Cytopathology

- What is useful and what value adds for the referring veterinarian?
- What are the difficult or controversial parts – I will cherry pick again!
- How does it complement histopathology?

Australian Animal Pathology Standards Program (AAPSP) 2013 Roadshow

A journey through the cytopathologist's World of thoughts and illusions – down the microscope everything is an artefact, but some are useful!

Professor Emeritus Paul Canfield, Faculty of Veterinary Science, University of Sydney

Cytology (cytopathology) – a bit of history

- Cytology: a science that has existed ever since the microscope was invented in the 1600's.
- Rudolph Virchow: In 1859, his book *Cell Pathology* became the foundation for all microscopic study of disease. Cytopathology is followed by histopathology in the late 1800's
- In veterinary diagnosis, cytopathology becomes a poor relation to histopathology, but rediscovered in the late 60's and especially in the 70's
- Cytopathology began again in the realms of the histopathologist, but was passed over to the clinical pathologists (not everywhere!) in the late 1970's and early 80's.
  - Now, with the growth of clinical specialists and the improvements in general practice cytopathology is practiced by all. (Christopher *et al Vet Clin Path* 2008: of 870 veterinary practitioners 48% did in-house evaluation, 22% did in-house followed by lab, and 30% sent directly to diagnostic lab)
- Cytopathology ***complements*** the use of the biopsy (histopathology)

Relative merits of cytology versus biopsy

CYTOLOGY

- Easy and quick, and often cheaper than a biopsy
- Requires little equipment to produce and stain smears
- Excellent visualisation of cells, agents of disease and acellular material under 100x oil objective (if you have a thin smear!)

BIOPSY

- Reasonably good for visualisation up to 40x
- Excellent for architecture (big picture!)
- Examines larger area of mass and more cells (therefore, less error?)
- Essential for examining margins and grading tumours

Biopsy is regarded as the 'gold standard', but how do the two correlate?

Chronological overview of cytology and histopathology correlative studies

- **1960s** - first important cytology paper for veterinary medicine *(Roszel JF: **Exfoliative cytology in diagnosis in malignant canine neoplasms.** Vet Scope 1967;12:14-20)*
- **Mid 1980s** - first cyto-histo correlative studies published (mainly investigating skin masses and superficial peripheral lymph nodes)- concentrating on inflammation vs neoplasia *(Griffiths GL., Lumsden JH., Valli VEO. **FNA cytology and histologic correlation in canine tumours**.Vet Clin Pathol 1984; 13:13-17 & Mills JN., Griffiths GL. **The accuracy of clinical diagnoses by fine needle aspiration cytology.** Aust Vet Journal 1984; 61: 269-271)*
- **Last decade** - routine ultrasonography and availability of other imaging-guided techniques has increased the investigation of deep intra-cavity lesions which has led to more correlative studies with histopathology

So, how do the correlative figures stack up?

- Skin masses: one study showed over 90% correlation, but most 75-90%
- Internal organ masses : studies show between 56-90% correlation, but is closer to 85%+ if FNAs are US-guided and splenic masses are excluded

- % Agreement for internal masses depends on whether you accept **partial** (pathological process) or **exact diagnoses** – *cytology has most problems with exact identification of some tumours*

Some correlation studies – 56-90%

	2004	*2003*	*2000*	*1986*	*1984*
First Author	Bonfati JSAP	Cohen JAVMA	Eich JAAHA	Menard CVJ	Mills AVJ
Title	Percutaneous FNB of deep thoracic and abdominal masses in dogs and cats	Evaluation of sensitivity & specificity of cytologic examination: 269 cases	Accuracy of intra-operative cytopath Dx cf with histopath	FNAB of malignant tumours in Dogs and Cats	Accuracy of clinical diagnoses by FNA cytology
Number of cytological specimens examined	152 (D,C)	269 (mainly D,C)	100 (D,C,H)	102 (D, C)	246 (D,C,H,R)
% Cytologic diagnoses in agreement with histopathologic diagnosis	89.4%	56.1%	83% to 90% if splenic masses excluded	69%	90.4%

General consensus: false-negatives (missing a diagnosis) were far more common than false positives (diagnosing a healthy animal with disease).

Why isn't cytopathology as accurate as histopathology?

1) ***Inherent limitations of the discipline*** – only examining cell detail in most cases
2) ***Limitations of collecting the sample*** (depends on method and timing) method
3) ***Limitations because of the type of lesion*** – some are just difficult to sample because of firmness or fragility
4) ***Individual limitations of the cytopathologist*** – error due to training or just having a bad day. However, this can also apply to the histopathologist! Need to think about how you think (and what can go wrong).

My approach to cytopathology heavily influenced by my past experiences and my personality

The 3 D's for diagnosis : detect, describe, deduce

The 5 pathological processes

Move from the general to the specific if I can't see a pattern

The 5 causes of disease

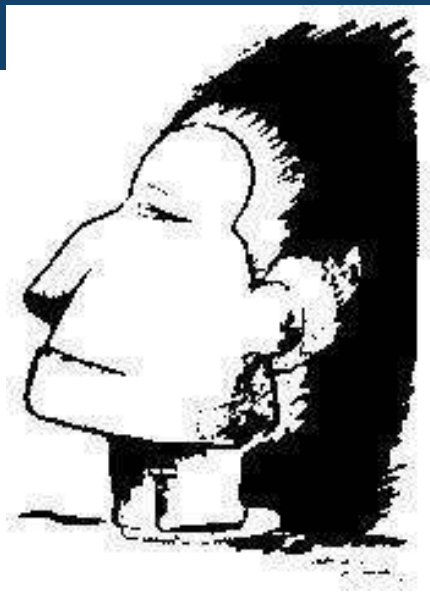
As you can see that left side of my brain predominates!

My 3 D's for Cytodiagnosis to keep me on 'the straight and narrow'

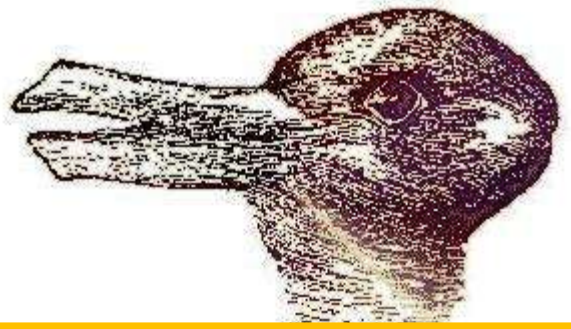
- **Detect (perception)**
  - **'sobjective'**, requires a good microscope and adequate eyesight for good sensory perception – I make sure that I look at slides at the best time of day for me!
- **Describe**
  - **objective**, you need to use a comprehensive glossary of terms – an 'anally retentive' personality is good for this (guess who has one of those?)
- **Deduce**
  - **subjective**, influenced by past experiences, your present mood and personality (The 3 'P's' significantly influence *diagnostic reasoning* for me) but I have developed a method based on a **template**!

Detect – sensory (visual) perception

- **A REMINDER FOR US ALL:**
  - Need a *good microscope*
  - Need a *set method* to examine a slide (so it becomes automatic)
  - Need a *good smear* so that you can look at the **monolayer area** with **minimal cell damage and blood contamination** (Isn't this the cytopathologist's mantra?)

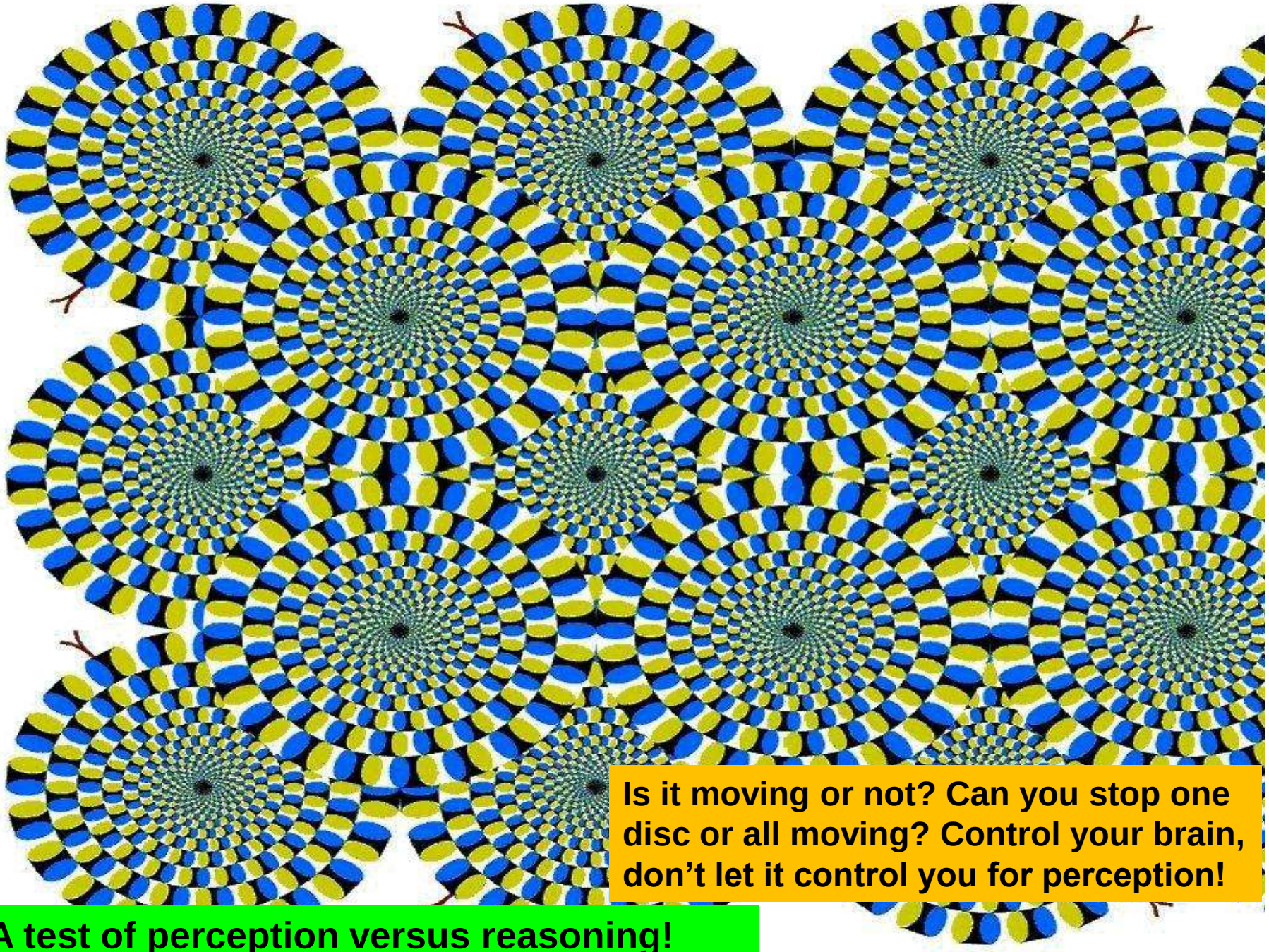


Effective Detection also requires an understanding of how **perception** works *ie how does the brain take in visual information and organise it*
And be wary, for sometimes what you see is not what it seems (*perception deception undermines good detection*)



can you trust what you see, smell or hear?
Perceptual organization: is it all innate or can some of it be learned? Both Pathologists and Clinicians integrate sensory perception in their brains **with a bias (**the three Ps influence decisions). That is because some of the signal goes directly to the amygdala (emotional centre) rather than to the cortex

"The world is full of obvious things which nobody by any chance ever observes."
Sherlock Holmes in *The Hound of the Baskervilles*



Is it moving or not? Can you stop one disc or all moving? Control your brain, don't let it control you for perception!

A test of perception versus reasoning!

Look at the chart and say the COLOUR not the word

YELLOW BLUE ORANGE
BLACK RED GREEN
PURPLE YELLOW RED
ORANGE GREEN BLACK
BLUE RED PURPLE
GREEN BLUE ORANGE

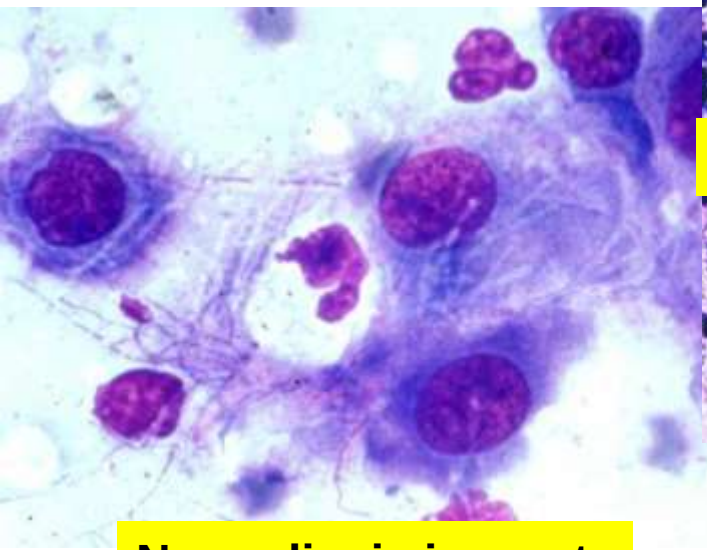
Left – Right Conflict

Your right brain tries to say the colour but your left brain insists on reading the word.

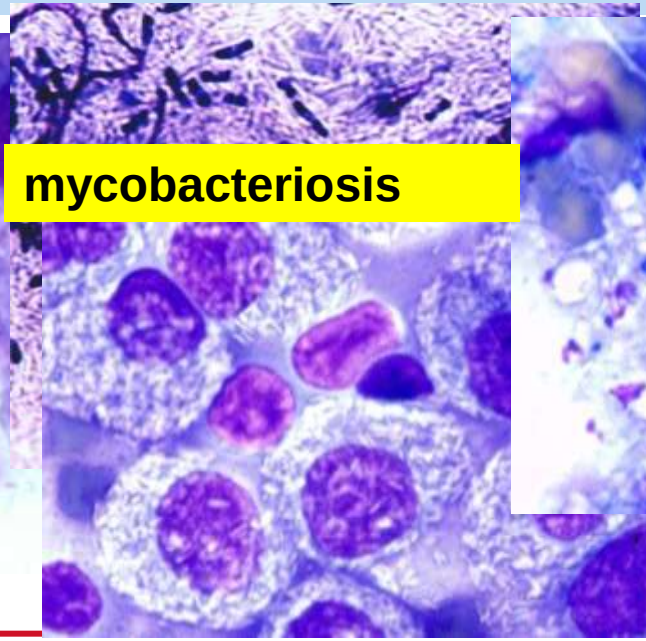
Perception depends on what part of the brain you are using!

That was a reminder about the limitations of sensory perception in both detection and interpretation

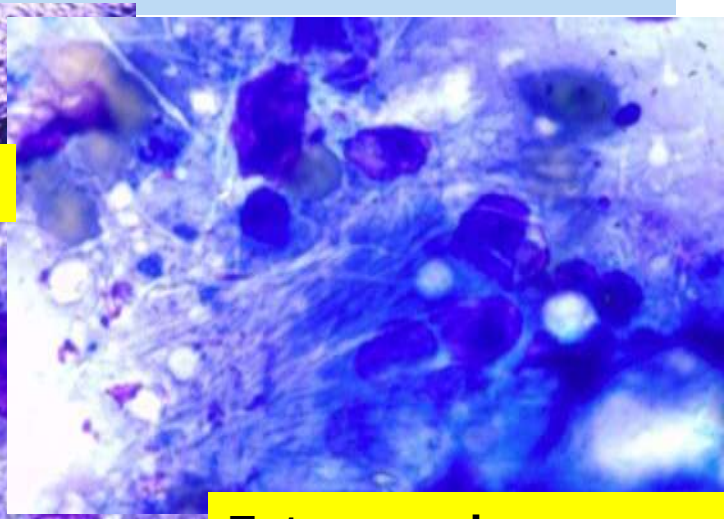
- When looking down the microscope your eyes detect shapes and colours of objects (and its context with surrounding objects) and then your brain tries to **pattern recognise** the object from past experiences (heuristics!)
- If it can't do this then it might try and search out for something similar (another heuristic!). If there is more than one similar object in memory then it needs to choose (part of the reasoning process). Of course, the brain may also say to you that it hasn't got a clue (panic or the 'Oh Sh**' feeling)!



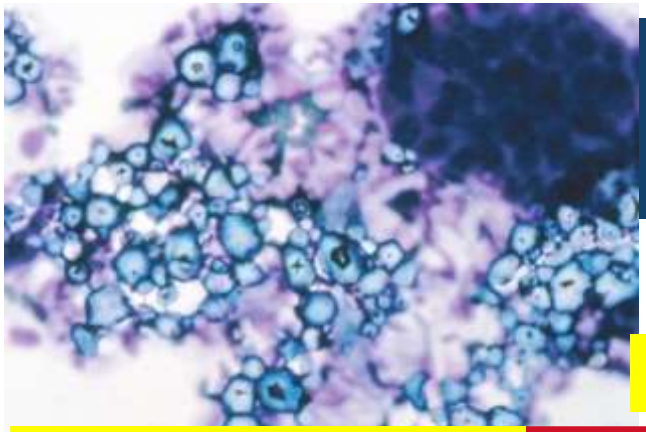
Nocardiosis in a cat



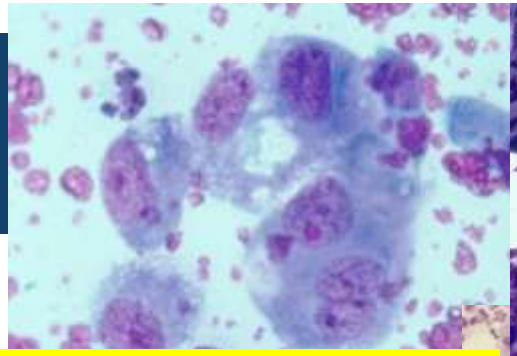
mycobacteriosis



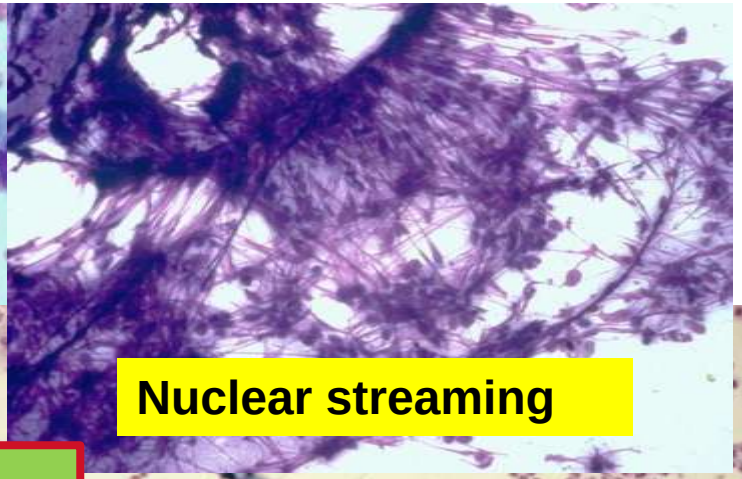
Fat necrosis



Glove powder

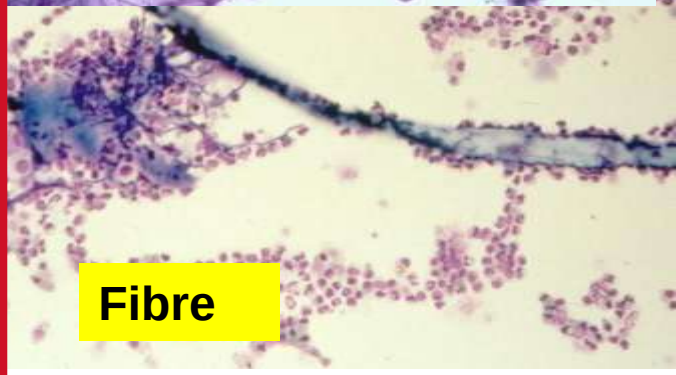


Ultrasound gel



Nuclear streaming

I was told very early on to be beware of 'false or misleading' artefacts on cytological smears!

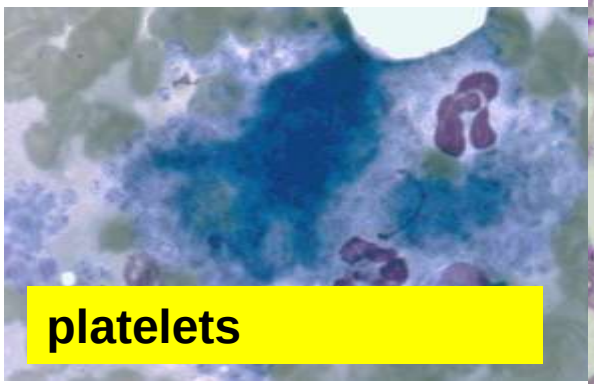


Fibre

Squashed insects

Haemoglobin crystals

Squames



platelets

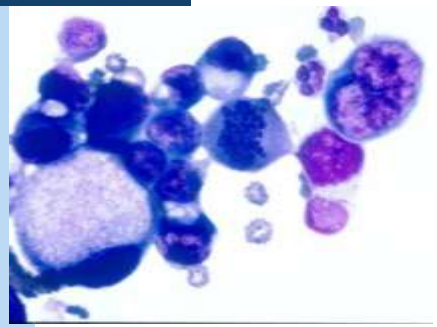
So, if my brain is constantly trying to pattern recognise when looking down the microscope how do I control it and minimise mistakes happening?

- Engage my left cerebral cortex and limbal system in the process of detection, description and deduction by being logical and sequential (even when I think I know the answer through pattern recognition!).

After I get excited about pattern recognizing, then I ask myself to detect (and describe) the evidence for the pattern:

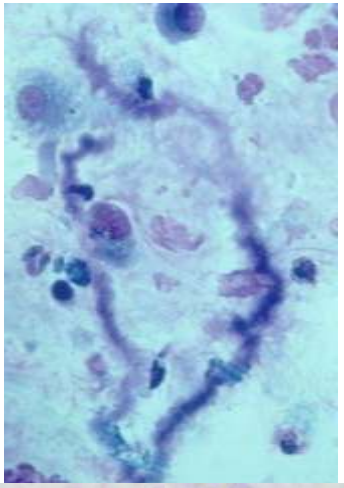
1. **What do the cells look like?**

   Constituent and infiltrating – **nuclei** and **cytoplasm**



2. **Is there acellular material present?**

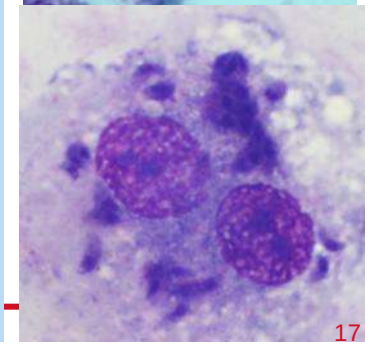
   Pigments, deposits, constituent material



3. **Can you detect agents of disease?**

   Foreign material?

   Living agents – parasites or microbes?



My template for moving from the general to the specific in the deductive process comes into play when I can't pattern recognise! Three steps:

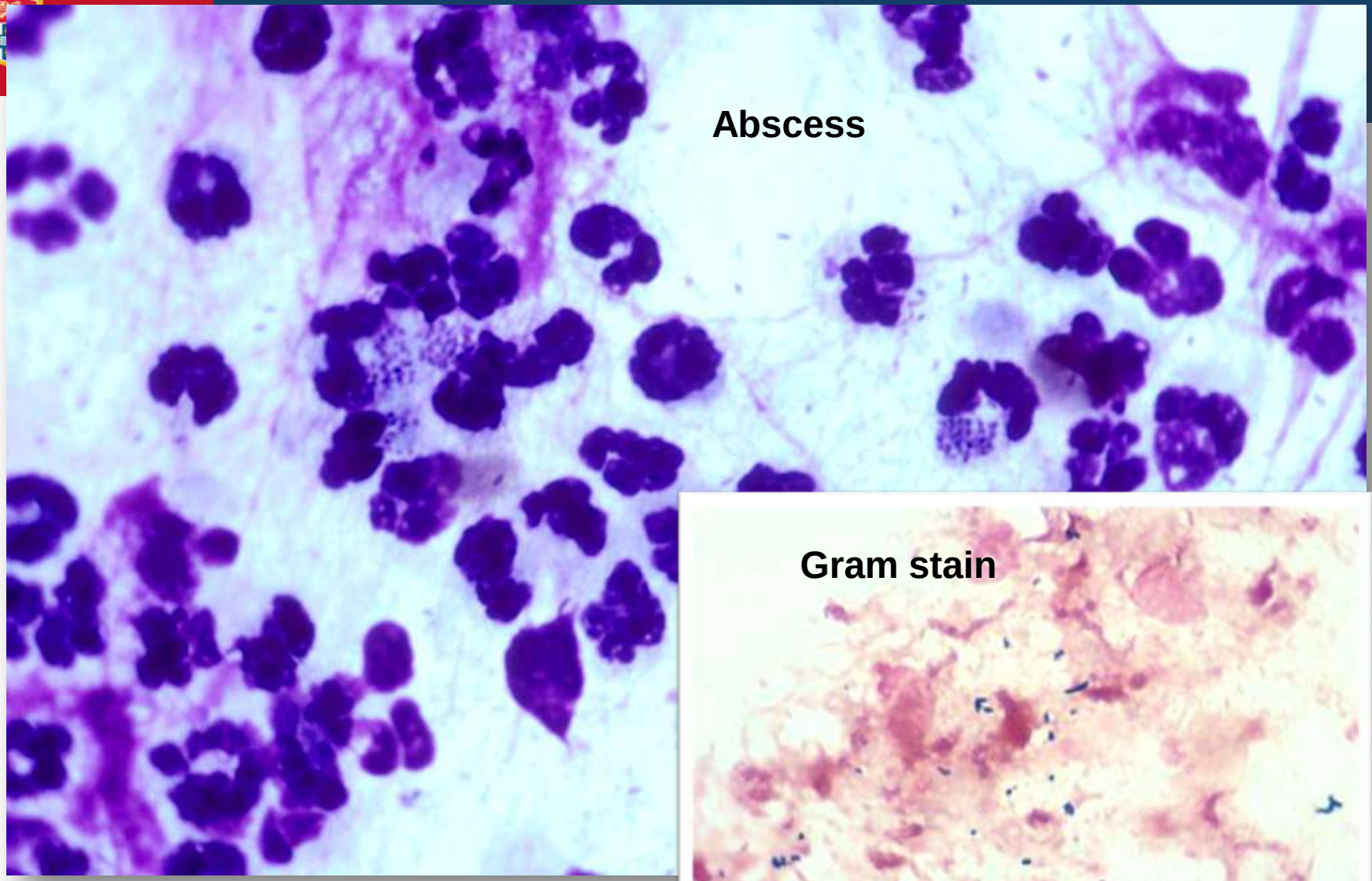
- **First** step: which of the 3 (5) pathological processes are occurring?
  - **Degenerative/non-inflammatory** (necrosis + deposits + many vascular changes); **Inflammatory;** Disorder of growth (most important is **neoplastic**)
- **Second** step: if I can detect more than one, which pathological process do I think is the *most important*? **First part of the morphological diagnosis**
- **Third** step: can I detect or determine an agent of disease? **(aetiological diagnosis):** I remember the list of five!
  - **Physical; Chemical; Infectious; Immune-based;Genetic?**

Why do I bother using a template?

"I consider that a man's brain originally is like a little empty attic, and you have to stock it with such furniture as you choose. A fool takes in all the lumber of every sort that he comes across, so that the knowledge which might be useful to him gets…diluted." Sherlock Holmes in *'A Study in Scarlet'*

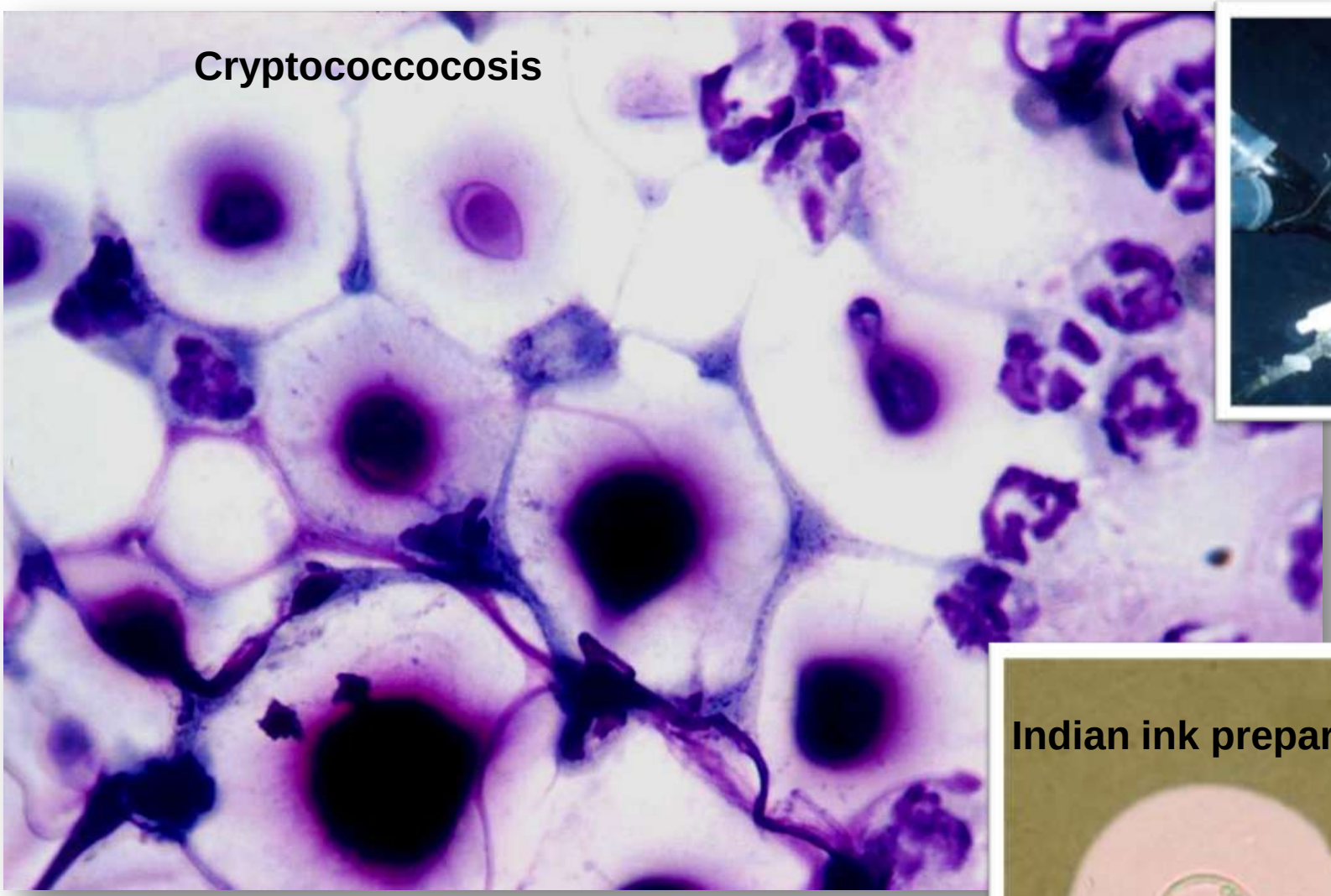
How do we value-add for the specialist and general practitioner in terms of cytological diagnosis? What can't they do themselves?

- Appreciating the limitations of cytopathology and the complementary nature of histopathology (the big picture view)
- A way forward if the slide is only partially diagnostic or even non-diagnostic
- If degenerative or inflammatory, an insight into aetio-pathogenesis (including the utilization of additional laboratory procedures)



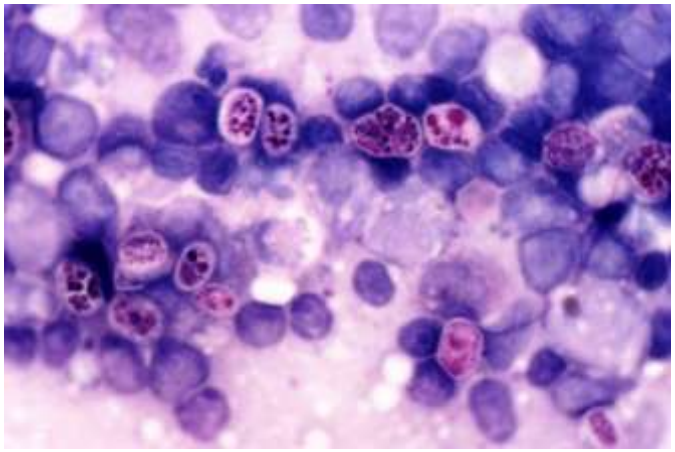
Cytopathology very good for detecting micro-organisms!

Cryptococcocosis

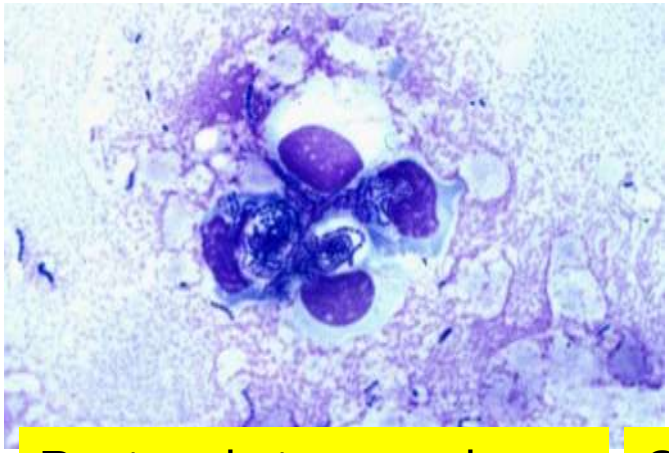


Indian ink preparation

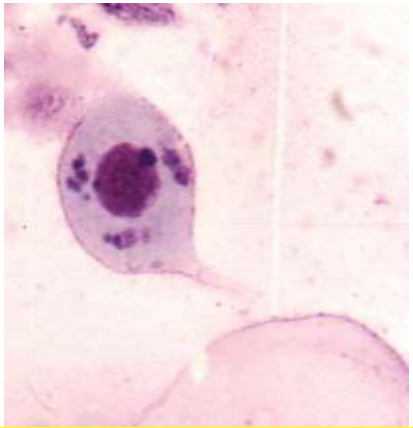
In fact, cytopathology beats histopathology hands down for detecting micro-organisms



Protothecosis in a Boxer colitis

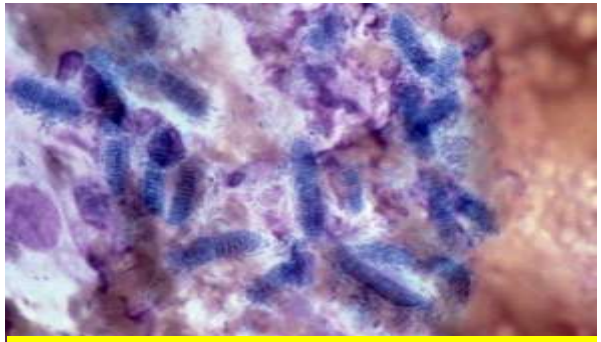


Ruptured uterus and Streptococcal peritonitis in a Cattle dog

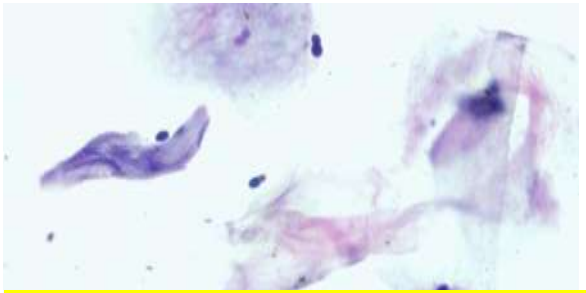


Conjunctival chlamydial inclusions in a cat

Conjunctival Distemper inclusions

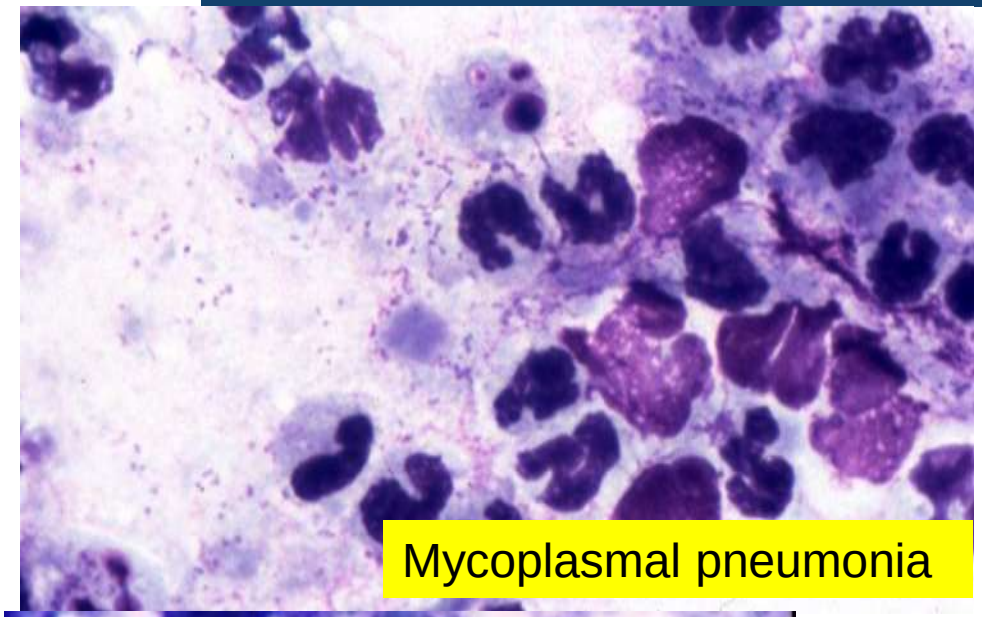


Simonsiella organisms in dog's mouth

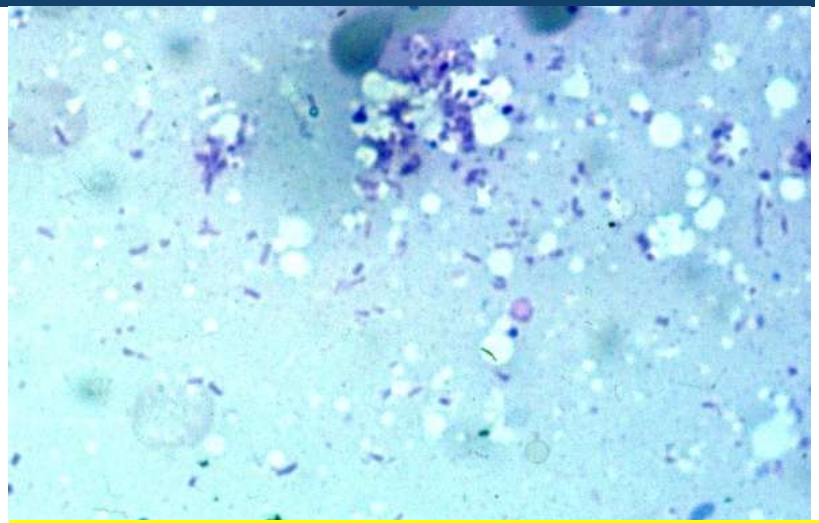


Malassezia in a canine ear canal

More micro-organisms detected through cytological examination – which one is the 'odd (wo)man out'!

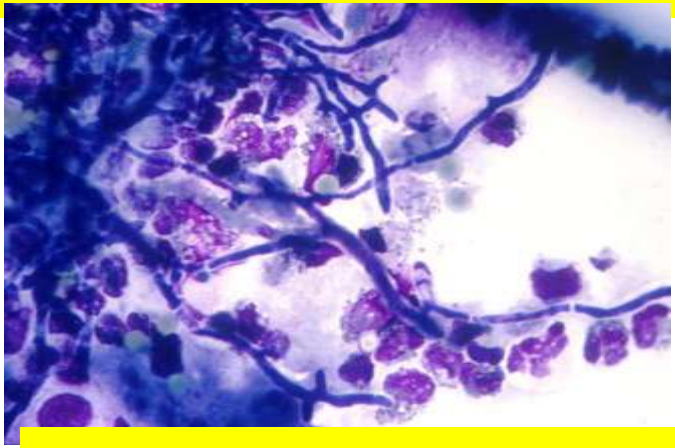


Mycoplasmal pneumonia

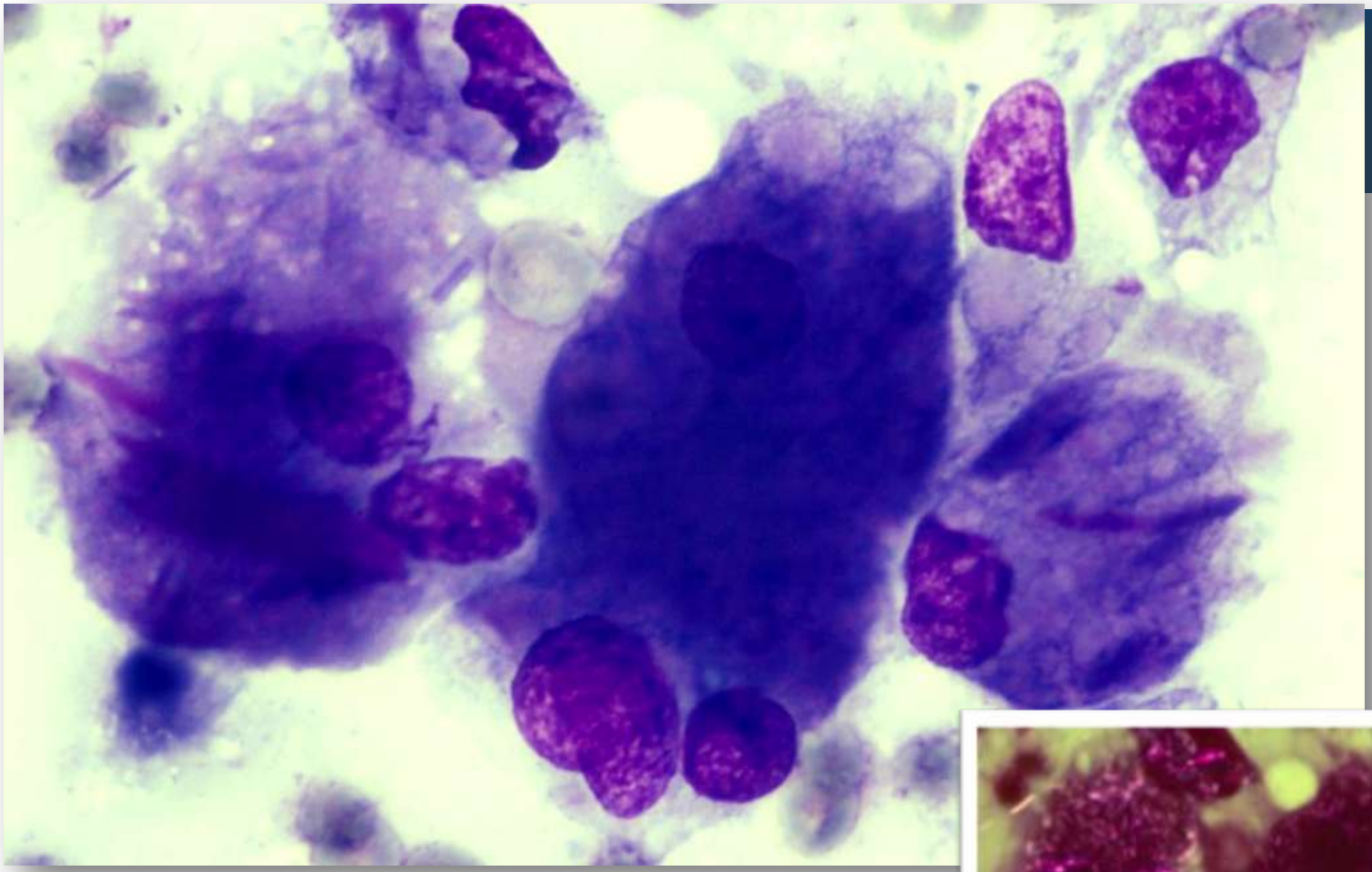


Free eosinophil granules in a cat's mouth

Pneumocystis carinii pneumonia in a dog

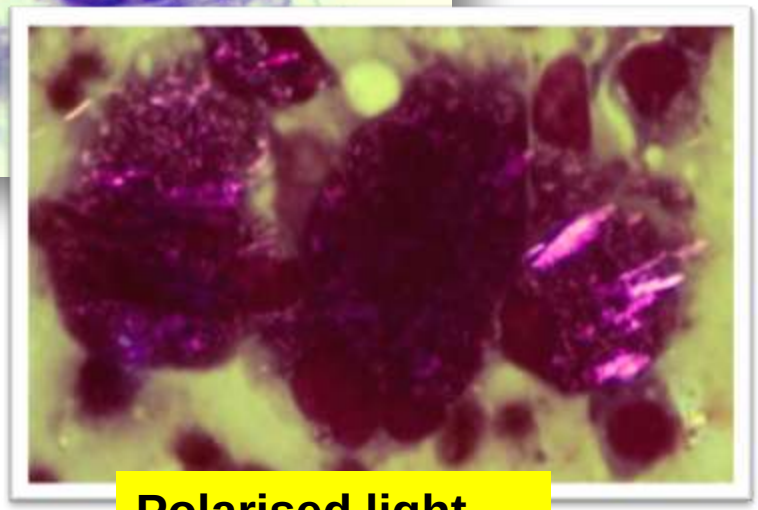


Aspergillosis – cystitis in a dog



Foreign body reaction

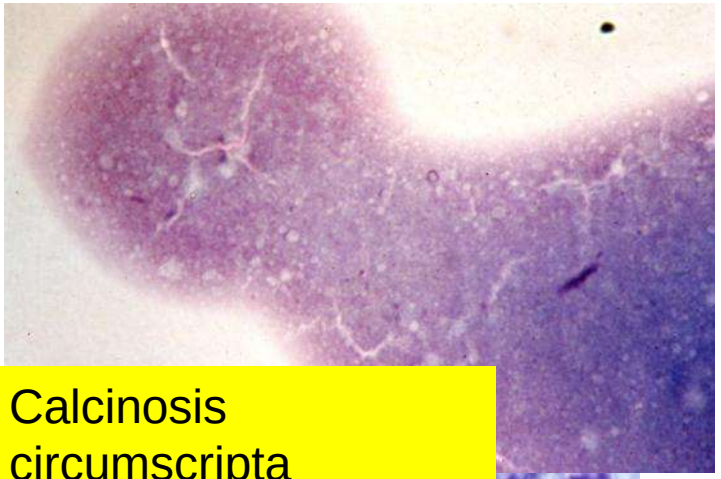
Specialised microscopic evaluation value adds for the referring veterinarian



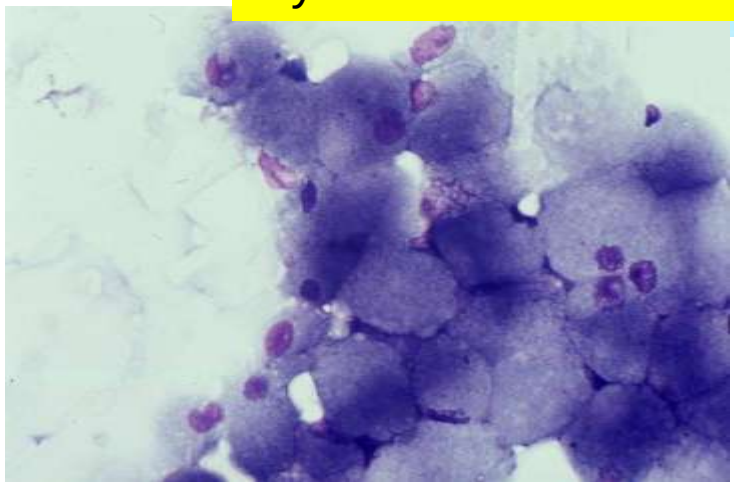
Polarised light

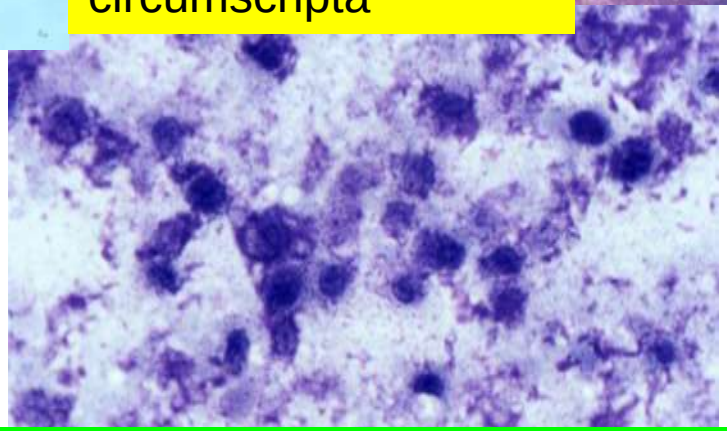
Primary degenerative lesions have necrotic cells, acellular material and usually few inflammatory cells

Cysts and cholesterol crystals



Calcinosis circumscripta





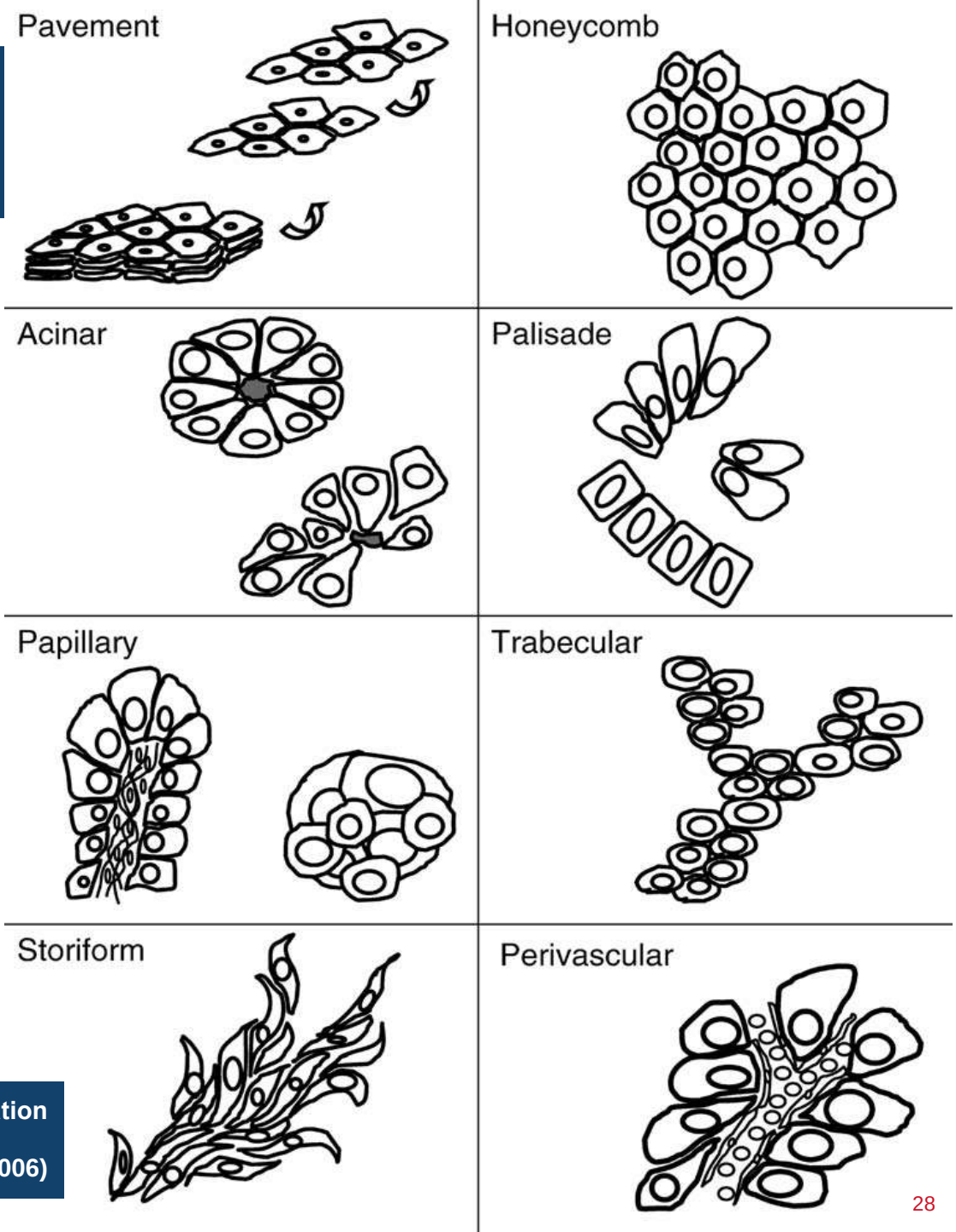
I am always wary of necrosis secondary to neoplasia or accompanying inflammation

How do we value add for the specialist and general practitioner in terms of cytological diagnosis? What can't they do themselves?

- Appreciating the limitations of cytopathology and the complementary nature of histopathology (the big picture view)
- A way forward if the slide is only partially diagnostic or even non-diagnostic
- If degenerative or inflammatory, an insight into aetio-pathogenesis (including the utilization of additional laboratory procedures)
- **Neoplasia!** Can be difficult for all of us! Need an effective template to move forward.
  - An appreciation of other disorders of growth and how they fit in with benign neoplasia useful
  - The differentiation of benign and malignant neoplasms and where limitations exist not always easy

'You can't make rules, only generalisations, for cells that break the rules in the first place!'

An appreciation of architecture of neoplasia through exposure to histopathology helps value add for epithelial and spindle cell disorders of growth!



More likely to be seen in tissue scrapings and imprints rather than FNA

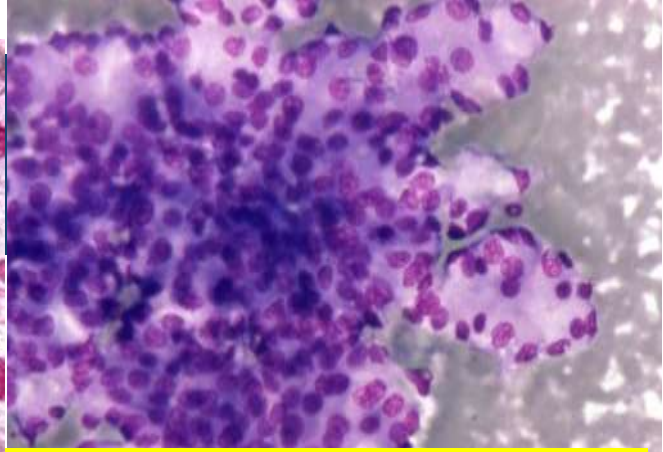
From 'Architectural patterns in cytology: correlation with histology' (Masserdotti Vet Clin Path 2006)

Solid tissue patterns for some epithelial and spindle cell neoplasms

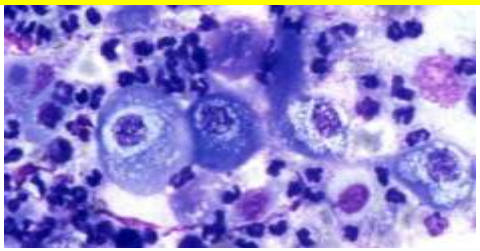
- **SCC, basal cell tumour**: pavement or palisade
- **TCC**: pavement, acinar or papillary
- **Intestinal, bronchial, bile duct carcinomas**: pallisade or acinar
- **Benign Prostatic Hyperplasia**: honeycomb or pallisade
- **Thyroid hyperplasia, adenoma or carcinoma**: honeycomb or acinar
- **Mammary gland adenoma or carcinoma**: pallisade, papillary or trabecular
- **Perianal gland hyperplasia, adenoma or carcinoma**: trabecular or perivascular
- **Liver carcinoma and testicular (Leydig/interstitial or Sertoli) tumours**: pallisade, trabecular or perivascular
- **Mesothelial hyperplasia and mesothelioma**: pavement or papillary
- **Sarcomas**: storiform or perivascular

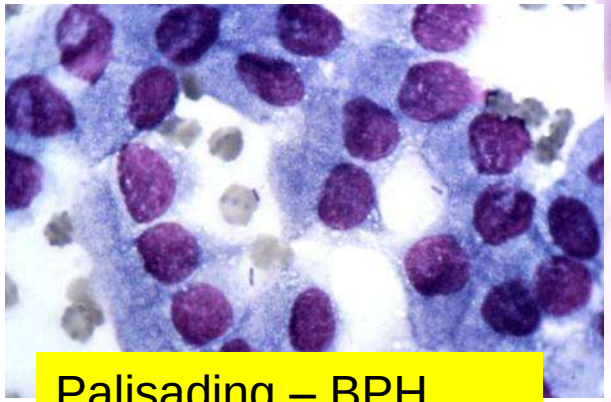
Pavement – mesothelium and SCC

Honeycomb – BPH

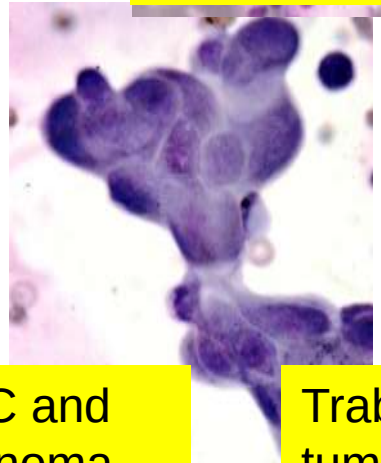


Acinar – thyroid carcinoma

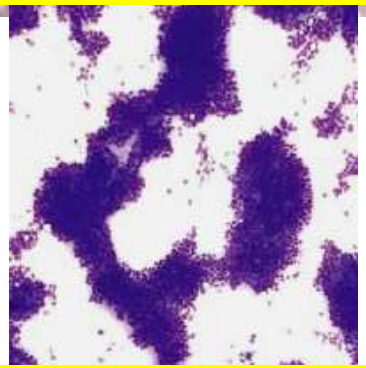




Palisading – BPH



Papillary?– BCC and mammary carcinoma



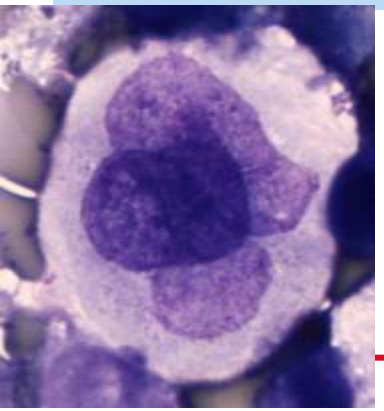
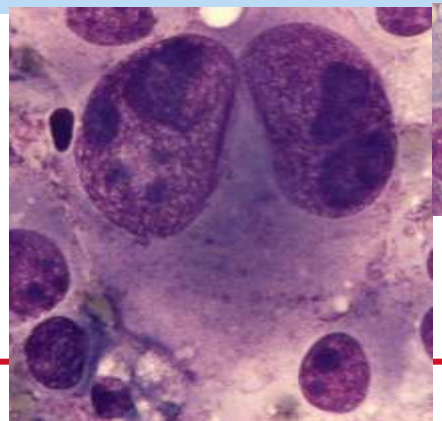
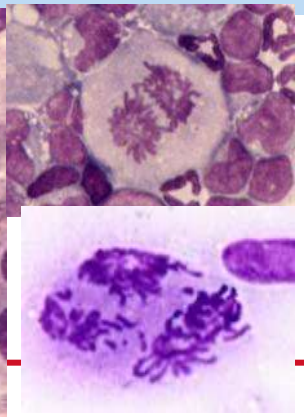
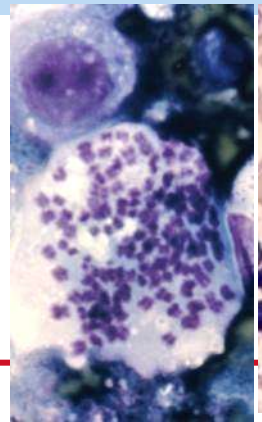
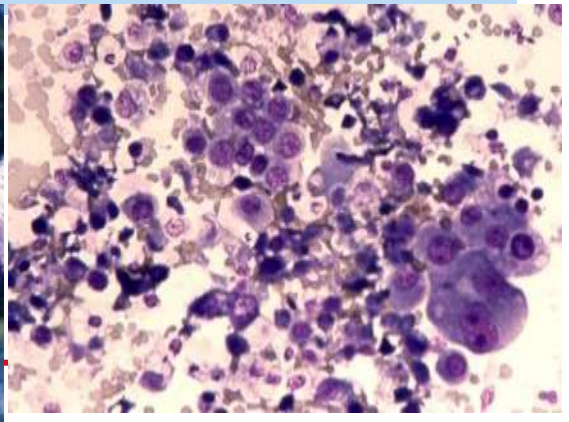
Trabecular – hepatoid tumour

Perivascular – haemangiopericytoma

Storiform – fibroma and fibrosarcoma

Features of Malignancy – of the below, what do you regard as key (we all have a favourite)? What others do you use?

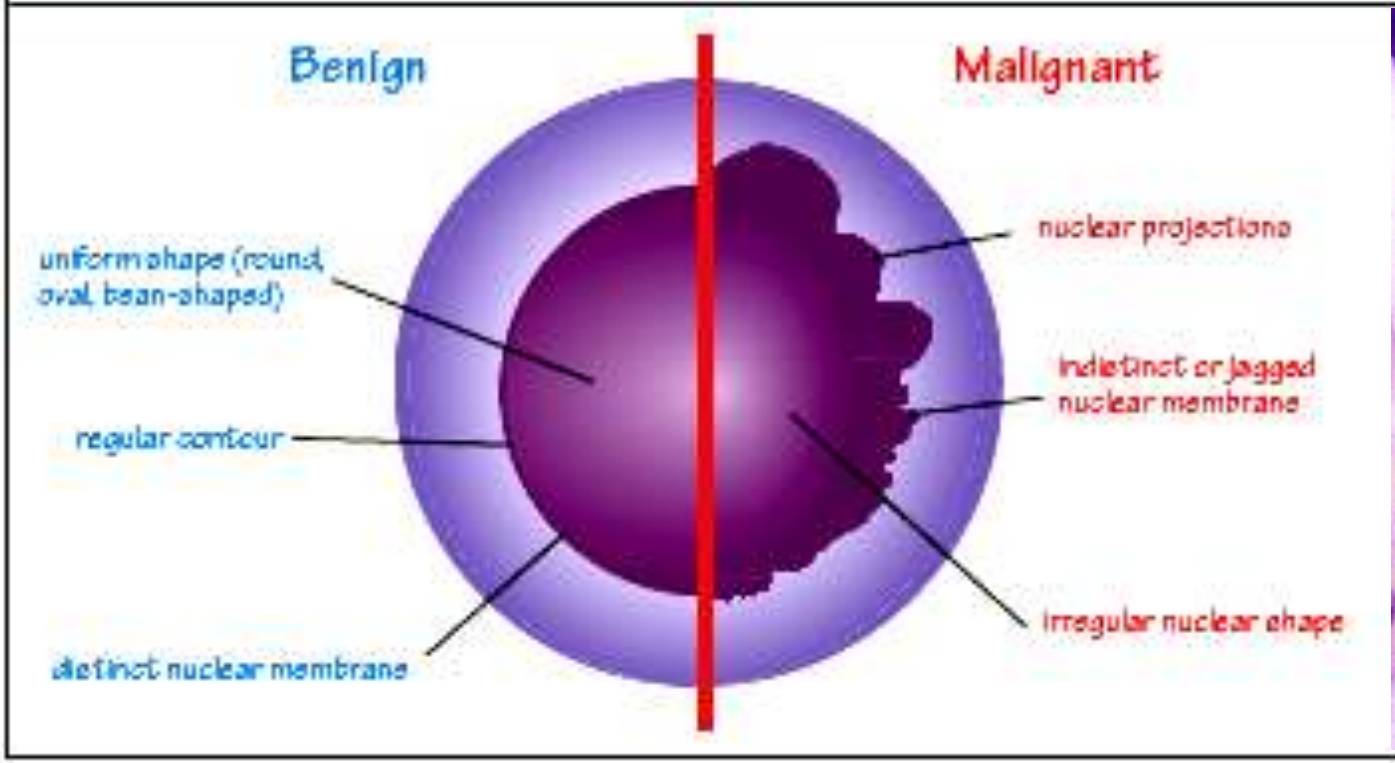
- **Nuclear details for malignancy**
  - Anisokaryosis (including giant nuclei and mutinucleate cells of variable sized nuclei)
  - Anisonucleolosis within a nucleus
  - Hyperchromaticity of nuclear material and increased N:C
  - Mitoses (normal and abnormal)
  - Nuclear moulding
  - Nuclear deterioration (apoptosis – single cell; necrosis– multiple cells)
- **Cytoplasmic features and any organisation of cells for histogenesis of a malignancy**

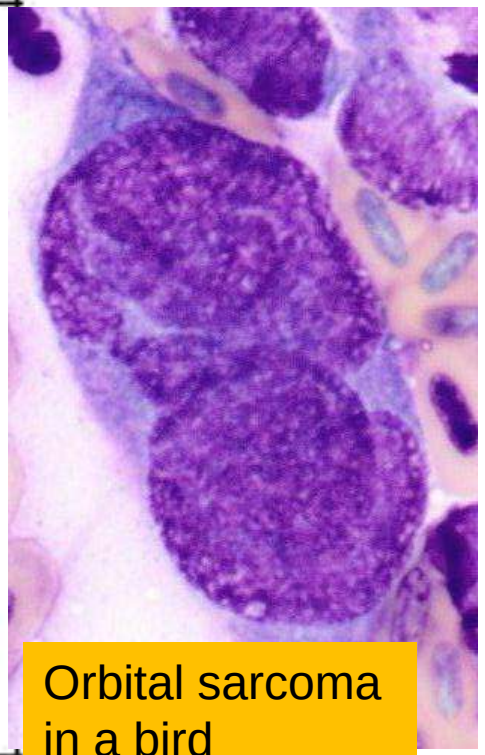






Nuclear contour and membrane changes in malignancy are best visualised in Papanicolaou stains?

Nuclear Contour and Nuclear Membrane

Benign	Malignant
Nucleus is round to oval (mesothelial cell, ventricular lining cell) or bean-shaped (reactive lymphocyte) with a regular nuclear contour. The nuclear membrane is prominent and distinct.	An irregular nuclear shape or contour may be present. The nucleus may be disintegrating, with rounded vesicle-like projections or "blebs" at the periphery. The nuclear membrane may be indistinct or jagged.





Orbital sarcoma in a bird

Those cytology stains

Table 1. Comparison of three fixation methods for cytologic specimens.*

Specimen Feature	Wet-fixation	Rehydration	Air-drying
Smearing technique	Less important	Less important	Important
Artifacts	If air-drying occurs	Minimal	With delayed or insufficient drying of wet specimens
Red blood cells	Present	Hemolyzed	Present
Cell loss	High	Moderate	Low
Cell size	Decreased, similar to histologic specimens	Slightly increased	Increased

*Modified form Orell,[31] Naib,[29] Stanley[42] and Yang.[47]

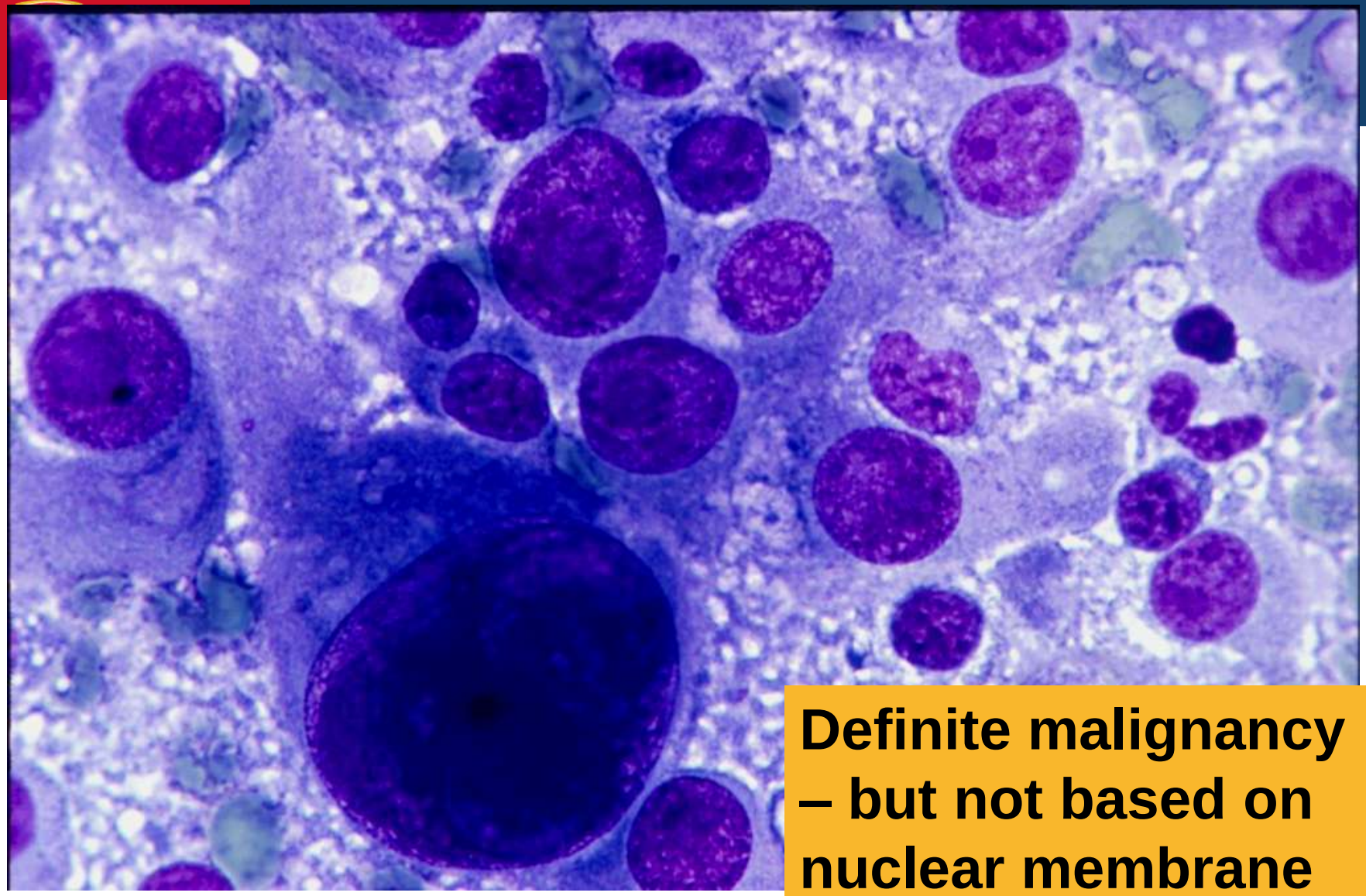
Table 2. Comparison of staining quality for three types of cytologic stains.*

Cell Feature	Hematoxylin and Eosin	Papanicolaou	Romanowsky
Tissue fragments	Good	Good	Poor
Nucleus	Excellent	Excellent	Fair
Nucleolus	Distinct	Excellent	Visible
Cytoplasm	Similar to histologic specimens	Keratin	Excellent
Extracellular matrix	Poor	Poor	Excellent

*Modified from Orell,[31] Naib,[29] Boon,[4] Rebar[36] and Yang.[47]

Rapid Staining Techniques in Cytopathology: A Review and Comparison of Modified Protocols for Hematoxylin and Eosin, Papanicolaou and Romanowsky Stains. E Jorundsson, JH Lumsden, RM Jacobs. *Vet Clin Pathol* **28:**100-108, 1999

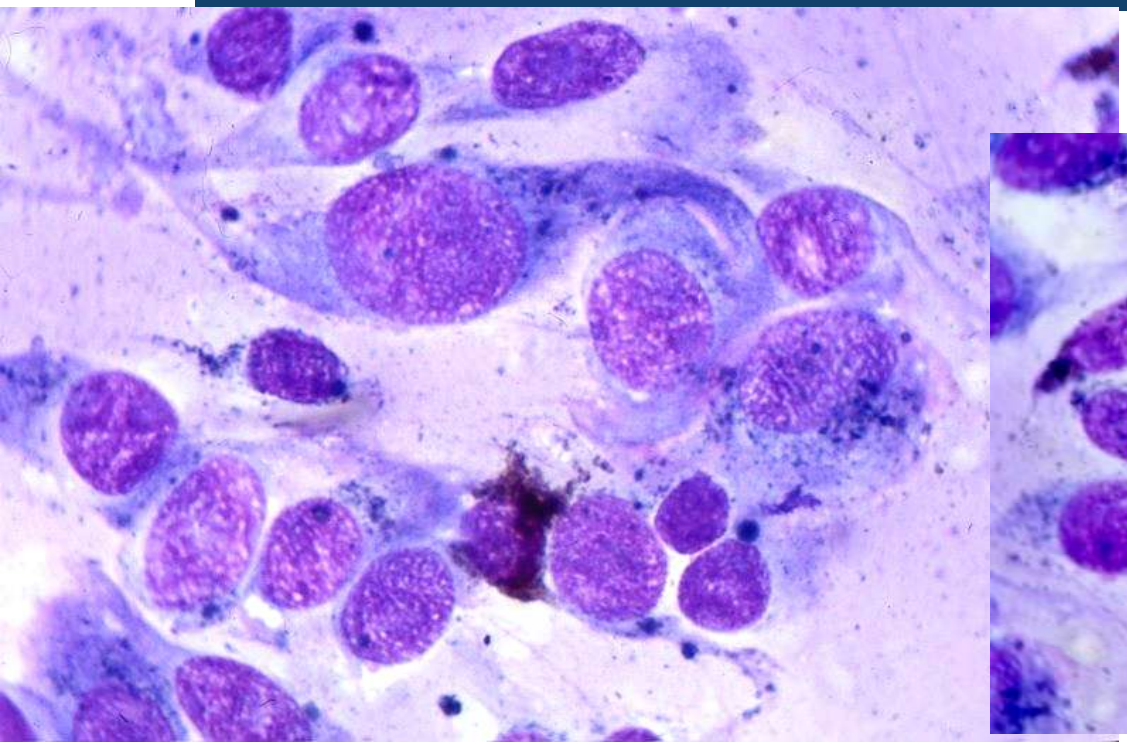
Romanowsky stains: combinations of the basic (cationic) dye, methylene blue; its breakdown products, azure A, B or C; and the acid (anionic) dye, eosinY). The only components necessary for the full Romanowsky effect are **azure B** and **eosinY**. ”The Romanowsky stain is polychromatic, i.e., it produces the”Romanowsky effect” by generating the ”new” colour, purple (mechanism not entirely understood).

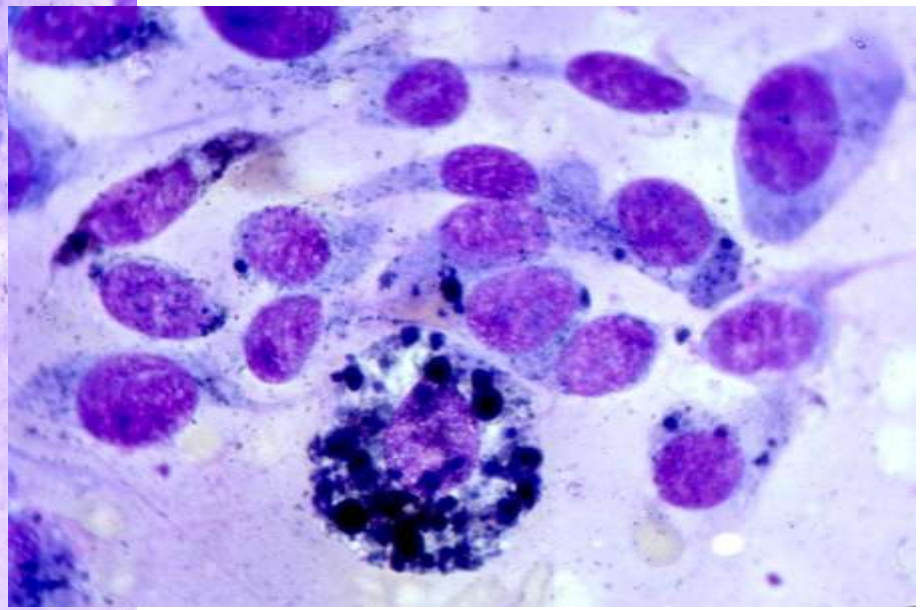


What hits you first?

Definite malignancy – but not based on nuclear membrane contour changes!

Digital mass in a dog – these can be tricky on pattern unless-





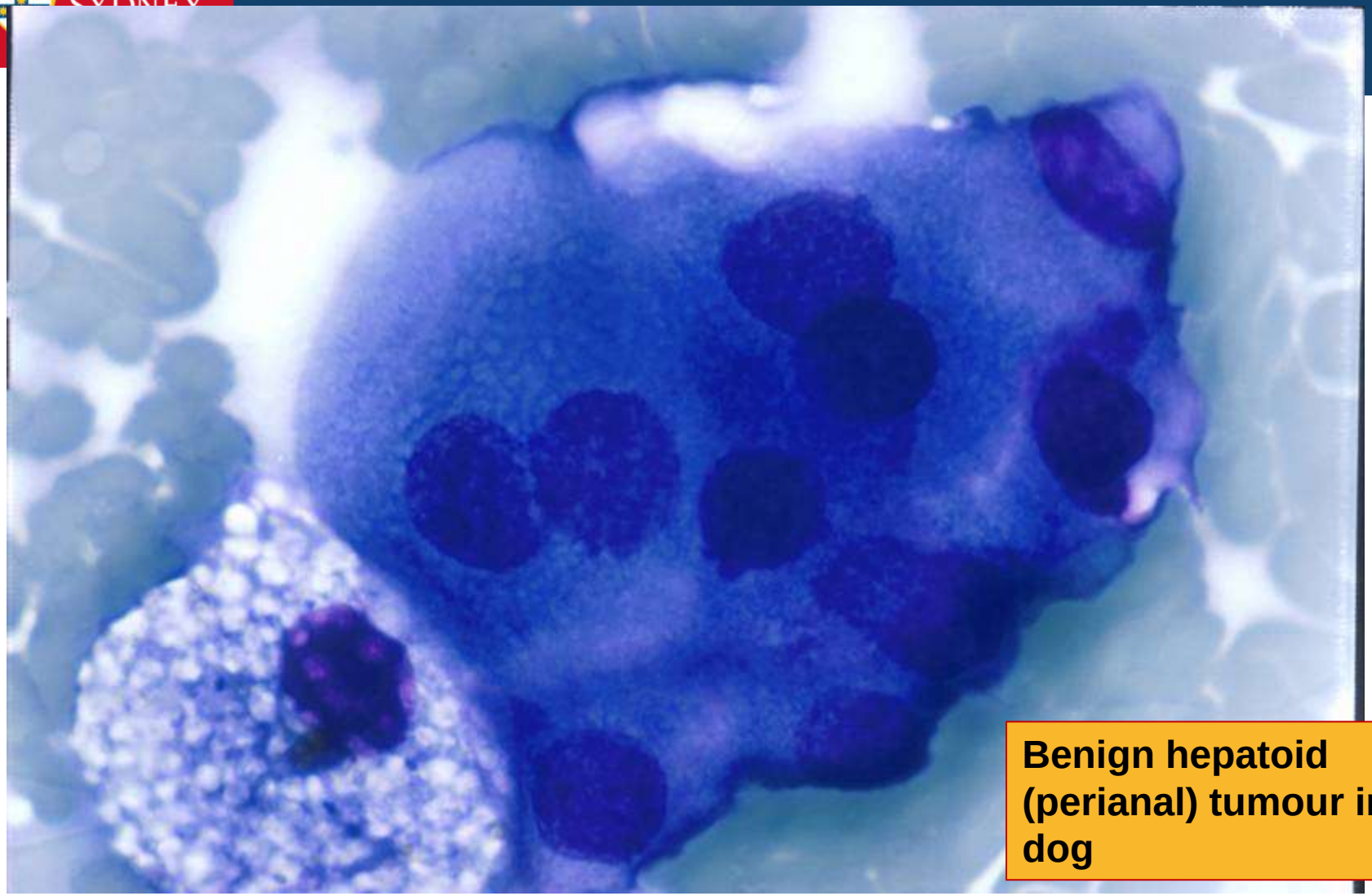
Does the nuclear detail dictate malignancy?

Does the cytoplasm give you a clue to the histogenesis?

Malignant melanoma

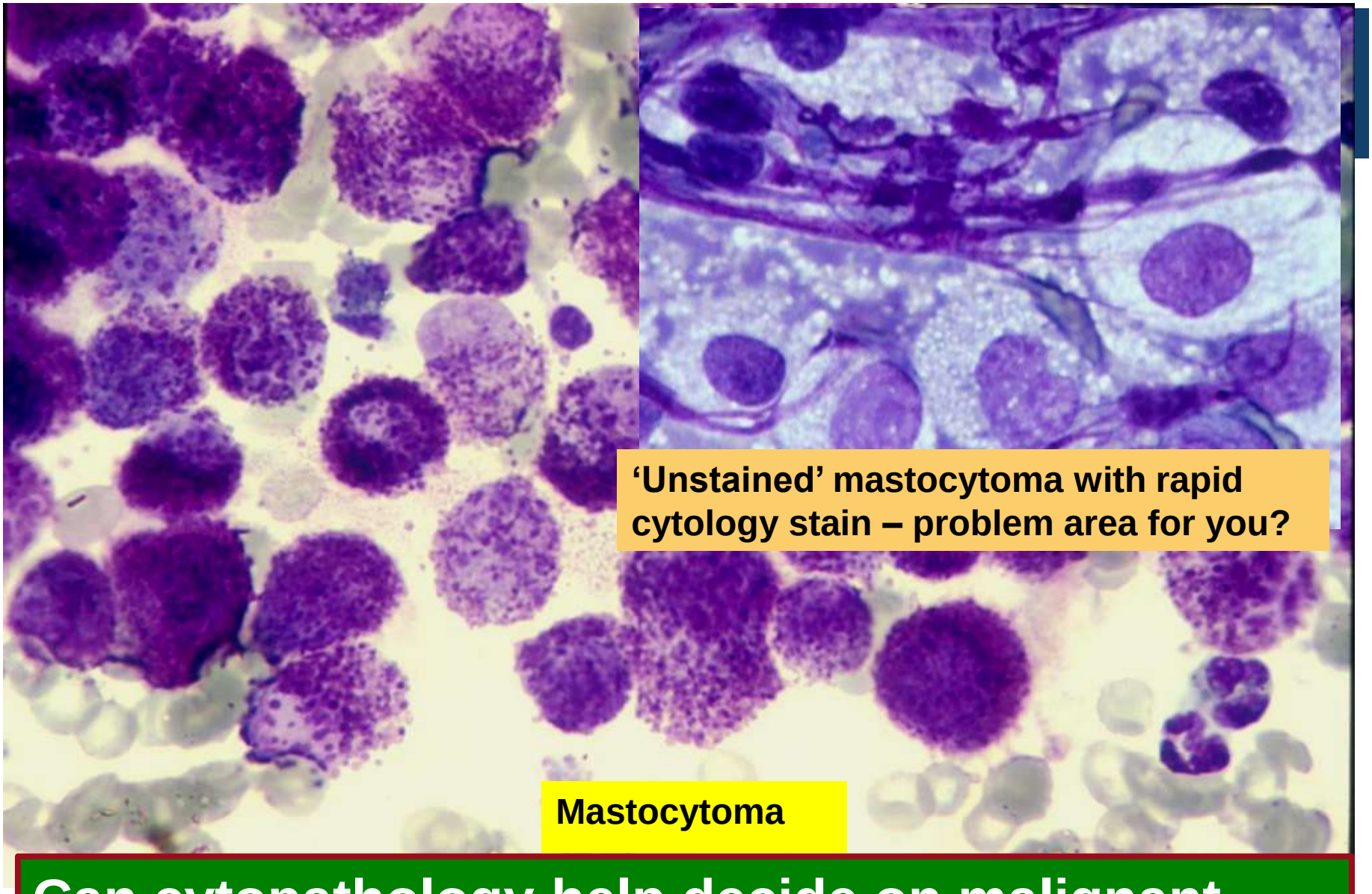
My problem areas for cytological interpretation

1. Is it benign or malignant?
2. Inflammation masking neoplasia
3. Hyperplastic/cystic from benign?
4. Reactive fibroblasts from neoplastic ones
5. Macrophages (epithelioid cells) from epithelial and perhaps other cells



Benign hepatoid (perianal) tumour in a dog

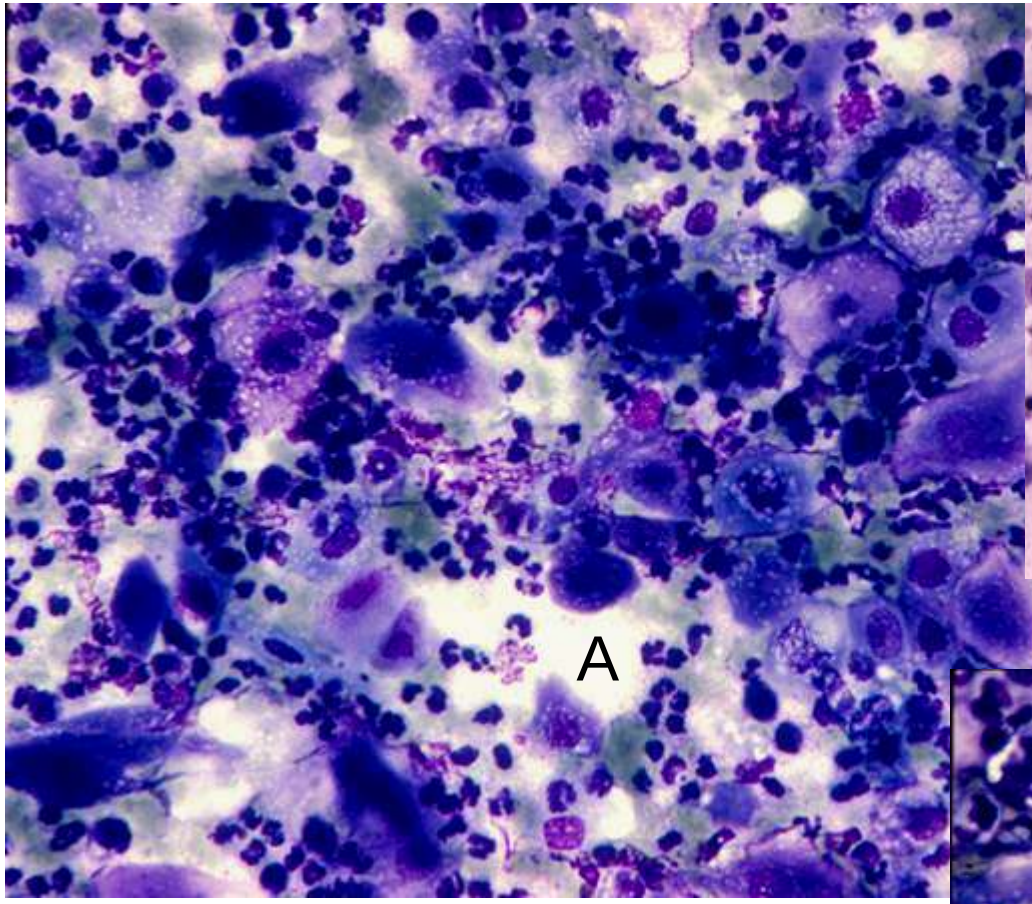
Problem area: benign or malignant? No nuclear or cytological variation, no can do!

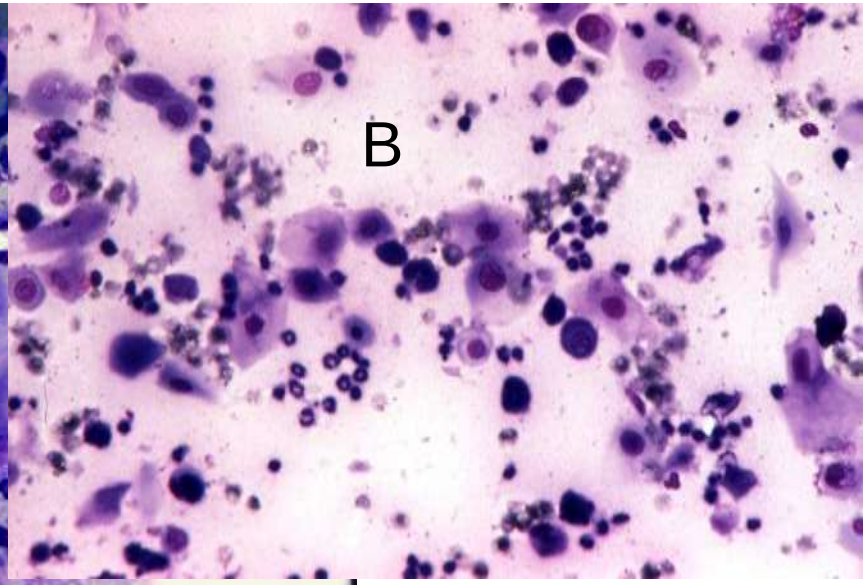


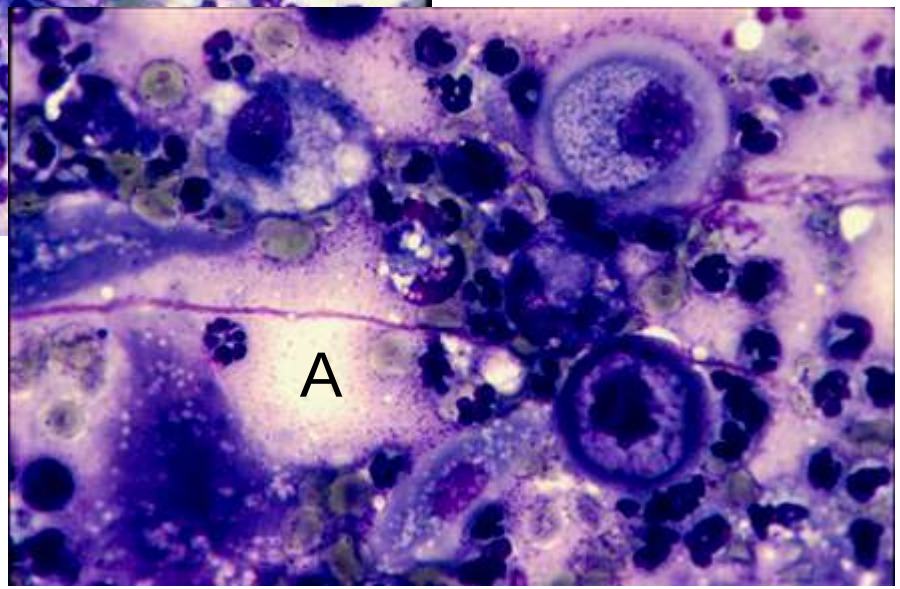
‘Unstained’ mastocytoma with rapid cytology stain – problem area for you?

Mastocytoma

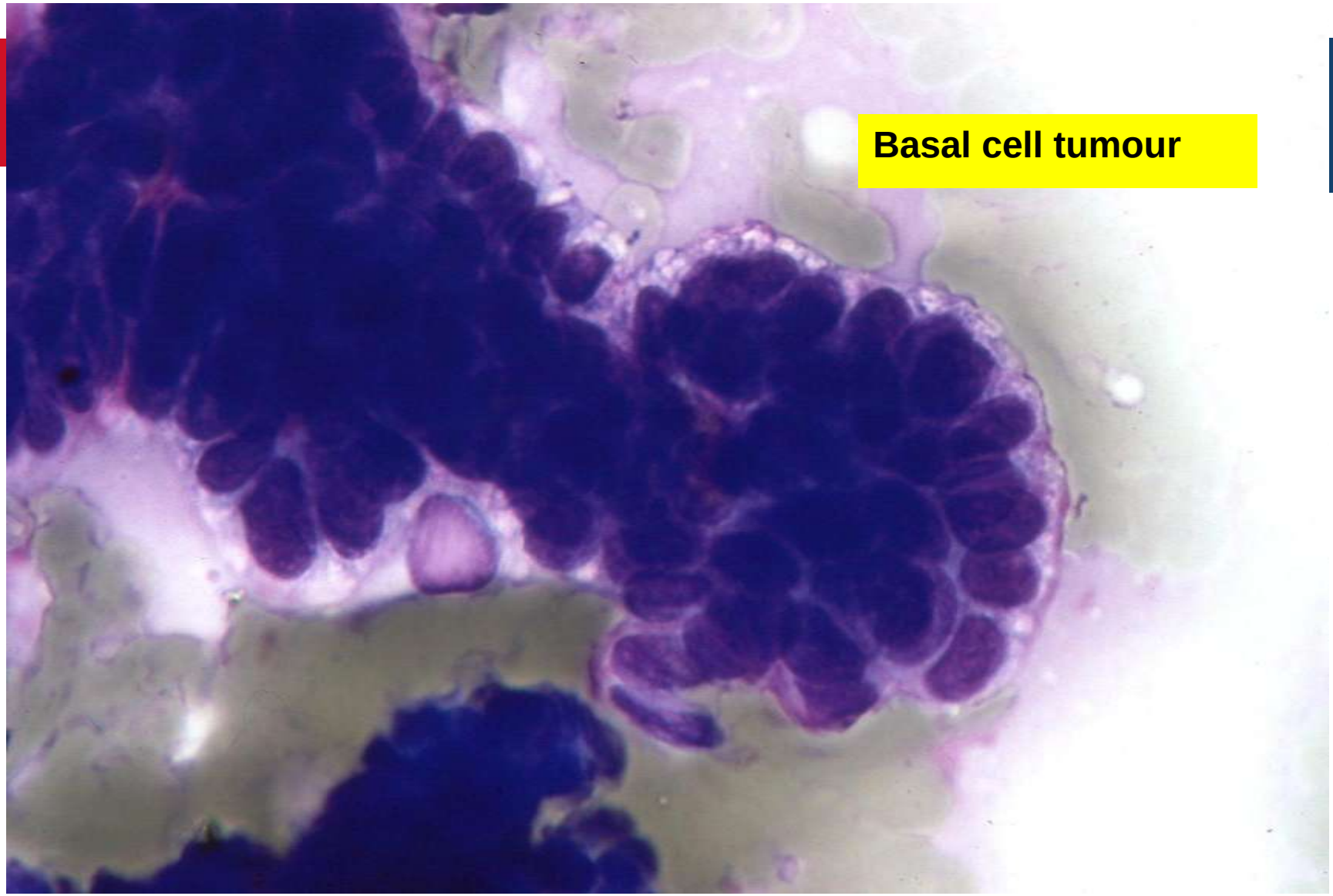
Can cytopathology help decide on malignant potential of mastocytomas?



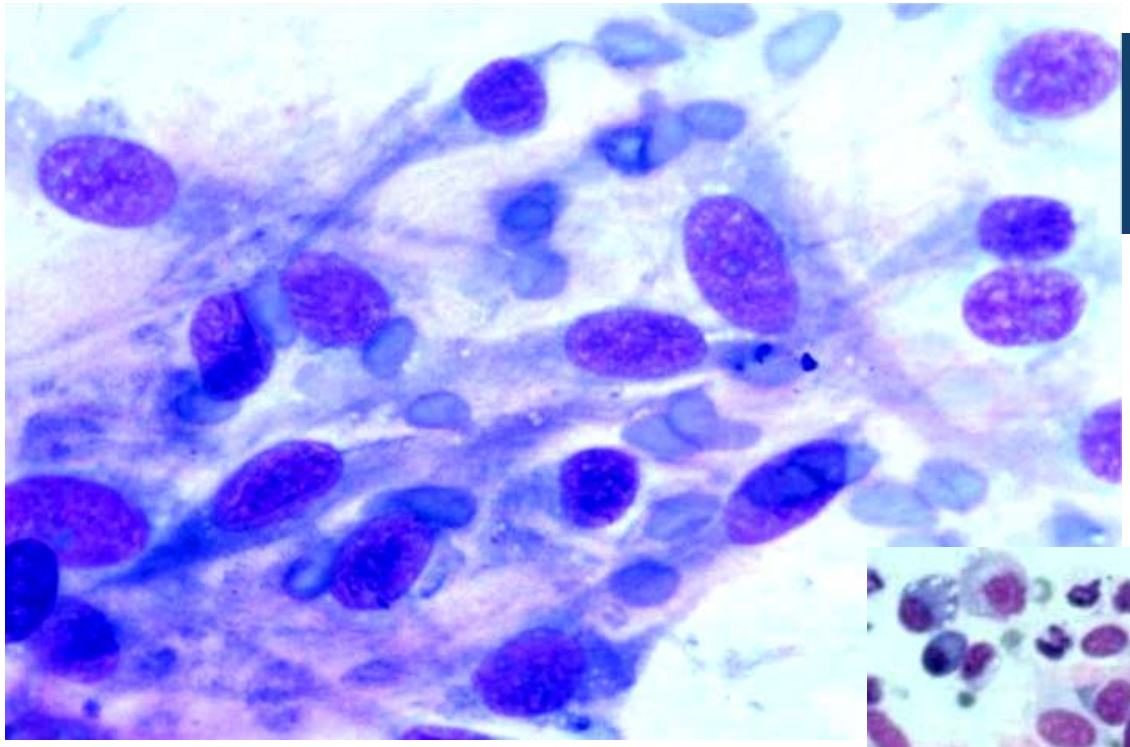




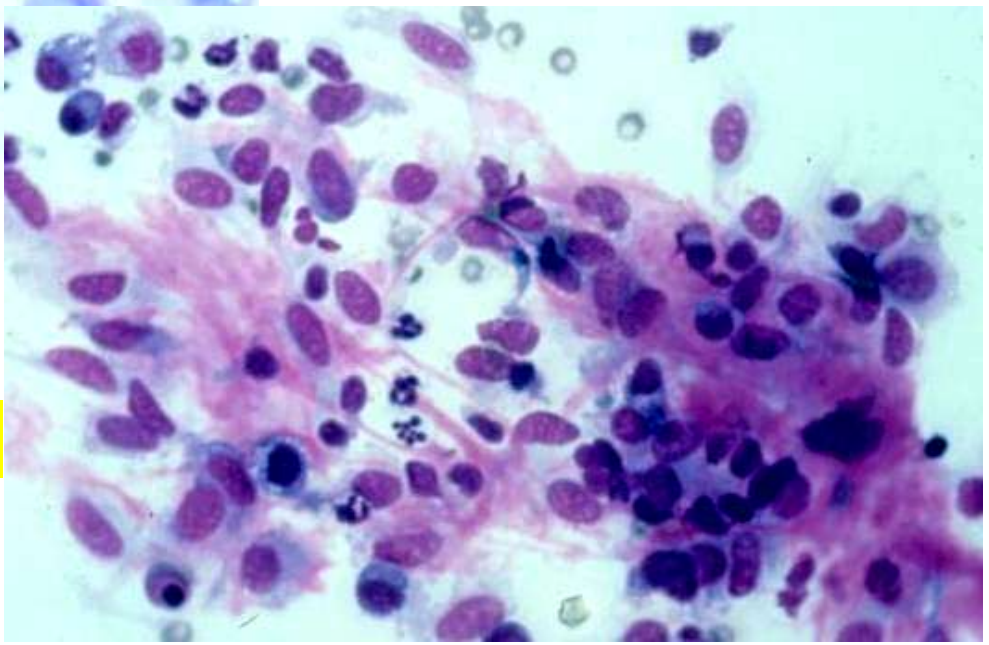
Inflammation and SCC x 2 – could slide B just be epitheliomatous hyperplasia/dysplasia with chronic dermatitis? Architecture would be useful!



Is this hyperplastic or neoplastic? Does anybody care?



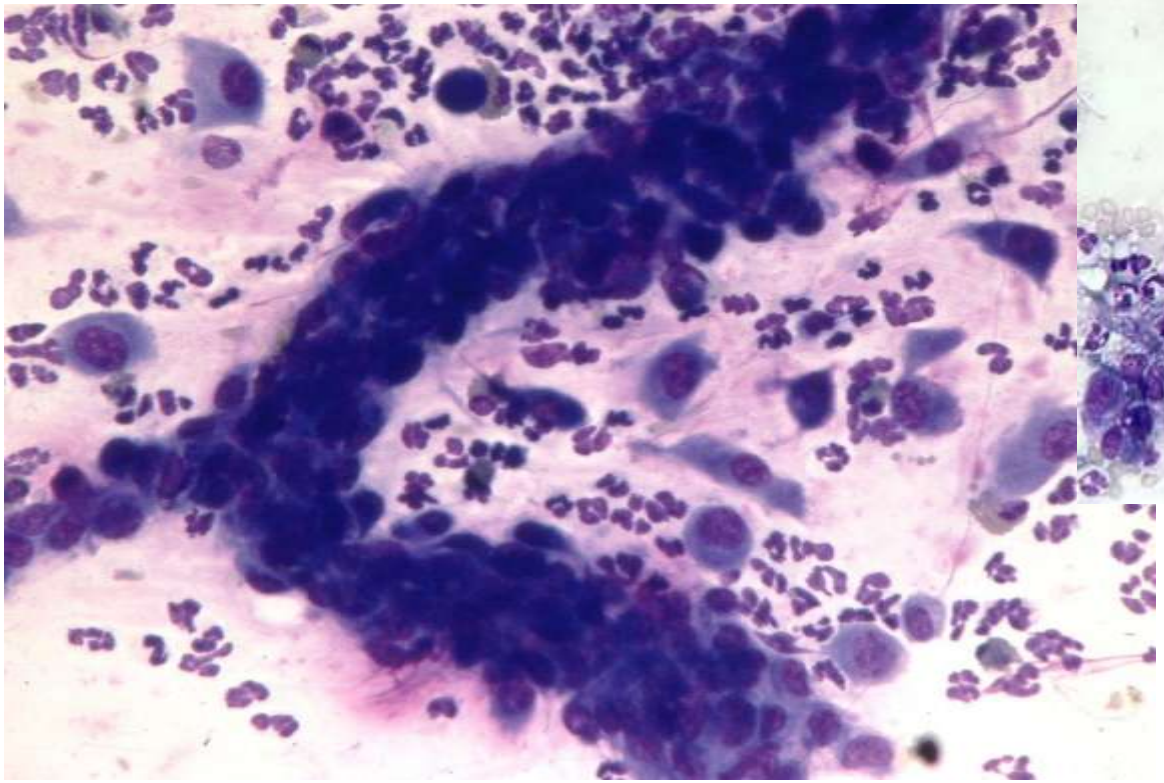
fibrosarcoma



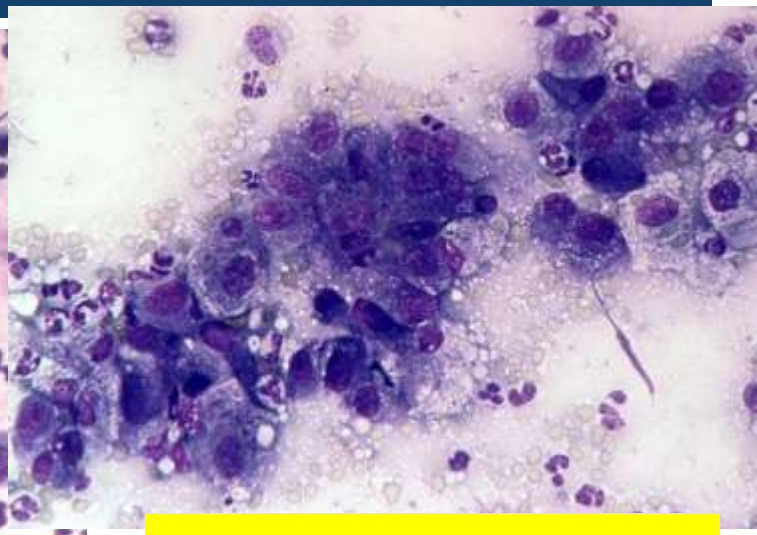
fibrosarcoma

More beautiful cytology smears, but are these spindle cells neoplastic or reactive?

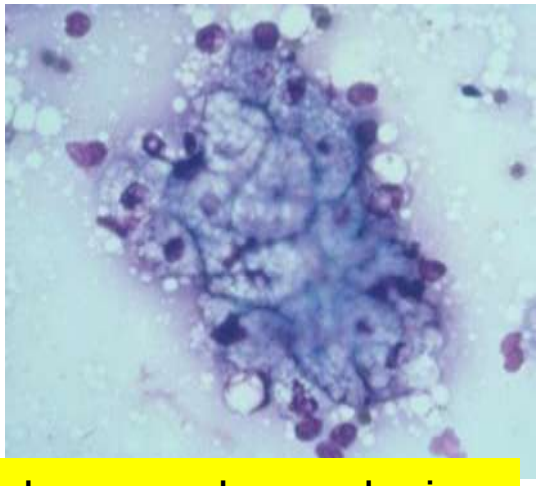
Are these cells epithelial?



Equine fungal granuloma



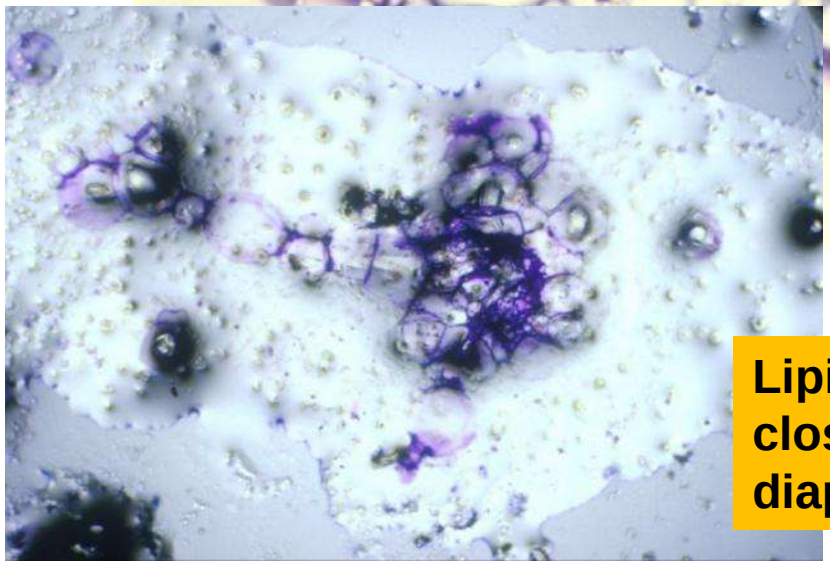
Chin granuloma in cat



Sebaceous hyperplasia

Lipoma

What about this one? Can we really value add for the practitioner?



Lipid viewed with a closed condenser diaphragm

Acknowledgements and further reading

- George Reppas for analysis of correlative data

- Cytology in Food Animal Practice (2007) Andrea A. Bohn, Robert J. Callan. *Vet Clinics (N Am) - Food Anim Prac* 23:443–479 (good for both solid tissue and fluids [includes bone marrow] – with an emphasis on fluids) – **solid tissue often done to detect lymphosarcoma or SCC and differentiate them from inflammatory lesions (mainly in skin and LN) in ruminants. Can also get liver aspirates to evaluate lipidosis in ruminants**
- Cytologic Diagnosis of Diseases of Rabbits, Guinea Pigs, and Rodents (2007) Michael M. Garner. *Vet Clinics (N Am) - Exot Anim Prac* 10: 25–49 – **covers mainly solid tissue cytology (tumours plus some inflammatory conditions) with a focus on pet rabbit, guinea pig, rat, mouse and hamster**

Common conditions in some laboratory animals kept as pets that can be diagnosed by cytopathology (Garner 2007)

Species	neoplasms	Inflammatory/infectious	Degenerative
Rabbit	•Malignant lymphoma •Soft tissue sarcoma •Lipoma •Thymoma •Uterine carcinoma •Basal cell/follicular •Mammary carcinoma •SCC •Testicular tumours	•Viral polyps of rectum, vulva and penile sheath •Squamous papilloma of skin (papillomavirus) •Skin and abdominal abscesses •Cutaneous treponemiasis (rabbit syphilis - spirochete *Treponema cuniculi*) – not reported in Australian rabbits?	
Guinea pig	•Malignant lymphoma •Lipidic tumours •Follicular tumours •Mammary carcinomas •Uterine leiomyoma •Bronchogenic tumours •Thyroid tumours •TCC	•Cervical lymphadenitis (streptococcal in young cavies; variable aetiology in older cavies)	•Hepatic lipidosis •Ovarian cysts
Rat	•Malignant lymphoma •Soft tissue sarcomas •Zymbal gland tumour of ear canal •Lipoma •Mammary fibroadenoma •Testicular (interstitial) tumour	•Vaginitis and pyometron •Bronchopneumonia •Pyoderma	
Mouse	Rare (malignant lymphoma, SCC and thyroid tumours most common)	Pyoderma (staphylococcal and ectoparasites)	

Cases for Discussion

- Can work through the cases on your own, in couples or more
- Use your own style, whether it be pattern recognition and working back or sequential, problem-oriented working forward
- Discussion will be along the lines:
    1. Can a diagnosis be offered and if so what are the key pieces of supporting information?
    2. What results can't be explained by the diagnosis?
    3. If a diagnosis can't be gleaned can you think of a way forward for the referring veterinarian to get a diagnosis (optional)?

Mel Gabor DPINSW: Adult male bovine, mid coast NSW. 2 steers died in last week. This animal was down with pale gums. Strongyle egg count of 80epg, negative for tapeworm, negative for coccidia.

HAEMATOLOGY	CASE	REF VALUES
Plasma appearance	Clear	Clear
Plasma protein (refract) g/L	79	65-85
Fibrinogen g/L	**11**	3-7
Pro/Fib ratio g/L	**6**	15-100
PCV L/L	0.26	0.23-0.44
Haemoglobin g/L	88	80-150
Erythrocytes $x10^{12}$/L	**3.34**	5-8
MCV fL	**78**	44-62
MCHC g/L	340	300-350
MCH pg	**26**	14-20
Leukocytes $x10^9$/L	**1.6**	4-12
Neutrophils (seg.) $x10^9$/L	**0.11**	0.6-4.0
Neutrophils (band) $x10^9$/L	0.0	0-0.2
Lymphocytes $x10^9$/L	**1.46**	2.5-7.5
Monocytes $x10^9$/L	0.03	0.03-0.84
Eosinophils $x10^9$/L	0.0	0.0-2.4
Basophils $x10^9$/L	0	0-0.2
Blood film: No retics seen on NMB blood film; Small inclusions in <0.1% of RBCs; Red blood cell inclusions consistent with Theileria; Fibrin clumps.		
Platelets $x10^9$/L	**29**	100-800

TEST	CASE	REF VALUES
AST IU/L	**294**	<120
GGT IU/L	0	<35
GLDH IU/L	4	<30
CK IU/L	**1640**	<300
Beta HOB µmol/L	0.08	<0.8
Bilirubin µmol/L	22.5	<24.0
Serum protein (biuret) g/L	66.2	60-85
Albumin (BCG) g/L	26.9	25-38
Globulins g/L	39.3	30-45
A:G ratio	0.7	0.7-1.1
Urea mmol/L	**12.8**	2.1-10.7
Creatinine µmol/L	146	50-186
Urea/Creat ratio	**0.09**	0.00-0.07
Calcium mmol/L	**1.81**	2.0-2.75
In Phosp mmol/L	1.53	0.8-2.8
Magnesium mmol/L	**0.69**	0.74-1.44

Although no mention of exposure in the history, the main ddx was listed as Toxicity: ptaquiloside (*Pteridium* spp. Bracken fern).

Likely conclusions*:* the underlying problem could be bone marrow depression due to bracken fern intoxication over a few weeks. The other changes can be explained by recumbency and perhaps local environmental conditions.

Possible reasons for changes*:* Elevated AST and CK due to recumbency? The mild azotaemia (urea) may be partly due to pre-renal causes. The marginal hypocalcaemia (correction for lowish albumin still gives 1.93 mmol/L) and hypomagnesaemia may be incidental or related to issues in the region? The aplastic anaemia (mild non-regen anaemia – only RBC's, marked leukopenia [all down] and thrombocytopenia) is likely due to bone marrow suppression. The increased fibrinogen may be related to downer cow and tissue damage. The theilerial organisms may be contributing to the anaemia, but not significantly (no real evidence for haemolysis)

Likely conclusions*:* the underlying problem could be bone marrow depression due to bracken fern intoxication over a few weeks. The other changes can be explained by recumbency and perhaps local environmental conditions.

Further testing*?* Monitoring after removal from bracken fern.

A five-year old, male neutered domestic short hair cat who was relocated to a new home and owner four weeks previously. Since relocation, he has been reluctant to eat, lethargic and appears timid of his new surroundings. The night before presentation, he had vomited several times, bringing up yellow-tinged froth. The new owner has also noted his urine to be an abnormal dark yellow colour. Upon initial physical examination, the cat appeared overweight (6.4kg) despite his recent inappetence. He seemed dull and lethargic, had reduced skin turgor and tacky mucous membranes. He was slightly tachypnoeic with an audible inspiratory stridor and also had a mild tachycardia (180bpm). Rectal temperature was normal. Mucous membranes and sclera appeared icteric. On abdominal palpation, the edge of the liver was just palpable ventrally, but was non-painful.

TEST	SAMPLE	REFERENCE VALUES
ALP u/l	**338**	<50
ALT u/l	**266**	<60
CK u/l	**598**	<200
Serum protein (biuret) g/l	66.2	53-76
Albumin (BCG) g/l	24.2	19-38
Globulins g/l	42	26-51
Total cholesterol mmol/l	1.79	1.9-3.9
Glucose mmol/l	**8.56**	3.6-6.6
Urea mmol/l	**4.47**	7.2-10.7
Creatinine µmol/l	124	90-180
Calcium mmol/l	1.98	1.75-2.6
Inorganic phosphate mmol/l	**0.87**	1.3-2.3
Sodium mmol/l	152.3	147-156
Potassium mmol/l	4.1	4.0-4.6
Chloride mmol/l	123.7	115-130

TEST	SAMPLE	REFERENCE VALUES
Plasma appearance	Icteric	Clear
PCV l/l	0.34	.30-.45
Plasma protein g/l (refractometer)	72	59-78
Haemoglobin g/l	119	80-140
Erythrocytes $x10^{12}$/l	7.85	6-10
MCV fL	43.3	40-45
MCHC g/l	350	310-350
MCH pg	15.2	13-17
Leukocytes $x10^{9}$/l	**14.1**	8-14
Neutrophils (seg.) $x10^{9}$/l	**12.97**	3.76-10.8
Neutrophils (band) $x10^{9}$/l	**0.56**	0-0.42
Lymphocytes $x10^{9}$/l	**0.42**	1.6-7.0
Monocytes $x10^{9}$/l	**0.00**	0.08.56
Eosinophils $x10^{9}$/l	**0.00**	0.16-1.4
Basophils $x10^{9}$/l	0.14	0-0.14
Platelets $x10^{9}$/l	Adequate (clumped)	300-700
Blood film: Moderate poikilocytosis, slight macroplatelets, some neutrophils appear vacuolated, 4 late normoblasts/100 WBCs.		

Urine (cystocentesis)	PH: 6.5
Appearance: cloudy	Glucose: -ve
Colour: dark yellow	Ketones: -ve
Specific gravity: 1.057	Blood: -ve
Protein (SSA): **trace**	Bilirubin: **4+**
Microscopic findings (centrifuged): much lipid, 1-3 white blood cells per high power field, 1-2 erythrocytes per high power field, scattered transitional epithelial cells.	

A five-year old, male neutered domestic short hair cat with vomiting and icterus

Likely conclusions: all the findings suggest that liver disease is a distinct possibility, with cholestatic mechanisms predominating. Although post-hepatic obstruction cannot be ruled out, intra-hepatic cholestasis is more likely because of the elevated ALT.

Postscript: Abdominal ultrasound-guided FNA of the liver showed extensive vacuolation of hepatocytes with some probable necrosis. A diagnosis of hepatic lipidosis was obtained from the laboratory. This was consistent with primary hepatic lipidosis, but secondary hepatic lipidosis could not be completely excluded.

The owners elected not to continue with treatment and the cat was destroyed. **At necropsy, and subsequent histopathology, hepatic lipidosis was confirmed. There was also a mild lymphocytic cholangitis and enteritis, and mild amyloid deposition in the pancreas.**

Possible reasons for changes: the elevated enzymes (marked elevation of ALP and moderate elevation of ALT) indicate marked cholestasis and moderate hepatocellular damage. The mildly elevated CK is more difficult to explain, but could be due to handling of the animal during examination (it is only MILD). The marginally low cholesterol is unusual and could be due to laboratory error in detection or perhaps just normal in this animal (reference ranges only cover 95% of the normal population). Reduced hepatic functional may cause hypocholesterolaemia, but this usually takes some time to develop because of good reserve capacity for production. The mildly elevated glucose (below renal threshold) could be due to stress (corticosteroid release) or perhaps a transient metabolic disturbance induced by conditions such as acute pancreatitis. The low urea is difficult to assess. It can occur due to emaciation or reduced functional hepatic mass, but the latter has to be severe. The low phosphate may be due to inappetence, but has been noted before in feline hepatic lipidosis (mechanism not elucidated). The haematological changes (a mild leukocytosis due to neutrophilia without left shift (the ratio is roughly 1:25), lymphopenia, absolute monocytopenia and eosinopenia) could be explained by stress (corticosteroid release effects on leukocytes) and toxicity/metabolic disturbances (toxic changes in neutrophils). Poikilocytosis is a non-specific change to intense or prolonged illness in the cat, but some haematologists believe it is more common in a variety of liver diseases. The presence of circulating nucleated erythroid cells in the absence of anaemia is difficult to explain (although numbers are low and probably not of great significance), but may be due to changes in the spleen, bone marrow or liver (increased BM leakage or perhaps decreased scavenging by splenic and the less important liver macrophages – could there have been splenic congestion and diminished capacity?). There is a possibility that the response could be appropriate and related to a compensated anaemia, but an increased reticulocyte count would need to confirm that.

The major urinary change is the significant bilirubinuria, which in the cat indicates significant conjugated bilirubin (most likely related to cholestasis and hence why the plasma was icteric). The trace protein at a specific gravity of 1.057 is probably not significant.

Likely conclusions and further investigation: all the findings suggest that liver disease is a distinct possibility, with cholestatic mechanisms predominating. Although post-hepatic obstruction cannot be ruled out, intra-hepatic cholestasis is more likely because of the elevated ALT. Further investigation of the liver could involve abdominal radiographs or ultrasonographs to determine the nature of the suspected hepatomegaly. Ultrasonography might be useful if fine needle cell aspiration is to be attempted. Additional hepatic testing might be considered (eg bile acids), but are probably not necessary.

Some clinical pathology results are not fully explained by liver disease and you may wish to follow those up by repeat testing (eg low urea, circulating nucleated erythroid cells) or additional testing (eg reticulocyte count).

It is possible that some of you may have considered pancreatitis (jaundice, vomiting, dehydration, neutrophilia, cholestasis, hyperglycaemia) and this could perhaps be investigated with abdominal ultrasonographs and pancreatic specific lipase (would this increase in vomiting alone?).

Clinical signs of respiratory disease may be followed up by thoracic radiographs. Some of you may wish to further investigate cardiac function. Clinical signs of dehydration were not supported by increased TPP, but it is feasible that there is still haemocentration (maybe the PCV is actually elevated and there could be an anaemia?).

(**postscript:** Abdominal ultrasound yielded a normal pancreas and diffusely increased echotexture of the liver with no evidence of large vessel biliary obstruction. From this, infiltrative disease of the liver was considered most probable and a fine needle cell aspiration performed. Results of this showed extensive vacuolation of hepatocytes with some probable necrosis. A diagnosis of hepatic lipidosis was obtained from the laboratory. This was consistent with primary hepatic lipidosis, but secondary hepatic lipidosis could not be completely excluded. Thoracic radiography did not identify any abnormalities.The owners elected not to continue with treatment and the cat was destroyed. At necropsy, and subsequent histopathology, hepatic lipidosis was confirmed. There was also a mild lymphocytic cholangitis and enteritis, and mild amyloid deposition in the pancreas. There were no other significant findings. A final diagnosis of hepatic lipidosis was provided.)

Gribbles Adelaide (Daren Hanshaw) Case study: Hx: cows with late-term abortion, pallor & jaundice

RBC	**1.73**	x 10 12 /L	(5.00 - 10.00)
Hb	**41**	g/L	(80 - 150)
Hct	**0.12**	L/L	(0.24 - 0.46)
MCV	**69**	fL	(40 - 60)
MCH	**24.0**	pg	(11 - 17)
MCHC	345	g/L	(300 - 360)
Reticulocytes	**4.6** %	80 x 10 ^9/L	
Nucleated RBCs	**60**	/100 WBCs	

WBC	7.6 x 10 ^9 /L		(4.0 - 12.0)
Neutrophils	74 %	**5.6** x10 ^9 /L	(0.6 - 4.0)
Band Forms	0 %	0.0 x10 ^9 /L	(< 0.2)
Lymphocytes	16 %	**1.2** x10 ^9 /L	(2.5 - 7.5)
Monocytes	6 %	0.5 x10 ^9 /L	(< 0.9)
Eosinophils	4 %	0.3 x10 ^9 /L	(< 2.5)
Platelets	259	x10 ^9 /L	(100 - 800)
Fibrinogen	5.7	g/L	(3.0 - 7.0)

TOTAL WBC corrected for presence of NRBC's

FILM MORPHOLOGY: RBC: **3+ anisocytosis, 2+ polychromasia, 1+ macrocytes, occasional basophilic stippling, numerous nucleated red cells**. WBC: Morphology normal. Platelets: appear adequate on the film.

Sodium	**155**	mmol/L	(132 - 152)
Potassium	5.2	mmol/L	(3.9 - 5.8)
Chloride	101	mmol/L	(95 - 110)
Bicarbonate	**35**	mmol/L	(20 - 30)
Na/K	29.8		
Urea	**12.8**	mmol/L	(2.1 - 9.6)
Creatinine	**152**	umol/L	(55 - 130)
Calcium	2.12	mmol/L	(2.00 - 3.00)
Phosphate	**3.30**	mmol/L	(1.29 - 2.26)
Magnesium	1.0	mmol/L	(0.5 - 1.5)

GLDH	**143**	U/L	(< 20)
B-OH Butyrate	0.8	mmol/L	(< 0.9)
Protein	**81**	g/L	(58 - 80)
Albumin	**43**	g/L	(22 - 36)
Globulin	38	g/L	(24 - 40)
T. Bilirubin	**77**	umol/L	(2 - 18)
Alk Phos	72	U/L	(35 - 350)
GGT	13	U/L	(< 36)
AST	**780**	U/L	(60 - 150)
CK	**1325**	U/L	(50 - 400)

SERUM INDICES (Clear/+/++/+++/++++)

Icterus index	**2+**
Lipaemia index	Clear
Haemolysis index	Clear

Likely conclusions: the animal has severe regenerative anaemia, probably due to a haemolytic crisis (but some bleeding cannot be excluded completely), which may have caused some hepatocyte and renal cell damage, the other changes could be related to this and dehydration. There is no indication as to cause – or is there?

My speculation: causation likely to be infectious (eg red cell parasites, clostridia) or chemical (exogenous oxidant?) because affecting an entire herd.

Postscript: Daren later told me that Theilerial organisms (PCR Ikeda) were detected on the blood smear.

Gribbles Adelaide (Daren Hanshaw) case study (MY INTERPRETATION)

Possible reasons for changes: the regenerative anaemia (severe drop in PCV, especially if you consider the animal to be dehydrated) could be related to haemolysis, especially if related to at least part of the hyperbilirubinaemia (although part is bound to be related to anorexia?). The anaemia is macrocytic (but not really hyperchromic, which could be due the regeneration and lack of ongoing haemolysis?) and there is basophilic stippling. The high levels of NRBCs, if related to the anaemia, fit in best with past haemolysis of perhaps one week or more duration. The mild neutrophilia with lymphocytopenia (N:L ratio is 4.7:1; normally less than 1.6) could be related to the regenerative anaemia and/or, because of the lymphocytopenia, due to corticosteroid release. The high sodium in conjunction with high protein and albumin (A:G is 1.1; normal should be 0.6-1.5?) suggest dehydration (derived osmolality is 320 and likely high). The high bicarb (metabolic alkalosis), might be related to abomasal reflux (HCl), which usually involves decreases in Cl^- and compensatory increases in bicarbonate (this could be the case as chloride is disproportionate to the Na change - *Corrected Cl = (normal Na/measured Na) x measured Cl; which in this case is 67 and still abnormal*). Because the Anion Gap is high (24.2 - normally less than 20 in a cow) there may be some other process affecting the acid/base balance. It would be tempting to suggest that renal disease may be occurring because of the mild azotaemia and the hyperphosphataemia (although dehydration is a pre-renal reason to be considered). The increased GLDH might suggest hepatocellular damage which could go along with nephrosis when you have such a severe drop in PCV (hypoperfusion). The hyperbilirubinaemia has been discussed. The elevated AST and CK could be due to recumbency, but some of the AST elevation may be due to hepatocyte damage.

Likely conclusions: the animal has severe regenerative anaemia, probably due to a haemolytic crisis (but some bleeding cannot be excluded completely), which may have caused some hepatocyte and possibly renal cell damage, the other changes could be related to this and dehydration. There is no indication as to cause.

Further testing: urinalysis to check for haemoglobinuria and to check for renal damage; conjugated to unconjugated bilirubin (contentious!); recheck haematology , electrolytes and acid base balance in other cows (or PM). Causation likely to be infectious (eg red cell parasites, clostridia, leptospirosis?) or chemical (exogenous oxidant?) because affecting herd.

Postscript: Daren later told me that Theilerial organisms were detected on the blood smear.

A 7-years-old desexed female kelpie dog with a 5 week history of vomiting, inappetence and lethargy. She was lethargic, anorectic, and appeared to have laboured respiration. Her mucous membranes were pale pink. She was underweight with reduced drinking but not clinically dehydrated. She had possible enlargement of the liver and of one or both prescapular lymph nodes.

TEST	SAMPLE	REF VALUES
AMYLASE IU/L	604	<1400
LIPASE IU/L	60	<100
ALP IU/L	**8070**	<110
ALT IU/L	**497**	<60
CK IU/L	108	<200
Serum protein (biuret) g/L	**47.6**	50-70
Albumin (BCG) g/L	**22.9**	23-43
Globulins g/L	**24.6**	27-44
Bilirubin µmol/L	**25.5**	1.2-8.1
Total cholesterol mmol/L	5.77	1.4-7.5
Glucose mmol/L	5.15	3.3-6.4
Urea mmol/L	3.78	3.0-10
Creatinine µmol/L	41	40-120
Calcium mmol/L	**1.9**	2.1-2.9
Inorganic phosphate mmol/L	1.3	0.8-1.6
Sodium mmol/L	140	137-150
Potassium mmol/L	4.1	3.3-4.8
Chloride mmol/L	116	105-120
Bicarbonate (TCO2) mmol/L	**26.5**	18-24

TEST	SAMPLE	REF VALUES
Plasma appearance	**Red**	Clear
PCV L/L	**0.34**	.37-.50
Plasma protein g/L (refract)	55	55-75
Haemoglobin g/L	122	100-150
Erythrocytes x1012/L	5.1	5-7
MCV fL	67	60-75
MCHC g/L	**359**	300-350
MCH pg	24	20-25
Leukocytes x109/L	**35.5**	7-12
Neutrophils (seg.) x109/L	**28.8**	4.1-9.4
Neutrophils (band) x109/L	**2.1**	0-.24
Lymphocytes x109/L	1.4	.91-3.6
Monocytes x109/L	**3.2**	.2-.96
Eosinophils x109/L	**0**	.14-1.2
Basophils x109/L	0	0-.36
Platelets x109/L	**170**	200-600
Reticulocyte % (uncorrected)	**3.0**	0-1.5
Blood film: **1 nucleated erythroid cell per 100 leukocytes. 1+ anisocytosis, 2+ polychromasia and poikilocytosis of erythrocytes. 1-2+ spherocytes. Macroplatelets present.**		

Colour and Urine appearance of **urine** (cystocentesis): yellow brown and clear	Glucose: -ve
Specific gravity: 1.025	Ketones: -ve
Protein (SSA): negative	Blood: trace
pH: 7.5	Bilirubin: **4+**
Microscopic findings: less than 1 leukocyte and erythrocyte per HPF. Some lipid and transitional epithelial cells	

Possible conclusions: the findings suggest that liver disease (and active-chronic in nature) is a distinct possibility. However, this does not explain the regenerative anaemia nor the thrombocytopenia (or the left shift). There is nothing definite to suggest why the lymph nodes are enlarged, but reaction to inflammation could be a possibility with the leukogram. The laboured respiration has not been explained satisfactorily. Could there be inflammation in the lungs?

(**Postscript:** Diagnostic imaging showed hepatomegaly, splenomegaly and possible nodules within the peritoneum. Diagnostic imaging of lungs was not performed. **Fine needle cell aspirates of lymph node, spleen and liver suggested lymphosarcoma**. The regenerative anaemia was considered to be immune-mediated secondary to neoplasia (a paraneoplastic phenomenon). The mild thrombocytopenia could have also been related to immune-mediated destruction, but utilisation secondary to neoplastic destruction of tissue could not be discounted. The neutrophilia with left shift could have been due to the haemolytic anaemia or due to inflammation secondary to neoplastic destruction of tissue. The owners decided not to opt for treatment of the animal and the outcome was unknown).

Possible reasons for changes: there is marked elevation of ALP and moderate elevation of ALT indicating cholestasis and hepatocellular damage. The low protein could be consistent with ongoing liver disease as both albumin and globulin production (except for gamma globulins) can be affected through advanced loss of hepatic function . The moderate hyperbilirubinaemia could suggest liver damage, but haemolytic anaemia as a cause cannot be ruled out. The mild hypocalcaemia once corrected for hypoalbuminaemia is 2.15, which is in the reference range. The metabolic alkalosis could be due to some of the vomiting, although there is not hypochloridaemia. Respiratory changes may be contributing? The haematological changes are challenging. The plasma is red and there is a regenerative anaemia (corrected reticulocyte count is 2.3%; absolute reticulocytes are 0.153 x 10^{12}/L [RR 0-0.08], polychromasia and anisocytosis on the blood film) with an elevated MCHC. This could be due to some haemolysis prior to or after collection of the sample. The spherocytes could suggest some damage to erythrocytes (immune mediated?). The leukogram suggests some corticosteroid response (monocytosis, eosinopenia and neutrophilia), but does not explain the left shift. This could be occurring due to true inflammatory demand or related to the anaemia (neutrophilia with left shift is not uncommon in the dog with an intense regenerative haemolytic anaemia. The macroplatelets suggest either increased destruction or utilisation of platelets. The level of platelets should not be causing a clinical problem. The 4+ bilirubinuria in a female dog is probably significant and related to the hepatic disease/anaemia.

Likely conclusions: the findings suggest that liver disease (and active-chronic in nature) is a distinct possibility. However, this does not explain the regenerative anaemia nor the thrombocytopenia (or the left shift). There is nothing definite to suggest why the lymph nodes are enlarged, but reaction to inflammation could be a possibility with the leukogram (perhaps FNA the nodes?). The laboured respiration has not been explained satisfactorily. Could there be inflammation in the lungs (perhaps imaging and bronchial washes might help)?

(**Postscript:** Diagnostic imaging showed hepatomegaly, splenomegaly and possible nodules within the peritoneum. Diagnostic imaging of lungs was not performed. Fine needle cell aspirates of lymph node, spleen and liver suggested lymphosarcoma. The regenerative anaemia was considered to be immune-mediated secondary to neoplasia. The mild thrombocytopenia could have also been related to immune-mediated destruction, but utilisation secondary to neoplastic destruction of tissue could not be discounted. The neutrophilia with left shift could have been due to the haemolytic anaemia or due to inflammation secondary to neoplastic destruction of tissue. The owners decided not to opt for treatment of the animal).

A three-years-old male Pomeranian was referred with a six months history of intermittent diarrhoea and occasional vomiting. On examination, the dog was depressed, thin, probably dehydrated and had a distinct peritoneal effusion.

TEST	SAMPLE	REF VALUES
Plasma appearance	Clear	Clear
PCV L/L	0.39	0.37-0.50
Plasma protein g/L	**52**	55-75
Leukocytes $x10^9$/L	11.1	7-12
Neutrophils (seg.) $x10^9$/L	8.77	4.1-9.4
Neutrophils (band) $x10^9$/L	0	0-0.24
Lymphocytes $x10^9$/L	1.22	0.9-3.6
Monocytes $x10^9$/L	0.89	0.2-1.0
Eosinophils $x10^9$/L	0.22	0.14-1.2
Basophils $x10^9$/L	0	0-0.4
Blood film: **1+ poikilocytosis of erythrocytes**		

TEST	SAMPLE	REFERENCE VALUES
CK IU/L	**426**	<200
Amylase IU/L	**1481**	<1400
ALP IU/L	22	<110
ALT IU/L	10	<60
Total protein g/L	**45**	50-70
Albumin g/L	23.4	23-43
Globulins g/L	**21.7**	27-44
Total cholesterol mmol/L	**7.88**	1.4-7.5
Glucose mmol/L	3.35	3.3-6.4
Urea mmol/L	**22**	3.0-10
Creatinine µmol/L	**179**	105-120
Calcium mmol/L	2.1	2.1-2.9
Inorganic phosphate mmol/L	**3.6**	0.8-1.6
Sodium mmol/L	142.1	137-150
Potassium mmol/L	**3.2**	3.3-4.8
Chloride mmol/L	111	105-120
Bicarbonate (TCO_2) mmol/L	20	18-24

Urinalysis (cystocentesis)	
Appearance: moderate turbidity	PH: 6.5
Colour: light yellow	Glucose: -ve
Specific gravity: 1.031	Ketones: -ve
Protein: **4+**	Blood: -ve
	Bilirubin: -ve
Microscopic findings: moderate numbers of lipid droplets, 1 erythrocyte per HPF and less than 1 leukocyte per HPF. Many sperm and less than 1 course granular cast per LPF.	

ABDOMINAL FLUID TEST	RESULT	REFERENCE VALUES
Appearance	clear and colourless	clear and colourless
Total protein g/L	<10	<25
Erythrocytes x 10^6/L	**10,000**	None
Nucleated cells x 10^6/L	200	<500
Smear	62% non-lytic neutrophils, 22% small lymphocytes, 16% monocytes, macrophages, mesothelial cells	Scattered mix of mononuclear cells and non-lytic (non-degenerate) neutrophils

Likely conclusions: it is likely that the dog has hypoproteinaemia and abdominal effusion due to renal disease. A 4+ protein would suggest a glomerular disease (protein losing glomerulopathy/nephropathy). The effusion is unlikely to be just due to the hypoproteinaemia (not low enough) and renal hypertension/electrolyte retention is probably contributing.

(**Postscript:** the dog was placed on a high quality protein diet, ACE inhibitor and given gastroprotective drugs because of the subsequent finding of melaena and suspected gastric bleeding. A poor prognosis was given, but the owner declined euthanasia. The outcome is not known)

Possible reasons for changes: most of the biochemical and haematological changes are mild, but the 4+ proteinuria in moderately concentrated urine really stands out. This could fit in with the hypoproteinaemia (due to hypoglobulinaemia and borderline hypoalbuminaemia – could be worse than what it seems because the animal is suspected of being dehydrated),. Because of the 4+ protein the specific gravity has probably been pushed up 3-4 units, so in actuality it is about 1.027-28. In a dehydrated dog this may indicate some impairment of renal concentrating ability. The mild azotaemia might be related to this, although in dogs azotaemia usually does not occur in tubular renal disease until the specific gravity is close to isosthenuria. However, glomerular disease can cause azotaemia from early on due to reduced clearance (GFR). The increased inorganic phosphate could be related to renal disease. The mild elevation in amylase could be due to reduced renal clearance (azotaemia and decreased GFR can cause increased globulin bound amylase) or upper gastrointestinal disease (especially vomiting) as there is little else to suggest pancreatitis (perhaps the occasional vomiting, perhaps a lower normal calcium [although correction for the level of albumin would make it normal]?). The increased cholesterol may be related to renal disease (increased beta lipoproteins). The hypokalaemia could have been related to the diarrhoea. The mild increase in CK is difficult to explain (struggling?), but is unlikely to be of consequence. The abdominal fluid could be classified as a pure transudate because of low nucleated cells and protein. However, some might call it a modified transudate because of the red cells present. The erythrocytes could be related to the abdominocentesis procedure or could be due to a disease process. This brings us back to the PCV, which although within the reference interval, could be lower than what it seems as the dog is probably dehydrated. Therefore a mild anaemia may exist. The cause of this is not apparent, but could be related to chronic disease. The slight (1+) poikilocytosis of erythrocytes may be related to chronic disease.

Likely conclusions, further testing and implications for management and prognosis?: it is likely that the dog has hypoproteinaemia and abdominal effusion due to renal disease. A 4+ protein would suggest a glomerular disease (protein losing glomerulopathy/nephropathy). The effusion is unlikely to be just due to the hypoproteinaemia (not low enough) and renal hypertension/electrolyte retention is probably contributing. A renal biopsy might be considered to delve into the problem further (if it is immune mediated, the animal could be treated with immunosuppressive drugs). Some of you might be concerned about pancreatitis and might want to do diagnostic imaging or a lipase to check. Some might be concerned about the gastrointestinal signs and want to do diagnostic imaging and faecal analysis. Some might want to investigate liver further with diagnostic imaging to detect if there was chronic liver disease (without liver enzyme increases) that could possibly contribute to the hypoproteinemia and the effusion through presinusoidal congestion. Some might wish to do a reticulocyte count to further investigate the possible anaemia. We cannot say for sure that blood loss is not occurring in the gut. All in all, management of this case would concentrate on the suspected glomerular disease and consequent hypoproteinaemia. Urine protein to creatinine ratios might be undertaken to monitor the proteinuria. Blood pressure determination and monitoring would be useful.

(**Postscript:** the dog was placed on a high quality protein diet, ACE inhibitor and given gastroprotective drugs because of the subsequent finding of melaena and suspected gastric bleeding. A poor prognosis was given, but the owner declined euthanasia. The outcome is not known)