interrelated.

i) observation of physical properties

ii) estimation of solute concentration

iii) chemical analysis

iv) sediment examination

v) optional examinations based on the results of the first 4 procedures

The way urine is collected will alter the results of urinalysis. Ideally, a 24 hour sample is necessary for accurate quantitative analysis, but in practice a single sample is analysed. Once the sample has been collected it should be analyzed quickly (within the first 2-3 hours - store at 4 degrees C if analysis cannot be done straight away).

A) Observation of Physical Properties

1) Volume - volume estimation ideally should be done on urine collected over a 24 hour period. This will allow the detection of increased volume (polyuria) or decreased volume (oliguria). Volume
changes can be transient or persistent (pathological)*. Normally an indication of volume is gained from
the measurement of specific gravity (or osmolality). The generalization is that low specific gravity means
high volume (exception: acute renal failure, oliguric phase of chronic renal failure).

*Transient polyuria (eg diuretic therapy, increased fluid intake, parental administration of fluids and
administration of ACTH and/or corticosteroids) or pathological (eg pyometra, hyperadrenocorticism,
diabetes insipidus, diabetes mellitus, polyuric phase of renal failure). Transient oliguria (eg decreased
water intake, high environmental temperature, hyperventilation in the dog) or pathological (eg shock,
fever, dehydration, urinary tract obstruction, oliguric phase of renal failure).

2) Colour - The normal colour of urine is dependent on the concentration of urochromes
(amorphous pigments and metabolic waste products). Normal urine for most of the domestic species is
varying shades of yellow or light amber (Note: horse's urine may turn brown on standing).

3) Turbidity - Normal urine is clear to mildly turbid. Turbidity, if present, indicates crystals, cells,
mucus and other formed elements in high concentrations. Turbidity may develop on standing of urine due
to the precipitation of crystals or bacterial overgrowth.

4) Odour - The normal odour of urine is derived from volatile organic acids. Abnormal odours
include ammonia and ketone bodies.

If abnormal gross characteristics are detected then the rest of urinalysis should detect the reasons for the
changes. If the gross characteristics are normal it does not mean that the urine is normal and the other
components of urinalysis should still be performed.

B) Solute Concentration
This gives an indication of the ability of the kidney tubules to dilute or concentrate excretory products (ie
a true function test). In addition, an estimation of solution concentration is essential for the correct
interpretation of levels of chemicals and cells.

Solute concentration can be determined by measuring the specific gravity of urine. Specific gravity is
dependent on the types of solutes, their numbers, molecular size and weights eg salts can affect specific
gravity significantly because of their low molecular weight and large numbers.

Specific gravity is measured today with a refractometer. If the urine is excessively turbid, it should be
centrifuged prior to determining specific gravity (if not the specific gravity may be falsely elevated by
002-004 units).

Solute concentration (as determined from specific gravity) is inversely related to urine volume in health
and in many disease processes. Isosthenuria can be defined as continued excretion of urine at the specific
gravity of the glomerular filtrate ie 1.008 - 1.012.

A specific gravity reading of 1.008 - 1.012 from a single urine sample may indicate:
1) a chance finding in a normal animal (eg the dog kidney is able to dilute urine to 1.001, and
concentrate urine to 1.060 [1.080 for the cat])
2) renal failure. Unlike (1), repeated urine samples will have a specific gravity of 1.008 to 1.012 or
close to it.

Proteinuria or glucosuria may elevate the specific gravity reading (usually by 001-005 units depending on
the levels). In renal failure with proteinuria this may take the reading out of the isosthenuric range.

Hyposthenuria can be defined as the continued excretion of urine of low specific gravity ie less than
1.008. A low specific gravity may be found occasionally in a normal animal but if it is constant it points
to such conditions as diabetes insipidus, psychogenic polydipsia and nephrogenic diabetes insipidus. At
times the specific gravity may be in the isosthenuric range for these conditions. Beware, healthy suckling
animals may have continued excretion or urine below 1.008!
Rather than measure specific gravity for an estimation of solute concentration, the osmolality of urine can be determined. This appears to give a better estimation of the solute concentration as it is dependent only on the number of molecules rather than their molecular size or weight. Osmolality is expressed in milliosmoles and is measured by an osmometer (the measurement commonly involves the degree of freezing point depression which is directly proportional to solute concentration). Urine osmolality assessment is normally done in conjunction with plasma/serum osmolality to determine the concentrating ability of the kidney (the ratio of urine to plasma osmolality on single blood and urine samples will provide an accurate assessment of renal concentrating ability eg a plasma reading of 297 and a urine reading of the same indicates no concentration by the kidney). Osmometers, however, are expensive and specific gravity will continue to be used for estimation of solute concentration in veterinary practice.

C) Chemical analysis of urine
Reagents strips and tablets are commonly used to detect chemicals in urine. They are screening tests and consequently have limitations. If more accurate measurement of a urinary chemical constituent is required, a 24 hour sample of urine should be collected.

NB: Chemical constituents of urine are determined according to concentration. Increases in concentration may occur due to increases in total amount of the chemical constituent excreted (true elevation) or be due to decreased urinary volume which is indicated by an increased specific gravity reading (relative or false elevation).

1) Protein - Urine from a normal animal has minimal levels of protein (mainly albumin). Reagent strips are used as a screening test for protein as they are sensitive to albumin but they are less sensitive to globulins (which will include Bence Jones protein). Highly alkaline urine and urine with high haemoglobin or myoglobin may give false positives on the reagent strip. Consequently, if a positive is recorded on the reagent strip, it is best to check it by another method eg sulfosalicylic acid (SSA) precipitation (nb all herbivores commonly have alkaline urine). SSA is less sensitive to albumin but detects all protein.

The magnitude of proteinuria can be assessed relatively accurately by determining the ratio of urine protein to urine creatinine (UP/UC or UPC). Protein is usually assessed by a microprotein method. A UPC ratio less than 0.5 is usually considered normal, a ratio between 0.5-1.0 is minimal or of questionable significance, whilst values equal or greater than 1.00 indicate significant proteinuria. Proteinurias related to glomerular disease usually give the greatest UPC ratios.

Proteinuria can be transient/physiological (eg exercise, estrus) or pathological (eg cardiac disease, glomerular/tubular damage, lower urinary or genital tract inflammation). Physiological causes of proteinuria usually produce minimal elevations (rarely greater than 1+).

NOTE: Haemorrhage: the protein strip may show a 1 to 3+ reading if the blood strip is showing a maximal reading.

2) Glucose - Glucose is passed by the glomerulus and usually completely reabsorbed by the tubules unless the renal threshold is exceeded (cats around 15-16 mmol/L, dogs and horses around 10-11 mmol/L, cattle around 5-6 mmol/L).

The 2 main methods of glucose estimation are reagent strips utilizing glucose oxidase and the Clinitest® reagent tablets utilizing the copper reducing power of glucose and other sugars. The reagent strips can give false negatives in the presence of high ketone or ascorbic acid levels. The Clinitest® may give false positives in the presence of antibiotics, salicylates and other sugars.

Glucosuria can be transient (eg stress, heavy meal of carbohydrate – related to transient hyperglycaemia) or pathological (most related to persistent hyperglycaemia). Transient glucosuria is particularly common in ruminants (as mentioned cows have a low renal threshold of about 5-6 mmol/L) and can occur not only in physiological changes but also in diseases that cause transient hyperglycaemia (eg neurological
diseases, bovine milk fever, ovine enterotoxaemia). Transient glucosuria in the cat can occur due to excitement or stress. By the time it is identified the hyperglycaemia may have disappeared.

In dogs, cats and horses glucosuria is usually related to diseases causing persistent hyperglycaemia, and, therefore, is persistent itself. In the dog and cat diabetes mellitus is the common cause but in horses it can occur in both hyperadrenocorticism and diabetes mellitus.

Glucosuria unrelated to hyperglycaemia is uncommon but in the dog, cat and horse has been recorded related to specific tubular diseases.

3) Ketones - Blood ketones increase whenever there is an increase in lipolysis or an interference with lipogenesis of the TCA cycle (ie defective carbohydrate metabolism). Since the renal threshold is low for ketones, ketonuria is often detected before a significant ketonaemia develops.

Ketonuria in dogs and cats in commonly related to diabetes mellitus. It may be seen also in puppies and kittens that have been starved (rarely in adults). In ruminants ketonuria is non-specific and can occur in starvation, high fat diet, bovine ketosis, ovine pregnancy toxaemia and in a variety of diseases that induce anorexia. Ketonuria is uncommon in the horse.

Ketones in the urine can be detected by reagent strips (specific for acetoacetate) or by Acetest® tablets (reacts with acetone and acetoacetate).

4) Bilirubin - Detected by the Ictotest® or by the bilirubin strip which are mainly reactive with conjugated bilirubin and rather insensitive to unconjugated bilirubin. The significance of bilirubinuria has been discussed in the laboratory evaluation of liver disease. In all common domestic species except for the dog bilirubinuria means conjugated hyperbilirubinaemia. In the dog, especially the male, bilirubinuria is not highly specific for conjugated hyperbilirubinaemia because of the low renal threshold and a renal capacity to conjugate and even breakdown haemoglobin to bilirubin. However, the presence of marked amounts of bilirubin in the urine of a dog usually means hyperbilirubinaemia.

5) pH - In the dog and cat the pH is normally 5.5-7.5 In the normal horse the pH is close to 8. Ruminants have a pH 7.4-8.4. Most young animals suckling, irrespective of species, will have acid urine.

Urine pH must be measured promptly and is the result of renal regulation of blood pH. It should not be used alone to evaluate acid/base status as it is heavily influenced by diet, therapy and urinary tract disease.

Aciduria occurs with a carnivorous diet, starvation, fever, metabolic acidosis and renal tubular acidosis. Occasionally, aciduria occurs with metabolic alkalosis because of the kidney concentrating more on correcting related electrolyte imbalances (hypochloraemia and hypokalaemia) and dehydration. This is called ‘paradoxical aciduria’, as it is unexpected, and can be seen in severe vomiting, gastric or abomasal reflux (the last two are so called ‘internal vomiting’ in herbivores) where both HCl and other electrolytes (such as potassium) can be lost to the body. Alkaline urine may be due to a herbivorous diet, cystitis (depends on the type of bacteria present - urea splitting bacteria will commonly produce alkaline urine), urine retention, metabolic alkalosis or extended standing of urine before testing (bacterial action).

6) Blood - The reagent strip test is based on the peroxidase activity of haemoglobin or myoglobin. The test is more sensitive to free haemoglobin than to haemoglobin within the erythrocyte although the two are supposed to be able to be distinguished on the strip (don’t rely on the strip for this - examine the sediment for erythrocytes).

A positive test means haematuria, haemoglobinuria or myoglobinuria. Haematuria can be distinguished on sediment examination while the other 2 can be distinguished on ammonium sulphate precipitation and plasma colour (plasma needs to be saturated with haemoglobin ie obviously red, before haemoglobin appears in urine. This is not the case with myoglobin as it is a smaller molecule and more easily passed by the kidney).
Haematuria indicates urogenital tract disease (especially if the sample has been voided), haemoglobinuria indicates intravascular haemolysis or lysed erythrocytes (may occur at low specific gravities), and myoglobinuria indicates muscle necrosis.

D) Sediment examination
What structures are present in the sediment will depend not only on disease but also on how the sample of urine is collected (ie voided, catheterisation or cystocentesis). Structures present in urine can be divided into 3 groups:

Group 1 consists of those formed elements that are present in health in low numbers. Increased numbers of these structures may have little significance unless indicated by other information eg most epithelial cells, many types of crystals, lipid, sperm, mucus, fungi, bacteria.

Group II, like group I, consists of formed elements present in health in low numbers. However, increased numbers commonly have significance eg casts, erythrocytes, leukocytes.

Group III consists of formed elements of sediment that are not normally present in urine eg neoplastic epithelial cells, unusual crystals, unusual casts. Their presence in any numbers are significant.

Below is information on elements that may be found in urine. Usually urine is concentrated by centrifugation prior to examination to ensure recognition of structures. In this laboratory 10mls of urine is centrifuged and the sediment is resuspended in about 0.5mls of supernatant. Other laboratories may centrifuge 5mls of urine and resuspend sediment in 0.5mls of supernatant: this will alter normal levels of cells presented below. Irrespective of the amount it is important to maintain a standard technique to ensure direct comparability of results.

1) Epithelial Cells

*Renal epithelium. In the dog and cat, their numbers are not a reliable indication of renal disease unless they are in clusters and associated with casts.

*Caudate epithelium. Primarily derived from the renal pelvis, ureter and prostatic urethra. Increased numbers may indicate disease of the areas mentioned if other indicators of disease are also present eg inflammatory cells.

*Transitional epithelium. Principally from the bladder. Their significance is questionable.

*Squamous epithelium. Derived from the lower urogenital tract and of little significance. They usually indicate contamination.

*Abnormal cells eg carcinoma cells. Significance obvious.

2) Erythrocytes
Indicates haematuria if greater than 5 per high powered (x 40 obj) field in moderately concentrated urine.

3) Leukocytes
Increased numbers (ie greater than 3-5 per high powered [x 40 obj] field) in moderately concentrated urine indicates inflammation in the urogenital tract. In cows, it has been suggested that greater than 8 per high powered [x 40 obj] field is more appropriate for a voided sample. Beware, leukocytes tend to disintegrate in alkaline or hypotonic urines that are left to stand (even if stored at 4oC). Consequently, in some bacterial cystitides with highly alkaline urine there could be few leukocytes present. Additionally, some bacteria may cause direct lysis of leukocytes and lead to false low numbers. Remember, a voided sample of urine may have leukocytes from the genital tract as well as the urinary tract.

4) casts
Casts are precipitates of protein formed in the distal convoluted tubules and the collecting tubules. They
conform to the shape of the tubules and may contain any material present in the tubular lumen at the time of formation. A small number of casts, especially hyaline casts, may be found in normal urine (up to one per low powered [10x objective] field).

The significance of cylindruria depends on the numbers and their types. However, in moderately concentrated urine greater that 1 cast per low powered (x 10 obj) field is suggestive of active renal damage, decreased urine flow or renal perfusion, or a response to renal damage. Persistent cylindruria is more significant than transient cylindruria in identifying renal damage.

*Hyaline casts. Increased numbers have been noted in mild renal tubular damage, fever conditions, and as a consequence of surgery or exercise.

*Granular casts. When hyaline casts have refractile particles embedded in them, they are called fine or course granular casts. The granules were thought to be derived from disintegrating tubular epithelium and, therefore, indicate tubular degeneration and necrosis, but recently the granules were shown to be composed of fractions of various serum proteins. Therefore, granular casts have the same significance as hyaline casts. These are the most common type in dogs.

*Waxy casts. They are thought to be derived from granular casts and indicate advanced renal disease. The presence of any waxy casts is significant.

*Fatty casts. Granular casts with a high degree of fat. These are the common type in the cat.

*Renal epithelial casts. Well preserved renal epithelium within the cast may indicate acute tubular damage. Any casts are deemed significant.

Casts may also contain erythrocytes and leukocytes (any number are significant and suggest cell origin in the tubules), and may be stained with bilirubin.

5) Lipid droplets
Increased amounts may occur in obesity, high fat diet, hypothyroidism and diabetes mellitus. Lipid may be seen in normal dog and cat urine (very common in the cat). The lipid is derived from the renal tubular epithelium.

6) Spermatozoa

7) Fungi
The yeasts and hyphae are nearly always contaminants. Significance will depend on the presence or absence of inflammation. Fungal cystitis can occur, mainly with Aspergillus spp, and should be accompanied by increased leukocytes.

8) Bacteria
The significance of bacteria in the urine will depend on determining an association with inflammation in the urogenital tract. Occasionally, leukocytes will not be obvious due to the destruction by bacterial toxins, low specific gravity or highly alkaline urine. Consequently, inappropriate bacteruria without pyuria should be investigated further. Cystocentesis should provide a sterile urine sample, whereas a voided urine sample will be invariably contaminated with bacteria from the lower urethra or genitalia

9) Crystals
Crystals are frequently found in urine and their precipitation is dependent on their concentration and the pH of the urine. They are usually of little significance but leucine/tyrosine/ammonium biurate (the last may occasionally occur in healthy dogs, especially Dalmatians) crystals may be indicative of chronic liver disease and/or porto-systemic shunting, oxalates (mainly calcium oxalate in the monohydrate form) of ethylene glycol toxicity, sulphonamides of nephrosis, and bilirubin or haemoglobin crystals of acute haemolytic states or haemorrhage.