

COLLEGE OF AGRICULTURE, SCHOOL OF VETERINARY MEDICINE

MODULE 2: VETERINARY SYSTEMIC AND CLINICAL PATHOLOGY

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PREFACE

Students who have completed the DVM program will be expected to have the Day 1 competencies of knowledge, skills and behavioral values to practice veterinary medicine independently. For this, the knowledge of Veterinary Pathology is very crucial and it is very vast, classified into different courses offered in the program. Module 2 provides the graduating veterinarians with reference materials in terms of Veterinary Systemic and Clinical Pathology courses. The module is divided into two sections: Section 1, *Systemic Pathology*, and Section 2, *Clinical Pathology.* Under systemic pathology, the subject matter is divided into ten chapters focusing on key concepts in the areas of pathologies of cardiovascular system, digestive system, urinary system, reproductive system, nervous system, endocrine glands, musculoskeletal system, the integument and sense organs. Veterinary clinical pathology is subdivided into two parts; Veterinary clinical pathology I and Veterinary clinical pathology II. Veterinary clinical pathology I covers Introduction to the course, hematology, examination of bone marrow, serum biochemistry and serum enzymology. Veterinary clinical pathology II emphasis about organ function tests (Liver and kidney), urinalysis, body water, electrolytes and fluid analysis, diagnostic cytology and the histopathological techniques.

The module uses harmonized DVM curriculum to select the course topics for the sake of explanation; however, those who need more practical examples are advised to refer to other books and laboratory manuals, which elaborate how to use some of those skills and methods in actual lessons. It is recommended that the users refer to some specific sections of Module 2 when they are involved in the national exit exam. The module offers general information regarding veterinary systemic and clinical pathology courses.

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SECTION 1: VETERINARY SYSTEMIC PATHOLOGY

By the end of this course, students will be able to:

- Know gross and microscopic appearances of lesions caused by various disease conditions
- Able to correlate gross and microscopic pathological changes with specific diseases for accurate diagnosis.
- Microscopic features of common abnormalities in different organ systems, tissues and cells, collecting and dispatching of pathological samples, necropsy procedures etc.
- Predict the application of the learned material in the control of animal diseases.

CHAPTER 1: PATHOLOGY OF CARDIOVASCULAR SYSTEM

DEVELOPMENT AL ANOMALIES

Persistent right aortic arch

This is a developmental anomaly of aorta in which the aorta develops from right arch present on right side of trachea and oesophagus. The ductus arteriosus forms a ring around trachea and esophagus by connecting aorta and pulmonary artery. This ring causes partial obstruction of trachea and/or esophagus.

Patent ductus arteriosus

The ductus arteriosus is a short blood vessel which connects pulmonary artery to aorta in foetal life for diversion of blood. Normally soon after birth this duct is sealed and remains in the form of a ligamentum arteriosum. But sometimes this ductus arteriosus remains open and blood is continuously shunted between aorta and pulmonary artery and may led to congestive heart failure, pulmonary hypertension and cyanosis due to mixing of venous and arterial blood.

Interventricular septal defects

In foetal life, there is no partition in ventricles and there is only one chamber which is divided into two right and left by inter ventricular septum. But when interventricular septum does not develop completely and due to defect in formation of complete partition, there is mixing of blood from both chambers. It is responsible for thickening of myocardium, roughening of endocardium and cyanosis.

Transposition of aorta

When there is a shift in position of aorta and pulmonary artery i.e. the aorta arises from right ventricle and pulmonary artery from left ventricle. This results in arterial blood in right and venous blood in left side and has no clinical significance. However, it may create problems when aorta arises from venous ventricle and pulmonary artery from arterial side.

Tetrad of Fallot

Tetrad of Fallot includes 4 developmental defects of cardiovascular system and is also known as *tetralogy of Fallot.*

i. Inter ventricular septal defect
ii. Pulmonary stenosis is characterized by narrowing of lumen of pulmonary artery at its origin due to fibrous tissue causing 'jet' effect.
iii. Hypertrophy of right ventricle
iv. Transposition of aorta.
v. Ectopia cardis When heart lies outside the thorax under the subcutaneous tissue of lower cervical region.

Interatrial septal defect

There is a developmental defect in inter atrial septa and remains as incomplete partition of atrium. It produces continuous overload on the right side of heart leading to pulmonary hypertension and hypertrophy of right side myocardium. However, a small defect in septum may persist throughout the life of animal without causing any clinical illness.

CARDIAC FAILURE

Cardiac failure is the inability of heart to maintain adequate blood supply leading to death. It can be divided into two types: Acute and chronic heart failure.

Acute cardiac failure

Acute cardiac failure is sudden failure of contraction of heart leading to death within minutes.

Etiology

- Anoxia
- Drugs/ poisons
- Shock
- Cardiac temponade
- Myocardial necrosis
- Sudden occlusion of aorta and/ or pulmonary artery.

Macroscopic and microscopic features

- Cardiac temponade
- Occlusive thrombus
- Pulmonary congestion
- Dialation of heart particularly of right ventricle
- Myocardial necrosis
- Centrilobular necrosis in liver "nut meg liver"
- In prolonged cases, congestion and oedema in visceral organs.

CHRONIC CARDIAC FAILURE

Chronic cardiac failure is the inability of heart to maintain balance between its output and venous return of blood. It can be further divided into two left and right sided heart failure.

LEFT SIDED HEART FAILURE

Left sided heart failure is caused by myocardial damage and characterized by congestion and oedema in lungs with hypertrophy of alveolar lining cells.

Etiology

- Myocardial degeneration/ necrosis
- Aortic and mitral valve disease
- Hypertension

Macroscopic and microscopic features

- Congestion and oedema in lungs
- Chronic dialation of heart
- Congestion of alveolar vessels
- Edema in lungs
- Hypertrophy of alveolar lining cells
- Alveolar macrophages contain hemosiderin pigment also called "heart failure cells"

RIGHT SIDED HEART FAILURE

Right-sided heart failure is caused by a disease of lungs or pulmonary vasculature and mostly occurs after a left sided heart failure.

Etiology

- Left sided heart failure
- Pulmonary lesions, congestion

Macroscopic and microscopic features

- Congestion of visceral organs
- Subcutis oedema and ascites
- Pulse in jugular vein
- "Nutmeg appearance"in liver due to centrilobular necrosis.
- Atrophy, necrosis and fibrosis in liver
- Congestion in visceral organs.

PERICARDITIS

Pericarditis is the inflammation of pericardium, the upper layer of heart. It may be serus, fibrinous or suppurative depending on the type of exudate.

Etiology/ Occurrence

- Pasteurellosis
- Salmonellosis in poultry.
- Hydropericardium syndrome in poultry
- Gout in poultry
- Traumal Foreign body *e.g.* Traumatic reticulo pericarditis (TRP).

Macroscopic and microscopic features

- Deposition of fibrin in between pericardium and heart gives an appearance of "bread and butter".
- In chronic- cases, pericardium becomes thick due to excessive fibrosis.
- Accumulation of fluid (clear, serus) in pericardial sac is termed as *Hydropericardium.*
- Presence of blood in pericardia I sac is known as *hemopericardium* and the excessive accumulation of blood leading to heart failure is termed as *cardiac temponade.*
- Accumulation of pus in pericardial sac is called as *pyopericardium.*
- Presence of gas in pericardial sac in termed as *pneumopericardium.*
- Hyperemia and haemorrhage in pericardium
- Deposition of fibrin, formation of fibrin network, infiltration of neutrophils, macrophages and lymphocytes.

MYOCARDITIS

Myocarditis is the inflammation of myocardium, the middle layer of heart. It may be suppurative, eosinophlic or lymphocytic depending on the type of the exudates.

Etiology

- Toxins *I* Poisons
- Bacterial Virus

- Parasites
- *Drugsl* Chemicals

Macroscopic and microscopic features

- Colour of myocardium may become dark red or cyanotic due to accumulation of blood.
- In suppurative myocarditis, one can find abscesses in myocardium from where yellow/green pus oozes out.
- Yellowish white streaks of necrosis in myocardium.
- Presence of cyst encapsulated by fibroplasia due to cysticercosis.
- Hyperemia and haemorrhages in myocardium.
- Infiltration of neutrophils, eosinophils or lymphocytes.
- Coagulative necrosis of muscle fibers.
- In chronic cases, proliferation of fibrous connective tissue.

ENDOCARDITIS

Endocarditis is the inflammation of the endocardium, the inner layer of heart.

Etiology/ Occurrence

- Chronic septicemic diseases like caused by *Actinomyces pyogenes, Erysipelothrix rhusiopathe*
- Staphylococci
- Streptococci
- *Pseudomonas aeruginosa*
- Clostridial infections.

Macroscopic and microscopic features

- Lesions in heart valves or wall of atrium/ ventricles.
- Presence of thrombi on endocardium.
- Vegetative/ cauliflower like growth on endocardium either in valves *(Valvular vegetative endocarditis e.g.* Swine erysepalas) or in wall *(Mural vegetative endocarditis).*

- Dilation of heart chambers.
- Infiltration of thrombocytes, neutophils, macrophages and lymphocytes.
- Masses of bacterial organisms can be seen.
- Underlying endocardium and myocardium shows the presence of fibrin network and infiltration of RBC, neutrophils and macrophages.

BRISKET DISEASF/ HIGH ALTITUDE DISEASE

Brisket disease is a condition of slow cardiac failure, which occurs at 2500 meters sea level or aoove where pressure of air is low.

Etiology

- Low oxygen in environment
- Decreased atmospheric pressure of air
- In native cattle morbidity rate is only 2% and in imported cattle at hills it is upto 40%.

Macroscopic and microscopic features

- Dilation of heart
- Hypertrophy of ventricular wall
- Chronic passive congestion in visceral organs
- Edema in sternal region in between forelegs.
- Nut meg liver due to chronic passive congestion
- Polycythemia
- Hypertrophy of muscle fibers in myocardium.

MULBERRY HEART DISEASE

It is characterized by firm contraction of heart and petechial hemorrhage on pericardium giving the appearance of mulberry.

Etiology

- Not known

- May be enterotoxaemia/ poisoning

Macroscopic and microscopic features

- Contraction of heart with petechial haemorrhage on pericardium looking like mulberry "Mulbery heart disease"
- Hydropericardium, hydroperitoneum and pulmonary oedema
- Edema fluid has high protein content resulting in clot formation
- Congestion of fundic portion of stomach.
- Congestion on serosa of visceral organs.

ARTERIOSCLEROSIS

Arteriosclerosis is hardening of arteries causing 3 types of diseases in arteries depending on their size and etiological factors viz., Atherosclerosis, medial sclerosis and arteriolosclerosis.

ATHEROSCLEROSIS

Atherosclerosis is characterized by hardening and thickening of intimal layer of large arteries and aorta due to proliferation of connective tissue, hyaline degeneration, infiltration of fat/ lipids and calcification. These intimal changes may lead to loss of elasticity of artery *(Athere* means mushy substance).

Etiology

- Exact cause is not clear
- Hypercholesterolemia and hyperlipidemia
- Hypertension

Macroscopic and microscopic features

- Fatty streaks running parallel in the direction of the artery.
- Intimal layer of aortal coronary arteries is elevated due to plaques which are white/yellow, fibrous and occluding the lumen of vessel.
- Occlusion of artery may lead to ischemia and infarction.

- Macrophages are filled with lipid droplets including cholesterol, fatty acids, triglycerides and phospholipids.
- Fragmented internal elastic lamina in the intimal layer of artery
- Proliferation of altered smooth muscles may become metaplastic to macrophages.
- Deposition of mucoid ground substance and collagen fibers
- Hyalinization of connective tissue "Fibrous plaques".
- Presence of some fat droplets in between the lesion

MEDIAL SCLEROSIS

Medial sclerosis involves medium sized muscular arteries and characterized by fatty degeneration and hyalinization of muscular tissue of medial arteries leading to necrosis. This is also known as *Monckeberg medial sclerosis.*

Etiology

- Old age
- Excessive administration of epinephrine (adrenaline).
- Nicotine
- Vitamin D toxicity.
- Hyperparathyroidism

Macroscopic and microscopic features

- Hardening of medium sized arteries
- Hyaline, fatty changes and calcification of arterial wall
- Fatty changes, hyalinization of muscular layer of medium sized arteries.
- Necrosis of myofibrils
- Calcification

ARTERIOLOSCLEROSIS

Arteriolosclerosis affects arterioles in kidneys, spleen and pancreas and is characterized by hyperplasia of intimal cells of arterioles producing concentric lamellations occluding their lumen.

Etiology

- Hypertension

Macroscopic and microscopic features

- No characteristic macroscopic lesion.
- Atrophy of organ, hardening.
- Proliferation of cells present in intima of blood vessels
- Swelling and necrosis of cells in medial layer leading to occlusion of lumen
- Calcification in chronic cases.

ARTERITIS

Arteritis is the inflammation of arteries characterized by infiltration of neutrophils, lymphocytes and macrophages in the media and intima of arterial wall.

Etiology

- Chemicals
- Thermal
- Virus *e.g.* Equine viral arteritis
- Pyogenic bacteria.
- Parasite *e.g. Strongylus vulgaris*

Macroscopic and microscopic features

- Hyperemia
- Conjunctivitis, oedema of eye
- Presence of thrombi in artery
- Presence of thrombi in artery involving intimal layer.
- Equine viral arteritis virus causes infiltration of lymphocytes and macrophages in media.
- Occlusion of 14men of arteries due to thickening of wall.
- In parasitic arteritis, parasitic thrombi may present along with inflammatory reaction in intimal layer.

ANEURYSM

Aneurysm is dilation of an artery or cardiac chamber leading to formation of sac.

Etiology

- Aflatoxin
- Infectious emboli
- Weak vessel wall due to rupture.
- Fracture or necrosis of medial layer of large blood vessel.
- Arteriolosclerosis

Macroscopic and microscopic features

- Fracture or necrosis of medial layer of large blood vessels permitting parallel blood circulation till the next division of blood vessel is called as *Dissecting aneurysm* or *false aneurysm.*
- Formation of sac in artery due to dilation, also known as *True aneurysm.*
- Rough intimal layer
- Wall of blood vessel damaged with inflammatory exudate.

PHLEBITIS

Phlebitis is the inflammation of veins characterized by presence of inflammatory exudate, thickening of the wall and dilation of the lumen.

Etiology/ Occurrence

- Naval infection in calves
- Uterine infections
- In jugular vein due to improper intravenous infection.
- *Varicose veins* are dilated and elongated veins following irregular and tortuous course
- *Telangiectasis* is marked dilation of veins particularly of sinusoidal capillaries in one or more lobules in liver.

Macroscopic feature

- Wall of vein is thickened.
- Vein contain large thick necrotic material
- Lumen dialated
- Inner surface of vein is rough and hyperemic.
- Infiltration of neutrophils in the wall of veins
- Sometimes calcification may also present.
- Wall of vein becomes thick due to inflammatory cells and/ or proliferation of fibrous tissue.

LYMPHANGITIS

Lymphangitis is the inflammation of lymph vessels characterized by aggregation of lymphocytes around lymphatics, oedema of dependent parts and distension of lymphatics.

Etiology/ Occurrence

- *Corynebacterium avis* causes caseous lymphangitis and lymphadenitis
- Equine epizootic lymphangitis

Macroscopic and microscopic features

- Distension of subcutaneous lymph vessels, nodules of lymphoid aggregates
- Edema due to failure of lymphatic drainage.
- Lymphoid aggregation around lymphatics.
- Lymphatics distended
- Edema of dependent tissue.

CHAPTER 2: RESPIRATORY SYSTEM

PATHOLOGY OF UPPER RESPIRATORY TRACT

In many infectious diseases, there is inflammation of mucosa of upper respiratory passage leading to nasal discharge which is catarrhal, purulent or fibrinous depending on the type of

infection. The infection may extend to lower parts of respiratory tract and reach in lungs causing pathological alterations. *Rhinitis* is the inflammation of nasal mucosa. *Sinusitis* is the inflammation of sinuses *e.g.* Frontal sinusitis in dehorned cattle. The larvae of botfly *Oestrus ovis* enters in nasal passage and migrate up to frontal sinuses and turbinate bones and cause mucopurulent inflammation. Similarly leeches *(Dinobdella ferox)* are known to cause nasal cavity inflammation in domestic animals and suck blood. Rhinitis caused by *Bordetella bronchiseptica* in pigs and characterized by mucopurulent exudate, disappearance of nasal septum, retarded growth of snout and plugging of passage by solidified exudate and dead tissue. This condition is known as *porcine atrophic rhinitis. Epistaxis* is bleeding from nasal passage due to trauma, neoplasm and ulcerative lesions as a result of infections. *Pharyngitis* is the inflammation of pharynix while *laryngitis* is the inflammation of larynx.

NASAL POLYPS

Nasal polyps are the inflammatory condition of respiratory mucosa resembling neoplastic growth caused by fungus and characterized by formation of new growth simulating benign neoplasm in nasal passage.

Etiology

- *Rhinosporidium sceberi*, a fungus most commonly prevalent in southern India.

Macroscopic and microscopic features

- Formation of a single polyp in respiratory mucosa, pedunculated, elongated, fills nasal cavity.
- Cauliflower like growth may cause bleeding.
- Fibrous covering by mucous membrane and heavily infilterated by neutrophils, lymphocytes, eosinophils, macrophages around fungus.

NASAL GRANULOMA

Nasal granuloma is the granulomatous inflammation of respiratory mucosa in nasal cavity caused by blood flukes and characterized by the presence of granulomatous growth filling the nasal passage causing obstruction.

Etiology

- *Schistosoma nasalis,* a blood fluke.
- Type II hypersensitivity reaction of nasal mucosa to plant pollens, fungi, mites etc.

Macroscopic and microscopic features

- Nasal pruritus
- Small tiny nodules on nasal mucosa later become cauliflower like growth filling the cavity and causing obstruction.
- Edema in lamina propria
- Infiltration of eosinophils, mast cells, lymphocytes and plasma cells and absence of epithelioid cells.
- Proliferation of fibroblasts.
- The lesion is covered by squamous epithelium.
- Mucous glands may have metaplastic pseudostratified columnar epithelium.

TRACHEITIS

Tracheitis is the inflammation of trachea. In canines, it is tracheobronchitis while in poultry it is manifested by laryngotracheitis.

Etiology

- Canine tracheobronchitis caused by adenovirus, influenza virus and herpes virus.
- Avian infectious laryngotracheitis (ILT) is caused by herpes virus.

Macroscopic and microscopic features

- Canine tracheobronchitis or *kennel cough* includes congestion of trachea and presence of catarrhal exudate.
- In poultry, haemorrhage in trachea and caseous plug in trachea towards larynx causing obstruction.
- Inclusion bodies in tracheal and bronchial epithelium in canines

- Haemorrhagic tracheitis, presence of intra nuclear basophilic inclusions in tracheal epithelial cells in infectious laryngotracheitis.

BRONCHITIS

Bronchitis is the inflammation of bronchi, characterized by catarrhal, suppurative, fibrinous or haemorrhagic exudate.

Etiology

- Bacteria *e.g.* Pasteurella
- Virus *e.g.* Infectious bronchitis in poultry
- Parasites
- Allergy/Inhalation of pollens etc.

Macroscopic and microscopic features

- Coughing, dyspnoea
- Mucous exudates in lumen
- Congestion and/ or haemorrhages in bronchi
- Presence of caseaous plugs at the point where bronchi enter in lungs in infectious bronchitis of poultry.
- Mucous exudate along with inflammatory cells in the lumen of bronchi.
- Hyperplasia and/or necrosis of bronchiolar epithelium
- Accumulation of mononuclear cells in the bronchial mucosa and in peribronchiolar area.

PATHOLOGY OF LUNGS

ATELECT ASIS

Atelectasis is the failure of alveoli to open or the alveoli are collapsed and thus do not have air.

Etiology

- Obstruction in bronchi/ bronchiole
- Pleuritis

- Atelectasis neonatorum in new born animals. In the absence of respiration, lung alveoli remain closed and thus sink in water indicating still birth.

Macroscopic and microscopic features

- Dull red in colour, hard area of lung like liver in consistency
- Atelectic lung sinks in water.
- Compressed alveoli.
- Absence of air spaces
- Collapsed bronchioles
- In inflammatory condition, exudate compresses alveoli

EMPHYSEMA

Emphysema is the increase in amount of air in lungs characterized by dilation of the alveolL It may be acute or chronic and focal or generalized.

Etiology

- Bronchitis
- Atelectasis in adjoining area of lung
- Pneumonia
- Allergy to dust, Pollens etc
- Pulmonary adenomatosis

Macroscopic and microscopic features

- Lungs are enlarged and flabby
- Imprints of ribs can be seen. Colour of lungs becomes pale.
- Cut surface is smooth and dry.
- Alveoli are distended.
- Some alveoli may rupture and form giant alveoli.
- Alveolar wall becomes thin due to stretching.
- Mild bronchitis.
- Hyperplasia of lymphoid tissue.

PULMONARY EDEMA

In pulmonary edema, there is accumulation of serous fluid in alveoli of lungs.

Etiology

- Bacteria
- Virus
- Allergy

Macroscopic and microscopic features

- Lungs become enlarged
- Weight of lungs increases
- Cut surface releases fluid and frothy exudate in trachea and/ or bronchi.
- Serous fluid accumulation in alveoli of lungs
- Fluid may also be seen in some bronchij bronchioles.
- Infiltration of inflammatory cells.
- Congestion of lungs.

PNEUMONIA

Pneumonia is the inflammation of .lungs characterized by congestion and consolidation of lungs. The pathological lesions in lungs are produced in a similar way irrespective of the type of etiological agent and include various stages like **congestion, red hepatization, grey hepatization** and **resolution**.

Stage of congestion: This stage of lung is characterized by active hyperemia and pulmonary oedema. The capillaries are distended with engorged blood and alveoli are filled with watery serous exudate. This requires 2 minutes to few hours to initiate the congestion.

Stage of red hepatization: This stage of lung is characterized by the consolidation of lungs due to accumulation of blood in blood vessels (congestion). The consolidated lungs are firm and looking like liver and hence the name "red hepatization". Such affected lung always sinks in

water. Alveoli are filled with serous or serofibrinous exudate giving hardness to lungs. In inflammatory condition; the neutrophils, macro phages and lymphocytes along with erythrocytes infilterate the affected area of lungs. This stage of red hepatization takes 2 days for development of firmness of lung.

Stage of grey hepatization: The lung remains hard but due to lysis and removal of erythrocytes, it becomes grey or less red in colour. Firmness/ hardness of lung remain same and thus, the name grey hepatization. There is increase in infiltration of inflammatory cells like macrophages, lymphocytes, epitheloid cells depending on the virulence of etiological agents.

Stage of resolution: After a week, the recovery starts in the form of resorption of fluid; autolized cells and debris is removed by phagocytic cells. The causative organism is neutralized or removed from the lungs through immunity of body. After few days the lung parenchyma becomes normal and starts functioning. If the causative agent is more virulent, it may cause death of animal due to respiratory failure or may cause permanent lesions like formation of scar, carnification, granuloma etc. There are various types of pneumonia caused by bacteria, virus, fungi, parasites, allergens, chemicals and all such affections of lungs are classified as under.

BRONCHOPNEUMONIA

Bronchopneumonia is the inflammation of lungs involving bronchi or bronchioles along with alveoli. It is thought to be spread through bronchogenous route and is the common type of pneumonia in animals.

Etiology

- Virus
- Bacteria
- Chemicals
- Mycoplasma
- Chlamydia
- Parasites
- Fungus

- Mainly through bronchogenous route.

Macroscopic and microscopic features

- Congestion and consolidation of anterior and ventral parts of lungs (Lobular pneumonia).
- Patchy lesions on one or several lobes and adjacent area shows emphysema.
- Mediastinallymphnodes are swollen.
- Congestion, oedema or haemorrhage in lung.
- Infiltration of neutrophils, mononuclear cells in and around bronchioles/ bronchi.
- Catarrhal inflammation of bronchi.
- Proliferation of bronchiolar epithelium

INTERSTITIAL PNEUMONIA

Interstitial pneumonia is the inflammation of the lungs characterized by thickening of alveolar septa due to serous/fibrinous exudate along with infiltration of neutrophils and/or mononuclear cells and proliferation of fibroblasts. It is also known as lobar pneumonia.

Etiology

- Bacteria
- Virus
- Chlamydia
- Parasites
- Mainly through hematogenous route.

Macroscopic and microscopic features

- Lungs are pale or dark red in colour.
- Edema, dripping of fluid from cut surface
- Alveoli may have serous or fibrinous exudate.
- Thickening of alveolar septa due to accumulation of exudate, inflammatory cells and in chronic cases, proliferation of fibrous tissue.
- Infiltration of mononuclear cells in alveolar septa.

FIBRINOUS PNEUMONIA

Fibrinous pneumonia is the inflammation of lungs characterized by the presence of fibrin in alveoli or bronchioles and may give rise to hyaline membrane formation over the surface of alveoli or bronchiole.

Etiology

- Bacteria
- Virus
- Parasites
- Toxin/ Poisons

Macroscopic and microscopic features

- Antero-ventral portion of lung is congested and consolidation.
- Colour of lungs become deep red due to congestion
- Surface of lungs is covered by fibrin sheet.
- Interlobular septa are prominent due to accumulation of plasma and fibrin.
- Principal exudate is fibrin, fills alveoli, bronchioles and bronchi.
- Congestion and/ or haemorrhages
- Infiltration of neutrophils, macrophages and giant cells
- Formation of eosinophilic false membrane of fibrin over the surface of alveoli and bronchiole and then known as *"hyaline membrane pneumonia"*.

VERMINOUS PNEUMONIA

Verminous pneumonia is caused by parasites and characterized by the presence of lesions of broncho pneumonia along with parasites or their larva.

Etiology

- *Metastrongylus apri* in pig.
- *Dictyocaulus filariae* in sheep and goat
- D. *viviparus* in cattle and buffaloes.
- *Capillaria aerophila* in dogs and cats.

- D. *arnfieldi* in horse and donkeys.

Macroscopic and microscopic features

- Multiple petechial haemorrhage in lungs at the site of parasite penetration.
- Mature worms in alveoli, bronchioles and bronchi.
- Mucopurulent exudate in alveoli/ bronchi.
- Pulmonary oedema, -emphysema.
- Dilation of bronchiole/ bronchi
- Lesions of chronic suppurative bronchiolitis
- Focal areas of inflammation in the vicinity of parasites and around bronchioles.
- Hyperplasia of bronchiolar epithelium.
- Infiltration of eosinophils and lymphocytes.

ASPIRATION PNEUMONIA

Aspiration pneumonia is caused by faulty medication through drenching which reaches in lungs instead of target place (digestive track) and characterized by necrosis and gangrene of lung paranchyma.

Etiology

- Drugs, food, foreign body and oil drench which reaches in lungs through trachea.
- Paresis of throat predisposes the animal for aspiration pneumonia.

Macroscopic and microscopic features

- Congestion and consolidation of anterior and ventral portion of lung.
- Affected part becomes green/ black in colour, moist gangrene.
- Affected lungs are often foul smelling.
- Presence of foreign body like heads of wheats, parts of com, oil, milk etc.
- Thrombosis of blood vessels.
- Necrosis in lungs.
- Presence of saprophytes, leucocytes and bacteria cause liquefaction and gangrene.
- Gangrenous lesions surrounded by intense inflammation.

- Congestion

MYCOTIC PNEUMONIA

Mycotic pneumonia is caused by a variety of fungi and characterized by the presence of chronic granulomatous lesions in lungs.

Etiology

- *Aspergillus fumigatus*
- *Blastomyces* sp.
- *Cryptococcus* sp.
- *Coccidioidomyces immitis*

Macroscopic and microscopic features

- Nodules in lungs
- On cut, cheese like caseative mass comes out from nodules.
- Caseation involves both bronchiole and alveoli.
- Such lesions may also present in trachea, bronchi and air sacs.
- Presence of granulomatus lesions *i.e.* caseative necrosis, macrophages, epithelioid cells, lymphocytes, giant cells, fibroblasts etc.
- Presence of branched hyphae of fungi in the necrosed area.

TUBERCULOUS PNEUMONIA

Tuberculous pneumonia is caused by *Mycobacterium* sp. and characterized by the presence of chronic granulomatous lesions in the lungs.

Etiology

- *Mycobacterium tuberculosis*
- M. *bovis*

Macroscopic and microscopic features

- Grey, white or light yellowish nodules in lungs.

- Nodules are hard, painful and/or calcified.
- Animal carcass is cachectic, weak or emaciated.
- On cut, the cheesy material comes out from the nodules.
- Presence of tubercle/ granuloma in lungs which comprises a central necrosed area surrounded by macrophages, epithelioid cells, lymphocytes, Langhan's giant cells and covered by fibrous covering.
- Acid-fast rod shaped bacteria may present in necrosed area.
- Central area may be calcified.

OVINE PULMONARY CARCINOMA (PREVIOUSLY PULMONARY ADENOMATOSIS)

Pulmonary adenomatosis is a slow viral disease of sheep and characterized by metaplasia of alveolar squamous epithelium to cuboidal and/ or columnar epithelium leading to glandular appearance of alveoli.

Etiology

- Retrovirus
- Pulmonary adenomatosis virus

Macroscopic and microscopic features

- Multiple focal areas of consolidation in lungs.
- Imprint of ribs on lungs.
- Congestion and hardening of mediastinal lymphnodes.
- Metaplasia of alveolar epithelium leading to formation of glandular structures in alveoli.
- Metaplasia of simple squamous epithelium to cuboidal or columnar epithelium which gives alveoli a gland like look.
- Mild inflammatory reaction.
- Proliferation of fibrous tissue.

HYPERSENSITIVITY PNEUMONITIS

Hypersensitivity pneumonitis is the inflammation of lung caused by an allergic reaction of antigen (allergen) and characterized by interstitial pneumonia, emphysema, hyaline membrane formation and hyperplasia of alveolar epithelium.

Etiology

- Allergens
- Parasites - *Dictyocaulus viviparous*
- Moldy hay
- Fungus- *Aspergillus* sp.

Macroscopic and micrqscopic features

- Lobes may contain small grey foci
- Presence of yellow and dense mucus in lumen of bronchi
- Excessive accumulation of air in lungs due to emphysema
- Presence of worms/ larvae.
- Extensive infiltration of lymphocytes, monocytes and eosinophils around the bronchi and bronchioles.
- Accumulation of catarrhal exudate in bronchi/ bronchiole.
- Emphysema as a result of widening of alveoli.
- Hyperplasia of bronchiolar musculature.
- Inflammatory cells in interalveolar septa may form small granulomas.
- Formation of hyaline membrane over alveolar and bronchiolar epithelium.

PNEUMOCONIOSIS

Pneumoconiosis is the granulomatous inflammation of lungs caused by aerogenous dust particles of sand, silica, beryllium, carbon or asbestos. It is also known as anthracosis.

Etiology

- Silica
- Asbestos
- Beryllium

- Bauxite
- Graphite
- Carbon
- Bronchogenous/ aerogenous administration of particles inhaled with air, mostly around mines/ factories.
- Generator smoke.

Macroscopic and microscopic features

- Dense fibrous nodules in lungs.
- Presence of carbon particles in tracheal bronchi mixed with mucous exudate.
- Granuloma formation around the particles of silica/ asbestos infilterated by macrophages, lymphocytes and giant cells
- Silica produces cellular reaction *'Silicosis'*.
- *Beryllium granuloma* looks like tubercule without caseation.
- *Asbestosis* is characterized by the presence of club shaped filaments bearing cells in lesion.

PATHOLOGY OF AIR SACS

AIR SACCULITIS

Air sacculitis is inflammation of air sacs caused by *E.coli,* Mycoplasma, reovirus etc. and characterized by thickening of the wall of air sacs and presence of cheesy exudates.

Etiology

- *Escherichia coli*
- *Mycoplasma gallisepticum*
- Avian reovirus

Macroscopic and microscopic features

- Thickening of the air sac wall, which becomes dirty and cloudy.
- Presence of cheesy exudate in air sacs, congestion of lungs.
- Fibrinous pericarditis
- Liver is covered with thin fibrinous membrane.

- Oedema and infiltration of neutrophils and lymphocytes in air sacs
- Caseous exudate in lungs and air sacs.

PATHOLOGY OF PLEURA

PLEURITIS

Pleuritis is the inflammation of pleura characterized by serous, fibrinous or purulent exudate. It is also known as *pleurisy.*

Etiology

- *Mycobacterium tuberculosis*
- *Mycoplasma mycoides*
- *Haemophilus suis*
- Organisms responsible for pneumonia/ traumatic pericarditis may also cause pleuritis.

Macroscopic and microscopic features

- Congestion of pleura
- Serous, fibrinous or purulent exudate.
- Accumulation of clear fluid in pleural thoracic cavity is called as *hydrothorax.*
- Presence of blood in thoracic cavity is known as *Hemothorax.*
- Suppurative exudate in thoracic cavity is known as *pyothorax.*
- Presence of air in pleural cavity is termed as *pneumothorax,* while presence of lymph in pleural cavity is called as *chylothorax.*
- Tuberculous pleuritis is characterized by small nodules on pleura and is known as *"pearly disease".*
- In chronic cases, development of fibrous tissue causes adhesions and is known as *adhesive pleuritis.*
- Congestion of blood vessels
- Infiltration of neutrophils and lymphocytes.
- Thickening of pleura due to oedema
- Proliferation of fibroblasts producing adhesive lesions.

CHAPTER 3: PATHOLOGY OF ALIMENTARY SYSTEM

DEVELOPMENT AL ANOMALIES

Epitheliogenesis imperfecta of tongue: Abnormal smooth surface of tongue due to small filiform papillae. It occurs as a defect in autosomal recessive gene and occurs in Holstein-Friesian cattle. This is also known as smooth tongue.

Cleft palate: This is most common congenital abnormality occurs due to failure of oral-nasal cavity to divide leaving cleft. It may also extend towards lips producing 'hare lip' condition.

Mega colon: There is distention of colon which abruptly terminate in rectum due to mutant gene in dogs.

Duplication of colon: In dog, the colon is duplicated from caecum to rectum and this defect is associated with malformation in the body of vertebrae T4 and Ts·

Atresia coli: In calf, the absence of colon occurs and the intestine terminates in blind caecum.

Atresia ani: This is absence of anal opening.

PATHOLOGY OF MOUTH CAVITY

STOMATITIS

Stomatitis is the inflammation of mucosa of oral cavity. It includes:

- ✓ *Gingivitis:* Inflammation of gums
- ✓ *Glossitis:* Inflammation of tongue
- ✓ *Cheilitis:* Inflammation of lips
- ✓ *Tonsilitis:* Inflammation of tonsil
- ✓ *Palatitis! Lampas:* Inflammation of palates

Etiology

- ➢ Trauma due to nails, wire, or any sharp object like needle
- ➢ Physical due to hot milk, medicines etc.

- Chemical- Alkali/ acids
- Microorganisms- Bacteria, virus, fungi

Macroscopic and microscopic features

- Catarrhal stomatitis: Mucous exudation in oral cavity.
- Vesicular stomatitis: Vesicles in oral mucosal *e.g.* FMD
- Erosive stomatitis: Erosions in oral mucosa *e.g.* Rinderpest
- Fibrinous stomatitis: False membrane in oral mucosa.
- Ulcerative stomatitis: Presence of ulcers in oral mucosa *e.g.* mucosal disease.
- Congestion of oral mucosa
- Presence of erosions, vesicles or ulcers
- Infiltration of neutrophils, lymphocytes and macrophages
- Presence of fibrinous exudate in the form of diphtheritic membrane.

PATHOLOGY OF ESOPHAGUS AND CROP CHOKE

Choke is complete or partial obstruction of esophagus either due to any foreign material or pressure from adjoining areas.

Etiology

- Beets, turnip, carrots, bone
- Abscess tumor of neck area

Macroscopic and microscopic features

- Tympany
- Gangrene, sapremia and toxaemia
- Sac like dilatation “Esophageal diverticulum"
- Perforation due to sharp bone ends
- Necrosis gangrene at a point of obstruction
- Congestion haemorrhage in perforated cases

ESOPHAGITIS

Esophagitis is the inflammation of esophagus caused by trauma, parasites etc. and characterized by catarrhal inflammation, ulceration or stenosis due to fibrosis.

Etiology

- Trauma due to foreign bodies
- Chemicals- Acids, alkalies
- Infection- Mucosal disease virus
- Parasite- *Spirocerca lupi*
- Nutritional- Vito A deficiency

Macroscopic and microscopic features

- Congestion
- Ulcer formation.
- Red streaks of catarrhal inflammation.
- Stenosis due to fibrous nodules or inflammatory exudate.
- Enlargement of glands.
- Congestion, haemorrhage
- Ulceration
- Infiltration of neutrophils, lymphocytes
- Sub-epithelial fibrosis/ nodules by *Spirocerea lupi.*

INGLUVITIS

Ingluvitis is the inflammation of crop caused by fungi and characterized by ulcerative or diphtheritic lesions.

Etiology

- *Candida albicans*
- *Monilia albicans*

Macroscopic and microscopic features

- Turkis towllike appearance in crop mucosa.

- Round and raised ulcers.
- In moniliasis, formation of diphtheritic membrane
- Necrotic and ulcerative lesions
- Fibrinous inflammation with infiltration of mononuclear cells

PATHOLOGY OF STOMACH

TYMPANY

Tympany is accumulation of gases in rumen due to failure of eructation as a result of obstruction or due to excessive production of gases characterized by distended rumen and dyspnoea. It is also known as *bloat.*

Etiology

- Choke of esophagus
- Sudden change in animal feed with high content of legumes.
- Excessive lush green fodder

Macroscopic and microscopic features

- Rumen is distended due to excessive accumulation of gases (C02, H2S, CO)
- Distended rumen compresses diaphragm to hinder respiration.
- Tarry colour blood, pale liver and rupture of diaphragm.
- On rupture of rumen gas comes out (dry tympany).
- The gas is trapped in small bubbles in the ruminal fluid forming fo~ and is not easily removed. This is known as *"frothy bloat",* which is produced by saponin and water soluble proteins and due to reduction in surface tension in the absence of fatty acids that favours froth formation.
- Haemorrhage in lungs, pericardium, trachea and lymphnodes
- Atelectasis in lungs.

RUMENITIS

Rumenitis is the inflammation of rumen in ruminant animals caused by change in diet, chemicals or drugs and characterized by seropurulent exudate or ulcer formation with or without parakeratosis.

Etiology

- Change in diet, corn or alfa-alfa hay.
- Chemicals/ drugs *e.g.* potassium antimony tarterate.
- *Spherophorus necrophorus* infection

Macroscopic and microscopic features

- Ulcers
- Spherical white nodules of 1-2 cm diameter size.
- Sloughing of mucosa.
- Seropurulent exudate
- Ulcers
- Infiltration of lymphocytes and neutrophils
- Fibrous nodules due to hyperplasia of fibroblasts
- Parakeratosis

RETICULITIS

Reticulitis is the inflammation of reticulum in ruminant animals caused by trauma/ perforation by foreign body including sharp object like needles, wires, etc. and characterized by abscess formation, adhesions, peritonitis and pericarditis.

Etiology

- Foreign body- sharp objects like needles, wires etc.

Macroscopic and microscopic features

- Perforation of reticulum by foreign body.
- Abscessation/ suppuration
- Peritonitis, adhesions of reticulum with diaphragm

- Pericarditis due to foreign body (traumatic reticulo pericarditis).
- Infiltration of neutrophils, macrophages, lymphocytes
- Proliferation of fibroblasts producing adhesions.
- Liquefactive necrosis.

OMASITIS

Omasitis is the inflammation of omasum in ruminant animals caused by *Actinobacillus* sp. and characterized by granulomatous inflammatory reaction.

Etiology

- *Actinobacillus ligneiresi*

Macroscopic and microscopic features

- Granulo.natous nodules in omasum
- Typical granuloma formation
- Sulfur granules of Actinobacillus in the centre of lesion.

ABOMASITIS

Abomasitis is the inflammation of abomasum in ruminants caused by chemicals/ drugs, bacteria, virus or parasites and characterized by congestion, edema and/ or haemorrhagic ulcers.

Etiology

- Chemicals/ drugs
- Bacteria *e.g. Clostridium septicum* cause of Braxy
- Virus *e.g.* Hog cholera, Mucosal disease
- Parasites *e.g. Theileria* sp.

Macroscopic and microscopic features

- Presence of ulcers (button ulcers in Hog cholera).
- Congestion, oedema of abomasal folds, haemorrhage in braxy.
- Catarrhal, haemorrhagic abomasits

- Presence of gram positive rods in case of braxy.
- Neutrophilic and lymphocytic infiltration.
- Congestion and haemorrhages.
- Ulceration with lymphocytic infiltration.

IMPACTION OF RUMEN AND RETICULUM

Impaction of rumen and reticulum is common in cattle and buffaloes caused by heavy carbohydrate diet and characterized by atony of rumen, indigestion, acidosis and haemorrhage on serous membranes.

Etiology

- Overfeeding of carbohydrate feed.
- Lack of water.
- Defective teeth or damaged tongue.
- Paralysis of rumen.

Macroscopic and microscopic features

- Atony of rumen due to lactic acid production.
- Rumen is filled with hard, caked undigested food with foul odour.
- Hemoconcentration, anuria, blood becomes dark in colour.
- Hemorrhage in lungs.
- Desquamation of ruminal epithelium.
- Lesions of acidosis/ toxicosis.

GASTRITIS

Gastritis is the inflammation of stomach in non-ruminant animals having simple stomach caused by chemicals/ drugs, bacteria, virus, parasite and characterized by congestion, edema, hemorrhage and ulceration. Inflammation of proventriculus in poultry is termed as proventriculitis.

Etiology

- Physical- overfeeding, trauma,
- Chemicals- Acid/ alkali
- Microorganisms such as bacteria, virus, fungi,
- Parasites *e.g. Trichostrongyles* sp., *Hemonchus* sp.
- Uremia

Macroscopic and microscopic features

- Congestion, oedema and haemorrhage of mucosal surface
- Thick mucous exudate in stomach
- Presence of vesicles/ ulcers on gastric mucosa
- Congestion and haemorrhage of gastric mucosa.
- Presence of ulcers/ necrosis.
- Infiltration of mononuclear cells.
- Lymphoid hyperplasia.

PATHOLOGY OF INTESTINES

CATARRHAL ENTERITIS

Catarrhal enteritis is characterized by increased number of goblet cells, congestion and infiltration of neutrophils and mononuclear cells in mucosa of intestine.

Etiology

- Physical- Foreign bodies and corase feed
- Chemical- drugs
- Microorganisms-E.coli, *Salmonella* sp., viruses
- Parasites- Coccidia

Macroscopic and microscopic features

- Presence of catarrhal exudate in lumen of intestine and congestion.
- Thickening of the wall of intestine.
- Diarrhoea.

- Presence of parasites in lumen of intestine.
- Increased number of goblet cells in intestinal villi, reduced length of villi.
- Congestion.
- Infiltration of polymorphonuclear and mononuclear cells.

HEMORRHAGIC ENTERITIS

Haemorrhagic enteritis is characterized by inflammation of the intestines along with haemorrhagic exudates.

Etiology

- Bacteria- E. *coli, Bacillus anthracis, Salmonella* sp.
- Virus- Coronavirus, BVD, MD, RP
- Parasites- Coccidia

Macroscopic and microscopic features

- Hemorrhagic exudate in intestines; blood mixed intestinal contents.
- Petechial or echymotic haemorrhage in mucosa and submucosa of intestine.
- Presence of erosions/ ulcers in mucosa.
- Hemorrhage in the mucosa of intestine
- Infiltration of neutrophils and mononuclear cells.
- Erosion or ulcers in intestinal mucosa
- Presence of coccidia in the mucosa

CHRONIC ENTERITIS

Chronic enteritis is the chronic inflammation of intestine characterized by proliferative changes like proliferation of fibrous tissue, infiltration of mononuclear cells and plasma cells in lamina propria leading to hardening of intestinal wall.

Etiology

- *Mycobacterium paratuberculosis* in bovines
- Intestinal helminths

- E. *coli* in poultry (Hjarre's disease)

Macroscopic and microscopic features

- Thickening of the wall of intestine (Corrugations in Johne's disease).
- Thick mucous cover over mucosa of intestine
- Transverse corrugations in the large intestine.
- Granulomatous nodules in duodenum.
- Small, round, raised necrotic foci on serosal surface of intestine covering whole length of intestine.
- Proliferation of fibrous tissue in lamina propria.
- Infiltration of macrophages, lymphocytes, plasma cells.
- Atrophy of intestinal glands.

NECROTIC ENTERITIS

Necrotic enteritis is characterized by necrosis of mucosal epithelium of intestine leading to erosions/ ulcer formation and exposition of underlying tissues.

Etiology

- Salmonella
- Rinderpest, rotavirus, cornovirus, Hog cholera virus.
- Coccidia, Histoplasma
- Niacin deficiency
- *Clostridium* sp. after coccidial infection in birds.

Macroscopic and microscopic features

- Necrotic patches in intestines.
- Fibrinous deposits over necrotic patches like bran deposits
- Swelling of mesenteric lymphnodes
- Ulcers in intestine.
- Congestion and infiltration of mononuclear cells.

- Necrosis and desquamation of intestinal villous epithelium, leading to exposed underlying tissue.
- Ulcers in mucosa.
- Proliferation of crypt epithelium, presence of abnormal epithelium over villous surface.

PARASITIC ENTERITIS

Parasitic enteritis is caused by parasites and characterized by catarrhal and/ or hemorrhagic exudate in intestine, presence of oval adult parasite and thickening of the wall of intestine.

Etiology

- Helminths
- Roundworms
- Tapeworms
- Protozoa
- Coccidia
- Histoplasma

Macroscopic and microscopic features

- Presence of parasite helminths in the lumen of intestine.
- Thickening of the wall of intestine.
- Catarrhal or haemorrhagic exudate in intestine.
- Presence of large number of goblet cells in mucosa of intestine.
- Congestion and/ or hemorrhage.
- Presence of parasite/ ova in the intestinal lumen
- Infiltration of eosinophils in mucosa and submucosa of the intestines.
- Coccidia can be seen on mucosal scrapings under microscope.

FIBRINOUS ENTERITIS

Fibrinous enteritis is the fibrinous inflammation of intestine characterized by presence of fibrinous exudate comprising of pseudomembrane in the mucosa of intestine.

Etiology

- *Salmonella choleraesuis*
- *Spherophorus necrophorus*

Macroscopic and microscopic features

- Presence of diphtheritic membrane over mucosa of intestine.
- Button ulcers
- Sometimes, diphtheritic membrane covers the faeces.
- Congestion and haemorrhage in intestine.
- Thickening of intestinal wall due to fibrinous exudate.
- Fibrin network in mucosa.

GRANULOMATOUS ENTERITIS

Granulomatous enteritis is caused by bacteria or fungi and characterized by granuloma formation in the intestines.

Etiology

- *Mycobacterium tuberculosis*
- Coli granuloma- E. *coli* in poultry (Hjarre's disease)
- Coccidioiodomycosis/ candidiasis.

Macroscopic and microscopic features

- Granulomatous about one cm diameter elevated/ raised areas on the serous surface of intestine.
- Thickening of the wall of intestine.
- Small, tiny, white necrotic nodules on serosa.
- Granuloma formation consisting of central necrosed area covered by lymphocytes, macrophages, epithelioid cells, giant cells and fibrous connective tissue
- Extensive proliferation of fibrus tissue.
- Presence of bacterial fungus in the lesion.

INTESTINAL OBSTRUCTION

Obstruction of intestines may occur as a result of foreign body, enterolith, piliconcretions, phytobezoars, polybezoars or due to hypermotility of intestines leading to intussusception, volvulus or torsion.

Piliconcretions: Piliconcretions are hair balls mostly found in stomach/ intestines of those animals having habit of licking. This vice is more common in suckling calves and in animals with pica related to phosphorus deficiency. The hairs are accumulated in stomach which becomes in rounded shape due to movements of stomach and look like balls. Such hair balls are not degradable in gastrointestinal tract and may cause obstruction.

Phytobezoars/ Polybezoars: Concretions formed in gastrointestinal tract as a result of deposition of salts around a nidus of undigested plants or polythenes. They may cause obstruction in gastrointestinal tract.

Foreign bodies: Foreign bodies like rubber balls, nuts, bones, stones, plastic and rubber materials, polythenes may obstruct the intestinal tract as they are not degradable in the gastrointestinal tract.

Hernia: Hernia is presence of intestinal loop in umbilical area, scrotum or inguinal cavity which causes passive congestion, oedema and obstruction in intestines.

Intussusception: Intussusception is telescoping of intestine means a portion of intestine enters in caudal segment due to violent peristaltic movement. It causes obstruction, passive congestion and oedema.

Volvulus: In volvulus, the loop of intestine passes through a tear in mesentry. It causes obstruction at both ends of loop.

Torsion: Torsion is twisting of intestine upon itself causing obstruction.

Enterolith: Concretions in intestines particularly in horses are responsible for obstruction of intestinal tract and are responsible for "colic in horse" and enterocolitis.

TYPHLITIS

Typhlitis is the inflammation of caecum. It is particularly important in poultry caused by protozoan parasites and characterized by haemorrhage, thickening of the wall, presence of cheesy exudates and/or necrotic ulcers.

Etiology

- *Eimeria tennella*
- *Histomonas melellgridis*

Macroscopic and microscopic features

- Haemorrhage in caecum, blood mixed contents.
- Thickening of the wall, with congestion and cheesy exudates.
- Presence of necrotic ulcers in caecum in case of histomoniasis which is further supported by round, depressed, yellowish green areas of necrosis in liver.
- Congestion, haemorrhage, necrosis
- Presence of protozoan parasites
- Necrotic hepatic lesions.

HEPATITIS

Hepatitis is the inflammation of liver. It may be acute or chronic. Acute hepatitis is characterized by the presence of degeneration and necrosis of hepatocytes and infiltration of neutrophils and mononuclear cells alongwith hyperemia and/or haemorrhage.

Etiology

- Bacteria- Necrobacillosis, *Salmonella,* E. *coli*
- Virus- ICH
- Chemicals- Carbon tetrachloride
- Parasites- *Fasciola gigantica, Fasciola hepatica*

Macroscopic and microscopic features

- Enlargement of liver.
- Congestion and/or haemorrhage.
- Presence of necrotic patches in liver.
- Presence of fibrinous diphtheritic membrane on liver.
- Cloudy swelling and/or fatty changes in liver.
- Congestion in blood vessels and in sinusoidal area.
- Infiltration of neutrophils, macrophages and lymphocytes.
- Necrosis of hepatic parenchyma.
- In acute toxic hepatitis there is necrosis of hepatocytes. According to location it can be classified as under which is helpful in making diagnosis.
- *Diffuse necrosis* covers a considerable area crossing over the lobular boundaries.
- *Focal necrosis* occupying only a part of lobule *e.g.* EHV induced aborted foetal liver.
- *Peripheral necrosis* is characterized by necrosis at the periphery of lobule which occurs due to presence of strong toxins in blood.
- *Midzonal necrosis* has necrosis of cells in midway of periphery and centre of lobule.
- *Centrilobular necrosis* is characterized by necrosis of hepatocytes around the central vein occurs due to stagnation of blood with toxaemia.
- *Paracentral necrosis* is characterized by necrosis of hepatocytes at one side of central vein *e.g.* Rift valley fever.

CIRRHOSIS

Cirrhosis is the chronic inflammation of liver characterized by extensive fibrosis, hepatic degeneration and necrosis.

Etiology

- Bacteria~ Salmonella, *Spherophorus necrophorous*
- Virus- Infectious canine hepatitis
- Chemicals- Carbon tetrachloride
- Parasites- *Fasdola hepatica,* F. *giantica*

- Poisons/ toxins- Aflatoxins
- Once cirrhosis of liver starts, it is not checked even after removal of the cause as the newly formed fibrous tissue itself acts as an irritant to cause further proliferation of fibroblasts.

Macroscopic and microscopic features

- Liver becomes hard and firm.
- Surface of liver becomes uneven and nodular.
- Size of liver becomes reduced due to atrophy.
- Colour becomes yellowish, grey.
- Increase in fibrous tissue within and around lobules.
- Infiltration of macrophages and lymphocytes.
- Central vein is either absent or placed eccentrically.
- Hepatocytes show degenerative and necrotic changes.
- *Biliary cirrhosis* is characterized by proliferation of fibrous tissue around the bile ducts encircling them *e.g. Fasciola giantica.*
- *Glissonian cirrhosis* is mostly confined to areas at a short distance beneath the capsule.
- *Pigment cirrhosis* is associated with yellow discolouration.
- *Central or cardiac cirrhosis* is increase in fibrous tissue around the central vein as a result of chronic passive congestion.
- *Parasitic cirrhosis* occurs due to damage caused by migration of parasites *e.g. Ascaris lumbricoides, Schistosoma* sp.

CHOLECYSTITIS

Cholecystitis is the inflammation of gall bladder characterized by congestion, thickening of wall and infiltration of mononuclear cells. Cholangitis is the inflammation of bile duct.

Etiology

- Parasites- *Fasciola* sp.
- Foreign bod y- Stones
- Bacteria- E. *coli.*

Macroscopic and microscopic features

- Thickening of the wall of gall bladder.
- On opening of gall bladder, there may be parasites/ stones/ foreign body.
- Contents of gall bladder may be watery or thick oily.
- Congestion
- Proliferation of fibrous tissue in the wall of gall bladder
- Infiltration of mononuclear cells
- Increased number of mucus secreting cells.

PANCREATITIS

Pancreatitis is the inflammation of pancreas characterized by necrosis of pancreatic tissue, infiltration of neutrophils and mononuclear cells and fibrous tissue proliferation.

Etiology

- Bacteria
- Virus- Reovirus in poultry
- Parasites

Macroscopic and microscopic features

- Pancreas becomes pale, swollen, oedematous.
- In chronic cases, atrophy of pancreas
- Pancreas becomes hard, firm, and fibrous.
- Necrosis of pancreatic cells.
- Edema, infiltration of leucocytes, haemorrhage.
- Fibrosis characterized by proliferation of fibroblasts.

PATHOLOGY OF PERITONIUM

Peritonitis is the inflammation of peritoneum characterized by suppurative, serofibrinous or nodular lesions.

Etiology

- Bacteria- Staphylococci, *Mycobacterium* sp.
- Virus
- Neoplasia
- Parasites

Macroscopic and microscopic features

- Serofibrinous, fibrinous, suppurative or granulomatous lesions
- Accumulation of clear fluid is known as *Hydroperitoneum* or *Ascites.*
- Presence of nodules in tuberculosis is also termed as *II Pearly disease".*
- Serofibrinous, suppurative or granulomatous lesions.
- Thickening of peritoneum, adhesions due to fibrosis.

CHAPTER 4: PATHOLOGY OF URINARY SYSTEM

DEVELOPMENT AL ANOMALIES

Aplasia: Absence of one or both kidneys. Absence of one kidney is observed in animals with compensatory hypertrophy of another kidney and such animals may survive well.

Hypoplasia: The size of kidneys remains small which don't grow properly due to defect in a single recessive autosomal gene.

Cyst in Kidney: Single or multiple cysts in pig and dog kidney are reported with tinged yellow in colour. They may arise from nephron due to its distension. Presence of multiple cysts is also termed as congenital polycystic kidney.

- *Type-I* cysts are formed due to dilation and hyperplasia of collecting tubules resulting in spongiform kidneys. In such neonates cystic bile ducts are also present.
- *Type-II* polycystic kidney is formed due to absence of collecting tubules and developmental failure of nephron. The cysts are thick walled with dense connective tissue and may involve one or both kidneys.
- *Type-III* cysts in kidneys occur due to multiple abnormalities during development. Cysts develop from tubules or Bowmen's capsule with part of glomeruli in cyst. This condition

is bilateral and causes considerable enlargement of kidney due to clear fluid or blood mixed fluid containing cysts.

FUNCTIONAL DISTURBANCES

Proteinuria: Presence of protein particularly albumin in urine. Protein is found as smooth, homogenous, pink staining precipitate also called as 'cast'. The presence of albumin in urine is indicative of damage in glomeruli. It is also characterized by oedema due to protein deficiency.

Hematuria: Presence of blood in urine giving bright red colour. It may occur due to damage in glomeruli, tubule or haemorrhage anywhere from glomeruli to urethra. The most important cause of hematuria is bracken fern toxicity.

Hemoglobinuria: When hemoglobin is present in urine without erythrocytes due to intravascular hemolysis. The urine becomes brownish red in colour. It must be differentiated from hematuria in which intact erythrocytes are present and settle down after some time leaving clear urine as supernatant. Hemoglobinuria is caused by various infections such as *Leptospira* sp., *Babesia* sp. or phosphorus deficiency in animals.

Anuria: Absence of urine is known as anuria which may be due to:

- Absence of urinary secretion due to glomerulonephritis
- Inelastic renal capsule unable to exert sufficient pressure required for glomerular filtration leading to nephrosis.
- Due to Hydronephrosis or calculi urine already secreted puts back pressure to prevent further secretion.
- Low blood pressure
- Dehydration
- Necrosis of tubular epithelium

Polyuria: Increased amount of urine leading to frequent urination caused due to diabetes insipedus, hormonal imbalance and polydypsia. In this condition, waste products are successfully eliminated.

Uremia: The presence of harmful waste products like uric acid, creatinine and urea in blood. Normally such waste products are removed by excretion through kidneys. But due to damage in kidneys or obstruction by inflammation, neoplasm, abscess and most importantly by presence of calculi urine remains in the system and causes uremia. Uremia is characterized by headache, vomiting, hyperirritability, convulsion, ulcers in oral cavity and stomach, normochromic and normocytic anemia, hemosiderosis and thrombocytopenia.

Glycosuria: Presence of glucose in urine. This is also known as diabetes mellitus, a metabolic disorder. It may occur due to insulin deficiency. This condition is not common in animals. However, it may occur in dogs as a result of hypoglycemia. It may occur in sheep due to enterotoxemia caused by *Clostridium welchii* type D.

Pyuria: Presence of pus in urine due to suppurative inflammation in urinary tract.

Ketonuria: Presence of ketone bodies in urine, which is common in diabetes mellitus, acetonemia, pregnancy toxemia and in starvation.

Oligouria: In this condition, there is decreased amount of urine, which occurs due to glomerulonephritis, obstruction in urinary passage, dehydration, low blood pressure and tubular damage.

NEPHROSIS

Nephrosis is the degeneration and necrosis of tubular epithelium without producing inflammatory reaction. It mostly includes acute tubular necrosis as a result of ischemia or toxic injury to kidney. Nephrosis is characterized by necrosis and sloughing of tubular epithelial cells exhibited by uremia, oligouria, anuria.

Etiology

- Hypotension
- Heavy metals

- Mycotoxins *e.g.* Ochratoxin
- Antibiotics *e.g.* Gentamicin

Macroscopic and microscopic features

- Swelling of kidneys
- Capsular surface smooth, pale and translucent.
- Vacuolation in tubular epithelium.
- Coagulative necrosis.
- Sloughing of tubular epithelium

GLOMERULONEPHRITIS

Glomerulonephritis is the inflammation of glomeruli primarily characterized by pale and enlarged kidneys with potential hemorrhage, oedema of glomeruli, congestion and infiltration of inflammatory cells. Due to presence of mesangial proliferation, it is also called as mesangio-proliferative glomerulonephritis (MPGN).

Etiology

- Streptococci infection
- Immune complexes
- Environmental pollutants such as - Organochlorine pesticides

Macroscopic and microscopic features

- Enlarged kidneys
- Edema, pale kidneys
- Petechiae on kidneys
- Proteinuria, uremia, hypercholesterolemia and increased creatinine level in blood.
- Edema of glomeruli leading to increase in size.
- Infiltration of neutrophils, macrophages.
- Compression of blood capillaries and absence of erythrocytes.
- Thrombosis and necrosis of glomerular capillaries.
- Based on type of lesions, it can be divided into 5 subtypes.

1. Type-I MPGN

- ✓ Proliferation of mesangial cells.
- ✓ Deposition of immune complexes containing IgG, IgM, IgA and C3
- ✓ Immune complexes penetrate vascular endothelium but not the basement membrane and are deposited in subendothelial region.
- ✓ Proliferation and swelling of endothelial cells.
- ✓ Immune complexes induce production of transforming growth factor (TGFB}) which increases production of fibronectin, collagen and proteoglycans leading to thickness of basement membrane; this is also known as "wire loop"lesions.

2. Type-II MPGN (Membranous)

- ✓ Deposition of immune complexes in basement membrane (lamina densa).
- ✓ Due to uncontrolled activation of complement.
- ✓ Proliferation of endothelium and mesangial cells.
- ✓ Demonstration of C3 component, no immunoglobulin.

3. Type III MPGN (Acute Proliferative)

- ✓ Subepithelial deposits of immune complexes and disruption of basement membrane.
- ✓ Swelling of epithelium and its proliferation forming "Epithelial cresent".
- ✓ Demonstration of IgG in subepithelial region.
- ✓ Congestion and edema of glomeruli.
- ✓ Infiltration of neutrophils, macrophages and lymphocytes.

4. Chronic glomerulonephritis

- ✓ Proliferation of epithelial and endothelial cells
- ✓ Reduplication, thickening and disorganization of glomerular basement membrane.
- ✓ Lumen of capillaries occluded.
- ✓ Entire glomerulus is replaced by Hyaline connective tissue.

5. Focal embolic glomerulonephritis

- ✓ Focal zone of necrosis in glomeruli
- ✓ Infiltration of neutrophiIs
- ✓ Proliferation of epithelial cells and formation of crescent.

INTERSTITIAL NEPHRITIS

Interstitial nephritis is the inflammation of kidney characterized by degeneration and necrosis of tubular epithelium, edema and infiltration of inflammatory cells in interstitium.

Etiology

- Ochratoxins and atrinin.
- Leptospira
- Toxins/ poisons *e.g.* Pesticides.
- Herpes virus
- Endogenous toxaemia *e.g.* Ketosis
- Immune complexes

Macroscopic and microscopic features

- Enlargement of kidneys
- Necrosis, congestion and hemorrhage
- Edema, congestion, hemorrhage
- Necrosis and degeneration of tubular epithelium
- Infiltration of inflammatory cells like neutrophils, macrophages and lymphocytes in interstitium.
- Loss of tubules, foci of mononuclear celis, fibrosis in chronic Cases
- Immune complexes are deposited in granular form causing degeneration of epithelial cells of tubules and mononuclear cell infiltration.

PYELONEPHRITIS

Pyelonephritis is the inflammation of renal pelvis and parenchyma i.e. tubules characterized by congestion, suppurative inflammation and fibrosis.

Etiology

- *Corynebacterium renale*
- *Staphylococcus au reus*
- E. *coli*
- *Actinomyces pyogenes*
- *Pseudomonas aeruginosa*

Macroscopic and microscopic features

- Congestion, hemorrhage and abscess formation in renal cortex, pelvis and ureters.
- Pyuria- Pus mixed urine in bladder.
- Enlargement of kidneys
- Congestion, hemorrhage
- Suppurative inflammation of pelvis and kidney parenchyma.
- Necrosis of collecting ducts.
- Purulent exudate in pelvis.
- Infiltration of neutrophils, lymphocytes and plasma cells in interstitium.

NEPHROSCLEROSIS

Nephrosclerosis is chronic fibrosis of kidney characterized by loss of glomeruli and tubules and extensive fibrosis.

Etiology

- Glomerulonephritis
- Interstitial nephritis
- Arteriolosclerosis

Macroscopic and microscopic features

- Hard, atrophied kidneys.
- Fibrous nodules on kidneys.

- Thickening of capsule.
- Small white firm kidneys.
- Ischemia, tubular atrophy
- Loss of glomeruli and tubules
- Extensive fibrosis
- Deposition of hyaline mass
- Infiltration of mononuclear cells.

UROLITHIASIS

Urolithiasis is the formation of stony precipitates anywhere in the urinary passage including kidneys, ureter, urinary bladder or urethra.

Etiology

- Bacterial infections
- Metabolic defects
- Vitamin A deficiency
- Hyperparathyroidism
- Mineral imbalance

Macroscopic and microscopic features

- Nephrosis, Hydronephrosis
- Distension of ureters
- Distension of ureters and urinary bladder
- Hard enlarged kidneys
- Presence of calculi! stone in kidney, ureter, bladder or urethra.
- Presence of crystals/ stone in lumen of tubules.
- Degeneration and necrosis of tubular epithelium.
- Hemorrhage
- Proliferation of fibrous tissue

There are various types of calculi, which differ in size, shape and composition. Some of them are as under:

Oxalate calculi are hard, light yellow, covered with sharp spines found in urinary bladder and formed due to calcium oxalate. It causes damage in urinary bladder leading to haemorrhage.

Uric acid calculi are composed of ammonium and sodium urates and uric acids, yellow to brown in colour, formed in acidic urine, spherical and irregular in shape and they are not radio opaque.

Phosphate calculi are white or grey in colour, chalky in consistency, soft, friable and can be crushed with mild pressure. They are mostly multiple in the form of sand like granules. They are composed of magnesium ammonium phosphate and occur as a result of bacterial infection.

Xanthine calculi are brownish red, concentrically laminated, fragile and irregular in shape. They rarely occur in animals.

Cystine calculi are small, soft with shiny and greasy in appearance, yellow in colour which becomes darker on air exposure. Insoluble amino acid cystine precipitates in bladder to form calculi. Such calculi may cause obstruction of urethra with cystinuria.

PATHOLOGY OF URETER

URETERITIS

Ureteritis is the inflammation of ureter characterized by enlargement, thickening of wall due to accumulation of urates, or calculi, pyonephrosis and pyelonephritis.

Etiology

- Tuberculosis
- Calculi
- Hydronephrosis
- Pyelonephritis

- Pyonephrosis

Macroscopic and microscopic features

- Deposits of whitish/ yellowish urates in ureter in poultry.
- Obstructions of ureter due to calculi lead to its enlargement and formation of diverticulum.
- Thickening of the wall due to congestion and infiltration of inflammatory cells.
- Extensive fibrosis with infiltration of mononuclear cells in chronic cases.

PATHOLOGY OF URINARY BLADDER

CYSTITIS

Cystitis is the inflammation of urinary bladder characterized by congestion and fibrinous, purulent or hemorrhagic exudates.

Etiology

- Urinary calculi
- Tuberculosis
- Blockage in urethra
- Bracken fern poisoning

Macroscopic and microscopic features

- Congestion, hemorrhage.
- Enlargement of urinary bladder.
- Thickening of the wall.
- Presence of small nodules on wall.
- Thickening of wall due to infiltration of neutrophils and macrophages.
- Granuloma in tuberculosis.
- Fibrosis
- Presence of neoplasm.

PATHOLOGY OF URETHRA

URETHRITIS

Inflammation of urethra is known as urethritis, which occurs as a result of catheter injury and calculi. It is characterized by congestion, obstruction, hydronephrosis and strictures.

Etiology

- Calculi
- Catheter injury
- *Trichomonas foetus* infection
- Picoma virus infection

Macroscopic and microscopic features

- Transient inflammation, congestion and hemorrhage.
- Strictures (male), diverticulum (female)
- Obstruction due to calculi, presence of calculi
- Thickening due to inflammatory exudate.

CHAPTER 5: PATHOLOGY OF FEMALE REPRODUCTIVE SYSTEM

DEVELOPMENTAL ANOMALIES

Agenesis: Absence of ovary, uterus, oviduct and cervix in females. It may be unilateral or bilateral.

Hypoplasia: Complete or partial lack of germ cells in ovaries. Hypoplasia of uterus is related with agenesis of gonads. Ovaries of freemartin are also hypoplastic. Hermaphrodite animal has ovary and testicular tissue both in the gonads.

Hermaphroditism: In hermaphrodites, there is presence of organs of both sexes in same individual animal. Both ovarian and testicular tissue occur in one animal leads to sterility in

animal (true hermaphrodite) while in pseudohermaphrodite the gonadal tissue of only one sex is present but there is some degree of development of opposite sex organs.

Uterus unicornis: Uterus unicornis is presence of only one horn of uterus instead of two, seen in animals with white heifer disease.

White heifer disease: White heifer disease occurs due to a single sex linked gene defect responsible for white coat colour. In such animals, there are normal ovaries, oviduct but uterus is incomplete and may lack communication with cervix. There is hypoplasia of cervix and vagina.

Uterus didelphys: Uterus didelphys is the occurrence of two cervix with two uterine bodies and single or double vagina. It occurs due to failure of Mullerian ducts to fuse at their distal end. Sometimes failure of fusion may affect only cervix and there is two cervix which is termed as *Cervix bifida.*

CYSTIC OVARIES

Cystic ovaries are defined as an ovary, which contains one or more clear cysts ranging from one to several centimeters in size.

Etiology

- Hormonal imbalance

Macroscopic and microscopic features

- Presence of cysts in ovaries.
- Hormonal imbalance of animal leads to sterility, continuous estrus, nymphomania due to follicular cyst.
- Lutein cysts may cause pyometra leading to pseudopregnancy.
- Follicular cyst
- Ova absent several layers of granulosa or a single layer of epithelium.
- Many follicular cysts are present
- Lutein cyst covered by fat containing granulosa cells.

OOPHORITIS

Oophoritis is the inflammation of ovary caused by trauma, infection and characterized by granulomatous or lymphocytic inflammation of ovary.

Etiology

- *Mycobacterium tuberculosis*
- Herpes virus

Macroscopic and microscopic features

- Hard, nodular lesions in ovary, encapsulated with fibrous tissue.
- Granuloma of tuberculosis through hematogenous infection.
- Infiltration of lymphocytes leading to lymphofollicular reaction in follicles.
- Atrophy or absence of ova.

SALPINGITIS

Salpingitis is the inflammation of oviduct or fallopian tube characterized by congestion, catarrhal or purulent exudates leading to distended lumen.

Etiology

- Mycoplasma
- Streptococci
- Tuberculosis *(Mycobacterium tuberculosis)*
- Trichomoniasis *(Trichomonas foetus)*

Macroscopic and microscopic features

- Congestion, abscess formation
- Distension of oviduct lumen due to accumulation of serous exudate which is known as *Hydrosalpinx.*
- Accumulation of pus in oviduct is termed as *Pyosalpinx.*
- Fibrosis, hardness

- Occlusion of lumen due to inflammatory exudate resulting in sterility.
- Inflammatory exudate is toxic to ova as well as sperms leading to sterility.
- Suppurative inflammation
- Infiltration of neutrophils, macrophages and lymphocytes
- Proliferation of fibrous tissue
- Debris of desquamated cells

METRITIS

Metritis is the inflammation of uterus characterized by suppurative exudate, hemorrhage and necrosis of uterus.

Etiology

- Actinomyces pyogenes
- *E.coli*
- Staphylococci
- Streptococci
- *Trichomonas foetus*
- *Campylobacter foetus*

Macroscopic and microscopic features

- Congestion, catarrhal or purulent exudate
- Hemorrhage
- Enlargement, oedema
- Oozing out of pus from uterus on pressure
- Seropurulent exudate in uterine wall.
- Edema
- Infiltration of macrophages and lymphocytes
- Desquamation of lining epithelium

PYOMETRA

Pyometra is an acute or chronic suppurative inflammation of uterus resulting in accumulation of pus in the uterus.

Etiology

- Occurs under the influence of progesterone.
- E. *coli*
- *Actinomyces pyogenes*
- *Proteus* spp.
- *Staphylococcus aureus*
- *Trichomonas foetus*

Macroscopic and microscopic features

- Discharge of thin cream like pus from vulva soiling the tail and perineal region.
- Pus discharge is more on sitting position of animal.
- Enlargement of abdomen due to distension of uterus.
- Uterus looking like as pregnant uterus as a result of accumulation of pus. This condition is also known as *Pseudocyesis* or *pseudopregnancy.*
- Rention of lutein cyst.
- Congestion, infiltration of neutrophils, lymphocytes and plasma cells.
- Necrosis of mucosal epithelium of uterus.
- Proliferation of endometrial epithelium.
- Edema, glandular hyperplasia.

ENDOMETRITIS

Endometritis is the inflammation of endometrium, the mucosa of uterus. It may be catarrhal or purulent and may occur after metritis.

Etiology

- *Trichomonas foetus*
- *Campylobacter foetus*
- Staphylococci
- Streptococci

- Organism enters in uterus as a result of coitus, artificial insemination or as iatrogenic infection.
- Strong chemicals/ medicines administered in uterus.

Macroscopic and microscopic features

- Catarrhal discharge from uterus containing desquamated cells.
- Sterility due to toxic environment of uterus to sperms.
- Congestion.
- Congestion
- Moderate infiltration of lymphocytes, plasma cells and neutrophils in mucosa.

CERVICITIS

Cervicitis is the inflammation of cervix as a result of either descending infection from uterus or ascending infection from vagina and characterized by catarrhal inflammation.

Etiology

- Etiological agents are similar as in endometritis.

Macroscopic and microscopic features

- Congestion
- Enlargement of cervix.
- Catarrhal inflammation of cervical mucosa.
- Hyperplasia of mucous glands with tall mucin containing epithelial cells.
- Presence of mucin in lumen.

VAGINITIS

Vaginitis is the inflammation of vagina characterized by congestion, granularity as a result of elevations in mucosa. This is also known as *infectious pustular vulvovaginitis* in cattle caused by herpes virus.

Etiology

- *Mycoplasma bovigenitalium*
- Bovine herpes virus-1 (BHV -1)
- Picoma virus
- *Trichomonas foetus*

Macroscopic and microscopic features

- Granular elevation in vaginal mucosa.
- Congestion
- Prolapse due to limitation.
- Accumulation of lymphocytes in sub epithelial region.
- Congestion

ABORTION

Abortion is expulsion of dead embryo or foetus before attaining normal gestation. There are two other terms related to abortion i.e. stillbirth and premature birth. *Stillbirth* is defined as expulsion of dead foetus on its full maturity while *premature birth* is birth of a live foetus before attaining full gestation period.

Etiology

- Brucellosis *(Brucella abortus,* B. *meletensis,* B. *ovis)*
- *Campylobacter foetus*
- *Salmonella abortus-equi*- mares
- Equine herpes virus- mares
- Bovine herpes virus-l- cattle
- *Chlamydia psittasci*
- *Trichomonas foetus*

- *Listeria monocytogenes (Listeria ivanovii)*
- *Leptospria* spp.
- *Mycobacterium tuberculosis*
- *Toxoplasma gondii*
- *Mycoplasma mycoides*
- Fungi- *Aspergillus* spp., *Coccidioides* spp. *Absidia* spp.
- Toxins/ poisons

Macroscopic and microscopic features

- Expulsion of dead foetus in early stage (3-4 month) of gestation (Trichomoniasis)
- Abortion in middle of gestation (Campylobacteriosis).
- Late abortions (7-9 months) occur due to Brucellosis, BHV-1 infection.
- Liver of foetus has necrotic foci, congestion.
- Stomach contents used for confirmation of Etiology.
- In some cases of abortion, there is retention of placenta *(e.g.* Brucellosis)
- Placenta becomes oedematous and necrotic (Placentitis)
- If the foetus becomes dead and not expelled outside the body due to non-opening of cervix, the dead foetus remains in uterus under sterile conditions. Such foetus undergoes autolysis and Iiquified. Liquid material is absorbed in uterus through lymph or blood and bones/ skin etc. remain in uterine horn sometimes causing irritation or damage to endometrium. Such foetus becomes shrunken with wrinkled skin and dried as mummy and are known as *"Mummified foetus"*.
- Necrotic hepatitis with lymphofoIlicular reaction in foetus (Brucellosis, BHV-l infection).
- Granulomatous lesions (tuberculosis, fungal infection), lymphofoIlicular reaction (mycoplasma, chlamydia).
- Demonstration/ isolation of causative organisms in foetal stomach contents.
- Liver of foetus icteric (leptospirosis)
- Endometritis in dam.
- Bronchopneumonia in foetus *e.g.* brucellosis.

RETAINED PLACENTA/PLACENTITIS

Retention of placenta occurs after abortion or parturition as a result of inflammation characterized by swelling, edema or fibrosis which prevents the separation of chorion from endometrium.

Etiology

- Lack of progesterone
- Infection *e.g.* Brucellosis, Trichomoniasis.

Macroscopic and microscopic features

- Retained placenta undergoes autolysis, putrefaction
- Toxaemia in dam.
- Endometritis, Pyometra
- Placenta is oedematous and congested
- Infiltration of neutrophils, mononuclear cells.
- Proliferation of fibroblasts.

MASTITIS

Mastitis is the inflammation of mammary gland characterized by oedema, haemorrhage and fibrosis of udder. Mastitis is always infectious and is a disease of lactating glands. There is no hematogenous infection and infections enter through teat canal to cause mastitis.

Etiology

- Bacteria *e.g. Streptococcus agalactiae, Streptococcus dysgalactiae, Staphylococcus au reus, Actinomyces pyogenes, Pseudomonas aeruginosa, Brucella abortus, Mycobacterium tuberculosis,* E. *coli, Pasteurella multocida* and many more.
- Virus *e.g.* FMD virus, pox virus, BHV-1
- Mycoplasma *e.g. Mycoplasma mycoides*
- Fungi *e.g. Candida ablicans, Trichosporon* spp. *Nocardia asteroids, CnJPtococcus neoformans.*

Macroscopic and microscopic features

- Edema of udder
- Flakes (coagulated milk proteins) in milk.
- Blood mixed milk
- Watery dirty grey or dark colour milk in animals, which are in dry period caused by *Actinomyces pyogenes* and are known as *"summer mastitis"*.
- Terminal atrophy or shrunken quarter
- Gangrene formation
- Congestion, haemorrhage
- Infiltration of neutrophils, macrophages, lymphocytes
- Necrosis of alveolar epithelium, hyperplasia of epithelial lining.
- Proliferation of fibrous tissue
- Increase in WBC count in milk (more than 100/ ml milk).

CHAPTER 6: PATHOLOGY OF MALE REPRODUCTIVE SYSTEM

DEVELOPMENTAL ANOMALIES

Testicular hypoplasia: Testicular hypoplasia occurs in animals with chromosomal abnormality such as XXY chromosomes or Klinefelter's syndrome. Hypoplasia is also seen in hermaphrodites and in animals with cryptorchidism.

Spermatocele: There is failure of development of mesonephric tubules and does not connect with vas deferens resulting into blind tubules filled with spermatozoa. Rupture of tubuies may lead to spermatic granuloma.

Cryptorchidism: The testicle fails to descend in scrotum through inguinal canal after birth and remains in abdominal cavity. This permanent retention of testicles in abdominal cavity causes their hypoplasia leading to lack of spermatogenesis. Such testes are more prone for development of neoplastic growth.

Phimosis: Phimosis is the failure of extension of penis from its sheath.

Paraphimosis: Paraphimosis is the failure of withdrawal of extended penis.

Hypospadias: In hypospadias, there is urethral opening in ventral side of the penis.

Epispadias: There is urthral opening on the dorsal side of the penis

Phallocampsis: Phallocampsis is the deviation of penis, which may be spiral *(Cork screw penis)* or ventral deviation *(rainbow penis).*

ORCHITIS

Orchitis is the inflammation of testes characterized by edema, necrosis and infiltration of neutrophils, macrophages, lymphocytes and proliferation of fibrous tissue leading to atrophy in chronic cases.

Etiology

- *Brucella* spp.
- *Campylobactor* spp.
- *Salmonella* spp.
- *Trichomonas* spp.
- *Corynebacterium pseudotuberculosis*
- *Actinomycess pyogenes*
- *Pseudomanas aeruginosa*
- *Actinomyces bovis*

Macroscopic and microscopic features

- Enlargement of testes, oedema.
- Accumulation of serous fluid in scrotal *sacl* tunica vaginalis is called as *hydrocele.*
- Enlargement of scrotum.
- Congestion
- Atrophy and hardening in chronic cases.
- Congestion

- Infiltration of neutrophils and mononuclear cells
- Necrosis of germinal cells
- Proliferation of fibrous tissue and infiltration of mononuclear cells.
- Granulomatous lesions in case of actinomycosis and tuberculosis
- Aspermatogenesis

EPIDIDYMITIS

Epididymitis is the inflammation of epididymis characterized by catarrhal or suppurative exudate with necrosis of lining epithelium.

Etiology

- *Brucella avis* in sheep
- Other organisms that cause orchitis which is preceded by epidiymitis

Macroscopic and microscopic features

- Enlargement of epididymis
- Edema of scrotum
- Accumulation of mucus and/ or purulent exudate in epididymis.
- Accumulation of serous exudate in scrotum.
- Necrosis of lining epithelium of epididymis
- Infiltration of neutrophils, macrophages and lymphocytes
- Edema
- Formation of granuloma in chronic cases.

FUNICULITIS

Funiculitis is inflammation of scirrhous cord characterized by enlargement of scrotum due to chronic abscess.

Etiology/ Occurrence

- Botryomycosis
- Actinomycosis
- Castration

- Unsanitary conditions

Macroscopic and microscopic features

- Enlargement of scrotum
- Hard swelling/ chronic abscess
- Chronic hyperplastic/ proliferative changes.
- Fibroplasia
- Infiltration of macrophages, lymphocytes, neutrophils around sulfur granules forming rosette.

SEMINAL VESICULITIS

Seminal vesiculitis is the inflammation of seminal vesicle characterized by metaplasia of the columnar epithelial lining to cornified stratified squamous epithelium.

Etiology

- *Pseudomonas aeruginosa*
- *Chlamydia psittasci*
- *Mycoplasma bovigenitalium*
- *Actinomyces pyogenes*
- *Corynebacterium renale*
- *Brucella abortus*
- E. *coli*

Macroscopic and microscopic features

- Melanosis in bulbourethral glands.
- Enlargement; hardness of seminal vesicle
- Metaplasia of columnar epithelium into severely cornified stratified squamous epithelium.
- Proliferation of melanoblasts/ melanocytes.

PROSTATITIS

Prostatitis is the inflammation of prostate gland by formation of painful abscess, atrophy, hyperplasia of epithelial cells, proliferation of fibroblasts and formation of cysts. It occurs in dogs.

Etiology

- Hormonal imbalance
- Pyogenic staphylococci, streptococci

Macroscopic and microscopic features

- Presence of abscess encapsulated by fibrous tissue
- Enlargement of prostate causing obstruction of urethra
- Obstruction in rectal passage
- Hematuria
- Infiltration of neutrophils and liquefied necrosis.
- Chronic inflammation is characterized by hyperplasia of glandular epithelium, fibroblasts and smooth muscle fibers.
- Cystic glandular hyperplasia.
- Infiltration of lymphocytes.

BALANOPOSTHITIS

Balanoposthitis is the inflammation of prepuce and glans penis characterized by phimosis or paraphimosis and pain during copulation. Balanitis is inflammation of glans penis and posthitis is inflammation of prepuce.

Etiology

- *Trichomonas foetus*
- BHV-l virus
- Vesicular exanthema virus
- *Mycoplasma* spp.
- *Pseudomonas aeruginosa*
- *Actinomyces pyogenes*

- *ConJnebacterium renale*

Macroscopic and microscopic features

- Phimosis and paraphimosis due to pain, adhesions
- Congestion
- Fibrinopurulent exudate
- Lymphocytic infiltration, congestion.

CHAPTER 7: PATHOLOGY OF NERVOUS SYSTEM

Nervous system is composed of brain, spinal cord, and peripheral nerves. The neuron is a basic functional unit of nervous system. Necrosis of neurons in brain is known as *encephalomalacia* while necrosis of neurons in spinal cord is termed as *myelomalacia.* If the necrosis occurs in gray matter it is known as *polioencephalomalacia* while necrosis of neurons in white matter is called as *leukoencephalomalacia.* There are three types of scavenger cells in nervous system known as *microglia, oligodendroglia* and *astrocytes.* Microlial cells surround the necrotic neurons and are known as *satellite cells* and the process is called as *satellitosis.* As the neuron dies, it is engulfed by microglial cell and this process is termed as *neuronophagia.* The necrosis of nerve fibers starts from myelin sheath and this change is called as *demyelination* or *Wallerian degeneration.* The brain and spinal cord is covered by meninges. The inflammation of meninges is termed as *meningitis. Meningoencephalitis* is used for inflammation of both meninges and brain. Inflammation of duramater is known as *pachymeningitis* and of piamater is termed as *leptomeningitis. Hydrocephalus* means accumulation of clear fluid in ventricles and in sub arachnoid space due to obstruction in drainage. Hydrocephalus occurs in neonatal calves due to influenza and parainfluenza virus and is termed as *congenital hydrocephalus.*

Some nutritional deficiency like vitamin A, folic acid, vitamin B12, niacin and zinc may also lead to hydrocephalus. Cerebeller hypoplasia has been observed due to bovine virus diarrhoea, hog cholera and feline panleukopenia virus. Some other congenital malformations are as under. *Anencephaly* means absence of brain. *Microencephaly* means small size of brain. *Cranioschisis*

is failure of cranium to fuse which results in hernia of meninges and known as *meningocele.* Hernia of meninges and brain is known as *meningoencephalocele.*

ENCEPHALITIS

Encephalitis is the inflammation of brain characterized by purulent/ lymphocytic or proliferative changes. Encephalomyelitis is the inflammation of brain as well as spinal cord.

Etiology

- Bacteria
- *Listeria monocytogenes* (L. *ivanovii)* main cause
- *Haemophius* spp.
- *Pasturella* spp.
- Virus
- Mycoplasma
- Strychnine poisoning

Macroscopic and microscopic features

- Congestion
- Hemorrhage
- Small, tiny abscess
- Necrosis also known as encephalomalacia.
- Involvement of spinal cord leads to encephalomyelitis and of meninges is termed as meningoencephalitis.
- Tiny or micro abscess in cerebrum
- Infiltration by neutrophils and lymphocytes.
- Perivascular cuffing in Virchow Robin space by lymphocytes
- Necrosis of neurons.
- Satellitosis, neuronophagia
- Pleocytosis- Increase in number of white blood cells in cerebrospinal fluid.

ENCEPHALOMALACIA

Encephalomalacia is the necrosis of nervous tissue in brain characterized by loss of normal architecture and soft friable liquified mass.

Etiology

- Deficiency of copper, thiamine, vitamin-E.
- Poisons: Bracken fern, lead, mercury, salt poisoning, enterotoxaemia, mycotoxins.

Macroscopic and microscopic features

- Encephalomalacia- necrosis in brain.
- Myelomalacia necrosis in spinal cord.
- Poliomalacia is necrosis in brain gray matter.
- Leukomalacia is necrosis in brain white matter.
- Soft, friable liquified mass in brain.
- Congestion.
- Liquefactive necrosis
- Surrounded by neurological cells/scavenger cells.
- Proliferation of small new capillaries

SPONGIFORM ENCEPHALOPATHY

Spongiform encephalopathy is characterized by the presence of vacuoles in grey and/or white matter.

Etiology

- Prion proteins
- Scrapie in sheep
- BSE in cattle

Macroscopic and microscopic features

- No characteristic gross lesion.
- Edema of brain or hydrocephalus
- Congestion

- Vacuolation in white and grey matter
- Vacuoles are usually in neurons, glial cells and in myelin
- Vacuoles are more extensive in medulla, pons and mid brain and gives brain "spongy form".

MENINGITIS

Meningitis is the inflammation of meninges, usually occurs along with encephalitis or encephalomyelitis and characterized by congestion and infiltration of neutrophils and mononuclear cells. Pachymeningitis is inflammation of durameter while leptomenigitis involves the piameter.

Etiology

- Virus *e.g.* swine fever
- Trauma
- Bacteria *e.g.* Pasturella, Listeria
- Toxoplasma
- Leptospira

Macroscopic and microscopic features

- Congestion
- Thickening of meninges.
- Petechial haemorrhage
- Infiltration of neutrophils and lymphocytes.
- Fibrosis

NEURITIS

Neuritis is the inflammation of nerves along with degenerative changes characterized by edema, infiltration of inflammatory cells.

Etiology

- Toxins

- Trauma
- Virus *e.g.* Marek's disease MD
- Lead and Mercury
- Bacteria *e.g.* Strangles
- Deficiency of vitamin E.

Macroscopic and microscopic features

- Wallerian degeneration
- Infiltration of neutrophils and lymphocytes.
- More destruction at distal end of the neuron.

CHAPTER 8: PATHOLOGY OF MUSCULOSKELETAL SYSTEM

PATHOLOGY OF MUSCLES

EQUINE RHABDOMYOLYSIS

It is also known as *Azoturia* or *Monday Morning Disease.* The disease occurs in well fed horse after a gap of holiday. Suddenly after walking few steps, the horse is unable to move further and feels pain with intense sweating and hardening of muscles.

Etiology

- Accumulation of lactic acid in muscles
- High glycogen storage
- Lack of oxygen supply

Macroscopic and microscopic features

- Hardening of muscle just like wood
- Urine is dark brown with *myoglobin-myoglobinuria*
- Tonic spasms in muscles
- Atrophy of affected muscles in chronic cases
- Necrosis of muscle fibers
- Edema

- Hyaline degeneration.
- Invasion of sarcolemma by macrophages and lymphocytes
- Degeneration and necrosis of tubular epithelium in kidneys.

WHITE MUSCLE DISEASE

Extensive coagulative necrosis of muscles is observed in calves possibly due to deficiency of vitamin E during 6 month of age.

Etiology

- Vitamin E deficiency
- Selenium deficiency
- Stress

Macroscopic and microscopic features

- Colour of muscle becomes pale pink, yellowish red, grey or white.
- Muscle becomes dry, inelastic and firm.
- Urine is brownj red or chocolate brown in colour because of myoglobin.
- Coagulative necrosis of muscles.
- In some muscle cells, cloudy swelling can be observed.
- Neutrophils, macrophages, lymphocytes and eosinophils may present.
- Calcium may be deposited in necrosed areas.

ACUTE MYOSITIS

Acute myositis is the acute inflammation of skeletal muscles characterized by the presence of serous, fibrinous or haemorrhagic exudates.

Etiology

- Trauma
- Vitamin *Ej* Selenium deficiency
- *Clostridium chauvoei*, the cause of black leg in cattle

Macroscopic and microscopic features

- Muscles become extremely moist.
- Colour becomes red, consistency is firm and tense.
- Swelling and accumulation of gas in muscles, crepitating sound on palpation.
- Muscle dark red/ black with gas mixed exudates.
- Presence of serous, fibrinous and/or haemorrhagic exudate.
- Infiltration of neutrophils, macrophages, lymphocytes, etc.
- Degenerative and necrotic changes in muscles.
- Presence of Gram positive rods in exudate.

HEMORRHAGIC MYOSITIS

Haemorrhagic myositis is characterized by the presence of large amount of blood and inflammation in muscles. It may occur due to trauma and muscle rupture.

Etiology

- Trauma
- Clostridial infections

Macroscopic and microscopic features

- Area becomes red/ cyanotic.
- On cut, large amount of blood comes out from muscles.
- On touch, the affected area is hard and painful to touch.
- Regionallymphnodes may become enlarged and swollen.
- Extravasation of blood in between the myofibrils.
- Infiltration of neutrophils, macrophages and lymphocytes in connective tissue between the muscle cells.

CHRONIC MYOSITIS

Chronic inflammation of muscle is characterized by necrosis, calcification and proliferation of fibrous connective tissue. In case of tuberculosis and pseudotuberculosis, there are multiple, focal nodules containing caseation and fibrous capsule.

Etiology

- *Mycobacterium tuberculosis*
- *Corynebacterium pseudotuberculosis*
- *Trichinella* spp. infection
- *Sarcosporidia* spp. infection

Macroscopic features

- Muscles become hard to touch
- Nodules can be seen.
- On cut the lesions of caseation and calcification observed.
- Caseative necrosis, infiltration of macrophages, lymphocytes and proliferation of fibrous tissue.
- Calcification can also be observed.
- In cases of pseudotuberculosis infiltration of neutrophils is seen.
- Extensive infiltration of eosinophils in sarcoporidia infection.

PATHOLOGY OF BONES

FIBROUS OSTEODYSTROPHY

Fibrous osteodystrophy occurs as excessive action of parathyroid hormone on bones and characterized by bone resorption with replacement by fibrous tissue, increased osteoid formation which does not get sufficient minerals for deposition and formation of cysts.

Etiology

- Hyperparathyroidism
- Dietary deficiency of calcium or excess of phosphorus
- Vitamin-D deficiency
- Excessive bran feeding (Disease in horses of flour millers).

Macroscopic and microscopic features

- Lack of calcification in bone
- Resorption of calcium from bone, fibrosis
- Bone becomes shoft, flexible and deformed
- Rubbery jaw due to involvement of facial bones
- Fibrous tissue hyperplasia in bones.
- Enlargement of Haversian canals.
- Boney tissue is replaced by fibroblasts, with osteoclastic giant cells lining the remaining bone tissue.

RICKETS

Rickets is failure of adequate deposition of calcium in bones of growing animals caused by deficiency of calcium and vitamin D and characterized by bending of limbs, enlargement of ends of long bones and skeletal deformities.

Etiology

- Vitamin D deficiency
- Calcium deficiency
- Deficiency of phosphorus

Macroscopic and microscopic features

- Bending of legs, bow legs
- Pot belly
- Enlarged costochondral articulation
- Softening of bones
- Increase in proliferating cartilage adjacent to the area of ossification and its disorderly arrangement.
- Disorderly penetration of cartilage by blood vessels
- Increased area of uncalcified osteoid tissue
- Fibrosis of marrow

OSTEOMALACIA

Osteomalacia is also known as *adult rickets.* It occurs in bone of adults caused by deficiency of vitamin D and calcium and characterized by softening of bones.

Etiology

- Vitamin-D deficiency
- Calcium-phosphorus ratio disturbance

Macroscopic and microscopic features

- Softening of bones
- Irregular diffuse thickening of bones
- Bone deformities
- Increase in osteoid tissue with failure of calcification
- Increase in osteoclastic activity

OSTEOPOROSIS

Osteoporosis is atrophy of bones caused by possibly hormonal imbalance and characterized by inadequate deposition of calcium, brittleness of bones due to its increased porosity.

Etiology

- Hormonal imbalance
- Vitamin C deficiency
- Copper deficiency

Macroscopic and microscopic features

- Inadequate calcium deposition
- Bone becomes brittle and porous
- Increased fragility of bones
- Widening of Haversian canals

- Increased activity of osteoclasts
- Decrease in zona compacta and thickness of bone trabeculae

OSTEOPETROSIS

Osteopetrosis is enlargement of bone caused by fluorosis or avian leukosis virus and characterized by increase in bony tissue. It is also known as *marble bone disease.*

Etiology

- Avian leukosis virus of retroviridae family
- Fluorosis

Macroscopic and microscopic features

- Enlargement of bone towards outside and inside.
- Reduced marrow cavity
- Bone becomes brittle, marbelling of bones.
- Cartilage is also calcified, surrounded by osteoid tissue.

OSTEOMYELITIS

Osteomyelitis is the inflammation of bone with bone marrow caused by trauma and pyogenic bacteria and characterized by destruction, replacement and excessive growth of new bone adjacent to the infected part.

Etiology

- Hematogenous infection
- Direct infection through trauma/ fracture
- *Actinomyces pyogenes,* A. *bovis*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*

Macroscopic and microscopic features

- Metastatic abscess in bone marrow

- Excessive growth of bone in adjacent area.
- Exostosis or endostosis.
- Infiltration of neutrophils
- Proliferation of osteoid tissue
- Demonstration of bacteria in pus

BONE FRACTURE AND REPAIR

Fracture is the break in the continuity of bone due to trauma. A fracture may be simple or compound depending on the severity of trauma. Healing of fracture occurs by reunion of the broken ends of bone through development and proliferation of fibroblasts, angioblasts, osteoid tissue and infiltration of calcium salts.

Etiology

- Trauma
- Accidents-automobile accidents.

Macroscopic and microscopic features

- Fracture can be identified by break in bones.
- Healing of fracture is characterized by development of callus at the site of reunion of break ends of bone.
- Callus may be soft or hard.
- Proliferation of fibroblasts, angioblasts and metaplasia of connective tissue to osteoid tissue.
- Areas of calcification in osteoid tissue

PULMONARY OSTEOARTHROPATHY

Pulmonary osteoarthropathy is a rare disease of dog, sheep, cat, horse, and lion caused by prolonged anoxia and characterized by cough, dyspnea, respiratory disturbances and formation of new bone leading to thickening and deformity of limbs.

Etiology

- Prolonged anoxia

- Toxaemia

Macroscopic and microscopic features

- Pneumonia
- New bone formation just beneath the periosteum in long bones.
- The proliferation of bone is irregular leading to rough surface.
- Bone becomes enlarged twice to its normal size.
- Heart worms in case of dogs.
- Bronchogenic carcinoma
- Granulomatous lesions of tuberculosis
- Chronic bronchiectasis
- Hyperplasia of osteoid tissue with no indication of any kind of neoplastic growth in bones.

SPONDYLITIS

Spondylitis is the inflammation of vertebrae caused by bacterial fungi and characterized by caseation, intra osseous abscess formation granulomatous lesions and fibrosis.

Etiology

- *Brucella abortus, Br. ovis, Br. meletensis*
- *Actinomyces bovis*
- *Coccidioidomyces* sp.

Macroscopic and microscopic features

- Intraosseous abscess
- Granuloma encapsulated by fibrous tissue involving one or two adjacent vertebrae.
- Local enlargement of bone.
- Granulomatous lesions with caseation
- Proliferation of osteoid tissue
- Infiteration of neutrophils in intraoseous abscess.

PATHOLOGY OF JOINTS

ARTHRITIS

Arthritis is the inflammation of joint caused by bacteria, virus, chlamydia, mycoplasma and immune complexes and characterized by serus, fibrinous, purulent or ankylosing lesions in joints.

Etiology

- Bacteria- E. *coli, Erysipelas rhusiopathae, Streptococus* sp., *Shigella* sp. *Corynebacterium ovis, Brucella* sp.
- Mycoplasma- *Mycoplasma mycoides, Mycoplasma sinoviae*
- Virus- Reovirus (Tenosynovitis in birds)
- Antigen antibody complexes
- Trauma

Macroscopic and microscopic features

- Swelling of joints with increase in synovial fluid.
- Difficulty in movement
- In chronic cases fusion of two bony processes leaving no joint (ankylosing)
- Synovial fluid diminishes, becomes dirty, thick in chronic illness
- Presence of increased number of leucocytes in synovial fluid.
- Serous, fibrinous or purulent exudate in joints.
- Thickening of synovial membrane
- Presence of plasma cells and immune complexes in synovial fluid.

CHAPTER 9: PATHOLOGY OF ENDOCRINE SYSTEM, EYES AND EAR

PATHOLOGY OF HYPOTHALAMUS

The lesions in hypothalamus may cause diabetes insipedus characterized by polydypsia and polyuria with low specific gravity of urine. It occurs due to deficiency of antidiuretic hormone vasopressin.

Etiology/ Occurrence

- Lesions in hypothalamus and/ or pituitary
- Adenoma and adenocarcinoma of pituitary
- Necrosis of hypothalamic nuclei due to larval migration

PATHOLOGY OF PITUITARY GLAND

HYPERPITUITARISM

Hyperpituitarism is increased secretion of hormone(s) from pituitary gland such as excessive secretion of somatotropic hormone may cause gigantism characterized by increased length of long bones, heavy and thick bones leading to large hands, feet, skull bones *(acromegaly).* Hyperpituitarism also increases adrenal cortical stimulating hormone leading to hyperplasia of adrenal cortex. Pituitary adenoma or adenocarcinoma is responsible for hyperpituitarism.

HYPOPITUITARISM

Hypopituitarism is decrease in pituitary hormone secretions due to atrophy, aplasia or hypoplasia of pituitary. Systemic diseases such as meningitis of bacterial or viral origin may also cause lesions in pituitary *e.g.* infectious canine hepatitis, hog cholera. It is characterized by dwarfism, genital hypoplasia and prolonged gestation period.

PATHOLOGY OF THYROID

HYPERTHYROIDISM

Hyperthyroidism is increased activity of thyroid gland leading to increased production of thyroxin characterized by tachycardia, increased basal metabolic rate, bulging of eyeballs and early maturity. It occurs due to presence of tumor in thyroid. Other signs included polydypsia, polyuria, and loss of weight, weakness, fatigue and hyperthermia.

HYPOTHYROIDISM

Hypothyroidism is reduced activity of thyroid gland characterized by decreased basal metabolic rate, obesity, retardation of growth and sexual development leading to cretinism. In adult, it is

characterized by myxomatous mucoid degeneration in subcutaneous region giving floppy and edematous appearance. Hypothyroidism is caused by aplasia or hypoplasia of thyroid gland.

Goiter: Goiter is enlargement of thyroid gland, which may be accompanied by hypo- or hyperthyroidism. The enlargement of thyroid is due to hyperplasia, inflammation, or proliferation of connective tissue. The hyperplasia of gland is characterized by increased height and number to epithelial cells in acini of gland. It may be caused by deficiency of iodine, thiouracil toxicity and by use of goiterogenic substances such as soybean and cabbage. The goiter has been classified into 6 forms described as under:

Hyperplastic goiter: Due to iodine deficiency, there is hyperplasia of thyroid gland with reduction in thyroxin production. It occurs due to increased level of thyrotropic hormone from pituitary gland.

Familial goiter: There is hyperplasia of thyroid gland with reduced thyroxin secretion caused by defective or absence of enzymes responsible for thyroxin synthesis. It is not related with iodine deficiency but have congenital basis of occurrence

Colloid goiter: Colloid goiter is enlargement and distention of acini filled with colloid and flat epithelium caused by deficiency of iodine.

Adenomatous goiter: This is characterized by nodular enlargement of thyroid gland, with one or many hard nodules of variable in size and is characteristic adenoma of gland.

Toxic goiter: Toxic goiter is characterized by exophthalmus due to hyperthyroidism, enlargement of thyroid due to hyperplasia, and occurs as a hypersecretion of thyrotropic hormone from pituitary.

Equine goiter: Equine goiter is caused by excessive iodine levels in feed and occurs in new born foals with weakness from a goiterous mare. These foals are having enlarged thyroid gland.

LYMPHOCYTIC THYRODITIS

Lymphocytic thyroditis is characterized by infiltration of lymphocytes in gland causing destruction and is caused by autoimmune mechanism. The infiltration of lymphocytes is so severe that gives lymphofollicular appearance.

PATHOLOGY OF PARATHYROID GLAND

HYPOPARATHYROIDISM

Hypoparathyroidism is decreased activity of parathyroid gland characterized by decreased concentration of blood calcium and tonic spasms of muscles. It occurs due to infection, neoplasms, low calcium diets and hypersecretion of thyrocalcitonin.

HYPERPARATHYROIDISM

Hyperparathyroidism is the increased activity of parathyroid gland characterized by weakness, polydypsia, polyurea, hypercalcemia nephrocalcinosis, demineralization of bones, metastatic calcification in soft tissues and fibrous osteodystrophy. It may occur in adenoma or adenocarcinoma of parathyroid and hyperplasia of gland. Hyperparathyroidism is also associated with renal disease and chronic hypocalcemia to produce more parathormone hormone.

PATHOLOGY OF ADENAL GLANDS

HYPOADRENOCORTICISM

Hypoadrenocorticism is decreased activity of adrenal cortex characterized by atrophy, necrosis and decreased hormones leading to low blood pressure, decreased blood volume, hypoglycemia, gastrointestinal malfunction and hyperpigmentation in skin. It may occur in tuberculosis, histoplasmosis, amyloidosis, neoplasms and drug toxicity.

HYPERADRENOCORTICISM

Hyperadrenocorticism is increased activity of adrenal cortex characterized by hyperplasia and neoplasia of the gland leading to alopecia, muscle weakness, pendulous abdomen, obesity, polyuria, polydipsia, lymphopenia, eosinophilia, neutrophila and excessive secretion of 17-ketogenic steroids.

PATHOLOGY OF PANCREAS

Pancreatic islets or islets of Langerhans' are responsible for production of insulin, deficiency of which may cause hyperglycemia or diabetes mellitus. It is characterized by polyuria, glycosuria, hyperglycemia, polydypsia, loss of secretary granules in b-cells of pancreatic islets. It is caused by inflammation of pancreas causing excocrine pancreatitis. This condition may lead to arteriosclerosis in blood vessels of animals.

PATHOLOGY OF PINEAL GLAND

The pineal gland is responsible for secretion of melatonin hormone which inhibits gonadotropic hormone synthesis and release by pituitary and thus plays an important role in seasonal estrus/ reproductive capacity of animals. Degeneration and necrosis of gland may cause its decreased function but are not well reported. Adenoma of gland may be associated with increased sexual libido and activity.

PATHOLOGY OF EYE

Blepheritis is the inflammation of eyelids while *conjunctivitis* is used to describe the inflammatory condition of conjunctiva and *keratitis* for cornea. Inward turning of eyelid is known as *entropion* which may result in keratitis or conjunctivitis. Conjunctivitis is also caused by double row of eye lashes *(disctichiasis).*

DEVELOPMENT AL ANOMALIES

Aphakia is the absence of lens.

Microphakia is the small size of lens.

Hypoplasia of optic nerve is underdeveloped optic nerve with absence of optic nerve layer and ganglion cell layer of retina.

Agenesis of optic nerve is absence of optic nerve.

Coloboma is the congenital defect in the continuity of one of the tunics of the eye *i.e.* iris.

Congenital anophthalmos is the absence of the eye which may occur due to vitamin A deficiency in dam.

Congenital microphathalmos is the decreased size of eyes and may occur due to maternal vitamin A deficiency

Congenital opacity of cornea occurs in cattle and dogs due to effect of inherited recessive gene trait

Hemeralopia is day blindness which may occur in dogs due to single autosomal recessive gene.

KERATOCONJUNCTIVITIS

Keratoconjunctivitis is the inflammation of cornea and conjunctiva characterized by congestion of eyes, blindness, opacity and corneal edema.

Etiology

- Penetrating foreign objects *e.g.* Awns of wheat
- *Moraxella bovis*
- *Mycoplasma* spp.
- BHV -1, poxvirus
- *Rickettsia conjunctivae*
- *Chlamydia* spp.
- *Thelazia* spp.
- Allergy

Macroscopic features

- Congestion of conjunctiva leading to redness "pink eye".
- Edema, pain
- Increased lacrimation (Decreased lacrimation also causes conjunctivitis)
- Corneal opacity

CATARACT

Cataract is opacity of lens and is classified as under: *Subcapsular cataract* is the opacity of lens due to abnormal proliferation of lens epithelium in anterior end as a result of injury. *Posterior polar cataract* is opacity of lens due to abnormal growth of lens epithelium at posterior face of lens. *Cortical cataract* is opacity of lens due to disorganization of the lens fibers. *Nuclear cataract* is the increased density of fibers of lens at the centre and occurs in old animals. *Morgagnian cataract* is the liquefaction of cortical substance and has not been observed in animals. *Congenital cataract* is seen in neonatal animals and occurs due to failure of closure of primary lens vesicle at the periphery of lens vesicle and is associated with Chediak - Higashi syndrome.

RETINITIS

Retinitis is the inflammation of retina caused by trauma, iritis, iridocyclitis and choroiditis. When it is associated with inflammation of choroids, it is known as chorioretinitis. It may lead to detachment of retina. *Iritis* is inflammation of iris. *Iridocyclitis* is the inflammation of iris and uvea. *Choroiditis* is inflammation of choroid. The *chorioretinitis* is characterized by glaucoma occurs in canine distemper, feline panleukopenia, toxoplasmosis, tuberculosis, COCcidioidomycosis, deficiency of vitamin A and bracken fern poisoning.

GLAUCOMA

Glaucoma is the intraoccular hypertension due to occlusion of the filtration angle and is caused by trauma, iridocyclitis, intraoccular hemorrhage and neoplasm. It may be unilateral or bilateral. It is characterized by enlargement of eye ball, opaque cornea and increase aqueous humor.

PATHOLOGY OF EAR

OTITIS EXTERN A

Otitis externa is inflammation of external ear caused by *Actinomyces bovis*, parasites and fungus and characterized by granulomatous inflammation.

Etiology

- *Actinomyces bovis*

- *Psoroptes communis* - mite
- *Otobius megnini* - tick
- Fungi- (otomycosis)
- Grass of wheat awns

Macroscopic and microscopic features

- Swelling and congestion leading to obstruction of ear canal.
- Excessive production of thick, tenacious and brownish wax.
- Granulomatous lesions filling the external auditory meatus
- Granulomatous lesions of actinomycosis in subcutaneous region around the cartilage.

OTITIS MEDIA

Otitis media is inflammation of middle ear including tympanic cavity and eustachian tube.

Etiology

- Infections from otitis externa or nasopharynx
- Mites
- Awns of wheat
- *Pasteurella* spp.

Macroscopic and microscopic features

- Occlusion of eustachian tube
- Purulent inflammation
- Suppurative inflammation

OTITIS INTERNA

Otitis interna is the inflammation of inner ear including membranous and osseous labyrinth. This is also known as *labyrinthitis.*

Etiology

- Infection from otitis media

- *Mycoplasma* spp.
- Mumps
- Measles

Macroscopic and microscopic features

- Disturbance in equilibrium
- Deafness
- Suppurative inflammation.

CHAPTER 10: PATHOLOGY OF HEMOPOITIC AND IMMUNE SYSTEM

DEVELOPMENT AL ANOMALIES

Hereditary anemia

Hereditary anemia has been reported in mice due to defects in erythropoiesis or reduced vitality of erythrocytes. Erythropenia along with leucopenia occurs in mouse foetus on 20th day of gestation due to defective autosomal chromosome 4. Sex linked anemia in mouse is hypochromic with deficient bone marrow and occurs in hemizygus males or homozygus females. This anemia occurs due to deficiency of iron as a result of poor absorption from gastrointestinal tract.

Autoimmune hemolytic anemia in foals

It occurs due to incompatible blood group antigens of male and female parents. The mare does not have that blood group antigen but foetus acquires it from father. The foetal blood exposed to dam through placental exchanges that leads to induction of antibody production in mares against foetal blood group antigen. These antibodies accumulate in colustrum and when foal suck the milk from mares, they are readily absorbed through G.I. tract of foals in blood and causes destruction of erythrocytes leading to anemia.

Congenital defects in lymphocytes

Congenital defects in lymphocytes are classified under stem cell aplasia/ agenesis leading to combined immunodeficiency with absence of both T- and B-Iymphocytes in Arabian foals. It occurs either due to inherited gene defect or there is differentiation/ maturation defects in

lymphocytes. It is characterized by agammaglobulinemia, lymphopenia, hypoplasia of thymus, lymphnodes and spleen.

Chediak Higashi Syndrome

This syndrome is related with defects in phagocytic cells such as defective neutrophils and monocytes. The defects are in chemotaxis, engulfment and killing of bacteria and associated with defective assembly of cytoplasmic microtubules responsible for degranulation and release of lysosomal enzymes, there is depression of superoxide anions leading to persistent bacterial infections.

ANEMIA

Anemia is the decrease in number of erythrocytes or hemoglobin concentration in erythrocytes per unit of blood and is characterized by pale mucus membrane, dyspnoea, cardiac hypertrophy and weakness. Anemia is classified according to morphological characteristics of erythrocytes and on the basis of causative factors. Morphologically, anemia is classified as macrocytic, normocytic and microcytic depending on the size of red blood cells and normochromic and hypochromic based on the presence of quantity of hemoglobin in RBC.

Macrocytic anemia is characterized by increased size of RBC and occurs due to acute blood loss or hemolysis resulting in excessive production and availability of immature erythrocytes in blood. Such cells also have reduced amount of hemoglobin and termed as hypochromic.

Macrocytic normochromic anemia is increase size of RBC with normal hemoglobin and has been observed in deficiency of folic acid, niacin and vitamin Bt2•

Normocytic anemias are most common in animals occurs due to neoplasia, irradiation and are also known as *aplastic anemia* as a result of aplasia or agenesis of RBC. Normocytic normochromic, normal size of RBC with normal hemoglobin anemia occurs as a result of depression of erythrogenesis.

Microcytic anemia is reduction in size of erythrocytes with decreased hemoglobin (Microcytic hypochromic) and occurs in deficiency of iron and pyridoxine or chronic blood loss. In anemia, the size of RBC varies markedly with some large and some small size and is known as *anisocytosis.* The presence of abnormal shape (elongated, angular, ovoid, distorted) of RBC is termed as *poikilocytosis.* In some blood smears, there are nucleated RBC's which are immature due to increased production to meet the demand. Sometimes, the erythrocytes are having minute dark spots known as *basophilic stippling* which occurs in acute blood loss. Some erythrocytes stain unevenly with some dark and light colour spots and are known as *polychromatophilia* which is an indication of active erythrogenesis. The denaturation and precipitation of hemoglobin leads to appearance of purplish granules in RBC near the cytoplasmic membrane which are known as "Heinz bodies". According to etiological factors, the anemia is classified as hemolytic, haemorrhagic or deficiency anemia.

HEMOLYTIC ANEMIA

Hemolytic anemia occurs due to excessive lysis of erythrocytes and characterized by icterus, hemoglobinuria, and presence of nucleated erythrocytes in blood and hemosiderosis in spleen.

Etiology

- Infections *e.g. Anaplasma* spp. *Babesia* spp., Equine infectious anemia virus
- Toxins/ poisons *e.g.* snake venom, chronic lead poisoning.
- Immune mechanisms *e.g.* autoimmunity against erythrocytes.

Macroscopic and microscopic features

- Pale mucous membranes
- Icterus
- Blood is thin, watery.
- Hemoglobinurea
- Decreased number of erythrocytes
- Presence of nucleated/ immature RBC in blood
- Hemosiderin laden cells in spleen

HEMORRHAGIC ANEMIA

Hemorrhagic anemia occurs due to severe haemorrhage, extravasation of blood and characterized by pale mucus membrane and hemorrhage in body.

Etiology

- Infections *e.g.* Acute septicemic diseases
- Toxins/ poisons *e.g.* Bracken fern poisoning
- Parasites *e.g. Hemonchus contortus*
- Deficiency *e.g.* vitamin C deficiency

Macroscopic and microscopic features

- Petechiae or Echymotic hemorrhage
- Pale mucous membrane
- Hematuria
- Hemorrhage in various tissues/ organs
- Macrocytic or normocytic characters of RBC
- Poikilocytosis
- Hyperplasia of bone marrow

DEFICIENCY ANEMIA

Deficiency anemia occurs as a result of deficiency of iron, copper, cobalt and vitamins and characterized by pale mucus membrane, weak and debilitated body and decreased number of erythrocytes with hypochromasia in blood.

Etiology

- Deficiency of iron
- Deficiency of copper
- Deficiency of cobalt
- Deficiency of vitamin B12, Pyridoxine, riboflavin and folic acid.
- Parasitic infestation may lead to deficiency.

Macroscopic and microscopic features

- Pale mucous membrane
- Thin watery blood with light red colour
- Weak and debilitated carcass
- Heavy parasitic load in gastrointestinal tract.
- Microcytic hypochromic erythrocytes.
- Poikilocytosis

TOXIC APLASTIC ANEMIA

Toxic aplastic anemia is agenesis or aplasia of hemopoietic tissues in bone marrow and there is lack of erythrocyte production. It is characterized by the absence of developmental stages of erythrocytes viz., normoblasts, megaloblasts etc.

Etiology

- Radiation *e.g.* X-rays, g rays, or UV rays
- Sulfonamides
- Bracken fern toxicity
- Uremia
- Feline panleukopenia

Macroscopic and microscopic features

- Pale mucuos membrane
- Weak and debilitated animal
- Dyspnoea
- Bone marrow becomes yellow/ fatty.
- Absence of developmental stages or RBC such as normoblasts, megaloblasts etc.
- Agranulocytosis i.e. Reduction of WBC in circulating blood.
- Bone marrow becomes fatty.

AUTOIMMUNE HEMOLYTIC ANEMIA

Autoimmune hemolytic anemia occurs as a result of destruction of erythrocytes by immune mechanisms developed against erythrocytes.

Etiology

- Autoimmune hemolytic anemia in foals.
- Antibodies produced against own RBC of an animal.
- Equine infectious anemia
- Anaplasmosis
- Systemic lupus erythematosus

Macroscopic and microscopic features

- Pale mucous membrane
- Enlargement of liver, spleen and lymphnodes
- Hemoglobinuria
- Lameness due to rheumatoid arthritis
- Erythrophagocytosis
- Demonstration of antibodies against own RBC in sera of animals.
- Active erythropoiesis
- Glomerulonephritis

POLYCYTHEMIA

Polycythemia is increase in number of erythrocytes in circulating blood. It may be relative increase as a result of dehydration or decrease in plasma volume or absolute due to anoxia.

Etiology

- Dehydration due to diarrhoea, vomiting and loss of fluid in oedemaj inflammation.
- Anoxia in high altitudes.
- Heart diseases *e.g.* Patent ductus arteriosus
- Severe pulmonary emphysema
- Erythroid leukemia

Macroscopic and microscopic features

- Dehydration, mucous membrane dry, sticky
- Pulmonary emphysema, fibrosis in lungs
- Increase hemoglobin concentration
- Increased number of erythrocytes
- Severe damage in lungs, congestion, emphysema, fibrosis

LEUCOCYTOSIS

Leucocytosis is increase in number of leucocytes in circulating blood caused by various infections. There is also increase in white blood cells in blood due to neoplastic condition and is known as *Leukemia.* As the leucocytes consist of neutrophils, lymphocytes eosinophils, monocytes and basophils; the increase in number of neutrophils is termed as *neutrophilia,* eosinophils as *eosinophilia*, lymphocytes as *lymphocytosis,* basophils as *basophilia* and of monocytes as *monocytosis.*

Etiology

- Infections
- Bacterial infection- neutrophilia
- Viral infections and chronic bacterial infections-lymphocytosis
- Parasites- eosinophilia
- Allergies- basophilia, lymphocytosis

Macroscopic and microscopic features

- No characteristic lesion.
- Reactive lymph node hyperplasia.
- Enlargement of lymphoid organs such as spleen, thymus and bursa.
- Increase in number of total leucocytes in blood
- Increase in absolute lymphocyte, absolute neutrophil, absolute eosinophil counts.
- Heperplastic lesions in lymphoid organs.

LEUCOPENIA

Leucopenia is decrease in number of whit~ blood cells. The leucocytes are neutrophils, lymphocytes monocytes, eosinophils and basophils. If there is decrease in number of all 5 cells of leucocytes, it is known as *panleucopenia.* The decrease in number of neutrophils is termed as *neutropenia* and lymphocytes as *lymphopenia.*

Etiology

- Congenital *e.g.* Chediak Higashi syndrome
- Infections *e.g.* Feline panleucopenia virus, infectious bursal disease virus
- Chemicals *e.g.* Pesticides, heavy metals
- Radiation *e.g.* X-rays

Macroscopic and microscopic features

- Atrophy of lymphoid organs
- Recurrent infections, vaccination failures, pyogenic disorders.
- Edema, hemorrhage in bursa, atrophy of bursa due to fibrosis in IBD infection.
- Decrease in total leucocyte count and absolute neutrophil and absolute lymphocyte counts
- Degeneration and necrosis of lymphoid cells in follicles of lymphoid organ
- Edema, necrosis, proliferation of fibrous tissue in bursa in IBD infection.

PATHOLOGY OF SPLEEN

SPLENITIS

Spleenitis is the inflammation of spleen characterized by enlargement, infiltration of inflammatory cells, proliferation of lymphoid follicles, congestion and oedema followed by proliferation of fibrous tissue.

Etiology

- Infections *e.g.* bacteria, virus
- Deficiency of vitamins and minerals
- Amyloidosis
- Immunodeficiency e.g.environmental pollution

Macroscopic and microscopic features

- Enlargement of spleen.
- Necrotic patches on spleen.
- In chronic cases or in immunological disorders.
- There is atrophy of spleen due to fibrosis.
- Necrotic patches and congestion leading to mottling.
- Congestion in spleen.
- Proliferation of lymphoid follicles/ cells.
- Edema.
- In atrophied spleen, proliferation of fibrous tissue, depletion of lymphoid cells/ follicles.

PATHOLOGY OF LYMPHNODES

LYMPHADENITIS

Lymphadenitis is the inflammation of lymphnodes characterized by enlargement atrophy, congestion proliferation of lymphoid cells/ depletion of lymphoid cells, oedema AND fibrosis of lymphnodes.

Etiology

- Infections *e.g.* Rinderpest
- Immunological disorders *e.g.* immuno-deficiency
- Deficiency *e.g.* Deficiency of protein
- Environmental pollution *e.g.* pesticides, heavy metals
- Tumors/ neoplasm *e.g.* lymphosarcoma

Macroscopic and microscopic features

- Enlargement of lymph nodes
- Congestion
- Edema
- In chronic cases- fibrosis

- Atrophy
- Congestion, edema, proliferation of lymphoid cells.
- In chronic cases, proliferation of fibrous tissue, depletion of lymphoid cells

PATHOLOGY OF THYMUS

THYMOMA/ THYMIC HYPERPLASIA

It is characterized by congestion and hyperplasia of lymphoid cells in thymus. The inflammation of thymus in chronic cases is characterized by atrophy and proliferation of fibrous tissue.

Etiology

- Immunological disorders
- Environmental pollution *e.g.* Pesticide, Heavy metals
- Toxins/poisons
- Aging *e.g.* in adult poultry thymus regresses

Macroscopic and microscopic features

- Congestion, reddening of thymus
- Edema
- Increase in size
- Atrophy, thinning like thread.
- Congestion, oedema
- Proliferation of lymphoid cells
- Depletion of lymphoid cells
- Proliferation of fibrous tissue

PATHOLOGY OF BURSA

BURSITIS

Bursitis is the inflammation of bursa of Fabricius in poultry characterized by edema, congestion, haemorrhage or atrophy and depletion of lymphoid cells.

Etiology

- Infectious Bursal disease virus (Birnavirus)
- Environmental pollution *e.g.* Pesticides, heavy metals.

Macroscopic and microscopic features

- Enlargement of bursa
- Congestion and/ or hemorrhage
- In chronic cases, atrophy and fibrosis
- Edema
- Depletion of lymphoid tissue
- Degeneration and necrosis of lymphoid cells
- Congestion and/or haemorrhage.
- Proliferation fibrous tissue.

SECTION 2: VETERINARY CLINICAL PATHOLOGY

PART 1: VETERINARY CLINICAL PATHOLOGY I

By the end of this course, students will be able to:

- Use different laboratory diagnostic tools and procedures
- Understand the pathophysiological changes underlying various disease conditions and their implications on the well-being of the animal.
- Perform various hematological, serum biochemical and enzymatic activity and interpret test results and correlate with history and clinical signs

CHAPTER 1: INTRODUCTION TO CLINICAL PATHOLOGY

- **Definition:** is subspecialty of pathology that deals with the use of ***laboratory method (hematology, clinical chemistry***, **cytology**, *urinalysis surgical pathology…)* for the diagnosis & treatment of disease.
- Without the laboratory examination of samples the diagnosis may not be complete
- Clinical pathology deals with **laboratory and other tests** /methods which enable the clinician ***to make a diagnosis*** or to help the clinician ***to confirm a diagnosis***.
- The samples that are examined include: blood, urine, faeces, exudates, skin scrapings, biopsy material.
- For a sample to be examined at the laboratory the following alternatives are very important:
  - ✓ **The clinical examinations** always precede the laboratory examination.
  - ✓ **The test should** be carefully chosen to give reliable result.
  - ✓ The correct type of sample must be collected for the test result to be reliable
  - ✓ The result must be correlated with the history and clinical signs

Interpretation of samples

- **Best interpretation of sample submitted to a laboratory depends on the following main points:**
  - ✓ **collected sample** (quantity and quality)

- ✓ **packaging skill**
- ✓ **shipment** (best if delivered by the owner, or vet or by courier service)
- ✓ **identifications of the sample**
- ✓ **facility of the laboratory**

The accuracy and validity of the laboratory investigation depends on:

1. ***Appropriate inquiry of history*** detailed physical examination and necropsy findings of both clinical and field cases.
2. **Selection of donor animals**.
   a. Animals in good state of preservation (advanced stages for bacterial diseases, early acute stages for viral diseases),
   b. As many animals as possible (from recently dead animals necropsy samples, from apparently healthy animals, etc.).
3. **Appropriate collection and handling of samples**.

- The samples collected should be ***appropriate*** for the intended purpose, ***adequate in number*** and amount, should be ***representative*** of the condition, should be ***collected aseptically***, (avoid cross-contamination between samples).
- Should be ***carefully packaged***, ***labeled***, and transmitted to the laboratory by the ***fastest practicable*** method, with the appropriate temperature control.
- All samples should be ***accompanied by a letter*** or submission form, which includes the name and address of the submitter, the origin of the material, the relevant history, animal identification.

4. **Technical proficiency of laboratory personnel**

The quality of the results depends on:

- Appropriate lab ware preparation. For microbial growth, lab wares should have to sterile.
- Ability to detect or describe the analyte or the results (turbidity determination for mycoplasma, use of hemadsorption or blind sub passages up to 5 times for viruses, etc.).
- Interpretation of the results.

5. **Documentation and recording**. This will serve to establish a data source for future uses.

<u>Preservation of specimens</u>

1. **Refrigeration**
   a. Natural ice:

- This method is adequate if :
  - ✓ the sample is properly packed and
  - ✓ the distance to the lab is not far
- ***preserve for only 24 hours (maximum)***
- The specimen must be in water-tight container (no direct contact with ice)

b. Dry ice:

- good if the specimen can be frozen without interfering with the lab procedure
- never place the dry ice in direct contact with the specimen unless freezing is not a problem
- Do not send dry ice in an airproof metal or glass container, because the ice volatilizes, and pressure may result in an explosion.

2. Chemical preservatives

i. For **blood cells** the most commonly used fixatives or preservatives are **absolute methyl and ethyl alcohols.**

ii. For **urine sample**, the commonly used preservatives are **40% formalin, toluene, thymol and 5% phenol.**

iii. **Mercuric chloride at 1mg per 5 ml** of rumen liqueur can be used as a preservative.

iv. For few days' preservation, feces should be mixed with equal quantities of 10% formalin and for long periods 70% alcohol may be used.

v. ***Fixing solution-*** never use chemicals to fix specimens designed to detect infectious agents

1. 10% formalin
2. 0.5% phenol
3. Others

Collection, Handling and Storage of Biological Samples

- During the collection and manipulation of biological samples, the use of disposable latex gloves, protection glasses and clothes is highly recommended.

- Even after the collection in the field, **all samples should be treated as a biological risk** until a complete screening has been made to access what infectious agents the animal might have carried thought its blood, feces or other samples.

Blood collection

- *Blood samples* – hematology/ pathology/culture/direct examination for bacteria, viruses, or protozoa, in which case it is usual to use anticoagulants
- May be taken for serology, which requires a clotted sample (***do not need anticoagulants***).
- Blood plasma is also used for some chemical analytic procedures.

ANTICOAGULANTS

- A great variety of anticoagulants may be used in preserving blood for hematologic/pathological examinations.
- **An anticoagulant is required if blood is to be used for**:
  - **blood cell counting/morphology study/culture/separation of plasma / blood transfusion**
- **The choice of anticoagulant and the ratio of blood to anticoagulant are of great importance**.
  - **Dry anticoagulants are used to avoid dilution of the collected blood.**

- **Anticoagulants should be mixed slowly and gently to avoid hemolysis.**
- The collection vials should be shaken by rotating in between the palms for proper mixing of the anticoagulants.

EDTA (Ethylene diaminetetra acetic acid)

- EDTA (especially K_2EDTA) best meets the requirements of good anticoagulant and is most frequently used in hematology.
- Two forms are used:
  - The dipotassium salt (K_2EDTA) and
  - The disodium salt (Na_2EDTA)
- The potassium salts (liquid or dry powder) are used in commercial tubes because they are more soluble.

Mode of action: prevents the coagulation of blood by combining with calcium ***(chelating***) and forms insoluble calcium salts.

- Refrigeration will extend the preservation to 24 hours.

Uses: Making a blood smear for **cell morphology studies**, **test for microfilaria**, **cell counts**, **BUN**, ***plasma protein***, **fibrinogen, glucose**, **determination of Comb's test**.

- EDTA preserves the staining and morphological characteristics or cellular details of **leukocytes**

Disadvantages:

- ***Excessive concentrations*** *of EDTA will cause shrinkage of RBC's and* ***erroneous PCV, MCV and MCHC*** *results (fill vacationer tubes with the required quantity of blood).*

- After several hours bovine lymphocytes may develop vacuoles and tend to resemble monocytes.
- **EDTA interferes with blood chemistry tests as follows:**
  - ✓ **Falsely decreases alkaline phosphates' by binding Mg^{++}.**
  - ✓ **Decreases the $C0_2$ combining power of blood.**
  - ✓ **Alters Na^{+}, K^{+} and Ca^{++} concentrations in plasma.**

HEPARIN

- It is a natural anticoagulant in the body, found in the liver (from the Greek "hepar" meaning liver), and may also be within basophiles and mast cells.
- Heparin is also called ***antithromboplastin or antithrombin***.
- **Mode of action:** It interferes with the formation and/or activity of **thrombin** and the activity of clotting factors IX, X, XI, XII (9-12).

- It has an affinity for blood proteins and prevents clotting by ***preventing conversion of Prothrombin to thrombin.***
- *Heparin is the anticoagulant of choice for* ***blood pH*** *and* ***blood gas analysis for acid base balance.***
- It may be used **for special trace element** studies and some cytology.
- ***Excessive heparin does not alter RBC volume.***

Disadvantages:

- It causes clumping of leukocytes

- It interferes with the staining of leukocytes hence not recommended for the study of morphology (It gives blue background and obscures the cellular morphology).
- ***Blood will clot in 8-12 hours because clotting is only delayed and not prevented***.
- It is not suitable for agglutination tests, coagulation studies (prothrombin time tests) or plasma fibrinogen determination (***Dysorder of hemostasis***).
- It may interfere with some automated biochemical analyses of plasma.

Collection of blood (sites of collection)

Blood is collected from animals through **puncture of veins** using syringe and needle. In laboratory animals or poultry, however, **it is collected directly from the heart**. The site of blood collection in different animal spps is as follows:

Cattle	jugular vein, ear vein
Horse/camel/shoats	jugular vein
Pig	ear vein, anterior venacava
Dog	cephalic vein, recurrent tarsal vein

Storage of blood samples

- It is always advisable to start the examination of blood ***immediately after collection***. But if delayed is expected, the blood samples should be kept in refrigerator at 4^0c for 24hr without much alteration.
- *The refrigerated blood samples should be taken out at least 30min before the start of examination* and the sample should be mixed properly with gentle shaking

Expiration of Samples for Blood Chemistry under Different Storage Temperatures (Santos 2006).

Exam	**Type of Sample**	**Expiration of the Sample**
Glucose	serum	immediate use only
Total Protein	serum or plasma	3 days 2-8°C, 1 week -10°C
Albumin	serum	3 days 2-8°C, 1 week -10°C

Amylase	serum or plasma (EDTA or heparin) or urine	24 hours 15-25°C, 2 months 2-8°C
AlkP	serum or plasma (heparin)	6 hours 2-8°C, several months -10°C
AST)	serum or plasma (EDTA or heparin	4 days 2-8°C, 1 week -10°C
Cholinesterase	serum or plasma (EDTA or heparin)	1 week 2-8°C
CK	serum or plasma (EDTA or heparin)	24 hours 15-25°C, 1 week 2-8°C
GGT	serum or plasma (EDTA or heparin)	2 weeks 2-8°C, 6 months -10°C
Lipase	serum or plasma (heparin)	24 hours 15-25°C, 3 weeks 2-8°C
SDH	serum or plasma (EDTA or heparin)	4 days 2-8°C, 1 week -10°C

Fibrinogen	plasma (citrate)	4 hours 2-8°C
Total Lipids	serum or plasma (EDTA)	10 days 2-8°C
Triglycerides	serum or plasma (EDTA or heparin)	3 days 2-8°C, 1 month -10°C
Cholesterol	serum or plasma (heparin)	1 week 2-8°C, 3 months -10°C
Creatinine	serum or plasma (EDTA or heparin) or urine	1 week 2-8°C

Total Bilirubin	serum	4 days 2-8°C safe from light, 3 months -10°C
Uric Acid	serum or plasma (EDTA or heparin) or urine	3 days 2-8°C, 1 week -10°C e 6 months -20°C
BUN	serum or plasma (EDTA or heparin) or urine	12 hours 15-25°C, 1 week 2-8°C, 3 months -10°C
Na	serum or plasma	1 week 2-8°C
K	serum or urine	1 week 2-8°C
Ca	serum or plasma (heparin) or urine	1 week 2-8°C, 2 months -10°C
Cl	serum or plasma (heparin) or urine	1 week 2-8°C, several months -10°C
P	serum or plasma (heparin)	2 days 15-25°C, 1 week 2-8°C
Mg	serum or plasma (heparin) or urine	24 hours 15-25°C, 2 weeks 2-8°C

Blood films /smears preparation & uses (*wet, thin and thick films)*

- ***Examination of a smear of peripheral blood is of particular value in aiding diagnosis and prognosis of acute, generalized and localized infections.***
- ***Blood disease (cytosis/cytopenia/leukemia …)*** can be diagnosed by examination of a blood smear
- Information relative to the states of the **erythrocytes** (size, shape, inclusion bodies associated with anemia may also be obtained).
- **Blood parasites** such as blood microfilaria, *anaplasma*, haemobartonella, eperythrozoa, *trypanosomes* and *babesia* organisms can be demonstrated by using properly prepared smears.
- Differential leukocyte count
- Estimation of platelet number

- Prepare blood film immediately from fresh blood or within an hour from blood collected with anticoagulant, ***as leukocytes and other cells have a tendency to degenerate rapidly.***
- Blood films can be prepared from whole blood and from plasma and buffy coat after centrifugation.
- Always new slides and cover slips need to be used.
- Old slides and cover slips can be dipped into 70 – 100% ethanol and whipped dry before use.
- Only hold the slides and cover slips on the edges.

Commonly three types of smears:

- Wet,
- Thin and
- Thick smear

Wet smear (**wet blood film)**

- Indications: quickest and easiest techniques of hemoparasite identification/ ***movement*** (trypanosomes and microfilariae) **but less sensitive** in detecting of ***low parasitemia.***
- **Materials:**
  - ✓ Blood (EDTA/Heparin)/Clean Microscope Slides& cover slips

- **Procedure:**
  - ✓ Place a drop of blood on a clean slide
  - ✓ Place a cover slip on it to spread the blood out
  - ✓ Examine the blood film for the presence of **motile** trypanosomes (*T. vivax* tend to shoot across the microscope field; *T. congolense* is **sluggish** but active with a tendency to adhere to RBC by anterior end and *T. brucie* displays a rapid movement in a confined area).

Thin and thick blood films

- ❖ Indications:
  - – To demonstrate Parasite(morphology) in peripheral blood
  - – Study the general trypanosome ***structure for species identification***

- *For the detection of other haemo-parasites (Babesia, Theileria, Anaplasma etc) in blood*
- thick smear is recommended when we suspect **low parasitemia**
- To study blood cell morphological abnormalities (size, shape, inclusions)

Thin film/Procedure

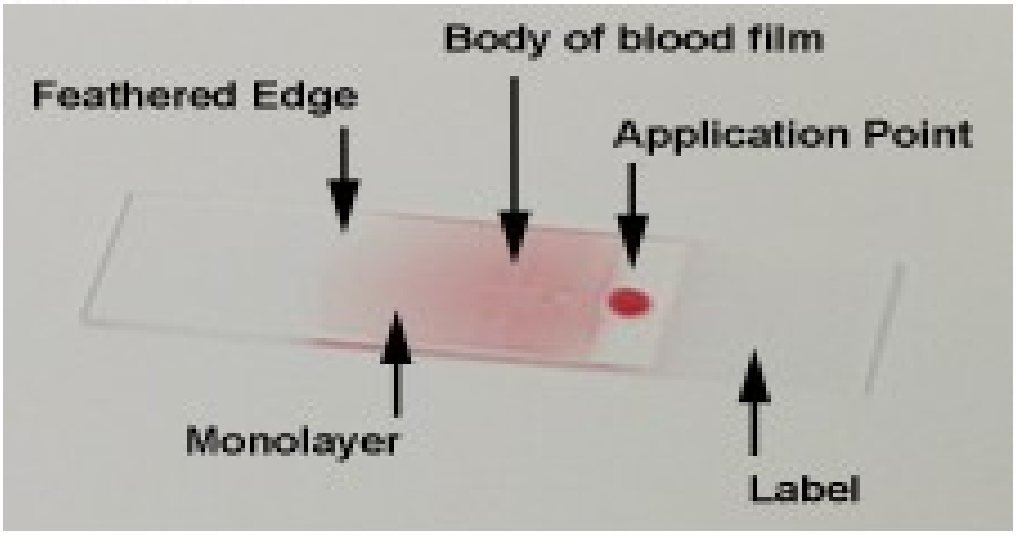
- Place a small drop of mixed blood near one end of a clean glass slide
- hold the spreader slide between thumb and forefinger, with the little finger
- At an angle of 30^0 after Allowing the blood to run to each end of the spreader (by capillary action) and then spread the blood along the slide in **a rapid, steady motion**.
- allow the smears to dry fast by waving in the air
- **Label** (on the thick end of the smear with a pencil).

- ***In general, the greater the angle, the thicker and shorter the film will be and*** *the smaller the angle the thinner* ***and longer the smear*****.**
- **A good slide film should have :**
  - ✓ Smooth appearance free from holes,
  - ✓ Straight borders and rainbow like appearance when seen against light.
  - ✓ ***It should be thick at one end and thin and feathered at the other***.

A blood film has following three zones:

- **Body-** Cell morphology, WBCs & platelet numbers are estimated
- **Monolayer-**RBC agglutination, RBCs for anisocytosis, poikilocytosis, polychromasia, Hgb concentration and RBC parasites
- **Feathered edge-** Microfilaria, phagocytized organisms and platelet clumping

Figure 2.



The components of the peripheral blood film slide.

Fixation of smears

- If the smear is **not stained immediately**, it should be fixed with alcohol and placed in a slide box and **covered or wrapped** in paper for protection.
- Commonly used fixatives (absolute methyl alcohol, **ethyl alcohol**) prevent **autolytic degeneration.**
- Immerse the films in methanol for 1-5 minutes.
- When long delay in staining the films is expected, fix for 15 minutes.

Stains and staining of blood smears

- **Romanowsky stains Type-** Leishman's staining /Wright's stain/Giemsa stain/Diff-quick
- **Supravital and Romanowsky stains (wright's stain) do not require fixation.**

Wright's stain

- **Procedure**
  - ✓ Mark area of the film with wax pencil
  - ✓ add few drops of stain and allow to act for 1 minute
  - ✓ Add an equal number of drops of buffer or neutral water. Mix gently without touching the smear till metallic film forms.
  - ✓ allow the diluted stain to act for 2-4 minutes
  - ✓ wash metallic film with neutral water and flood with water
  - ✓ dry it and examine

Giemsa stain

- **Giemsa stain is valuable for**:
  - ✓ staining large number of slides simultaneously, for the staining of thick film and
  - ✓ for the diagnosis of parasitic diseases.
  - ✓ It is not as good as wright's stain for routine hematological procedures because
    - ➢ **the staining time is longer,**
    - ➢ **preliminary fixation is required and**
    - ➢ **the neutrophilic granular detail is not good.**
- **Preparation**: take 0.3g of Giemsa stain in mortar. Add 25ml of glycerine in small amounts while grinding. Add 25 ml acetone free methyl alcohol. This makes stock solution.
- **Procedure**
  - ✓ Fix the blood film for 3-5 minutes in methyle alcohol and air dry.
  - ✓ Prepare working solution by adding 1ml of giemsa stain to 9ml neutral distilled water or buffered distilled water (PH 6.8).
  - ✓ Place the blood film in coplin jar having diluted stain for 30-45 minutes
  - ✓ Wash the film with neutral water, then drain dry and examine.

Examination of blood smear

- Inspect the blood smear **under low power** to note distribution of cells, and select a portion of smear ***near the thin end*** where the cells don't overlap.
- ***Switch to the oil immersion objective for making the rest of the examination***

Causes of incorrectly stained blood smear:

A. ***Entire smear is too blue;*** *granules of neutrophils are over stained and larger than normal and granules of eosinophils are deep grey.*

- – Buffered water or stain is two alkali.
- – Excessive thickness of the smear.
- – Alkaline residue on the slide.
- – Insufficient washing.
- – Prolonged staining.

B. Nuclei of leucocytes are pale blue and erythrocytes and granules of eosinophils are usually red.
   - Buffered water or **stain is too acids**.
   - Acid residue on slide.

C. ***Entire smear has a pale stain***
   - **Under staining**
   - Weak stain.
   - Excessive washing on allowing buffered distilled water to stand on slide.
   - Using worm or hot buffered distilled water for washing slide.

D. Variations on staining on different areas of the smear
   - Buffered water was unevenly applied.
   - Acid or alkali residue on slide.
   - Water wasn't properly drained from slide after washing.

E. ***Precipitated stain***
   - Lack of through washing.
   - Precipitate hasn't properly filtered.
   - Evaporation of alcoholic stain may be due to excessive staining time, slide titled so that stain runs to one end or to titling stain of slide before washing.

CHAPTER 2: ERYTHROCYTE PRODUCTION, MORPHOLOGY (PER SPECIES), & ABNORMAL MORPHOLOGY (*THEIR INTERPRETATIONS*)

ERYTHROPOIESIS

1. In mammals, erythropoiesis occurs **extravascularly in bone marrow parenchyma**. In **avian species**, erythropoiesis occurs within the **vascular sinuses of the bone marrow** (intravascular or intrasinusoidal development).

2. Characteristic morphologic changes take place during maturation from the rubriblast to the mature erythrocyte.

– a. Cells become smaller.

– b. Nuclei become smaller and their chromatin is more aggregated:

- The nucleus is extruded at the metarubricyte state, and a reticulocyte is formed.

- Cytoplasmic color changes from blue to orange as hemoglobin is formed and RNA is lost.
- **Reticulocytes** of most species remain in the bone marrow (venous sinus) for two to three days before release and ultimately mature in the peripheral blood or spleen.
- In health, the reticulocytes **of cattle and horses** mature in the bone marrow; mature erythrocytes are released.

Regulation of erythropoiesis (Erythropoietin)

- Erythropoietin is a glycoprotein hormone produced by the *cells in the interstitium of the kidney in* response to reduced oxygen tension (hypoxia).
- *Erythropoietin's principal site of action is the bone marrow*.

The effects of erythropoietin include:-

- Stimulation of growth and differentiation of *both erythroid and platelet progenitors*
- *Induction of hemoglobin synthesis in dividing erythrocyte precursors*
- Increased erythropoietin release can ultimately lead to increased hematocrit, total red blood cell (RBC) numbers, and platelet count.

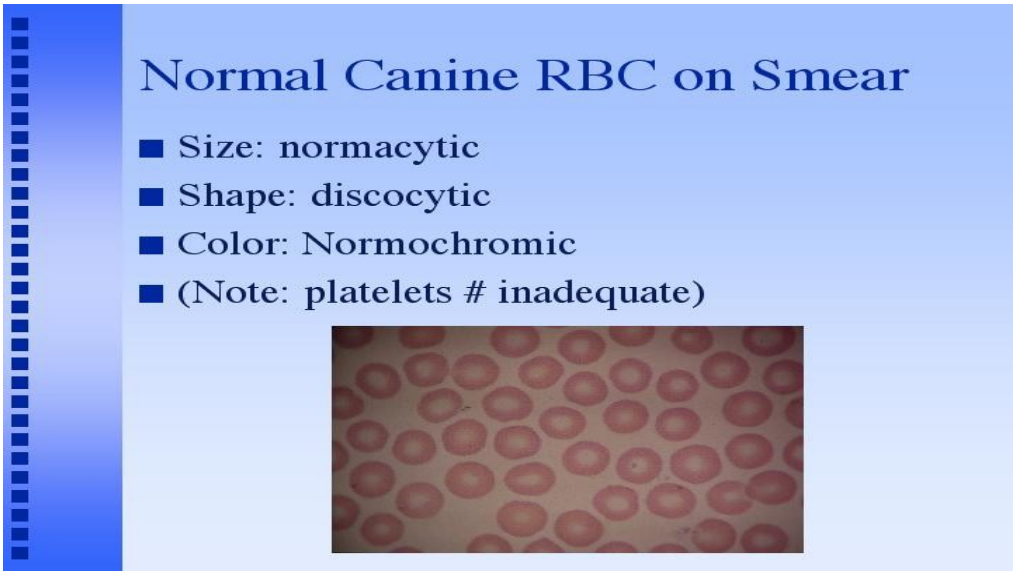
ERYTHROCYTE DESTRUCTION

- A. average lifespan cow, 160 days; sheep, 150 days; horse, 145 days; dog, 110 days; pig, 86 days; cat, 70 days; bird, approximately 35 days.
- B. Aging of erythrocytes is accompanied by changes in enzyme content and cell membrane structure that make the cells less capable of survival and subject to removal by the spleen.
- In health, senescent erythrocytes are removed from circulation by Phagocytosis by macrophages.
- The erythrocyte releases its hemoglobin (split into heme and globin).
- Globin is broken down to its constituent amino acids, which are reutilized.
- After releasing the iron (conserved), heme is cleaved by **heme oxygenase** to carbon monoxide and biliverdin.
- Biliverdin is reduced by **biliverdin reductase** to bilirubin
- *Numbers of circulating RBCs are affected by*:
  - ✓ Changes in plasma volume
  - ✓ Rate of RBC destruction or loss

- ✓ Splenic contraction (sinosoidal/non-sinusoidal spleen)
- ✓ Erythropoietin (EPO) secretion and the rate of bone marrow production

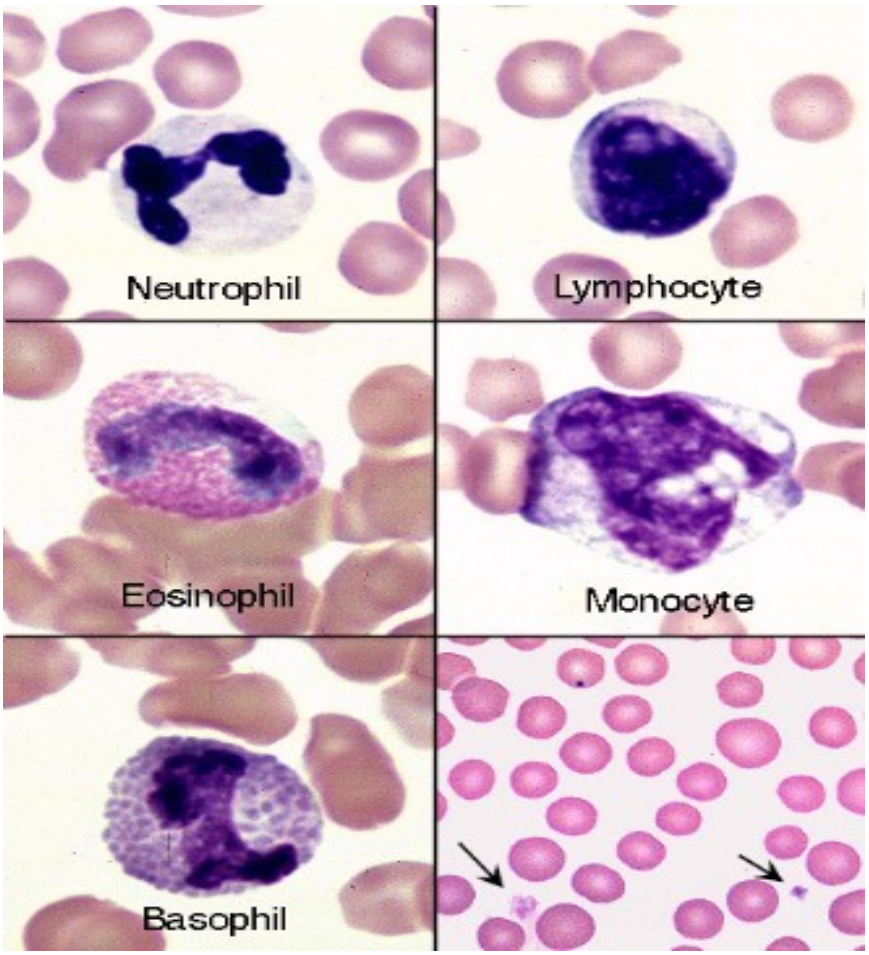
Erythrocyte morphology (different Spps)

- ***Canine erythrocytes*** average 7 µm in diameter (largest), are **uniform in size**, and have **central pallor** (biconcave disk).
- ***Feline erythrocytes*** average 5.8 µm in diameter, have mild **anisocytosis** (i.e., variation in size), and exhibit **very slight central** *pallo*r. Crenation is commonly observed. Howell-Jolly bodies (nuclear remnants) occur in up to 1% of the erythrocytes. ***Mild Rouleaux formation*** (Stacking one on other) also may be present.
- Bovine erythrocytes average 5.5 µm in diameter. Anisocytosis is common, and ***central pallor is usually slight***. Crenation is **common.**



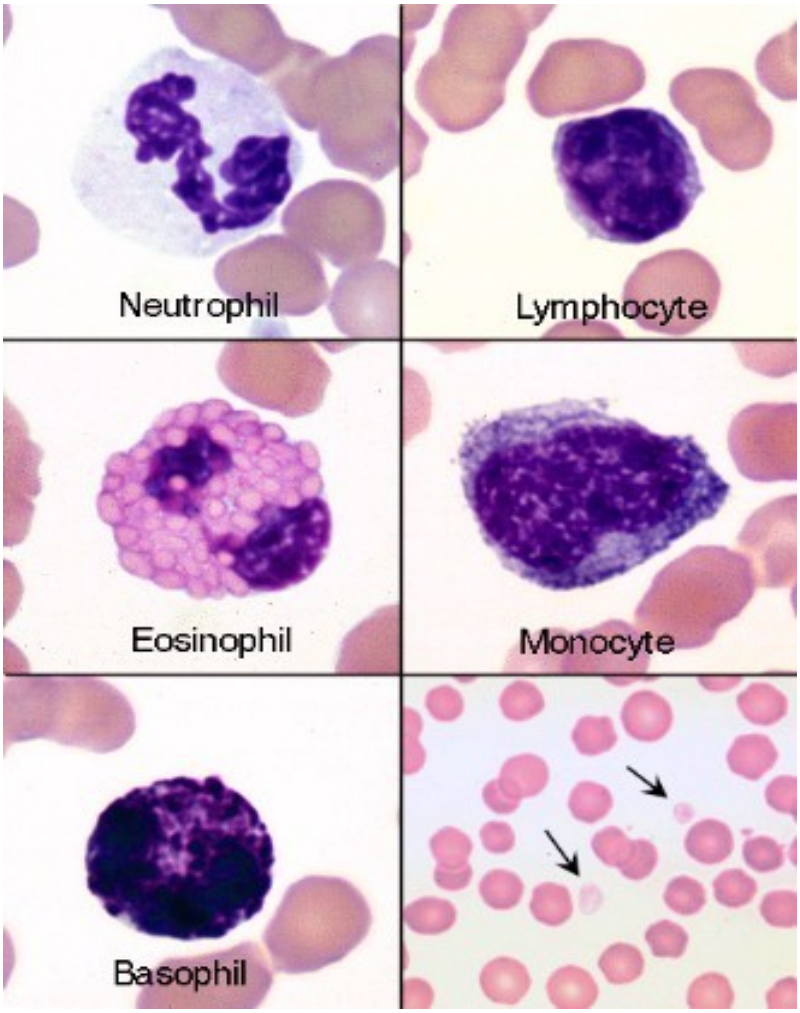
- **Equine erythrocytes average** 5.7 µm in diameter and lack central pallor. Rouleaux formation is common.
- Porcine erythrocytes average 6 µm in diameter and often exhibit poikilocytosis.
- **Ovine erythrocytes** are similar to those of the cow but smaller. The average diameter is 4.5 µm.
- **Caprine erythrocytes** are the smallest blood cells of the domestic animals. They are usually less than 4 µm in diameter. Anisocytosis and poikilocytosis are common.
- **Camelidae** (camel, llama, alpaca, etc.) erythrocytes are thin (approximately 1.1 µm) and ellipsoidal in shape with an average diameter of 6.5 µm, and usually **have high erythrocyte counts**.

- Avian erythrocytes are oval and nucleated with an average size 12 × 6 µm.



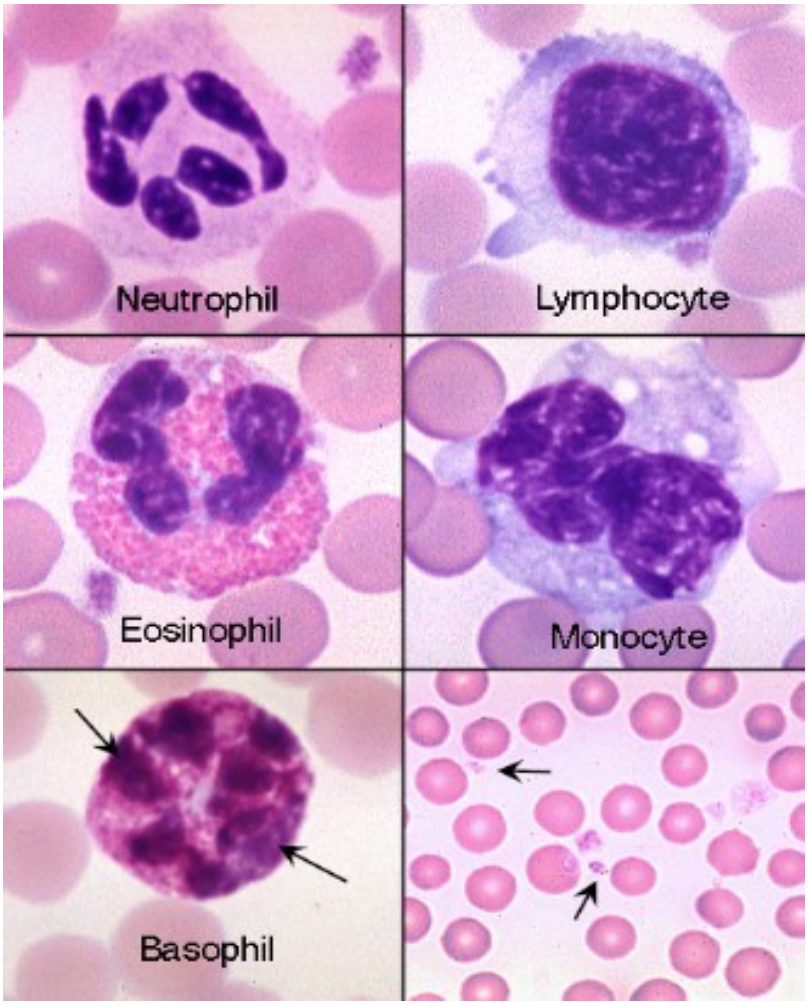
Horse red cells

- Horse red cells are similar to those of cats with regard to size and degree of anisocytosis.
- Compared to other species, horse red cells have a marked tendency to form **rouleaux.**
- Polychromatophilic red cells are absent in blood of non-anemic horses, **and are rare even in blood of horses with regenerative anemia (***always remember when evaluating anemia in horse***).**
- Equine platelets are smooth discs with faint granules. Platelet size is small and uniform.



Cow red cells

- Cow red cells are similar to cats and horses with regard to size and degree of anisocytosis but ***they are resistant to rouleaux formation***
- The latter characteristic is the reason that cow blood is very slow to settle out on standing.
- ***Cow red cells have a small area of central pallor.***
- Platelets in ruminant blood are small, moderately variable in size, and distinctly granulated.
- Lymphocytes outnumber neutrophils in healthy cows, and are more variable in appearance compared to those of other species



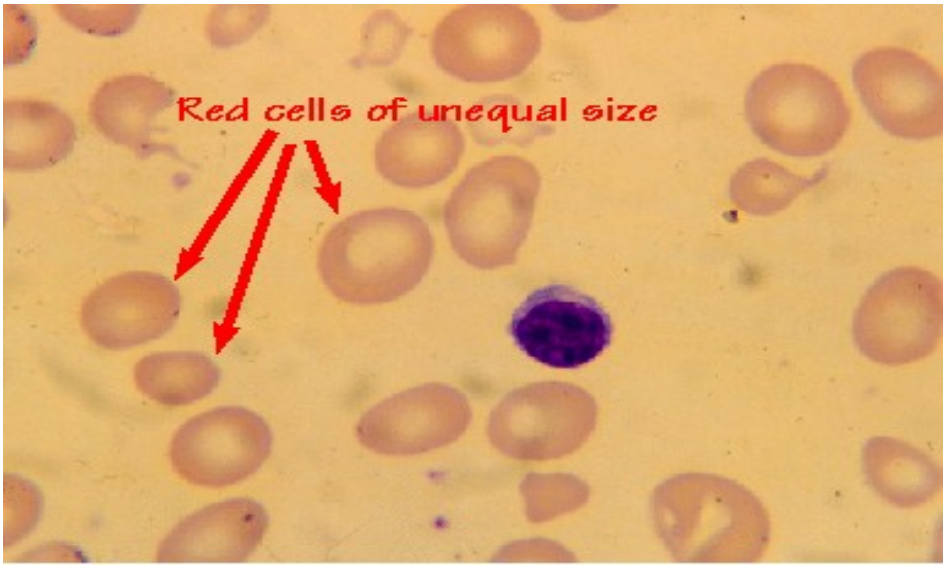
RBC abnormal morphologies & disorders/diseases associated with

Variation in Size *of RBC*: Know as *Anisocytosis*

- can be- Macrocytic (abnormally **larg**e RBCs) or Microcytic **(small** RBCs)
- *Anisocytosis* is variation in cell size. Although mild anisocytosis can be observed in erythrocytes of some healthy animals (dogs and cats), more **pronounced anisocytosis** is associated with a variety of conditions.
- These include:
  - **regenerative anemia's/ some hemolytic anemia's**
  - **Heinz-body anemia, and breed-associated variations in RBC size**
- In Regenerative anemia ***Immature RBCs are*** visible as **larger** erythrocytes that have blue-**grey** cytoplasm.
- These cells are termed ***macrocytic polychromatophilic erythrocytes .***

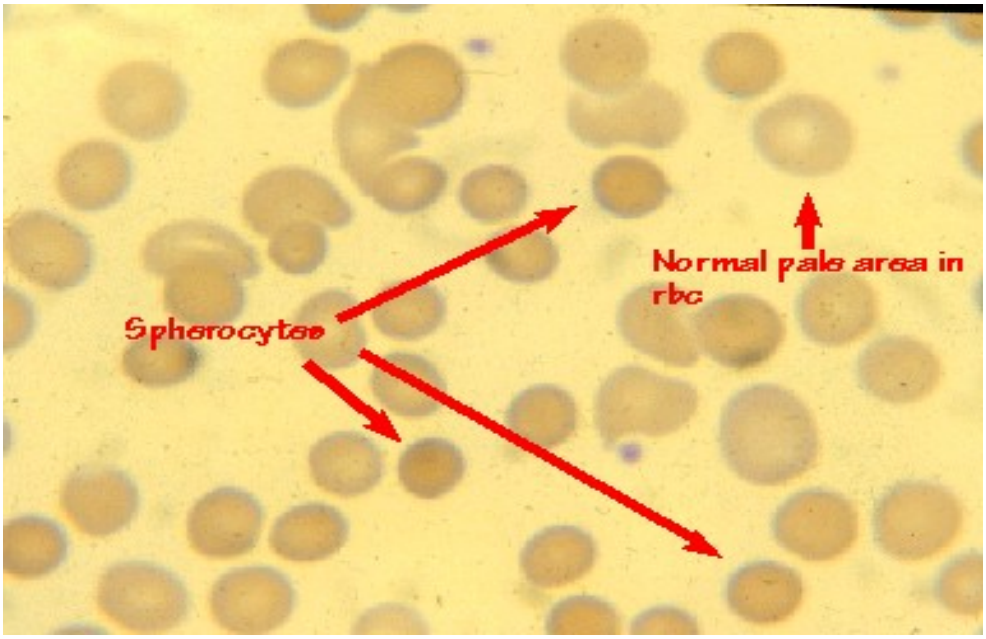
Microcytic RBCs

- In Iron deficiency anemia RBCs are visible as **small** erythrocytes that have **pale cytoplasm *(Hypochromia*)**
- ***Hypochromic RBCs*** have a large area of **central pallor**(exception dog) due to reduced hemoglobin content.
-
- Hypochromia and poikilocytosis are evident.



Spherocytes & interpretations

- Spherocytes are small, spherical erythrocytes that ***lack central pallor***.
  - Small RBCs lacking central pallor but stained darkly
  - *Seen in dogs and suggest **immune mediated hemolytic anemia** (IMHA)
  - Also seen in transfused blood, **blood parasites (Anaplasma) and Zn toxicity**.
- Spherocytes are formed when macrophages within the liver and spleen remove a portion of a damaged erythrocyte's cell membrane.
- ***This generally occurs after the erythrocyte's surface has been coated with either antibody or complement.***
- Because a portion of the membrane is removed, the resulting RBC has reduced cell membrane surface area relative to cytoplasmic volume.
- Therefore, spherocytes appear as smaller, dense cells that lack central pallor.



RBCs SHAPE abnormalities, their interpretation & associated diseases

- **Poikilocytes -** RBCs having abnormal shape
- *Poikilocytes* are abnormally shaped erythrocytes. *Poikilocyte* is a general term used to describe any specific change in shape in an erythrocyte.

Leptocytes, and conditions they are associated with?

- *Leptocytes are thin flat hypochromic appearing erythrocytes*
- *Leptocytes are thin erythrocytes with an increased amount of cell membrane relative to the* total cell volume (↑membrane/volume ratio)
- They are thin; thus they are **folded over**.
- **Seen in severe iron deficiency anemia/liver disease** (portosystemic shunt)

Target cells (codocytes), and their significance

- A *target cell is a subclass of leptocyte with a distribution of hemoglobin to the center(* **bull eye***) and* periphery of the erythrocyte, forming a target-like structure.

- Animals with liver disease, iron-lacking anemia, or immune-mediated hemolytic anemia may have increased numbers of circulating target cells

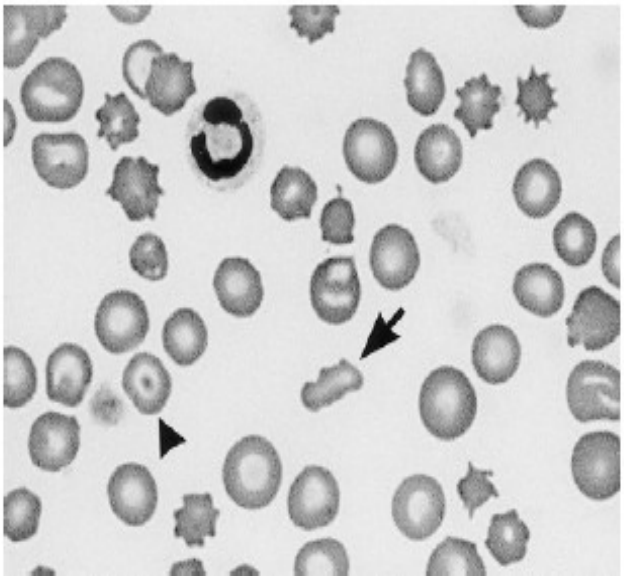
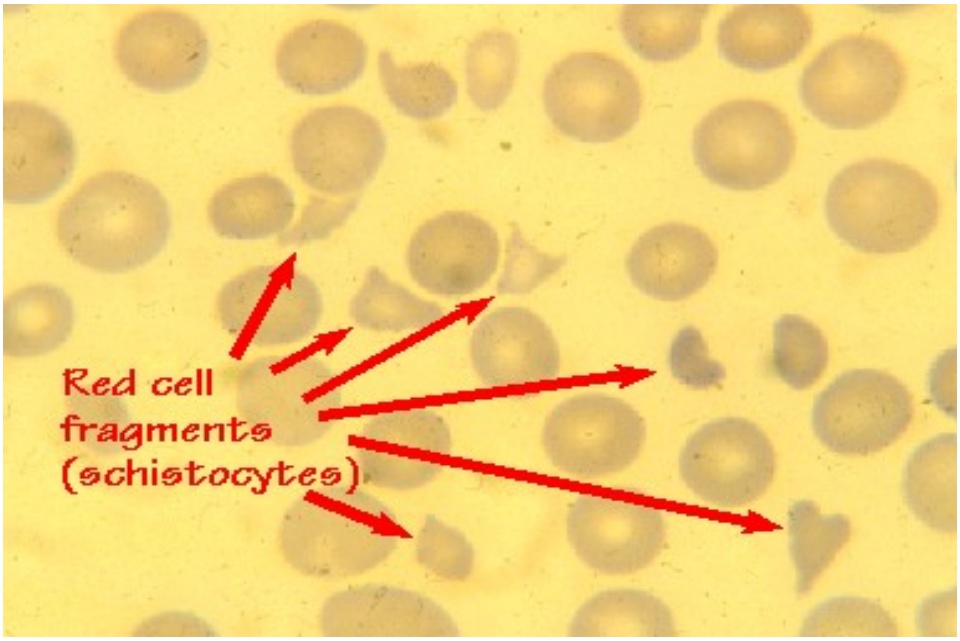


Figure 2-2 Peripheral blood smear from a dog diagnosed with splenic hemangiosarcoma. A schistocyte is identified by the arrow. The arrowhead points to a target cell.



The morphologic appearance of schistocytes

- *Schistocytes are fragments of damaged erythrocytes that appear as crescents, triangles, or* other irregular shapes.
- **How are schistocytes formed?**
- Schistocytes are formed secondary to mechanical trauma in the vasculature. Physical trauma to the erythrocyte membrane as it passes through fibrin deposits within vessel

walls, as seen with disseminated intravascular coagulation (DIC), can result in schistocyte formation.

Diseases associated with schistocytes

- Schistocytes have been associated with a number of fibrin-forming conditions, including
  - Hemangiosarcoma
  - vasculitis
  - congestive heart failure
  - DIC, myelofibrosis, microangiopathic anemia, and glomerulonephritis.

Stomatocytes, and their significance

- *Stomatocytes are a type of leptocyte that is **bowl** shaped when three dimensional.*
- *When* folded, however, stomatocytes appear to have a central crescentic clearing that is a slit or is mouth shaped.
- Stomatocytes may be seen in liver disease, chronic anemias, and hereditary stomatocytosis.

Acanthocytes, and diseases associated with them

- *Acanthocytes are erythrocytes with several unevenly spaced, irregular projections or spicules* that form secondary to an altered membrane lipid and cholesterol content
- Acanthocytes are associated with diseases that alter lipid and cholesterol
  - liver disease
  - endocrine disease
  - malabsorptive diseases, and hemangiosarcoma, especially involving the liver.

Variation in color among RBCs & its significances

- Hypochromasis/polychromasia

- **Hypochromasia, hypocromatic (**Decreased staining intensity of RBCs caused by decreased [Hgb]
- **Its significance**
- *Hypochromia* is ***reduced cytoplasmic staining with increased central pallor in the erythrocyte***. This is a result of **decreased amounts of hemoglobin within the RBC**.
- ***Any factor that interferes with the production of hemoglobin can result in hypochromia, including***:
- iron deficiency

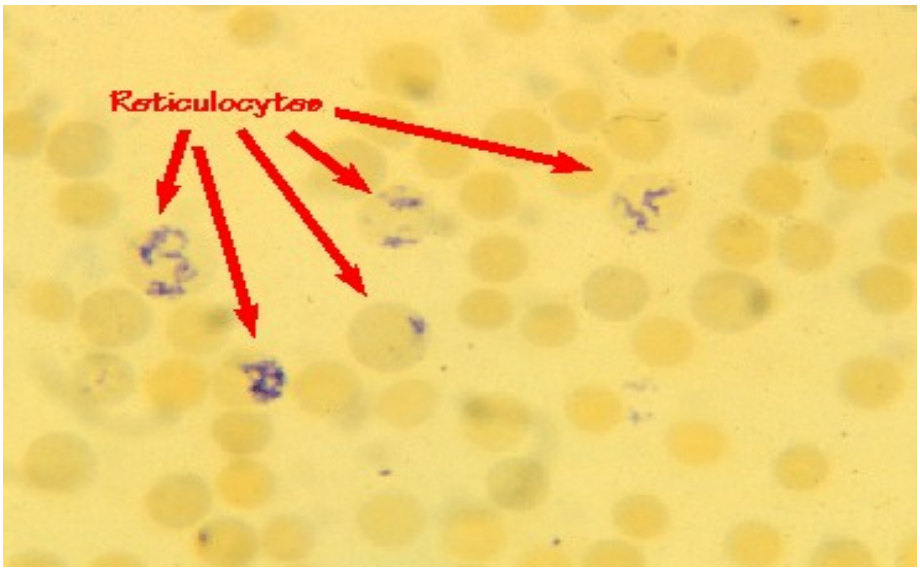
- Lead toxicosis. However, iron deficiency is the most common cause of hypochromia

Polychromasia in RBC and its significance

- *Polychromasia* is the variation in cell staining (change to bluish gray) observed in erythrocytes stained with Romanowsky-type stains due to the presence of residual RNA.
- ***Polychromasia is used as an index of regeneration in anemia***

Reticulocytes

- Reticulocytes are immature, anucleate(non nucleated), circulating erythrocytes that contain ***residual cytoplasmic ribonucleic acid (RNA),*** mitochondria, and organelles (also known as *reticulum*) that are visible **when stained with new methylene blue**.
- Reticulocytes in the peripheral blood constitute a reliable index of **new red cell production**
- *Reticulocytes appear bluish on a Romanowsky stain (Wright's, Diff-Quik) and are referred to as polychromatophilic cell.*
- It takes approximately 2 to 3 days for reticulocytes to be produced and released from the bone marrow in response to anemia.
- It takes 5 to 7 days for reticulocytes to reach a maximum response.
- **The significance of reticulocytes in blood smears & SPPs variation**
- Presence of reticulocytes is used as an index of regeneration in anemia
- Reticulocyte enumeration as an index of regenerative anemia works well for dogs, cow & pigs
- But tends to be grossly inaccurate in the cat where large number of reticulocytes are produced with small amount of residual RNA known as *punctuate reticulocytes*
- Cats also release reticulocytes with large area of RNA known as *aggregate reticulocytes* & so enumeration of reticulocytes as index of regeneration is prone to error
- In the horse, which ***does not release reticulocytes*** into the peripheral blood, the dtermination of increased erythrocytosis is largely dependent on changes in RBC numbers & association of increased mean cell volume



Nucleated erythrocytes (nRBCs)

- Nucleated erythrocytes are RBC precursors that have not yet expelled their nuclei. Nucleated RBCs include rubriblasts , prorubricytes, rubricytes, and metarubricytes,. Usually, nRBCs refer to Metarubricytes
- **The significance of increased numbers of nRBCs in peripheral blood**
- Nucleated erythrocytes are normally present in **the bone marrow**.
- Increased numbers of nRBCs can be released from the bone marrow with both:
    - Strongly regenerative anemias
    - Increased **erythropoietin** secretion
    - This condition is known as *appropriate metarubricytosis*. Appropriate metarubricytosis is accompanied by reticulocytosis

INCLUSION BODIES IN ERYTHROCYTES AND THEIR INTERPRETATIONS

- **Howell-Jolly bodies**
- Howell-Jolly bodies are ***remnant portions of nuclear material that are found within the cytoplasm of erythrocytes*(** left behind when the nucleus was extruded from the erythroblast)
- These bodies are ***basophilic and spherical***.
- They are intensely staining, perfectly round, **ecentrically** (mostly) located bodies in the RBCs
- Howell-Jolly bodies are usually observed with:
- accelerated erythrocyte production

- Or after splenectomy (Seen in the peripheral blood when the spleen has been removed, or is not functioning properly /hyposplenism).

- ***Their numbers do increase to detectable levels in regenerative anemias*** (responding anemia)

Heinz bodies and their interpretation

- Heinz bodies are *precipitates of denatured hemoglobin* ***attached to the erythrocyte membrane***
- Round structures of denatured hemoglobin ***attached to cell membrane (protruded outside)***
- In circulation, RBCs are constantly confronted with oxidants. The key antioxidant in the RBC system is ***reduced glutathione***.
- Reduced glutathione may be exhausted by oxidant attack from macrophage or drugs. This allows denaturation of the globin chains which form intraerythrocytic precipitate called *Heinz bodies*.
- These bodies occur following *oxidation of hemoglobin*.
- Heinz bodies are found in low numbers in health in certain species (e.g., cat)
- or may be associated with **hemolytic anemias** & infections.

Rouleax formation and its significance

- *Rouleaux* are clusters of erythrocytes that line up (stack of coins).
- A mild degree of rouleaux formation can be observed in healthy dogs and cats. More pronounced rouleaux formation, however, has been associated with ***neoplastic and inflammatory disease***, usually caused by **increased plasma protein** that alters the erythrocyte surface charge.
  - Seen in increased **serum fibrinogen and γ globulin**
  - Conditions which do this include infections, inflammatory and connective tissue disorders, and cancers.

EVALUATION OF ERYTHROCYTES NUMBERS AND OXYGEN CARRYING CAPACITY

- **The complete blood count**
- The complete blood count (CBC) is a set of tests done on **blood** and plasma for cost effective screening of abnormalities. **The CBC should include the following**
- **Packed cell volume (PCV**) and total erythrocyte count

- **Total plasma protein (TPP**) concentration
- **Total leukocyte count (TLC**)
- **Hemoglobin concentration, erythrocytic indices** (**MCV**, **MCH** and **MCHC)**
- Blood smear examination: differential leukocyte count (DLC), erythrocytes morphology, reticulocyte count, and platelet count(hemostasis).

Total erythrocyte count

- Total RBC count could only reflect the total number of RBCs in the circulating blood and **does not indicate either the oxygen carrying capacity** or **amount of haemoglobin available**.
- ***Therefore, the count should be made inconjunction to:***
  - ***haemoglobin content***
  - ***PCV determination***
  - ***Erythrocyte indices.***

<u>Note:</u> Decrease in one or more of the peripheral erythrocyte parameter indicates the presence of anemia but not the type of anemia.

- Rather they are used to determine the erythrocytic (Wintrobe indices), which are in turn used to classify the type anemia(***complement by smear/morphology***).
- Direct visual counting is of limited value because of the large error involved.
- Electronic counters such as **Coulter counter**; **Sysmex Hematology analysers** provide more rapid and accurate results.
- The main use of RBC count is that it allows determination of MCV and MCH.
- For manual counting the red cell pipette gives a dilution of 1:200 when blood is drawn to the 0.5 marks and the diluent to the 101 mark.
- **The diluent for RBCs is Hayem's solution**. The diluted blood is then discharged to the hemocytometer chambers and counted.
- The inherent error is about plus or minus 20%.
- The number of RBCs decrease in **anemic conditions** and increase in **polycythemia**.

Determination of Packed cell volume (PCV)

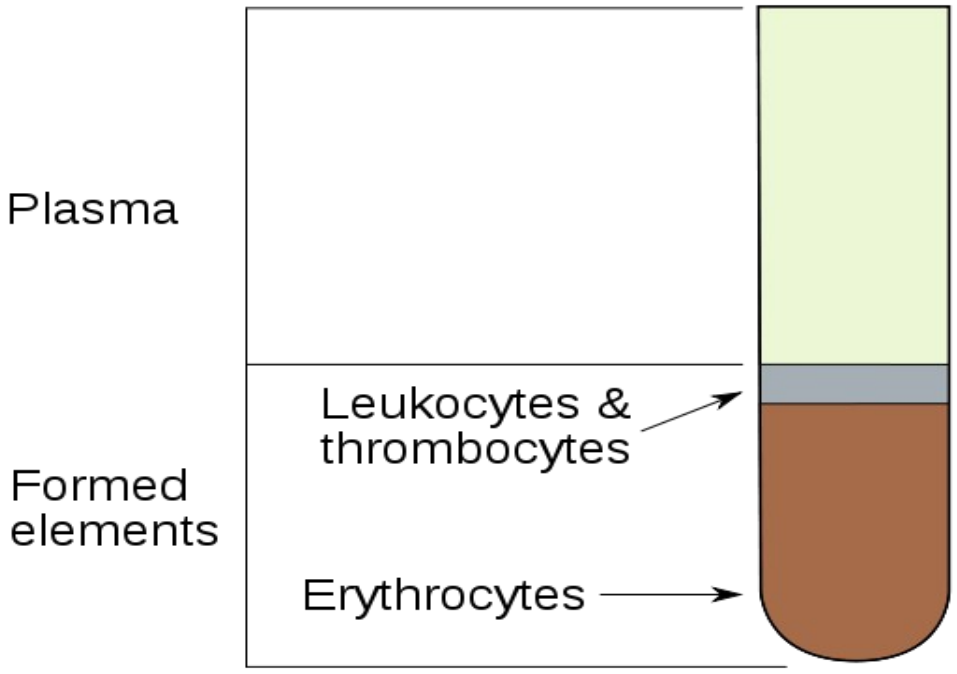
- **Hematocrit method:** literally, it meant to separate blood into components by centrifugation. After centrifugation blood is separated **into three** distinct parts as follow:
  - ✓ 1) The mass of erythrocytes at the bottom, which is referred to as **packed cell volume**

- ✓ 2) A white or gray layer of leukocytes and thrombocytes immediately above the red cell mass that is referred to **as buffy coat**
- ✓ 3) **The blood plasma**

- A **capillary hematocrit** tube approximately 7cm in length and having a bore of about 1mm is recommended.
- Capillary tubes containing anticoagulant can be purchase and used to collect blood directly and plain tubes are available for use on blood already containing anticoagulant.
- The tubes are filled by capillary action, the outside is carefully dried with a piece of gause, and the opposite end of the tube is sealed with special clay.
- These sealed tubes are placed in a special high-speed centrifuge by putting the sealed end near the outside rim of the centrifuge
- Then centrifuged for the length of time prescribed by the manufacturer (**5 min. at 10.000 rpm if no manufacturer information).**

Read the PCV through a special reader as follows:

- **Place the bottom of the RBCs column** (which is just above the sealed end) exactly on the base line of the reader (0).
- Move the reader until the top of the plasma layer exactly means 100.
- Observe a line on the reader, which passes across the top of the RBCs layer, read the P.C.V. from the scale at the point where this line meets it



- **Lower PCV usually emanates** from **anemia, suboptimal nutrition** and **hemodilution**
- **Higher PCV result from plycythemia vera** and **dehydration (hemoconcentration).**
- However, certain factors need to be considered before interpreting the results of PCV.
    - **altitude**, **season**, **emotional influence**, **accuracy of determination** and **physiological anemia** of young animals.

Buffy coat: corresponds to the height of the leukocytes and thrombocytes layer above the red cells.

- In a normal animal, the height of buffy coat is between **0.5 to 1.2mm.**
- Each 0.1mm of the first 1mm of the buffy coat is equal to 1, 000 leukocyte where as each 0.1mm beyond the first 1.0mm is equal to about 2, 000 leukocytes.
- Generally, a buffy coat of less than 0.5mm would suggest **leucopenia,** while buffy coat of over 1.5mm would suggest **leukocytosis.**

The plasma component: the volume of plasma normally exceeds 50% of whole blood.

- The **color of plasma** is used to estimate **icterus index.**
- Yellowish coloration indicates the presence of **bilirubin** and can result from:
    - 1) **hemolytic diseases**
    - 2) **hepathopathy**
    - 3) **bile – duct obstruction**. The degree of yellow pigmentation of plasma is referred to as icterus index.
- The icterus index is determined by matching by the plasma color in the tube with standards prepared from **potassium dichromate**.
- The icterus index is recorded in units from 0 to 100.
- One unit is defined as the color produced by one part of potassium dichromate in 10, 000 parts of distilled water.
- The normal icterus index score is
    - <5 units in dogs and cats and icterus index values over 7.5 units are definitely abnormal.
    - In cattle the normal range is between 5 and 15
    - in equines it is between 7.5 and 20.

Hemoglobin Determination

- It provides **the most direct indication of the oxygen carrying capacity of blood** and should be approximately **one – third** of the **PCV under normal condition.**
- It provides determination of MCV and MCHC.
- Several practical techniques have been developed to determine the concentration of Hb.
- **Acid hematin methods**
- **Direct methods**
- **oxyhemoglobin method and cyanmethehemoglobin method**

A. Acid – hematin method

- The principle of determination of Hb using this method is based on the conversion of **hemoglobin into acid hematin using diluited HCl.**
- The resulting **brownish - yellow color** depends on the **amount** of hemoglobin in the blood and is matched with standard color in a **colorimeter** or **Sahil's hemoglobinometer**.

Sources of error in Acid hematin methods:

- 1). **Non hemoglobin substances such as proteins and lipids normaly present in plasma** and **cell stroma may influence the color of the diluents**
- 2). It is difficult to match the sample accurately with the brown standard
- 3). Individual variation in matching the color…etc.

B. Direct matching methods

- Two principal methods have been widely used (the **Tallquvis**t hemoglobin scale and the **Dare** hemoglobinometer).
- **Tallquvist method** -a drop of blood is placed on a piece of white absorbent paper, and the paper with its drop of blood in inserted into the central perforation of a serial color chart.
- The intensity of red color is matched with the most suitable color on the paper scale, and hemoglobin read as the value that this scale represents.
- This is **rapid, simple and inexpensive method that is used as screening test** in the field where accuracy is not highly needed.
- **Dare hemoglobinometer method** - utilizes a drop of blood in a capillary chamber between small glass plates and matched with a permanent red glass standard.

C-Cyanomethemoglobin method

- It is the most frequently used and accurate method of determination of hemoglobin concentration.
- To perform the test about 5ml of a diluent (**cyanomethemoglobin reagent)** is added to 0.02 ml of blood in a cuvette.
- The mixture is allowed to stand for about 10 minutes after inverting several times.
- The cuvette is placed into ***spectrophotometer for reading***.
- The percent transmission or optical density at 540µm is recorded and compared with readings obtained from standard cyanomethemoglobin.
- This in effect will convert the value to **grams per deciliter** of blood.
- The accuracy is approximately plus or minus 5%.
- **The concentration of hemoglobin decreases in anemic conditions** and **nutritional deficiencies** (such as ***iron*** deficiency, ***copper*** deficiency, ***vitamin*** deficiency, ***protein*** deficiency, etc).

The major red blood cell indices & their uses to characterize anemia

- They are calculated values that help us to **figure out the relative size and hemoglobin concentration of RBCs.**
- They including **mean corpuscular volume (MCV**), ***mean corpuscular hemoglobin (MCH)***, mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).
- Note:- **both reticulocyte count and RBC morphology are extremely important** in determining if an anemia is ***regenerative or nonregenerative.***

MCV = mean corpuscular volume

- It represents the **average volume** of a single RBC in femtoliter (10^{-15} L).
- The MCV is determined most accurately by **direct measurements** using **electronic cell counters**.
- It can also be determined indirectly by dividing the HCT by total erythrocyte count and multiplying it with 10.

  i.e. **MCV = $\frac{\text{HCT (PCV)}}{\text{RBC count}}$ X 10**

- MCV varies greatly depending on **species of animals**.

- **Mammals have smaller RBCs than do birds, reptiles or amphibians.**
- Generally species with larger RBCs have **lower RBC counts**, resulting in similar HCT but higher MCV.

High MCV & associated conditions

- High MCV are usually associated with **regenerative anemias**. Such anemia results from hemolytic diseases including **babesiosis, bacillary hemoglobinuria, trypanosomosis, etc.**
- It could also occur in myeloproliferative disorders such as in case of feline leukemia.
- **Macrocytosis** which accounts for **higher values of MCV** is more likely to occur in response **to hemolytic anemia** than in ***response to hemorrhage.***
- This is because reticulocytes produce and released in response to severe anemia (**stress reticulocytes**) are much larger than mature RBCs.
- Four or five days are needed to elapse for peak reticulocye response to occur, and then the newly produced larger cells comprise a high percentage of total RBCs in the circulation and are responsible for increased MCV.
- Note that fetal and neonatal RBCs are larger than that of adults and so is the MCV and it declines during maturation.

Lower MCV & related conditions

- **Lower MCV occurs under conditions of iron deficiency.**
- Such ***microcytic iron deficiency*** **anemia** results from chronic hemorrhages.
- Depending on the initial MCV and the magnitude of ongoing blood loss, one or more months is required before the MCV decreases below reference ranges.
- **Young animals tend to have smaller erythrocytes and a corresponding low MCV (don't confose with neonatals).**
- *Microcytosis,* or decreased MCV, can be seen in animals with **portosystemic shunts.**
- Iron deficiency causes decreased MCV.
- The body iron reserve must be depleted and microcytes must comprise higher percentage of circulating RBCs for detectable decrease in MCV to occur.
- **Copper deficiency** can cause similar conditions in some species of animals.

Mean Cell Hemoglobin Concentration (MCHC)

- It represents the **average concentration of hemoglobin within erythrocytes.**

- It can be calculated by dividing the hemoglobin concentration (in grams per deciliter) of blood by the HCT (as percentage) and multiplying by 100.
- That is,

$$MCHC = \frac{\text{Hb Con.}}{\text{HCT}} \times 100$$

- HCHC is expressed **as gram per deciliter** of erythrocytes.
- **It is the most accurate of the erythrocyte indices because it does not require erythrocyte count.**
- It reflects the oxygen carrying capacity of erythrocytes.

Low MCHC & associated conditions

- MCHC decreases in ***regenerative anemias***, especially in those with high percentage of stress recticulocytes.
- ***Low MCHC is also common in chronic iron deficiency*** (eg. In chronic hemorrhages such as occurs in parasitemia).
- Such anemias are termed **hypochromic anemia**.

Higher values of MCHC & associated conditions

Some causes of hyperchromasia (increased MCHC)

- **a**. **Absolute increases in MCHC**, or actual increased hemoglobin within erythrocytes, are not usually observed and often are artifactual.
- **b**. **Intravascular or invitro hemolysis** is the most common artifact that causes increased MCHC.
- **c. Hyperlipidemia or Heinz bodies** may cause falsely increased hemoglobin concentration due to *interferen*

Mean Cell Hemoglobin (MCH)

- It is calculated by the hemoglobin value (in grams per deciliter) of blood by the erythrocyte count (in millions per microliter) and multiplying by 10.
- The MCH provides no added value, because it depends on the MCV and MCHC.
- **It usually correlates directly with the MCV except in animals with macrocytic hypochromic erythrocytes**.

$$MCH = \frac{\text{Hb Con.}}{\text{MCV}} \times 10$$

Depending on the above indices anemia can be classified as:

- Macrocytic hypochromic
- Macrocytic normochromic
- Microcyticn
- Microcytic hypochromic
- Normocytic normochromic

Various Causes of the Different Types Anemia

Normocytic normochromic

- acute hemolysis before reticulocyte rewsponse
- acute hemorrhage before iron depletion
- chronic inflammation and neoplasia
- early iron deficiency
- chronic renal diseases
- aplastic and hypoplastic bone marrow
- Vitamin B_{12} deficiency

Macrocytic hypochromic

- regenerative anemia (heloysis)

Macrocytic normochromic

- Regenerative anemia
- FeLV infections
- Folate deficiency
- Congenital dyserythropoiesis
- Erythroleukemia

Microcytic normochromic/hypochromic

- chronic iron deficiency
- anemia of chronic diseases
- copper deficiency
- pyridoxine deficiency

Erythrocyte Sedimentation Rate

- Erythrocyte sedimentation rate (ESR) involves measuring **how fast red *cells will settle out on standing***, a measurement which depends to some extent on plasma viscosity, which alters **when *inflammatory proteins are present*.**
- It is an old-fashioned test, but still favored by some general practitioners as a general indicator as to whether a patient is actually ill or not.
- *However, results are extremely species specific, and the test has no place in veterinary medicine*.
- In particular, the tendency of equine erythrocytes to form rouleaux means that the cells sediment extremely quickly, and the result is almost entirely a factor of the PCV.
- **Feline cells often behave in a very similar manner**.
- The erythrocytes of ruminants show little tendency to form rouleau in drawn blood (no sedimentation in healthy ruminants)
- Thus, rouleax formation and significant ESR is a good indicator of grave disease conditions in ruminants specially cattle.
- A declining rate suggests that there is healing, an increasing rate suggests that the disease is uncontrolled and a continuing abnormal rate indicates that recovery has not yet taken place.
- **Elevated ESR is anticipated in:**

  - All acute generalized infections

  -localized acute inflammatory diseases of serous membranes

  - Chronic localized infections/- traumatic injury

  - Inflammation of skin/- malignant neoplasia

  - Chronic interstitial nephritis (dogs), etc

Erythrocyte fragility test

- ***Indication-to detect the resistance of erythrocytes to haemolysis***
- the resistance of erythrocytes is known as decrease or increase in disease situations.
- It is well known that the resistance of erythrocytes to haemolysis may be increased or decreased in disease.
- The use of such an evaluation should be considered in prolonged deficiency and dietary studies when the haematopoietic system might be adversely affected

- The erythrocyte fragility is tested by exposing freshly drown erythrocytes to **hypotonic saline solution of various strength**.
- The resultant swelling and haemolysis in a lower dilution are an index of cell stability in relation to hypotonic solution.
- ***In general dogs and chickens have the greatest resistance among domestic animals, whereas goats have the least.***

ANEMIA

- **Define anemia and explain the difference between relative anemia and absolute anemia.**
- Definition - decrease in the hematocrit (PCV), erythrocyte count, and hemoglobin concentration.

True or absolute anemia -**decrease in erythrocyte mass** in the body (PCV, Hb and RBC counts are below their reference values).

Relative anemia

- ❖ Low erythrocyte values may also be present in blood when the **total body erythrocyte mass is normal** (relative anemia).
- ❖ This can result from **overhydration, resulting in erythrocyte dilution**
- ❖ **from splenic sequestration of erythrocytes**, as occurs with ***splenic relaxation during anesthesia***
- ❖ **Heparin induced erythrocyte agglutination** in horses and varies causes of **splenomegally.**

- *Relative anemia* is not associated with a true reduction in the total red blood cell (RBC) mass, but rather is caused by a dilutional effect that can be observed after ***aggressive fluid therapy or other cause of increased plasma volume.***
- Anemia is basically the result of either an **excessive loss**, or a **deficient production** of red cells.
- ❖ **An excessive loss of blood** from the peripheral blood occurs in either of **the two ways**:
  - A loss from the vascular system by hemorrhage- **hemorrhagic anemia**
  - A loss due to decreased red cell life span (red cell **lysis**)- **hemolytic A**
- ❖ **Inadequate production** of red cells by marrow also falls into two categories

- **Reduced proliferation of red cell precursors**
- **Defective synthesis of hemoglobin**

Classification of anemia:

- Anemia may be classified using different criteria:
- It can be classified using
  - **Erythrocyte indices** (morphological classification as Macrocytic/microcytic/hyperchromic/hypochromic)
  - **Etiological classification**
  - **Response of bone marrow** (**regenerative** *versus* **generative anemia**).
  - Also, both ***reticulocyte count and RBC morphology are extremely important in determining if an anemia is regenerative or non regenerative*.**

Some common causes of non regenerative anemia.

- **A**. **Blood loss of less** than 3 days (a regenerative anemia that has not yet had time to show a regenerative response)
- **B**. **Erythropoietic suppression**
  - (**1) Anemia of chronic disease (anemia of inflammatory disease)**
  - **(2) Renal failure**
  - (3**) Metabolic disease**
  - (**4) Drugs and toxins**
- **C. Infections (e.g., parvoviruses)**
- D. **Bone marrow disease:**
  - (1) **Myelofibrosis**
  - (2) **Pure red cell aplasia**
  - (3) **Marrow necrosis**
  - (4**) Neoplasia**
  - **E. Radiation**
- **Hemorrhagic anemia & diagnostic approach**
  - If external easy
  - If the blood confined **within the body**, (**internal**) laboratory and other diagnostic tests should be done.

- The **gastrointestinal and urogenital tracts** are common sites of occult hemorrhage.
- Tests that may assist in the diagnosis gastrointestinal hemorrhage include:

- **The occult blood test in the feces** (a test for the presence of microscopic amounts of blood in the feces secondary to bleeding in the digestive tract)
- **Faecal examination for parasite/ova and *diagnostic imaging* to identify tumors or ulcers**
- ***Urinalysis* and *diagnostic imaging* of the urinary tract may assist in the diagnosis of *renal or bladder hemorrhage***

❖ **Note:-After acute hemorrhage, hematocrit initially is within the normal reference interval (cellular components and plasma are lost in equal).**

- The body begins to replace plasma volume a few hours after blood loss, with total replacement of plasma volume in 2 to 3 days.
- Hematocrit begins to drop as plasma volume is restored as a result of hemodilution.

➢ **Polychromasia is an indicator of increased bone marrow production of erythrocytes and is observed within 2 to 3 days after acute blood loss.**

- The most reliable laboratory indicators of regenerative anemia are:-
  - A. **Reticulocytosis**
  - **B. Polychromasia**
  - **C. Anisocytosis/increased RDW**
  - **D. Macrocytosis**

➢ ***An* absolute increase in reticulocyte numbers is the best indicator of a regenerative bone marrow response to anemia.**

Hemolytic anemia

- Extravascular hemolysis and (breakdown by macrophages within the spleen and liver).
- Intravascular hemolysis (rupture within the vascular system).
  - a. Oxidative injury
  - b. Membrane alteration
  - c. Physical damage
  - d. Immune-mediated lysis

- e. Parasitism (e.g., babesiosis)

Dx- blood smear for hemoparasites

Immunological teste for antibodies/ complements…

None regenerative anemia & Lab indications

- **Reduced proliferation of red cell precursors (**intoxications, viral infections, irradiation or immune reactions)
- **Lack evidence of bone marrow response to anemia** (eg *the absolute reticulocyte count in blood is not increased or is minimally increased for the degree of anemia*).
- **Disappearance of cells from the marrow** (destruction of stem cells or alternately, the cellular production line may be blocked at some point and numerous precursors may be present).
- ***Bone marrow biopsies are often required to delineate the nature of none regenerative anemia.***
- Total marrow aplasia or hypoplasia will give rise to pancytopenia, (deficit in all circulating cells).
- The marrow suppression may be reversible or irreversible. Feline

Iron deficiency anemia & lab indicators

- **Impaired absorption/Excessive loss/Decrease iron intake (diet)**

- Iron is essential component of hemoglobin, myoglobin, the cytochrome enzymes of mitochondria and is required as ***a cofactor for function of other enzymes.***
- Thus, while anemia is the principal sign of iron deficiency, there are diverse effects, which include
  - **Neutropenia**, decreased capacity for killing ingested bacteria
  - **impaired T-cell response** …etc.
- **Hematologically**, the anemia is
  - classically **hypochromic & microcytic** in all species
  - accompanied by **neutropenia with hyper segmentation** & **marked thrombocytosis of small platelets**.
  - RBCs are not only small & poorly saturated but also distorted in shape.

- Serum iron is generally low (9µmol/L –dog) & the total iron binding capacity is decreased (**must be measured & indicative if correlate with hemotological values**)

Polycythemia (erythrocytosis): lab. diagnosis and management

- An increase in the RBC count, packed cell volume (PCV), and hemoglobin concentration.
- **Classifications**
  - Relative
  - Absolute.
- Absolute erythrocytosis can be classified as:
  - Primary
  - Secondary
- The secondary causes further defined as:
  - Physiologically appropriate
  - Inappropriate (Descriptive classification of erythrocytosis)
- Clinical signs of erythrocytosis include:
  - Plethora (eg. Skin and mucus membrane erythema)/ brick red or cyanotic mucous membranes
  - polyuria/polydipsia
  - Episodic bleeding diatheses (eg. Epistaxis,hematuria, melena, hematochezia, hematemesis)
  - CNS disturbances such as mentation changes (eg. Lethargy, dementia), posterior weakness, ataxia, blindness and seizures.
  - low exercise tolerance, increased size and tortuosity of retinal and

- Signs are the result of hyperviscosity caused by increased RBC mass. Blood flow slow, resulting in distention of small vessels and capillaries and increasing the risk of hypoxia, thrombosis and vessel rupture.
- Polyuria and poludipsia have been proposed to be linked to an erythrocytosis-induced disturbance of urine concentrating ability of the kidney, and impaired vasopressin release has been documented.

Relative erythrocytosis

- Associated with an ***elevated venous PCV but normal RBC mass***. Fluid balance derangement (deceased plasma volume/ ***decreased intake, severe dehydration, cutaneous losses***), or vascular redistribution such as hemorrhagic gastroenteritis.
- Severe pain - splenic contracture - injection of ***concentrated splenic*** RBCs).
- Moderate increase in PCV, total protein concentration in plasma and prerenal azotemia may also be present are laboratory findings
- **Relative erythrocytosis is most often recognized at the physical and clinical evaluation**.
- Resolution of relative erythrocytosis occurs ***after volume repletion or removal of the inciting cause***.

Absolute erythrocytosis (primary Vs secondary)

- **Primary erythrocytosis - *myeloproliferative disorder*** - neoplastic erythroid stem cells that require little or no EPO for clonal expansion, growth and differentiation.
  - In dogs and cats, primary erythrocytosis is considered a rare neoplastic disease of young to middle-aged
- **Laboratory features:**
  - Marked increase in PCV with erythroid hyperplasia in marrow.
  - *Clinical evidence of hypoxemia is not apparent*.
  - *Erythropoietin levels are normal or reduced*
- **Secondary erythrocytosis** is caused by:
  - Altered regulatory activity
  - Excessive production of EPO
- If systemic hypoxia is present, the increased EPO production results in an increased RBC mass capable of increased oxygen-carrying capacity, which represents **appropriate**, compensatory response.
- Decreased 2,3 diphosphoglycerate (2,3 DPG) levels in RBCs
- High altitude environments
- Massive obesity

Appropriate secondary erythrocytosis most often results from:

- Cardiovascular disease
- Chronic pulmonary disease

- Hemoglobinopathy, such as methemoglobin reductase deficiency (rare in veterinary patients)
- Neurologic disease causing depressed respiratory center function and decrseased alveolar ventilation.

- **Laboratory features:** Moderate to marked increase in PCV and marrow erythroid hyperplasia with ***decreased arterial PO_2 content***. Erythropoietin levels are increased.
- Inappropriate secondary erythrocytosis is characterized by:
  - Increased EPO level *without evidence of a hypoxic stimulus*
  - Neoplasms of the kidney, lung, liver, uterus, ovaries, adrenal glands, thymus, and CSN
  - Renal cysts, hydronephrosis, renal transplantation have also been reported as causes in humans.
- **Laboratory features:** Moderate or marked increase in PCV with marrow erythroid hyperplasia and ***a normal arterial PO_2 content***. Erythropoietin levels plasma are increased

Executive notes

- **Reticulocyte counts are not used to evaluate the bone marrow response in equine anemia.**
- Because horses do not release reticulocytes from the bone marrow into circulation in significant numbers, reticulocyte counts cannot be used as an index of regeneration in this species.
- *Methods to evaluate bone marrow responses in horses include* ***repeated hematocrits****,* ***bone marrow evaluation****, and* ***red cell distribution width (RDW)*****.**
- In health, cattle do not release reticulocytes from the bone marrow in significant numbers; normal erythrocyte maturation occurs in the bone marrow.
- Low numbers of circulating reticulocytes (approximately 1% or greater) are associated with a regenerative response to anemia.
- During strongly regenerative anemias, cattle can have basophilic stippling in erythrocytes. These cases usually have associated reticulocytosis.

- Sheep erythrocytes (3.2-6.0 μ) and goat erythrocytes (2.5-3.9 μ) are smaller than cattle erythrocytes (4-8 μ). This can cause difficulties in obtaining accurate erythrocyte counts with cell counters. The thresholds must be adjusted for the smaller erythrocyte size.

CHAPTER 3: LEUKOCYTES

MORPHOLOGY, KINETICS, FUNCTION, EVALUATION OF THE DISORDERS & SIGNIFICANCES

- ***Leukon*:** All leukocytes in animal, includind **leukocyte precursors**, **lymphocytes in blood and lymph vessels**, and **tissue leukocytes**
- **Leukopoiesis** is a part of hematopoiesis, which is complex system involving stem cells that are capable of self-renewal or differentiation towards committed cells.
    A. **bone marrow** contains ***precursors*** for the **neutrophils, eosinophils, basophils**, **monocytes, lymphocytes** and **mast cell** (tissue leukocytes)
    B. **lymph nodes, spleen and thymus** contains precursors for **B &T lymphocytes and null lymphocytes**
    C. **leukocytes in blood** are in transit from site of production to sites of function or
    D. **tissue leukocytes**
- ***Most of the leukocytes (neutrophils, eosinophils, monocytes, basophils) provide non – specific immunity.***
- **Their action is innate** – it is **inherited** by the animals and is effective against a broad range of pathogens.
- Part of the innate response is inflammation, a wide ranging physiologic response that “marshals” the body to resist the effects of infection and trauma.
- **Neutrophils and lymphocytes** are the most numerous WBCs in the blood of healthy domestic animals
- In **pets and equines** about **70% or more** of the WBCS are **neutrophils** while about **65% - 75%** of the WBCs in **ruminants** are **lymphocytes.**
- **But they are nearly equal in swines**
- **Evaluation of the leukogram** is a part CBC and includes both **quantitative techniques** and **qualitative morphological or functional assays.**

- A specific disease is rarely diagnosed through leukocyte evaluation but the informatioin gathered is useful for:
  - ✓ ***Narrowening of the differential diagnosis***
  - ✓ **Predicting the severity of the disease** (**toxic neutrophils** » **severe conditions** with **guarded prognosis**; **degenerative left shift** is a marker of **severe conditions with poor prognosis)**
- **Monitoring response to treatment**
- **Formulating prognosis** (generally ***marked leukocytosis*** with **mature neutrophilia** is indicator of ***good prognosis)***

Disorders of leukocytes & associated conditions

Leukocytosis

- An increase in total leukocyte number is called leukocytosis. It may result from a number of conditions:

A. *Physiological Leukocytosis*

- **Frightened animals** » **epinephrine** which will act on the **smooth muscles of**
  - 1) **pulmonary and splenic capillaries** to **cause mature neutrophilia**
  - 2) lymphatics to end in lymphocytosis.

B. Stress Response

- Under ***stress*** animals will release high concentration of **corticosteroids**. The corticosteroids will cause:
  1. **decreased adherence of neutrophils to the endothelium of capillaries**,
  2. ***increased movement of neutrophils from marginal neutrophil pools to the central neutrophil pool,***
  3. ***increased release of mature neutrophils from bone marrow to the peripheral circulation and***
  4. **lysis of lymphocytes**.
- These all conditions will culminate in neutrophilia and lymphopenia. Monocytosis and eosinophilia are also possible findings.

C. Leukocytosis Due To Acute Generalized Infections

- ***Leukocytosis marked by* neutrophilia*, monocytosis and changes in other WBCs is the usual finding in acute generalized infections***

D. Left shift

- This is the appearance in the peripheral blood of **more immature components** of the maturation pool
- *Left shift* indicates the presence of neutrophil precursors in blood, typically increased numbers of ***band neutrophils*** and rarely ***metamyelocytes*** and earlier precursors.
- When there is a great demand for neutrophils in the tissue younger stages (metamyecytes, myelocytes and rarely progranulocytes) may be present in the left shift.
- **Causes: - infections agents (pyogenic bacterial or fungi)**
    - Noninfectious causes of inflammation, such as **tissue necrosis** and **immune-mediated disease and neoplasia**
    - **Glucocorticoids and endotoxin may caused mild left shift**

Regenerative Vs degenerative left shift

- **Regenerative left shift** -In typical Regenerative left shift, the proportion of **various immature neutrophils** is **orderly** (pyramidal distribution)
    - with the most immature cells being the least numerous.
    - Usually the mature neutrophils outnumber the immature cells.
    - It could also be **slight left shift** (with neutrophilia that contains ≤ 2% bands); **moderate left shift** (neutrophilia with some bands and metamyelocytes) and **marked left shift** (neutrophilia with band cells, metamyelocytes and myelocytes).
- Degenerative - young neutrophilic granulocytes exceeding mature ones.
    - **Clinical significance**: - degenerative left shift is of greater concern than a regenerative » marrow granulopoiesis is unable to maintain an appropriate segmented neutrophil (granulopoietic suppression)

Right shift

- **increased numbers of hypersegmented neutrophils**, with five or more nuclear lobes.
- Hypersegmentation of neutrophils usually occurs as an aging change, when neutrophils circulate in blood for a longer time.
    - Glucocorticoid therapy or hyperadrenocorticism(inhibit the emigration).
    - vitamin B12 maladsorptive disorder, myelodysplasia , idiopathic neutrophil hypersegmentation in horses

Toxic neutrophils

- Toxic neutrophils are characterized by:
  - **Dohle body (gray mass in the cytoplasm)**
  - **Cytoplasmic basophilia**
  - **Toxic granulation (large eosinophilic granules)**
  - **Vacuolization**
- The presence of toxic neutrophils indicates toxaemia – the presence of toxins in the blood stream that causes morphologic damage to the cells.

D. Leukomoid Response

- This is leukocytosis characterized by marked (extreme) elevation **of leukocyte number.**
- The elevation can reach up to 50,000 to 100, 000 leukocyte per µL of blood. **It is nearly similar to leukemia**.
- **The leukocytosis is caused by neutrophilia and monocytosis but neutrophilia is more marked than monocytosis**.
- Leukomoid responses occur when continuous stimulus to bone marrow precursor.
- Selective differentiation into myeloblasts and monoblasts — monopoiesis and granulopoiesis — neutrophilia with marked to extreme regenerative left shift and monocytosis (secretion of monokines by the proliferating monocytes to stimulate fibroblast proliferation and encapsulation).
  - ✓ Eg. suppurative inflammations (pyometra, peritonitis), multiple encapsulated localized pyogenic infections, acute coliform mastitis, etct.
- ***A decrease in number of leukocytes is termed leucopenia. This can also be caused by a number of factors***:
  - ✓ **Leukocyte destroying viruses (panleukopeia in cats**)
  - ✓ **Overwhelming(Very intense)bacterial infections** (foal salmonellosis, septicemic pasteurellosis, peracute black leg, etc)
  - ✓ **Shock**
  - ✓ **Bone marrow hypoplasia** (by drugs like CAF, barbiturates)
- **Generally leucopenia is a marker of poor prognosis.**

Kinetics of Leukocytes

- ***Neutrophils*** *(segmentors, polymorphonuclear cells, PMNs)*
- An increase in number of neutrophils is called neutrophilia and it could be the result of :

√ Physiologic neutrophilia: occurs when there is release of epinephrine in angry and frightened animals (MNP → CNP).

. .***Physiological***

. **Vigorous exercise**

. **Pregnancy**

. Newborn

- ***acute inflammatory neutrophilia***:-this results from changes in neutrophil kinetics that are caused by **acute inflammatory mediators.**
- The net **result** is an **increase circulatory neutrophils pool** that may contain left shift

Note- Inflammatory neutrophilia: occurs in both acute infectious and non – infectious conditions (due to increased granulopoiesis and

- ***Inflammation or necrosis***
- **Metabolic disorders** e.g. diabetic ketoacidosis, uremia, and eclampsia
- **Steroid therapy/ Acute hemorrhage or hemolysis**

- ***Steroid – induced neutrophilia:*** occurs as a result of both exogenous and endogenous corticisteroids. Neutrophilia results from:
  - Movement of neutrophils from MNP to CNP
  - Decreased diapedesis
  - Increased release of mature neutrophils from bone marrow
- *The magnitude of inflammatory neutrophilias varies among species, and interpretation of specific concentrations differ among species*
- *Rank of the domestic animals by their ability to develop maximal degrees of neutrophilia during inflammation.*
  - Dog, Cat, Horse, Cattle in decreasing order.
  - ***The inflammatory bovine neutrophilia results primarily from increased production (granulocytic hyperplasia) and not from stored pool as cattle have small storage pool.***
- A decreased number of neutrophils is called **neutropenia**. This condition can occur in:
- Inflammatory neutropenia: as a result of overwhelming bacterial infection
- Destruction (canine distemper, CAF)

- **C**= Cyclophosphamide **A**= Doxorubicin Hydrochloride (***Adriamycin)*** **F**= Fluorouracil
- Shock due to movement of neutrophils from CNP to MNP

The general mechanisms (with appropriate examples) that cause neutropenia

a) **Excessive emigration to tissues during inflammation (acute bacterial infection, sepsis (The presence of pus-forming bacteria or their toxins in the blood or tissues**), **necrosis)**

b) **Endotoxin-induced margination** (equine salmonellosis)

c) **Decreased marrow granulopoiesis**

(1) Viruses (feline leukemia virus [FeLV], feline immunodeficiency virus [FIV], **parvoviruses**

(2) Cellular infiltration of marrow (myelophthistic conditions, e.g., lymphosarcoma, myelofibrosis, granulomatous disease)

(3) **Toxins (**bracken fern in cattle, estrogen and sulfadiazine in dogs)

Mononuclear Cells disorder

- **Lymphocytes**:
  - **T- Lymphocytes account for 65-80%** of peripheral blood lymphocytes and are functionally divided into **T helper cells** (**predominate in blood**) and **T suppressor cells** (**predominate in marrow**)
  - **B- lymphocytes**: these have endogenously produced **Ig** molecules on the cell surface, which act as receptors for specific antigens.
- **Lymphocytosis:-** absolute lymphocyte count greater than normal values.
  - Levels are higher in infancy and gradually decrease toward adult levels.

Conditions associated with lymphocytosis

a. Inflammatory-induced antigenic stimulation
   i. Noninfectious
b. Vaccination (antigenic stimulation)
c. Physiologic leukogram (epinephrine-induced shift from lymphoid tissues)
d. Hypoadrenocorticism (lack of glucocorticoids)
e. Persistent lymphocytosis in cattle infected with bovine leukemia virus (BoLV)

f. Lymphoid leukemias

Conditions associated with lymphopenia

a) Stress or hyperadrenocorticism (endogenous glucocorticoids)
b) Glucocorticoid therapy
c) Acute inflammation
d) Viral infection
e) Decreased recirculation of lymphocytes from lymphoid tissues: Chylous effusions caused by anterior mediastinal mass, congestive heart failure, or thoracic duct leakage
f) f. Decreased recirculation of lymphocytes from intestinal lymphatics:
    (1) Lymphangiectasia
    (2) Intestinal neoplasm
    (3) Granulomatous inflammation

LABORATORY EVALUATION OF LEUKOCYTES (analytical principles and methods)

- ***Leukogram***
  - The *leukogram* consists of the laboratory tests used in the routine evaluation of leukocytes on the complete blood count (CBC).

Total leukocyte count

- ***Principles of determining leukocyte determination (WBC count)***
- The *total leukocyte concentration* is measured by hemocytometer.
- Hemocytometer (**manual/automated**) is used to determine WBC by diluting the blood and then dispensing the diluted blood into the hemocytometer chamber.
- The manaul method is very time consuming and is relatively inaccurate, with up to ***a 20% margin of error***.
- *Automated cell counters*, which make precise dilutions and count the leukocytes in a larger volume of blood, provide more accurate results (<5% error).
- The leukocyte diluent contains a lysing agent that ruptures the erythrocyte so thet do notinterfere with nucleated cell counting.
- The chamber is examined with a microscope, and the leukocytes are counted within 0.9µl volume of fluid demarcated by the nine large squares.

- To obtain the leukocyte concentration, the number of leukocytes counted is first multiplied by 1.1 to calculate a concentration in the diluted blood. This producted is then multiplied by the dilution factor (eg,100) to calculate the WBC in the blood.
- Counts should be done in both chambers of the hemocytometer and the mean of the two values should be used.
- Note: advanced clinical pathology laboratories used automated counters.
- *The next step is to determine the relative percentage of each leukocyte type using differential leukocyte count*

Estimation of the number WBCs from stained blood smears.

- Determination of the average number of WBCs in 10 fields.
  - The number can be corrected by multiplying the result by 1500 to get the number of cells per microliter.
- Alternatively, The WBCs can be counted in 30 adjacent oil immersion fields.
  - The average number of leukocytes per 1000 RBCs (3 fields) is found by dividing this number by 10.
  - The total number of leukocytes is estimated by multiplying the number leukocytes per 1000 erythrocytes by 7000.
- This method of estimation depends on the assumption that:
  - in normal animals there is one leukocyte for every 1000 RBCs
  - in the monolayer area there about 1000 RBCs in 3 fields.
- For example, using oil immersion objective 15 leukocytes were counted in 30 adjacent fields of a monolayer area of blood films from a dog.
- The estimated total WBCs is 15/3/10 X 7000 = 3500/µL. The most accurate methods for enumeration of leukocytes are automated methods and manual counting using hemacytometer.

Differential Leukocyte Count

- The “differential” leukocyte count is used to determine the number of each WBC type in a specimen.
- Differential leukocyte count can be performed either electronically using automated machines or by microscopic examination of stained blood smears.
- There are two types of differentials:

- relative
- absolute.

- The relative differential count is determined from stained smears on which at least 100 cells are counted and classified. For greater accuracy enumeration of 200 consecutive leukocytes is carried out and the percentage of each cell type encountered determined.
- The absolute differential count provides a more consistent evaluation of the number of each type of leukocyte present.
- The absolute differential count is calculated by multiplying the total leukocyte count by the relative differential count expressed as a decimal.
- It is the absolute differential count which is used for interpretation of the results of count
- Example: if a relative neutrophil count is 65% in dogs and if the total leukocyte count is 9,000/µL, the absolute count is 9000 X 0.65 = 5,850/µL.
- The main purpose of differential leukocyte count is to monitor the differential response of these cells to various disease conditions and follow their kinetics in the body of animals.
- **Note: -** When the total leukocyte count is within the reference interval, a percentage distribution based on the differential counting ***of 100 leukocytes is typically performed***.
- When significant *leukocytosis* is present, more leukocytes must be counted to accurately reflect leukocytes present in low numbers.
- One **rule of thumb** is to count 100 leukocytes for each 20,000 cells/microliter (µl) increment of the total leukocyte count. For example, when the total leukocyte count is 40,000/µl, a 200-cell differential would be performed.
- If there is significant *leukopenia*, however, it can be difficult to find 100 leukocytes on the smear. Therefore a percentage distribution of leukocytes may be obtained from a differential count of 50 or even 25 leukocytes
- ***The absolute concentration of each leukocyte type (not their percentages) is compared to appropriate reference intervals for that animal to detect any increases or decreases.***
- ***In addition, microscopic changes in the morphology of leukocytes are noted on the stained blood smear, typically using the 100× oil-objective lens***

CHAPTER 4: PLATELETS

❖ **Platelets are small cytoplasmic fragments of megakarycytes derived from pluripotent hematopoeitic stem cells**

- **Mamalian platelets share the following:**
  - **A phospholipid membrane containing glycoprotein important in cell-cell interaction & important for coagulation**
  - **A cytoskeleten(actin, myocin) allows for shape change & pseudopod formation with activation**
  - **α-granules that stores numerous proteins involved hemostasis & vessel repair**
  - **Dense granules containing, Ca 2+, Ma2+, ADP, ATP & serotonin, all of which are secreted with approprait stimuli**
- **Platelate production-*megakarypoiesis & thrombopoiesis***
- ***Megakarypoiesis* –proliferation & maturation of megakaryocyte**
  - **Occurs in bone marrow & resident myeloid progenitor cells in response to Tpo (thrombopoietin)**
- ***Thrombopoiesis*-formation of thrombocytes from megakarycyte &their delivery to circulation (is also mediated by Tpo)**
  - **Thrombopoies is occurs in bone marrow & at other sites of hematopoiesis (spleen). It also occurs in the lung from megakaryocytes lodged after circulation**
- Tpo is produced in hepatocytes, renal tubular epithelium & stromal cells of the bone marrow
  - The major function of platelets is to help repair vascular damage & prevent hemorrhage (plugs)
  - **Non-hemostatic function-platelets** are important in inflammation & wound repair. They interact with leukocytes & release vasoactive amine, cytokines, mitogens & growth factors

Microscopic features of platelets

- In blood films platelets are present individually or in small clumps
- Clumps usually result from in vitro platelet activation, but anticogulant induced agglutination of nonhuman platelets have been reported
  - Clumps are most frequent in feline & bovine samples
  - Clumps may be found via out film but are best found at feathered edge, sides & base.

Shape

- non activated platelets are discoid, mostly round, oval or elongated in blood film from non-anticoagulated blood
- Platelets become sleroid with EDTA(also with citrate but lesser)
- Elongated platelets, which probabily represent undivided megakaryocytes pseudopod segments(proplatelets) may be more numerous during stimulated thrombopoiesis
- Activated platelets may have peripheral pseudopodia

Size- in most healthy individuals' platelets is uniform in size

- ***Feline platelets*** are larger & more variable than any other domestic animals, may exceed than feline erythrocytes
- **Giant platelets** (shift platelets, stress platelets, megaplatelets, megathrombocytes) are larger than normal but not larger than erythocytes
- Their increase suggest accelerated thrombopoiesis , usually because of destructive or consumptive thrombocytopenia (congenital disorders, clonal hemic disorders, splenic contraction)

Analytical principles & methods

- Quantitative & qualitative regarding platelets are routine part of mammalian CBC
- **Thrombogram**: those portions of CBC related to platelates

A. Blood film evaluation
    - Microscopic evaluation of platelets on stained blood film is an important part of thrombogram, especially in bleeding patients or when thrombocytopenia or extrem thrombocytosis is present
    - **I**t serves three major purpose

B. It enables detection of platelet clumps, which may falsely decrease [platelet]

C. It enables evaluation of platelets for abnormal features

D. It enables evaluation of plateltes for abnormal features

B. platelet concentration (platelet count)- is number of platletes per unit volume of blood

Blood film evaluation of platelets

1. Scan the feathered edge & smear body for **platelet clumps**. If clumps are present markedly under estimate the true [platelets]

2. Estimating the individual platelets - this requires first finding the appropriate counting window, where **leukocytes are flattened** like fried eggs to expose nuclear & cytoplasmic detail.

- appropriate window contain field of similar blood thickness
- smear thickness can be judged by the degree to which cells are flattened out (fried egg appearance)than cells proximity to each
- if the thicker areas of the smear will be evaluated [platele] be overstimated

- If platelets are well distribute, estimate the average number of platelets per 1000X field in at least 5 (preferably 10) fields
- Converte the average number of platelets per fielr to estimate [platelet] by using the following conversion factor
  - 1 platelet/1000X field= 15,000- 20,000 platelets/µl
  - Eg. If the mean number per 1000X is 2, then the estimated [platelet] = 30,000-40,000.
- The estimated [platelet] should then be referred to specific Spps references
  - ***To exclude thrombocytopenia, dogs, cats, & cattle should generally have at least 8-10 platelets/1000x field & horses should at least have 5 platelets/1000X field***
- EDTA anticoagulat is common but when EDTA-induce platelet clumping citrated venous blood (1 part citrate, 9 part blood) can be used.
- Heparin samples should not be used (platelet clumping)
- Platelets can easily be activated during blood collection (clumping)
  - Slow & poor vein puncture techniques
  - Inadequate mixing of blood & anticoagulant
- Average storage 8hrs room temp. & 48hrs at 4oc(vary greatly)
- Hemocytometer method
  - Platelets are enumerated in defined grid region after sample dilution & lysis of RBC
  - Labour intensive & had less precision

Thrombocytopenia

- Is pathologic state in which [platelet] is less than a valid lower reference limit (platelet clumping must be absent)

- It marked significant is the potentiation of bleeding when [platelet] are markedly decreased

The clinical presentation of thrombocytopenia

- The clinical impression cannot easily be differentiated based on clinical presentation.
- Signs are petechiae, purpura, and ecchymosis. This type of bleeding is often associated with mucous membranes or friction areas such as the axilla or inguinal areas.
- Other, nonspecific signs may include hematuria, epistaxis, hematochezia, and hemoptysis.

Diseases & conditions

- Pseudo-thrombocytopenia
  - May occur when not all platelets are not counted (clump)
  - When blood collection platelet activation & aggregation
- Decreased platelet production
  - Generalized bone marrow disease or megakaryocytes specific disease
  - ***Bone marrow aspirate or core samples conclude absence of megakarycytes***
- Drugs
  - Antineoplastics
  - Estrogens(sertoli cell tumor, granulosa cell…dogs)
  - Bracken furn (aplastic anemia with thrombocytopenia)
- Infections- BVD/ canine distempet/parvo
- Decreased platelet survival (accelerate platelet destruction or consumption)
  - Platelet consumption (hemostasis)
  - Platelet destruction – usually immune-mediated destruction
- Blood loss (acute & severe)
- Platelet activation with accelerated consumption or use Localized (controlled) intravascular coagulation (hemangiosarcoma, thrombosis, hemorrhage)
- DIC- uncontrolled activation of coagulation cascade

Buccal mucosal bleeding time

- The BMBT is used when platelet dysfunction (thrombocytopathia, von Willebrand's disease) or endothelial cell dysfunction is suspected.
- In dogs or cats with platelet counts less than 70,000/µl, the BMBT is often prolonged.

- If petechiae are present, a BMBT will add no additional information and need not be performed because petechiae are specific for a primary system dysfunction.
- Therefore, in effect, the use of the BMBT is reserved for nonthrombocytopenic patients with evidence of primary hemostatic disorders
- Tests for primary hemostasis involve evaluation of platelet numbers & the functional assessment of vonWillebrand factors. Identification of blood vessel wall problems require appropriate clinical sigs

PART 2: VETERINARY CLINICAL PATHOLOGY-II

By the end of this course, students will be able to:

- Understand the major pathophysiological changes in diseased animals
- Be able to perform different organ function tests
- Be able to perform and interpret urinalysis
- Be able to perform and interpret water and electrolyte analysis
- Be able to perform and interpret diagnostic cytology.
- Be able to perform tissue processing techniques and interpret test results and correlate with history and clinical findings.

CHAPTER 1: DIGESTIVE SYSTEM FUNCTION TESTS

- ✓ **Introduction**
- ✓ **Liver function tests**
- ✓ **Pancreatic function tests**
- ✓ **Rumen function tests**

Introduction

- Damage and/or dysfunction associated with certain organs or tissues (principally the **kidneys, liver, pancreas and musculo-skeletal system**) can affect quite a wide variety of biochemical constituents and aspects of metabolism.
- **Clinical chemistry** measures a variety of analytes in the fluid component of blood (serum or plasma).
- These measurements can give an indication of the health of the body as a whole or pointing to diseases involving specific organs.

1. LIVER FUNCTION TESTS

Functions of liver

- Liver is largest and most complex internal organ
- All blood flow from intestine and pancreas reaches liver via portal venous system
- Liver is a multifunctional organ that is involved in diverse body functions.

1. **<u>Metabolic Functions</u>**

- ✓ Liver actively participates in carbohydrate metabolism, lipid, protein, and mineral and vitamin metabolisms.

2. **Excretory Functions**

- ✓ Bilirubin, cholesterol, drug metabolites are excreted in bile into intestine.

3. **Protective functions**

- ✓ Kupffer cells of liver perform phagocytosis of bacteria or other foreign material from blood to eliminate foreign compounds.
- ✓ **Detoxification** – converts noxious or insoluble compounds into less toxic or more water soluble forms
- ✓ For example: ammonia is detoxified to urea and metabolism of xenobiotics.

4. **Synthetic functions**

- Synthesis of plasma proteins (albumin and prothrombin), cholesterol, triglycerides and lipoproteins.

5. **Storage functions**

- ✓ Glycogen, Fat soluble vitamins (A, D, E, K) and B_{12}

6. **Serum enzymes**

- ✓ Acting as markers of liver damage

Liver function Tests

- Liver function tests (LFT) are helpful to detect the abnormalities and extent of liver damage.
- Noninvasive methods for screening of liver dysfunction
- Assess severity and allow prediction of outcome
- Disease and treatment follow up
- Indicating the broad diagnostic category of the liver disease
- The use of laboratory tests is necessary for liver disease diagnosis; definitive etiological diagnosis is usually only possible by means of a liver biopsy.
- All laboratory tests are aids to diagnosis and must be carefully interpreted in conjunction with clinical and other available data.
- The results and interpretation of such tests, however, depend on

1) the nature of the lesion

- ✓ bile acids, arginase and GGT gives a sensitive indicator of cholestasis and/or hepatocellular necrosis

2) the duration and severity of the disease, and

3) species variations.

- Serum chemistry can be used to detect several types of hepatic abnormalities. This includes:-

- ✓ Injury or necrosis to the hepatocytes
- ✓ Alteration in the synthetic or excretory functions
- ✓ Cholestasis and
- ✓ Altered portal circulation

Broadly classified as:

1. Tests to **detect hepatic injury**
    - Mild or severe; acute or chronic
    - Nature of liver injury (hepatocellular or cholestasis)
    - More specifically, AST, ALT, and alkaline phosphatase are called the liver enzymes and they typically are used to detect damage or injury to the liver (not its function).
2. Tests to assess **hepatic function**

- The **true** liver function tests (LFT's) include albumin, bilirubin, bile acids, ammonia, PT, and glucose.

Classification of LFTs

Group I: Markers of hepatocellular injury

- □ Alanine aminotransferase (ALT)
- □ Aspartate aminotransferase (AST)

Group II: Markers of cholestasis

- □ Alkaline phosphatase (ALP)
- □ g-glutamyltransferase (GGT)
- □ **Membrane bound enzymes**

Group III: Markers of liver dysfunction

- □ Serum bilirubin: total and conjugated

- Urine: bile salts and urobilinogen
- Total protein, serum albumin and albumin/globulin ratio
- Prothrombin Time

Liver damage

- A number of different enzymes are released into the plasma when the liver is damaged; their usefulness varies from species to species.
- The interpretation of elevated values of enzymes in plasma is dependent not only on the tissue and site of origin but also on the half-time of clearance of the enzyme.
- The magnitude of the increase in serum enzymatic activity depends on the number of hepatocytes affected, severity of injury and the serum half-life of the enzyme.

AST (Aspartate Aminotransferase)

- Formerly SGOT or serum glutamic oxaloacetic transaminase)
- Found in mitochondria and cytoplasm of all cells: (with high conc. in cardiac and skeletal muscle, liver, kidney and erythrocytes)
- Acute/medium-term damage, most species (but not very liver-specific).
- Normal level = < 100 IU/L, (Half-life = 17 hours) (in horse upper limit is grater than 100 IU/L)
- Erythrocytes contains AST so increased levels may occur with in invivo or invitro hemolysis. So what?
- A marker of hepatocellular damage
- High serum levels are observed in:
  - Chronic hepatitis, cirrhosis and liver cancer
- The presence of AST in many tissues precludes the use of these enzymes as an organ–specific enzyme for diagnosis but it can be used in conjunction with other enzymes.
- NB: sometimes the rise could be:

✓ Physiological: during exercise (150 IU/L)

✓ Artefactual: in-vitro AST release from RBC, if there is hemolysis

ALT (Alanine aminotransferase)

- Formerly called **Serum glutamic-pyruvic transaminase (SGPT).**
- In pets, it is virtually used for diagnosis of hepatic damages.
- Normal conc. is < 100 IU/L (in dogs and cats: it's liver specific)

- Marked increase >400 IU/L in small animals indicate sever liver necrosis
- More liver-specific than AST
- Acute/medium-term damage, dogs and cats.
- But in large animals: ALT is not liver specific, its level in cardiac and skeletal muscles exceeds the level in the liver.
- High serum levels in acute hepatitis
- Minor elevation in cirrhosis
- Appears in plasma many days before clinical signs appear
- N.B. In liver damage, both enzymes are increased but ALT increases more.

ALP (Alkaline phosphatase)

- osteoblasts, chondroblasts, hepathocytes, renal tubules, intestine wall, mammary gland, placenta
- Present on hepatocyte membrane
- A non-specific marker of liver disease (Produced by bone osteoblasts (for bone calcification))
- Moderate rises in acute/medium-term damage in all species, but not very liver-specific.
- Very marked increases accompany **biliary obstruction**.
- Of the tests available for testing of **biliary obstruction** the serum ALP test is preferred.
- ALP is not commonly included in the biochemical panels for large animals (cattle, horse, sheep and goats).

✓ Wide reference interval in this spp. (not sensitive indicator of bilary diseases).

✓ GGT is preferred indicator of cholestasis.

- ALP level in plasma increases when there is obstruction of biliary tract (in dogs), liver damage (in cats) and drug administration; Bone diseases (rickets, osteomalacia, 20 hyperparathyroidism, etc) / tumor.
- Each tissue has its own isoenzyme that can be identified by electrophoresis.
- High levels are observed in:
    - Extrahepatic obstruction (obstructive jaundice) and intrahepatic cholestasis
- Very high levels are observed in:
    - Bone diseases

GGT (gamma-glutamyl transpeptidase)

- It occurs mainly in the cells of liver, kidneys, pancreas and prostate. However most cells (including myocytes) have some GGT activities.
- Most GGT observed in serum originate from liver while GGT from kidney do not appear in serum rather they are found in urine.
- In large animals: an increase in serum GGT level is due to long term liver damage and obstruction to hepato-biliary tracts and
- induction of enzyme synthesis by drugs (without cell damage)
- is an enzyme widely distributed in a variety of equine tissues.
- Specific activity of GGT in the horse is highest in the kidney, pancreas and liver.
- Serum GGT activity is used as a diagnostic criterion for hepatobiliary diseases in cattle, sheep and horses. (little use in small animals).
- GGT is more specific indicator of cholestasis than ALP, except in the cat.

Arginase

- ✓ Found in cytoplasm of hepatocytes (liver specific).
- ✓ Is a specific indicator of hepatic disease because it is not found in appreciable quantities in other organs.
- ✓ Arginase has a short blood half-life, which makes it useful for the diagnosis of acute hepatic disease but not for less severe forms.

SDH (sorbitol dehydrogenase)

- ✓ Acute damage, horses (and esp. ruminants).
- ✓ Almost completely selective as an indicator of liver damage in sheep and cattle

GDH (glutamate dehydrogenease)

- ✓ Acute damage, horses and ruminants.
- ✓ Liver specific in birds.

LDH (Lactate dehydrogenase)

- ✓ Very non-specific, little diagnostic usefulness.
- ✓ It is abundant in liver, kidney, muscle and myocardium

Liver dysfunction

- This is not necessarily accompanied by the enzyme changes typical of liver damage, as dysfunction may occur without actual cellular disruption, and more chronic cases of liver failure may have little hepatic tissue left to release enzymes into the circulation.

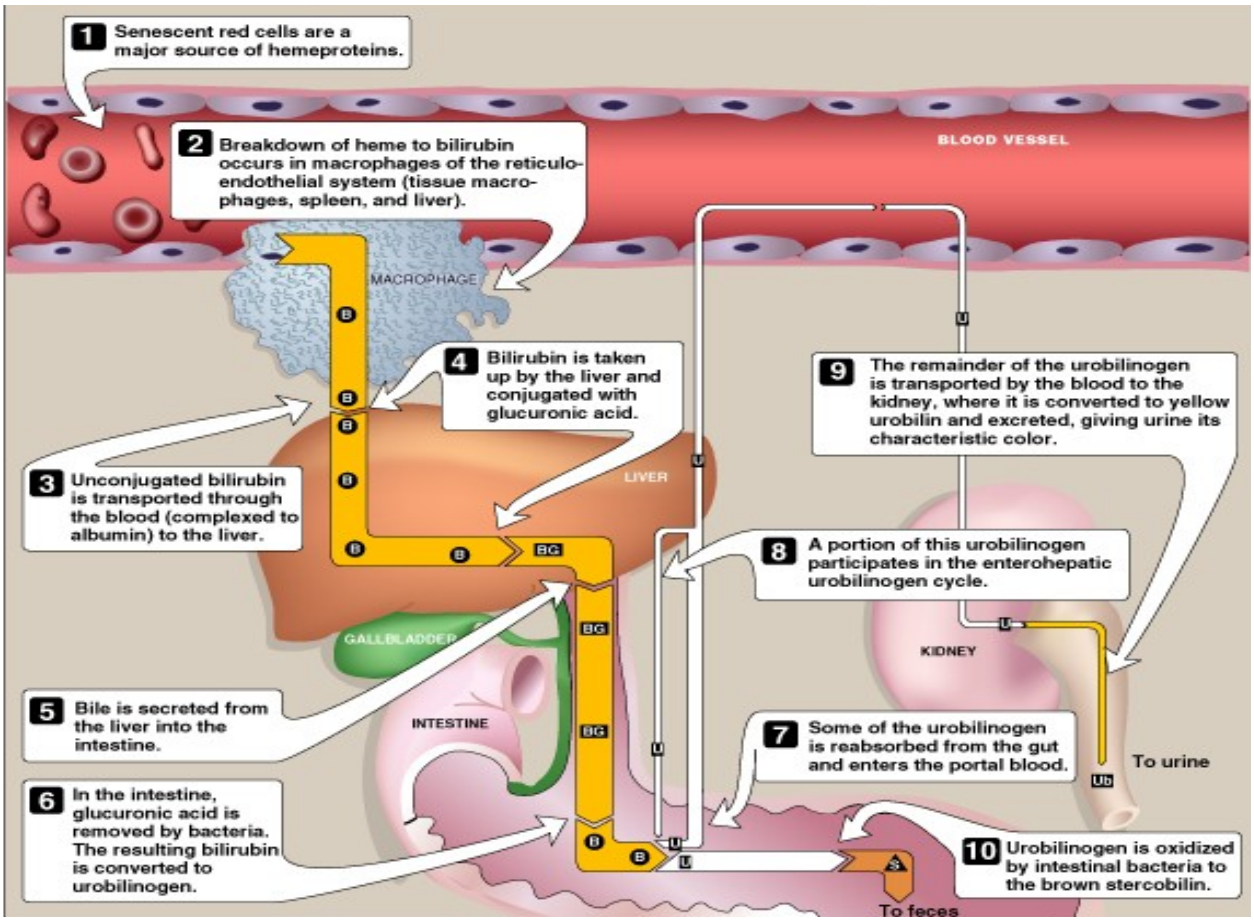
- The liver has many different functions, and it is unusual for all of them to be affected equally.
- Often one or more functions may be severely deficient while others may be virtually unaffected, depending on the actual pathology involved and the chronicity of the condition.
- 70% or more of the functional hepatocytes must be lost before alterations of hepatic function are detectable by serum biochemical testing.
- Mechanism of decreased functional liver mass:
- Heptocellular injury or necrosis
- Cirrhosis (Replacement by fibrous CT)
- Hepatic atrophy

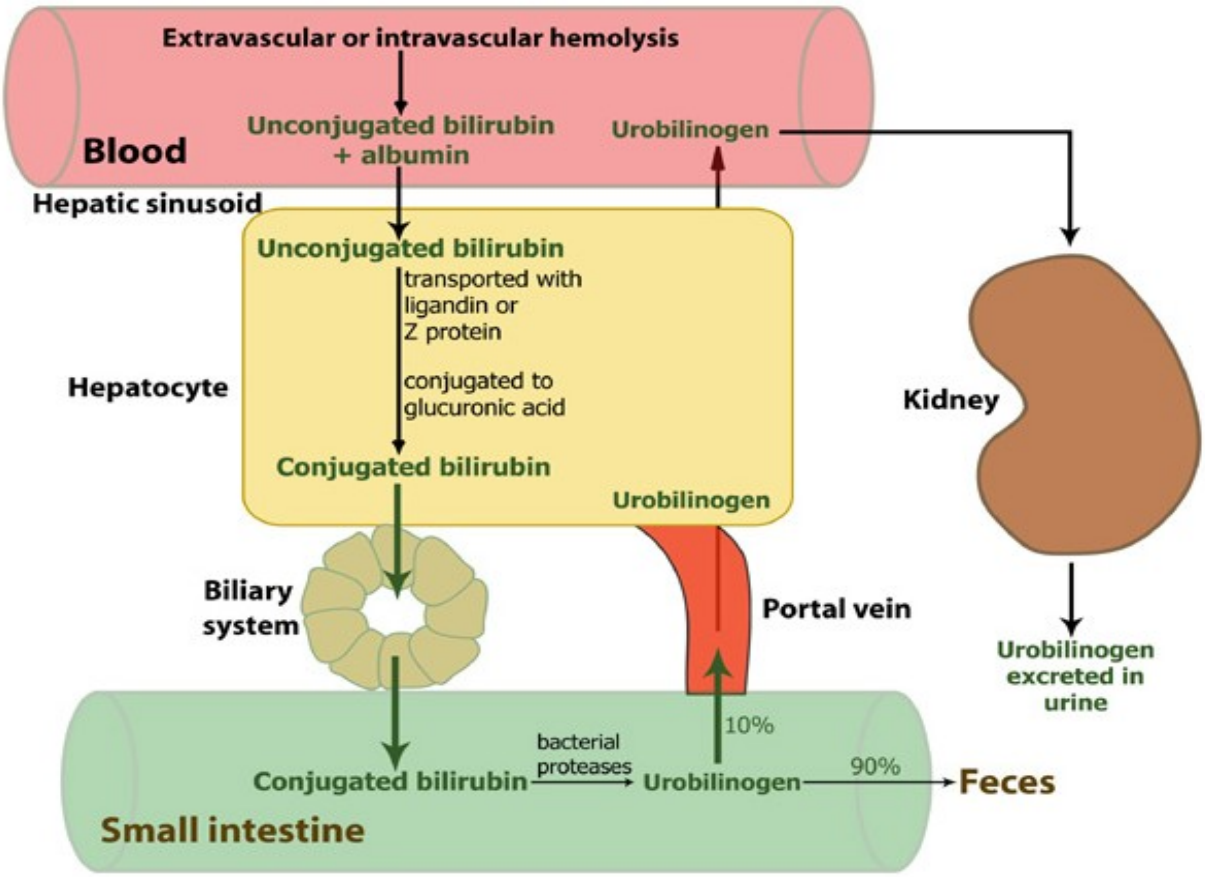
Common liver function tests includes:-

a. Tests of peripheral blood **uptake**, **conjugation**, and **secretion**: serum bilirubin and sulfobromophthalein (BSP) excretion test.
b. **Tests of portal blood clearance**: ammonia tolerance and bile acids.
c. **Tests of hepatic synthesis**: serum glucose, albumin, urea, and clotting factors.

Bilirubin

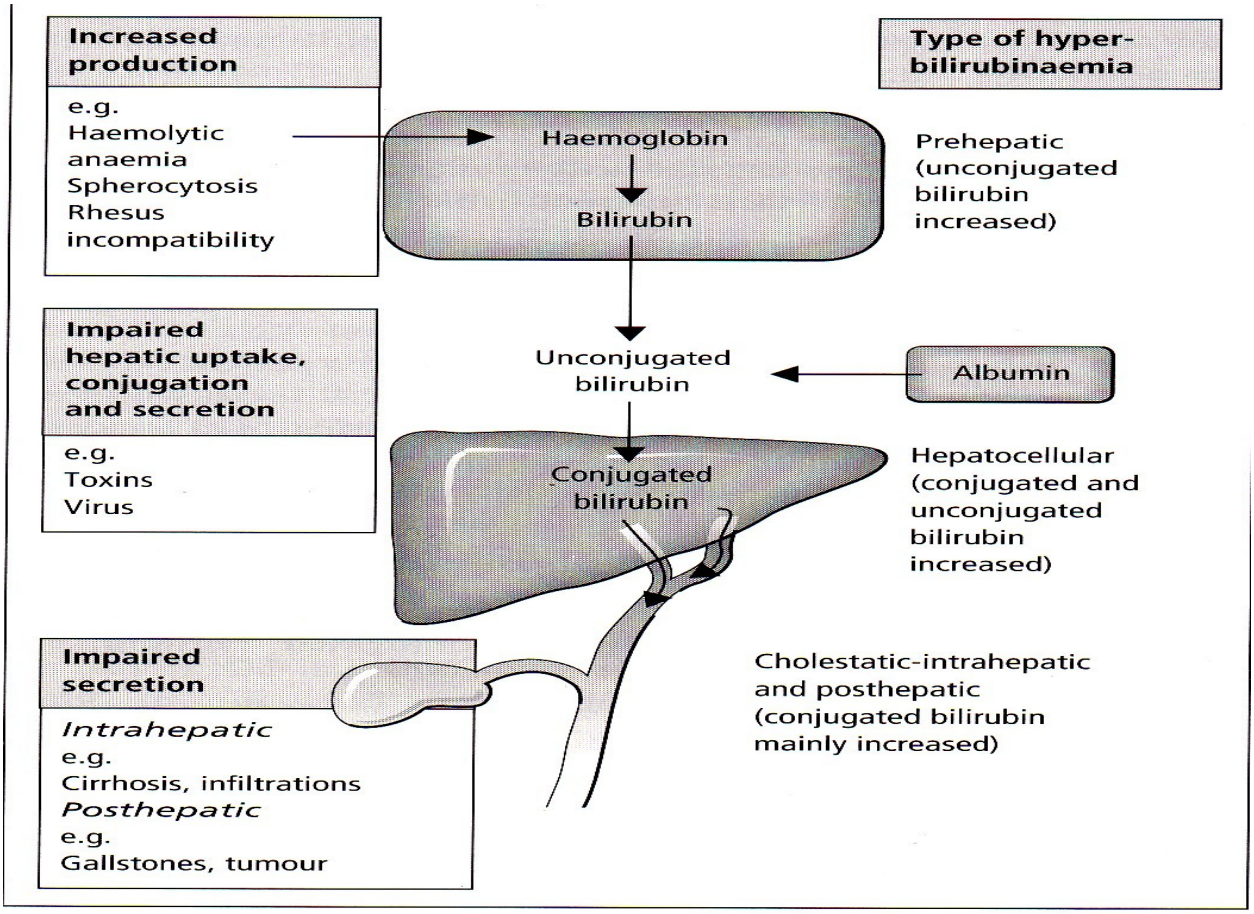
- A byproduct of red blood cell breakdown
- It is the yellowish pigment observed in jaundice
- High bilirubin levels are observed in:
  - Gallstones, acute and chronic hepatitis
- Birds lack biliverdin reductase and don’t form significant quantities of bilirubin.


1 Senescent red cells are a major source of hemeproteins.
2 Breakdown of heme to bilirubin occurs in macrophages of the reticulo-endothelial system (tissue macrophages, spleen, and liver).
BLOOD VESSEL
MACROPHAGE
3 Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.
4 Bilirubin is taken up by the liver and conjugated with glucuronic acid.
LIVER
9 The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.
8 A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.
GALLBLADDER
KIDNEY
5 Bile is secreted from the liver into the intestine.
INTESTINE
7 Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.
To urine
6 In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.
10 Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
To feces



Jaundice (hyperbilirubinaemia)

- Hyperbilirubinemia occurs in many diseases of cattle and in most cases is related to a failure of the liver to remove unconjugated bilirubin from the serum rather than to a failure of the liver to excrete conjugated Bilirubin.
- The basic cause of the jaundice ± haemolytic (`pre-hepatic'), hepatic, or bile duct obstruction (`post-hepatic').



Haemolytic disease

- The patient will inevitably be anaemic!
- The anaemia is usually regenerative and not hypochromic.
- Plasma bilirubin is usually only slightly/moderately elevated
- Liver enzymes will be normal (except in copper poisoning).

Hepatic disease

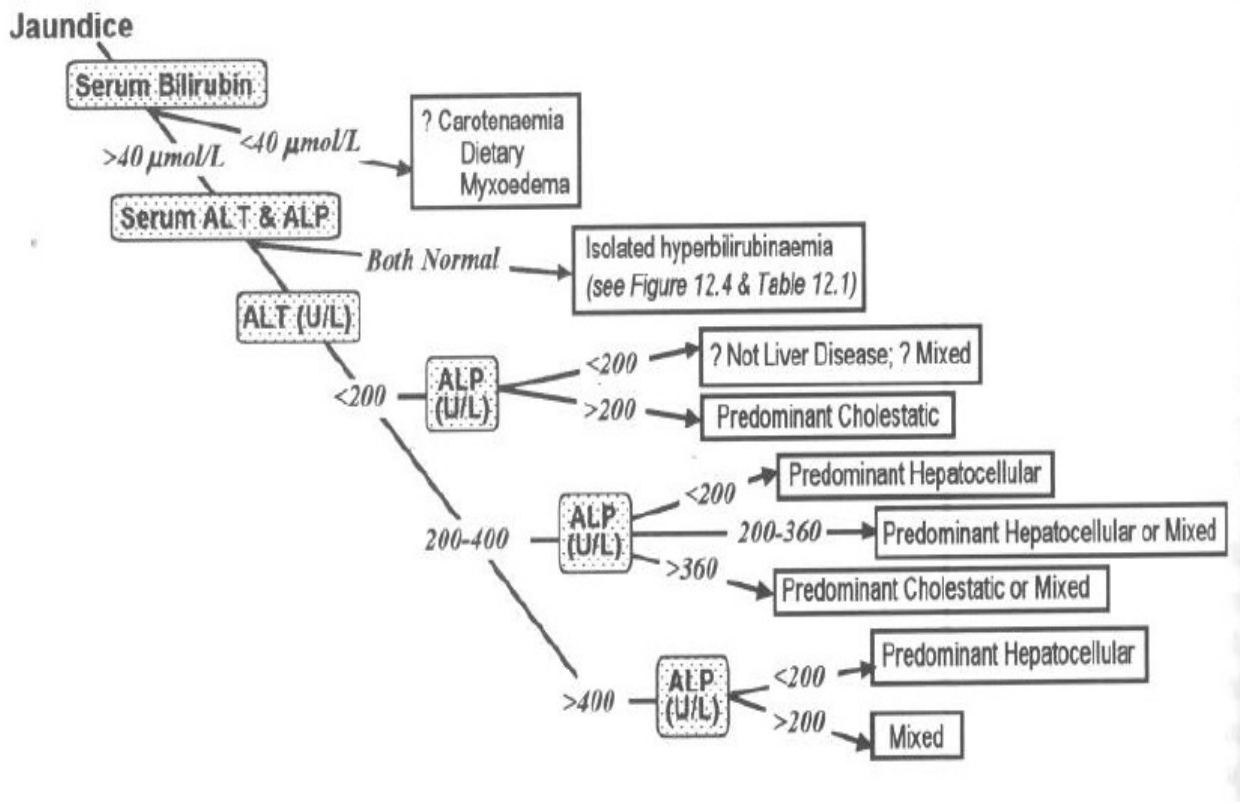
- **Hepatic parenchymal enzymes** (ALT, SDH, GDH, depending on the species) and **ALP** (gGT in large animals) will be elevated in parallel.
- In small animals: **ALT** activity is often extremely high (1000±4000 iu/l)
- while **ALP** activity is only moderately elevated (1000±4000 iu/l).
- Occasionally in certain chronic liver conditions there may be no change in liver enzymes, but

- the hepatic aetiology is demonstrated by other signs of liver dysfunction (such as hypoalbuminaemia) and the absence of any evidence of either haemolysis or biliary tract obstruction.

Biliary tract obstruction

- In an extra-hepatic obstruction the hepatic parenchymal enzymes will be normal or only slightly increased, while **ALP** activity is extremely high (10,000±50,000 iu/l).
- Where the obstruction is intra-hepatic the results will also reflect the hepatic disease, and the parenchymal enzymes may also be relatively high.

Lab. Investigation of jaundice



Urea

- May be low if urea cycle is impaired, but this is not invariably the case.
- Decreased BUN can occur as a result of: Liver failure, Anabolic steroids, and Low-protein diet.

Ammonia

- Inevitably high if urea cycle is impaired.
- The microbial deamination of aminoacids in the intestinal tract is the major source of ammonia which is absorbed by the intestine into portal venous blood and converted into urea by the liver.
- The concentration of blood ammonia can be an indication of functional hepatic mass.
- In healthy cattle, the plasma ammonia:urea concentration ratio is 9:1 and the plasma ammonia:glucose concentration 11:1.
- In hepatic disease, a plasma ammonia:glucose ratio 40:1 or plasma ammonia:urea ratio 30:1.

Bile acids

- Bile acids are synthesized in the liver from cholesterol.
- They are excreted in the bile where they aid in digestion and reabsorbed from the intestine into portal circulation and recycled (enterohepatic circulaton).
- The rise in total serum bile acid concentration usually correlates well with the severity of liver disease.
- Portosystemic shunt
- Loss of functional hepatic mass result increased bile acid conc. Due to decreased bile acid recycling.
- Cholestasis causes reflux of bile acids into the blood stream.
- In **cattle**, there is extreme variability among all types and ages of animals and the variation is even greater in beef cattle than in dairy cattle.
- In order to be specific for liver damage in cattle, the value determined for a single sample would have to be more than 126 µmol/L in beef cattle, or more than 88µ mol/L in lactating dairy cattle.
- In cattle, total serum bile acids are more specific and sensitive indicators of a wide variety of hepatic disease and are significantly correlated with the degree of illness compared to other tests of hepatic function.

<u>Albumin</u>

- The most abundant protein synthesized by the liver

- Normal serum levels: 3.5 – 5 g/dL
- Synthesis depends on the extent of functioning liver cell mass
- Longer half-life: 20 days
- Low when synthetic ability is impaired.
- Its levels decrease in all chronic liver diseases
- However, half-life is quite long; therefore hypoalbuminaemia is only seen in long-standing conditions.

Prothrombin time

- Prothrombin: synthesized by the liver, a marker of liver function
- Half-life: 6 hrs. (indicates the present function of the liver)
- PT is prolonged only when liver loses more than 80% of its reserve capacity.
- prolonged when synthetic ability (fibrinogen, prothriombin, factor V, VII and X) is impaired or
- impairment of the flow of bile reduces the digestion and absorption of the fat-soluble vitamin K and VIt K dependent factors (prothrombin, factor VII, IX & X).
- Half-life is shorter than albumin; nevertheless, again hypoprothrombinaemia is a feature of chronic conditions.

Activity: Do liver enzyme tests evaluate liver function?

2. **PANCREATIC FUNCTION TESTS**

Exocrine Pancrease

- Laboratory evaluation of the exocrine pancrease is directed toward two categories of diseases:-
    1. Inflammation and necrosis (Pancreatitis)
    2. Exocrine insufficiency (Maldigestion)

Pancreatic damage

- Occurs primarily in dog and occassionally in cat.
- not described in herbivores/ farm animals
- Inflammatory and degenerative changes are detected post mortem in some cattle but are rarely diagnosed clinically because of a **lack of clinical** and **laboratory findings.**

- Clinical signs may include anorexia, depression, vomiting, and possibly marked abdominal pain.

Primary tests

- **Serum enzymes**
- Enzymes produced and stored in acinar cells may leak into plasma during pancreatic injury, resulting in increased serum activity.

(1) **Amylase**. Usually very high relatively early in the disease process

(2) **Lipase**. Increases more slowly but remains elevated for longer.

- Because of its more limited tissue distribution, **lipase** is considered superior to amylase for the diagnosis of pancreatitis in dogs.
- Extrapancreatic sources, such as the small intestinal and gastric mucosa.
- Both amylase and lipase are eliminated by the kidneys
- Amylase and lipase determinations seem **to have drawbacks** for the diagnosis of canine pancreatitis.

(3) **IRT (immunoreactive trypsin)**

- ✓ This will also be <u>high</u> but it offers no particular diagnostic benefit over amylase and lipase (and takes a great deal longer to measure).

(4) Pancreatic lipase immunoreactivity (PLI): high sensitivity

- ✓ The best test available thus far for the diagnosis of canine pancreatitis.

Secondary tests

(1) **Calcium**. Some cases of pancreatitis are significantly hypocalcaemic. Why?

(2) <u>Incidental findings</u>: an increase in ALT, ALP and Bilirubin (concurrent hepatitis).

Exocrine pancreatic dysfunction

- Once again this applies almost <u>exclusively to the dog</u>.
- The condition is not described in herbivores, and it is extremely rare in the cat.

Tests

(1) **Faecal trypsin** and demonstration of **<u>undigested food</u> elements**.

- Faecal Trypsin will be low or absent, and fat globules, starch granules and muscle fibers will be seen in the faeces.

(2) **IRT:** Will be low

Laboratory detection of exocrine pancreatic insufficiency

1. **Fecal analyses**

- **Bulky feces and steatorrhea are clinical findings**

a. Examination of fecal smears

✓ Sudan staining (Undigested, neutral fat)

✓ Lugol's iodine staining (starch granules)

✓ Unstained fecal smears (undigested muscle fibers)

b. Fecal proteolytic activity

2. Serum IRT: low

a. Fecal smears

Fat test (Sudan staining)

- **Purpose: To determine the presence of fat substance in the fecal sample**
- **Reagent preparation: Sudan III or Sudan IV stain**
- **Sample required: Feces**

Procedure

• Mix a small portion of feces with water or saline on the slide

• Combine the feces with equal parts of Sudan III or IV stain

• Examine under the low power objective (×10) after placing a cover glass on the mixture

Result

- Neutral fat will appear as orange as red globules, which vary in size and shape and indicate deficiency of pancreatic lipase.

Lugol's iodine staining (starch granules)

- Starch granules appear as blue-black structures with blue green fringes.
- The presence of fecal starch indicates amylase deficiency.

Unstained fecal smears (undigested muscle fibers)

✓ Microscopically presence of undigested muscle fibers of animals with meat diet indicates protease deficiency

b. Fecal proteolytic activity

Trypsin

Film test

- Test for proteolytic activity employing gelatin digestion (for small animals)

Procedure

- ✓ Bring 9ml of 5% sodium bicarbonate and mix well with the feces
- ✓ Place ½ to ¼ of the mixture in a vial or test tube, place a narrow strip of gelatin coated radiographic film in the vial
- ✓ Incubate at 37°C for half an hour
- ✓ Wash the film under a gentle stream of tap water

Result

- A cleared area the film indicates the presence of Trypsin
- With a deficiency of trypsin the **emulsion** will only be water marked or partially removed.

Gelatin Test Method

- This test is more sensitive than the film test method

Procedure

- ✓ Warm two test tube each containing 2ml of 7.5% gelatin in 37°C to liquefy
- ✓ Mix well 9ml of water with feces
- ✓ Add 2ml of the feces mixture to as described above to one gelatin tube
- ✓ Add 2ml of 5% sodium bicarbonate (NaHCO3) to the other gelatin tube for the control, mix well
- ✓ Incubate both tubes at 37°C for 1hr
- ✓ Refrigerate or place in cold water (4°C) for 20 minutes

Result

- Failure to gel formation indicates the presence of trypsin, which has digested the gelatin i.e No pancreatic insufficiency
- Gel formation (coagulation) indicates the absence of trypsin. i.e pancreatic insufficiency.

3. **RUMEN FUNCTION TEST**

- Rumen fluid is collected by **stomach tube**; keep it at room temperature for 9 hours before examination.

Gross examination

- **Color**: Color of the rumen content fluid depends up on the **type of the feed**.
- The color should be from green to brownish green.

- It will be **green** in grazing animals yellow brown in those that feed on silage or straw.
- Abnormally it may be greenish black in decomposition

Consistency

- It is slightly viscous under normal condition.
- It will be watery in case of acidosis and foamy in foamy bloat.

Odor

- Under normal condition the odor of the rumen is aromatic odor and non replant
- Penetrating odor in acidosis
- Foul smelling in decomposition

Total acidity of rumen contents

- The pH of the rumen fluid varies from 5.5 – 7 which may be affected by saliva which contains bicarbonate.
- Alkaline in active rumen during starvation and urea poisoning
- the bacteria responsible for digestion and production of acid are weak and alkaline is predominates.
- **Indicator or dip stick test**
- Insert an indicator /deep stick in to the fluid and observe the color change.

Bacteria Examination

- **Purpose: To determine the dominate bacteria in the ruminal fluid**
- **Method: Gram stain**
- **Materials: Gram staining dyes (CV, iodine, safranin, methanol or acetone), slide, microscope**

Procedure

- ✓ Take a rumen fluid on the slide and make a smear
- ✓ Dry and stain with gram stain
- ✓ Observe under oil immersion for the presence or absence of the microorganisms.
- ✓ Observe the gram negative and gram positive bacteria, write the dominate and the ratio
- ✓ Under normal condition **gram negative bacteria** are the dominate
- ✓ Under **acidosis** condition Gram positive bacteria predominantly present

Microscopic Examination of Rumen Content Protozoan's

- There are two form of protozoa to be predominate in rumen fluid:

- ✓ Ciliates
- ✓ Flagellates

- The ciliates are important because massive in numbers than the flagellates

Procedure

- Take one or two drops of rumen fluid on the slide
- Cover with cover slip and warm around 13°C
- Observe under microscope
- Grade the number of protozoa

Result

- If it is abundant---------- + + +
- If it is moderate ---------- + +
- If it is few ---------- +
- If no protozoa ---------- -ve
- There is no digestion in the rumen in the death of protozoa.
- Therefore, observe the proportion of death to alive one, in case of acidosis, No digestion i.e., no protozoa

Cellulose Digestion Test

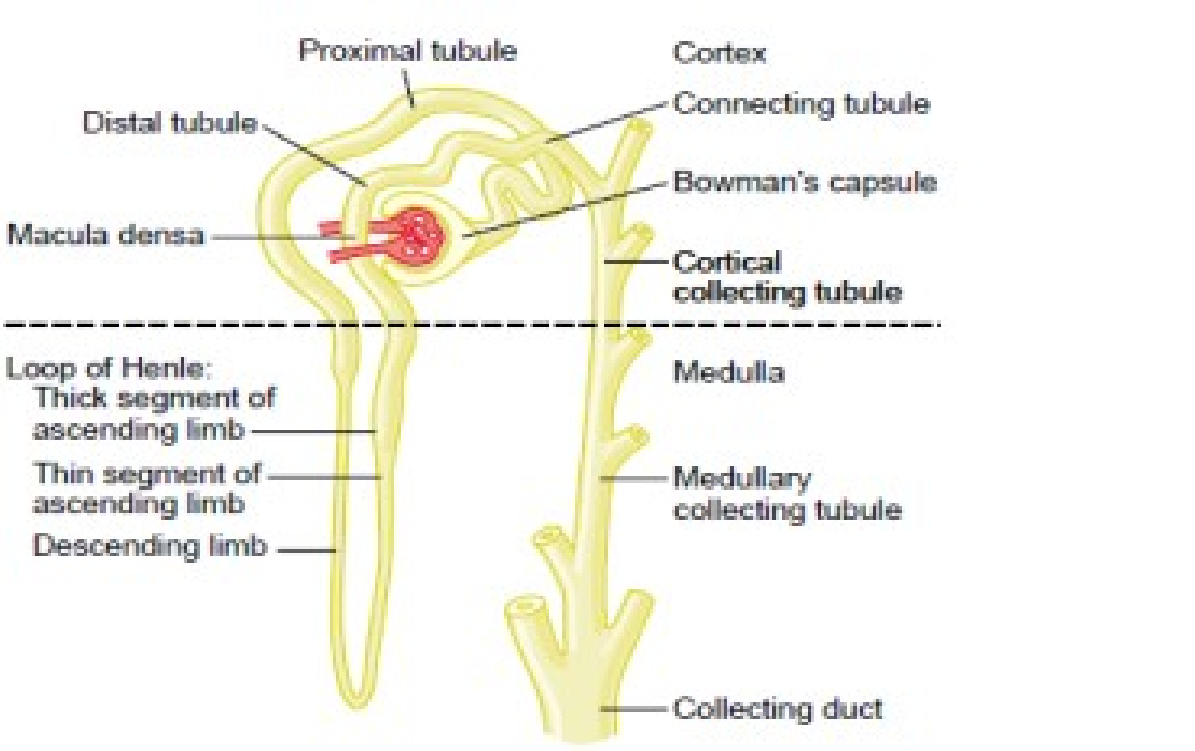
Procedure

- ✓ Take 10ml of rumen content in the test tube
- ✓ Add about 0.3ml of 10% glucose solution
- ✓ Suspend cotton thread to the solution and incubate in 37°C for 48 hrs
- ✓ The expected result is the action of microflora on the thread

Result

- If the rumen is active, there will be break down of the thread

4. URINARY SYSTEM FUNCTION TESTS



- The **kidneys**, the body's natural filtration system perform many vital functions:-

▪ **Excretion of waste**-production of urine

✓ Eliminating of metabolic waste products (Urea, creatinine, uric acid…etc)

✓ Elimination of foreign material (drugs)

▪ **Control of volume and composition of ECF**

✓ regulating the body's water and electrolyte balance, and

✓ maintaining the pH (acidity/alkalinity) of the body's fluids.

▪ **Endocrine function**:- (Renin, erythropoiten and calcitriol (activation of Vitamin D).

- Laboratory tests of the **urine** and **blood** can provide valuable information about kidney pathology and the adequacy of renal function.

- Renal functions or disorders can be assessed by glomerular function tests and tubular function tests (Urinalysis).

➢ **Derangement:-**

- ✓ decreased excretion of waste products and
- ✓ hence their accumulation in the body or
- ✓ loss of some vital nutrient from the body.

- Based on the level of these **excretory products** and **nutrients in the urine** as well as in **blood** we can make an accurate calculation to interpret the efficiency of the kidney to undertake its various functions.

Why test Renal Function?

- ✓ To identify renal dysfunction
- ✓ To diagnose renal disease
- ✓ To monitor disease progress
- ✓ To monitor treatment response

- Renal function tests evaluate the functional capability of the kidney and, in general, assess:
  1) Glomerular filtration and
  2) Tubular function
- These tests depend on whether they are based on the examination of serum, urine or both, and assess either function or the presence of injury.

What gets filtered in the glomerulus?

Freely Filitered

- Water
- Na+, K+, Cl-,

HCO- 3,Ca++,Mg+, PO-4 etc.

- Glucose
- Urea
- Creatinine
- Uric acid

None filtered

- Protein
- Immunoglobin
- Ferritin
- Blood cells

Tubular Reabsorption

- **Urea**, **creatinin**e and **uric acid**, are poorly reabsorbed and are therefore excreted in large amounts in the **urine**.
- **Electrolytes**, such as **sodium ions**, **chloride ions**, and **bicarbonate ions**, are **highly reabsorbed.**
- **Amino acids** and glucose, are **completely reabsorbed** from the tubules

Renal dysfunction

- ***Serum Creatinine Concentration***

✓ The serum creatinine concentration is the **most reliable** simple biochemical test of glomerular function.

✓ More renal-specific, little influence by pre-renal effects.

✓ However, these analytes **do not** usually increase until more than about 60% of nephrons are dysfunctional, so they are **not much** of an early warning system.

- The principle of the test is based on the fact that **creatinine** when present in biological fluids reacts with **picric acid** to form creatinine picrate.
- **Creatinine + alkaline picrate solution**



- **Bright orange/red colored complex**

  absorbs light at 485nm

✓ The creatinine picrate is a colored product and the absorbance of the product is measured **spectrophtometrically**.

✓ The concentration of the creatinine in the solution or sample is finally determined by the **Beer's law**. (*the absorbance of a solution is proportional to its concentration*).

- ✓ Since ingestion of meat can increase the serum creatinine concentration by **30%** following meat meal in few hours,
- ✓ creatinine measurement needs to be made after overnight fasting in pets.
- ✓ Mild increase in serum creatinine has also been observed following strenuous exercise.
- ✓ serum creatinine concentration inversely related to the GFR.
- ✓ Consequently, a normal serum creatinine does not necessarily imply normal renal function but a change in serum creatinine usually indicates a change in GFR.
- ✓ Some degree of change in serum creatinine can occur under conditions of increased muscle bulk.
- ✓ Decrease in serum creatinine can occur if there is:
    - wasting diseases
    - immediately after surgery
    - starvation
    - treatment with corticosteroids
- ✓ However, change in serum creatinine under these conditions is well within the **normal limits** and does not cause diagnostic confusion.

- ❑ ***Serum BUN (*Blood Urea Nitrogen) *level***

AZOTEMIA

- an abnormal concentration of urea, creatinine, and other non protein nitrogenous substances in blood, plasma, or serum.
- urea is synthesized in the liver in all domestic animals and also in the rumen in ruminants.
- It is primarily a byproduct of the deamination of amino acids.
- Eliminate in urine as a major nitrogen waste product (85%).
- Often used an **index** of renal function along with plasma creatinine.
- Not entirely renal-specific, also increases with pre-renal effects.
- Blood urea level can be changed independently to **renal disease**:
- High protein intake
- GIT hemorrhage
- Hypovolumia, burns

- Dehydration
- Catabolic state
- **Reduced:** starvation, low protein diet and sever liver disease
- Thus, BU needs to be compared to creatinine to determine **true renal dysfunction**.
- The levels of urea and creatinine almost always are paralleled to each other.
- Serum urea concentration is often used as an index of renal glomerular function but **serum creatinine** is more accurate measurement.
- The test is based on the principle that **urease enzymes** break down urea if present in the samples into ammonia.
- The ammonia produced reacts with **salicylates** and **hypochlorite** and produces green colored products.
- The absorbance of the product is measured spectrophotometrically and the final concentration of urea is determined by the **Beer's law**.
- Urea production is increased by high protein intake, in catabolic states, and by absorption of amino acids and peptides after some hemorrhages.
- Conversely production is decreased in patients with low protein intake and liver diseases.

Urinalysis

- General urine Examination
- It is a general test for evaluation of renal function
- Fresh sample= valid sample
- **Physical**
- **Chemical and**
- **Microscopic examination**

❑ **Urine collection**

- ✓ Cystocentesis
- ✓ Catheterization
- ✓ Free catch

- Urine collected by free catch is usually sufficient for most analysis especially if the result is negative.
- if there are certain changes in urinary cell counts, protein assay results and bacteriological culture result :

- Urine should be: collected either by cystocentesis or catheterization.
- Urinalysis should be completed on **freshly** collected urine;
- Refrigerated specimens are acceptable for up to 6 hours.

Gross examination of urine /physical examination/

- ***Color and transparency***

- The color and transparency of urine is determined in the test tubes or urinometer cylinder.
- The color and transparency of urine should always be recorded.
- Under normal conditions the color of urine is **yellowish** to **amber** due to the presence of urochromes (urobilins).
- Abnormal color may develop as a result of the following conditions:

- **Urine Color Change under Disease Conditions**

COLOR	CONDITION

- Color less/ pale yellow → Diluted urine/ polyuria
- Dark yellow → Concentrated urine/dehydration/ fever/acute nephritis
- Greenish yellow → High bilirubin content
- Red/ wine/brown → Hematuria/ hemoglobin/ myoglobin
- Brown/brownish black → Hemoglobin/ bile up on standing
- Except in equines the urine has **transparent** appearance under normal conditions.
- In equines urine has **thick** and **cloudy appearance** due to the presence of mucus and calcium carbonate crystals.
- In other species **cloudy urine** can be due to **inflammatory cells**, epithelial cells, bacteria, **fats** or mucus which can be further differentiated by microscopy.

Odor

- Normal urine has in offensive odor.
- Odor can provide clues to certain urine abnormalities.

Characteristic Odor of Urine commonly Observed

- Ammonia odor- Presence of urea splitting bacteria
- Fruity odor- Acetone or ketone bodies
- Sweet odor- Glucose or other sugars
- Foul odor- Pus / inflammation

Chemical properties

- ***Specific gravity***

✓ Specific gravity is the ratio of the mass of a solution compared with the mass of an equal volume of water.

✓ The specific gravity is a clinically useful test

✓ since loss of concentrating ability is one of the first signs of renal tubular diseases

✓ It is the measure of the concentrating ability of the tubules and good indicator of the action of the renal system on glomerular filtrate.

✓ Specific gravity can be measured by urinometer but indicator **paper strips** can also be used.

✓ The specific gravity of the glomerular filtrate lower and is usually within range of **1.008 – 1.012**.

✓ The specific gravity can vary depending upon the hydration status of animals from as low as **1.001** to as high as **1.060**.

✓ The evaluation of urine specific gravity should be done concurrently with assessment of serum urea level or creatinine in the serum.

✓ For example if blood urea nitrogen or creatinine is increased and the specific gravity of urine is less than normal

✓ *primary renal complications* are the usual cause lowered specific gravity.

✓ But if the specific gravity is increased along with increased level of blood urea nitrogen or creatinine in serum,

✓ the possible factor responsible is *prerenal diseases*.

- Decreased specific gravity can occur due to:
  - diuresis
  - over hydration
  - Fluid therapy
  - Renal diseases (nephritis, pyelonephritis)
- Increased specific gravity results from:
  - Dehydration (vomition, diarrhea)
  - Fever
  - Edema (circulatory failure)

- Shock

❑ **pH**

✓ Urine pH is a crude reflection of the acid – base state of the body and is influenced by diet.

✓ Herbivores have an alkaline urine pH while

✓ Carnivores and omnivores can have either acidic or alkaline urine depending on the amount of animal proteins in the diet.

✓ Alterations from normal pH are commonly an indication of a systemic acid – base imbalance than that of localized diseases of urinary system.

▪ **Normal Urine pH of Domestic Animals**

✓ Ruminants- alkaline

✓ Porcine- alkaline/acidic

✓ Pets- acidic

✓ Equines- alkaline

✓ Primates- alkaline / acidic

• Any young nursing animals of any species have acidic urine.

• The pH of urine is determined by:

✓ The lithmus paper

✓ Hydrion pH paper strips

✓ pH meter

❑ Pathologically increased acidity indicates:

- acidosis
- starvation
- fever
- prolonged muscular activity
- acid therapy

► Increased urine alkalinity could be due to:

- alkalosis
- urine retention in the bladder due to obstruction
- cystitis
- alkaline therapy

- **Protein assays**

- Under normal conditions urine is **free from proteins.**
- The presence of proteins in the urine is always considered pathological except at the time of parturition, pregnancy estrus, etc.
- Detection of protein in the urine has clinical significance and any persistent proteinuria should be investigated.
- The presence of proteins in the urine can be determined by:
  - **Robert's method**

✓ This method incorporates strong acids such as nitric acids
✓ The strong acids react with proteins and precipitate them out the solution
✓ the test is **simple** and can be performed in a test tube.
✓ The reagent is placed in a test tube and urine is gently added to it
✓ The presence of protein is indicated by formation of **white ring** or precipitate at the junction of the reagent and urine
✓ The **density of the ring** is proportional to the amount of the protein in the urine.
✓ It can be rated from + 1 to +4.
✓ **Principle: Precipitation of protein by strong acid**
✓ **Robert's reagent: Conc. Nitric acid – 1 part**
✓ Saturated Magnesium sulphate – 5 part (770gm to 1 litre)

Procedure

✓ Place 2ml of Robert's reagent in a test tube
✓ Layer 2ml of clear urine on the reagent by inclining the tube and allowing the urine to run slowly dawn the side from along dropped or pipett.
❖ A positive test is indicated by a **white ring at the zone of concentration**, which should be read against a dark back ground and reported as

- **Negative** - No ring at the zone of concentration.
- **Trace** - barely precipitate ring
- + Distinct narrow ring
- ++ Wider definite ring
- +++ Very wide ring
- ++++ Thick, dense ring occupies most or all of urine layer

- **Note:** In many clinical laboratories**, Robert's test is routine method** as it is simple, quick and easy to read even when only a small amount of protein presents.

➢ **The use of reagent strips**

- The strips contain **tetrabromophenol blue** as indicator and a **citrate** as a buffer on bibulous strip of cellulose.
- The basis of the test reaction depends on the fact that, at fixed pH of the paper, the indicator can have one color in the presence of protein and another in the absence of proteins.
- The buffer maintains the pH of the strip at acidic (3) and
- At this pH the tetrabromophenol blue indicator turns **green or blue** in the presence of proteins and turns **yellow** in the absence of proteins
- The **intensity of green or blue** color observed is proportional to the amount of protein present in the urine

❑ **Result interpretation**

- Once it is established that the magnitude of proteinuria is abnormal, its cause and source should be determined.
- Proteinuria in the presence of RBC or inflammatory cells (WBC) in the urine is difficult to interpret.
- Hemorrhage causes leakage of RBC + plasma proteins into the urine.
- Inflammation causes leakage of WBC + plasma proteins into urine.
- Both WBC + proteins and RBC + proteins can occur any where along the urinary tracts.
- Proteinuria in the absence of these cells could be renal or glomerular proteinuria that is due to:
    - acute glomerulonephritis
    - pyelonephritis
    - Amyloidosis
- These are marked by severe proteinuria that is dominated by albumin and indicate altered permeability of glomeruli
- It could also be due to tubular proteinuria that is characterized by:

✓ mild to moderate proteinuria that is dominated by globulins or

✓ post renal proteinuria that results from:

- cystitis
- prostatitis
- urethritis
- urolithiasis

- **Glucose Assay**

- Normally no glucose is found in the urine.
- However, if the load in the blood exceeds the renal threshold, glucose may appear in the urine.
- Tubular damages and degenerations can also cause leakage of glucose.
- Several methods are available to assay glucose in the urine:

Benedicts test

- **Method: Qualitative method**
- **Principle: Reducing sugars** present in the urine **react** with the **copper sulphate to reduce the copric ions to cuprous oxide giving a** color change depending on the amount of reducing substances present.
- **Reagent used**
- Benedict's reagent: Sodium sulphate ----------173g
- Sodium carbonate ---------- 100g
- Copper sulphate ---------- 17.3g
- Distilled water ---------- 1000 liter

Procedure

- ✓ Place 4ml of benedict's solution in a test tube
- ✓ Add 1ml of urine and mix very well
- ✓ Place the test tube in boiling **water for 5min** and observe the color change.

Result

- Negative ------ blue
- + ---------- trace light (light green)
- ++ ---------- green (significant)
- +++ ---------- moderate orange to yellow
- ++++ ---------- brick red (high concentration, serious)

- **Colorimetric strips**

- The colorimetric strips contain **glucose oxidase**, **peroxidase** and **indicator**.
- When the strips are immersed into the urine specimens if present in the urine glucose is oxidized by the enzyme,
- **glucose oxidase** to yield gluconic acid and hydrogen peroxide.
- The hydrogen peroxide is hydrolyzed by peroxidase to release free oxygen.
- The **oxygen radicals** released oxidizes the indicator in the strips and cause change of color.
- The presence of glucose is marked by development of **blue color.**

►The presence of glucose in the urine is could be due to:

- tubular damage
- enterotoxaemia in sheep
- hyperglycemia / diabetes in pets

Acetone (Ketone body) Assay

- Ketone bodies are results of **accelerated** break down of lipids and fatty acids as an energy sources.
- In canine ketonemia is seen frequently in malnourished dogs and in advanced cases of diabetes.
- In ruminants it occurs in ketosis.
- The determination of ketone bodies in the urine can be done by:

Ross test

- **Principle:** Sodium nitroprusside decomposed to sodium ferrocyanide, sodium nitrite and ferric hydroxide in an alkaline solution.
- These products are oxidizing agents that form a complex having **a purple color** in the presence of di – acetic acid and acetone

Reagent

- It consists of a powdered mixture of: Sodium nitroprusside ---------- 1 part
- Ammonium sulphate ---------- 100 parts

Procedure

✓ Place half of the powdered reagent in a dry test tube

- ✓ Add 5ml of urine to the tube and agitate
- ✓ Add 1ml to 2ml of conc. ammonium hydroxide (NH4OH) to form a layer above the mixture
  - If ketone bodies are present, a **purple to black** color will appear.
  - The **intensity of the color** is dependent up on the amount of the ketone bodies.

Result

- Trace ---------- very slight purple color
- + ---------- slight purple color
- ++ ---------- moderate purple color
- +++ ---------- dark purple color
- ++++ ---------- dark purple color or black

Ketostix (deep stick) method

- Routinely used method; the reagents and principle of the test is similar to that of the Ross test.

►Ketonuria indicates:

In pets: diabetes

Starvation

In herbivores: ketosis

Prolonged anorexia

Hypoglycemia

Microscopic Examination of Urine Sample

- It is of great clinical importance and should never been omitted.
- Examination of urine sediment for **casts**, **cells** (RBC, WBC, epithelial) and **bacteria**

Method

• Agitate the urine to suspend any sediment that may settled to bottom

• Fill the centrifuge tube with urine and centrifuge for 3min at low rate of speed

- Pour a drop of sediment on a glass slide, and cover with a cover glass
- Examine under microscope with the lower power objective

• Finding should be reported as few, many or abundant

• If necessary stain with new methylene blue.

- The presence of more than **5 WBC** may suggest:-
- ✓ Infection, pyelonephritis or inflammation of the genitourinary tract
- Large number of **RBCs** in the urine may be associated with **renal disease**, **lower urinary tract disease**, **external disease**, physiological causes including exercise.
- **Epithelial cells** increased in bladder inflammation

Activities

1. Why renal function test?
2. What are the tests used for assessing glomerular function?
3. Write the principle behind Roberts test?
4. Which test of serum level of waste substance is prefered to detect glomerular function.
5. Write the components of urialysis?

CHAPTER 2: CARDIOVASCULAR SYSTEM FUNCTION TESTS

- Oedema
- Extracellular fluid (CSF, transudate and exudate)

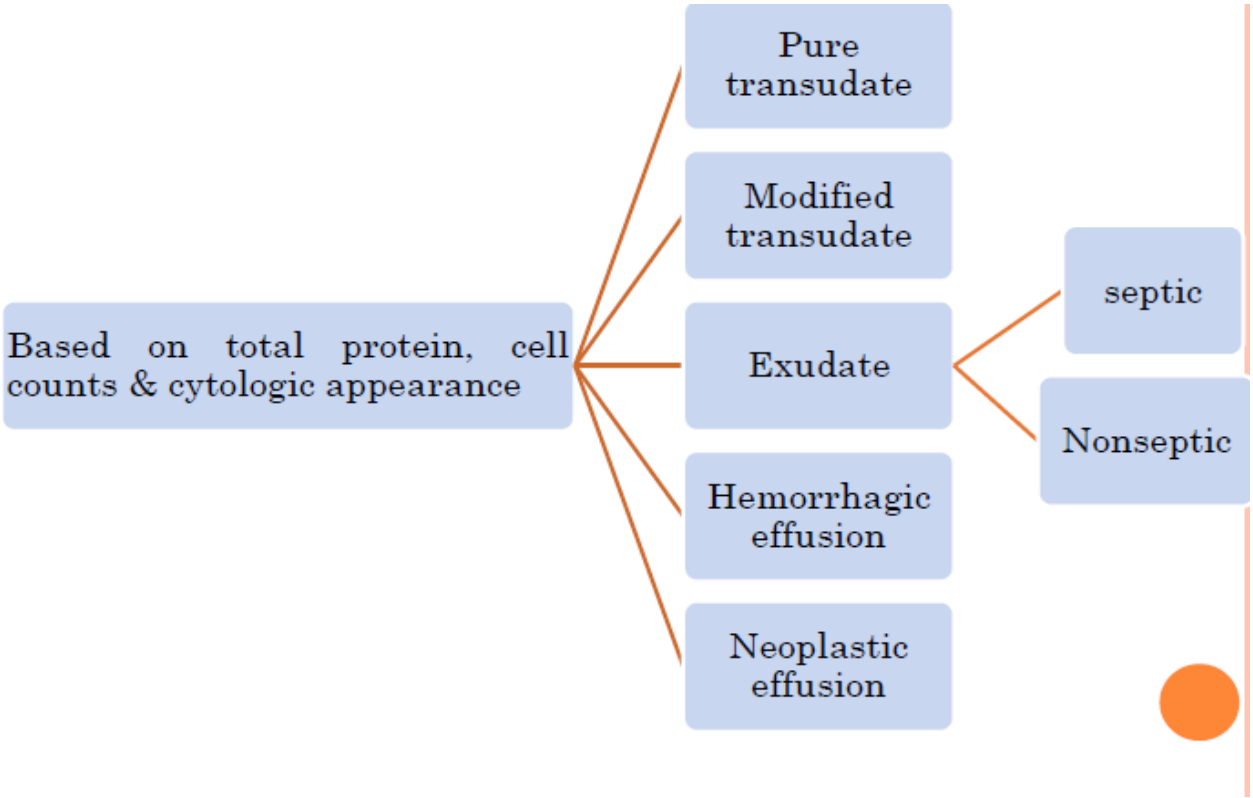
INTRODUCTION

- Small amount of fluid in body cavities for lubrication.
- **An effusion is** the abnormal or increased accumulation of this fluid in any of the body cavities that are lined by mesothelial cells.
- Thoracic, pericardial and abdominal cavities
- Effusions develop during trauma, neoplasia, cardiovascular problems, hypoalbumenia, infectious/inflammatory diseases
- Due to the major importance of albumin in retaining water in the plasma, **hypoalbuminaemic** patients are almost always oedematous and/or ascitic.
- Dietary deficiencies are seldom serious enough to cause major oedema unless **extreme malnutrition** has occurred, and so the three main places to look are **liver, kidney and gut**.
- Renal protein loss is easy to demonstrate on a urine sample.
- Liver failure can be demonstrated by liver function tests.

- Malabsorption/protein-losing enteropathy cases are usually at least intermittently diarrhoeic

Peritoneal and pleural fluids

- Some nine different types of fluid may be encountered in the abdominal cavity, each of which has its own characteristic **composition.**
- Thus, recording information about Gross appearance of the fluid when the sample is collected should be standard practice.
- Whenever ascitic or thoracic fluid is detected, a sample of the fluid should be drained off **for identification** into sterile universal bottles.
- Such fluids are in no danger of clotting and no anticoagulant is necessary.
- EDTA makes protein estimation impossible and does not preserve cells.
- It kills bacteria making the sample useless for culture.



True transudate

- This fluid forms as a result of severe hypoproteinaemia
- It accumulates in the **peritoneal** and **pleural** cavities.
- It is **clear**, **colorless** and **non-viscous**; may look just like tap of water.

- There is no change on centrifugation and little or no frothing when shaken.
- **Total protein:** Less than 25 g/l, with negligible albumin.
- **Total white cell count:** Less than 1x103/µl.
- **Cytology:** Often no cells at all, or a very few mesothelial cells.
- the two likely causes are **nephrotic syndrome** or **protein-losing enteropathy**

Modified transudate

- This fluid forms as a result of **impaired circulation.**
- Common causes of modified transudates include cardiac disease, neoplasms, hepatic disease, and FIP
- **Physical appearance**.

✓ Clear, perhaps slightly viscous, and either colourless or very slightly yellow-tinged.

✓ There is no change on centrifugation, but the fluid will froth when shaken.

- **Total protein:** Around 25 - 50 g/l, about half of which will be albumin.
- **Total white cell count:** (1-7) x 103/µl.
- **Cytology**. A few mesothelial cells, possibly a very few erythrocytes also, and the occasional neutrophil or lymphocyte.
- The two likely causes are congestive heart failure and liver disease.

➢ **Cardiac disease**

- Fluid accumulate in both the chest and the abdomen, and there may also be oedema in subcutaneous tissues.
- E.g. Right-sided congestive heart failure(RSHF) -> congestion in the systemic circulation. In the kidneys the increase in hydrostatic pressure is offset by the reduced flow of blood and urine output is reduced.
- The increased back pressure to the glomerulus causes increased permeability and escape of plasma protein into the urine.
- In ruminants there is subcutaneous edema occurring in the brisket region, under the jaw and along the ventral midline.
- Acute congestive heart failure occasionally produces a blood stained modified transudate due to leakage of erythrocytes from a congested spleen.

Hepatic disease

- Fluid accumulates only in the abdomen, as impaired local circulation is the main precipitating factor.
- E.g. Hepatic congestion due to RSHF results in an inevitable venous congestion in the portal system and is accompanied by impaired digestion and absorption and terminally by diarrhea.
- Low plasma albumin and high serum bile acid concentration as evidences of liver dysfunction.

Exudate

- Exudates develop because of ***increased vascular permeability caused by inflammation.***
- The fluid varies in color but is usually **turbid** because of high cell count.
- The nucleated cell count is usually greater than 5,000 to 7,000 cells/μL.
- Neutrophils are usually the predominant cells
- Numbers of other cell types vary according to the type and severity of the irritant and duration of the disease process.
- **Nonseptic exudates** are caused by irritants such as bile, urine, and pancreatic enzymes or because of tissue necrosis (e.g. neoplasms, lung lobe torsion).
- Neutrophils in such effusions are **non-degenerate**.
- **Septic exudates** are caused by microorganisms which may or may not be evident cytologically.
- Some bacteria produce toxins that cause *neutrophilic degeneration but many organisms do not.*
- Therefore, any exudate should be examined carefully for the presence of infectious agents and/or be cultured.

CHAPTER 3: CEREBROSPINAL FLUID EXAMINATION

- ❖ **Cerebrospinal fluid** (**CSF**)
- ❖ The CSF is essentially an ultra-filtrate of plasma.
- ✓ Clear, colorless bodily fluid found in the brain and spine
- ✓ It is produced in the choroid plexus of the brain:
- ▪ basic mechanical and immunological protection to the brain inside the skull, and

- it serves a vital function in cerebral autoregulation of cerebral blood flow

❖ **CSF serves several purposes**:

- **Protection**

✓ CSF protects the brain tissue from injury when jolted or hit.

✓ In certain situations such as auto accidents or sports injuries

- **Chemical stability**

✓ CSF flows throughout the inner ventricular system in the brain and is absorbed back into the bloodstream

✓ Rinsing the metabolic waste from the central nervous system through the blood–brain barrier_

✓ slight changes can cause problems or damage to the nervous system

❑ **Prevention of brain ischemia**

✓ The prevention of brain ischemia is made by decreasing the amount of CSF in the limited space inside the skull

✓ This decreases total intracranial pressure and facilitates blood perfusion

❑ **Clearing waste**

✓ plays an important role in flushing metabolic toxins or waste from the brain's tissues.

- Collection and evaluation of CSF is of most importance in investigation of D+
- diagnosis of various diseases with involvement of central nervous system (brain and spinal cord)
- now-a-days CSF is frequently examined for diagnostic purpose

✓ for understanding the **severity** and **nature** of the disease process involving the CNS.

❑ CSF analysis may be used to help diagnose a wide variety of diseases and conditions affecting the **central nervous system**

❑ **They may be divided into four main categories:**

✓ **Infectious diseases** such as meningitis and encephalitis testing is used to determine if infection is caused by

- Bacteria, viruses or, less commonly by tuberculosis, fungi or parasites, and
- to distinguish it from other conditions or
- to detect infections of or near the spinal cord or to investigate a fever of unknown origin.

✓ Bleeding (hemorrhaging) within the brain or skull

- ✓ Diseases that cause inflammation or other immune responses such as the production of antibodies—include:

- autoimmune disorders, such as Guillain-Barré syndrome or sarcoidosis, or diseases that cause the destruction of myelin, such as multiple sclerosis
- ✓ Tumors located within the central nervous system (primary) or that spread to the central nervous system (metastatic cancer)

❖ **When is it ordered?**

- CSF analysis may be ordered when a condition or disease involving central nervous system
- when the animal has suffered trauma to the brain or spinal cord
- cancer that may have spread into the central nervous system or has signs or symptoms suggestive of central nervous system involvement.

❖ **Collection of CSF**

- For collection of CSF the material required:
- 3 inch and 16 G spinal needle with a stylet, cotton swab, sterilized test tubes, sharp razor etc
- sterilized condition and aseptic precautions should be taken during collection.

❖ **Methods of collection**

- The CSF is collected from two sites:

A. **Cisterna magna** or **atlanto-occipital puncture** in:

- horse, cat and dog.

B. **Sub lumbar** or **lumbosacral** puncture in:

- cow, sheep and goat

❖ **A. Atlanto occipital puncture:**

- The collection can be done either in the standing position or in the **lateral recumbency** with following steps,

1. Restrain the animal, casting with rope.
2. Sedation of the animal by giving local anesthesia
   - such as 2% Xylocain, Lignocain and tranquilization with Siquil or Largactil.

3. Flex the head by bending and stretching the neck, so that it forms a 900 angle with the longitudinal axis of the neck and hold it in this position firmly.

4. Clip, clean and sterilize the selected area.

5. Use a 3-4” long and 16 G spinal needle with a stylet and insert it slowly at the cranial edge

- Needle enters into atlanto-occipital joint, remove the stylet so that the CSF flows out and collect approximately 1 to 2 ml of CSF.

❖ **Sub lumbar puncture:**

✓ The collection is done in the standing position

1.Presurgical and aseptic precautions are taken

- the depression between the dorsal process of last lumbar vertebra and cephalic end of median crest of sacrum is palpated.
- Needle is passed and punctured at this site, insert the needle vertically, then slightly oblique by applying gradual pressure in forward and
- backward directions as the needle enters in subarachnoid space comparatively less resistance is felt.

2. Animal must be tied firmly to avoid damage to

the spinal cord.

- CSF collection is done by removing the stylet, apply a syringe and suck the fluid.

❖ **Examination of CSF**

- The CSF is examined for following tests

I. Physical examination

II. Chemical examination

Table 1. Normal cerebrospinal fluid analysis findings

Parameter	Findings
Colour and clarity	Clear and transparent
Total protein content	Cisternal tap: < 25 mg/100ml Lumbar tap: < 45 mg/100ml
Total cell count	Erythrocytes: 0/µl Leukocytes: < 5/µl
Differential cell count	Lymphocytes: 60-70% Monocytes: 30-40% Segmented white blood cells: <1%

❖ **Physical examination of CSF**

➢ **Colour**

✓ Clear, watery and transparent → Normal

✓ Red → Puncture of blood vessel during collection or heamorrhage

✓ Dull red / brownish → Intracranial hemorrhage, cranium fracture

✓ Yellow (xanthochromic) → Presence of bile pigments (jaundice) or subarachnoid hemorrhage in the absence of jaundice.

✓ Toxoplasmosis and cryptococcosis

✓ Grayish or greenish → Due to infection leading to pus formation

❖ **Turbidity**

✓ Clear, transparent → Normal

✓ Hazy, ground glass like → Presence of cells/white clots appearance (pleocytosis)

✓ Cloudy/purulent → Encephalitis, bacterial meningitis.

✓ Red turbid → Puncture of blood vessel during collection.

- ❖ **Coagulation**
- ✓ No coagulation → Normal
- ✓ Coagulation → Presence of abnormal amount of proteins especially fibrinogen in cases of meningitis
- ✓ Blood (in large quantities) → Internal hemorrhage or improper collection.

Chemical examination

a. Proteins: Normal protein content of CSF is 12-40 mg/100 ml and most of it is albumin.

b. Glucose

- The concentration of the glucose in CSF is approximately 60-70 % of blood glucose level and ranges from 40-80 mg/100 ml in normal CSF
- ❖ The glucose level in CSF depends upon the

1. Blood glucose levels,
2. Selective permeability of the blood to CSF barrier,
3. Presence or absence of glycolytic barrier

- An increased glucose level in the CSF is termed as "**hyper glycorrhacia"** and is seen in association with any disease having a hyperglycemia (Diabetes mellitus),
- encephalitis, spinal cord compression, brain tumors or brain abscess.
- ❑ A decreased glucose level in the CSF is termed as 'hypo glycorrhacia' and is associated with systemic hypoglycemia or acute pyogenic infection.

C. Chlorides: normal CSF values in domestic animals

- ranges between 650-850 mg/100ml.
- ❖ Lower values are seen in pyogenic meningitis, protracted vomiting, advanced pneumonia
- normally higher values of chlorides in CSF are recorded than in serum.

d. Sodium: Slightly higher in CSF than in blood in salt poisoning cases.

E. Cholesterol:

- ✓ Hemorrhages in the CNS, tumors**,** meningitis and brain abscess lead to an increase in cholesterol content.
- ✓ Usually normal cholesterol level is very low and values recorded in Horse: 0.36 - 0.55 mg/dl and Goat: 0.51 mg/dl.
- ❑ ***Diseases and Conditions That Cause Hypercholesterolemia***
- ➢ **Increased Cholesterol Production**

- *By hepatocytes:*
- Nephrotic syndrome or protein-losing nephropathy
- *By enterocytes:*

➢ **Decreased Lipolysis or Intravascular Processing of Lipoproteins**

- Hypothyroidism
- Nephrotic syndrome or protein-losing nephropathy

F. Determination of enzymes

- Enzymes don't normally cross the blood brain barrier and so elevated CSF levels suggest tissue damage in the CNS.
- Increased levels of CSF GPT (ALT): 20.1(9-46 unit) and GOT (AST): 13.7 (2-32 units)

✓ observed in dogs suffering from **distemper** with involvement of CNS, **purulent meningitis** and **cerebral infarction.**

❖ **Lactic dehydrogenase**

- Enzyme level of CSF are also increased in bacterial meningitis, metastatic carcinoma, lymphoid tumor, subarachnoid hemorrhage and cerebral infarction.
- A marked elevation in the CPK (creatinine phosphokinase) is also seen in certain neurological conditions.

G. Calcium: Normally the calcium is lower in CSF than in serum.

- Increased level of protein bound calcium in CSF indicates disturbance in blood brain barrier.

❖ **Chemical Examination of CSF**

❑ **Foam test**

✓ Take CSF in test tube and shake the test tube at least for 5 minutes

❑ **Observation**

✓ Slight foam that disappears after few minutes;

✓ more foam that remains Protein level Increased

❑ **Inference**

✓ Normal Protein levels

❖ **Sulfosalicylic acid test (SSA)**

✓ 3 ml of 3% SSA + 1 ml CSF mix and allow to Stand

❑ **Observation**

✓ Increase in the turbidity

❑ **Inference**

✓ Presence of proteins

❖ **Nonne - Apelt test**

✓ 1 ml saturated ammonia solution + 1 ml CSF Do not mix. Allow to stand

❑ **Observation**

✓ White to grayish ring at the junction of two fluids

❑ **Inference**

✓ ↑***globuli*** in CSF seen in:

- Encephalitis, Meningitis, Neoplasia, Hemorrhage, Hydrocephalus, tissue destruction, Uremia, Toxoplasmosis, Pneumonia

❖ **Cytological examination**

- The total cell counts of the CSF must be estimated
- With in **20 minutes** of collection, since the cells degenerate rapidly.
- The estimation of the number of cells is done as for the determination of WBC's of the blood.
- The total number of cells which are obtained are then multiplied by 0.6 to get number of cells in one cu mm of the CSF.

✓ **Normal counts**:

- Cattle, sheep and pig 0 - 15 cells/ cu mm
- Dog upto 25 cells/cu mm
- Horse upto 23 cells/cu mm
- **Pleocytosis** or increased number of WBC's are seen in inflammatory conditions brain, spinal cord or meninges, abscess of brain or

✓ spinal cord, encephalitis, chronic inflammatory conditions, toxic or degenerative conditions

❖ **Differential Count:**

✓ Prepare the smear from CSF, dry it and stain

✓ Examine under Microscope

- neutrophilia indicates pyogenic or bacterial infection,
- abscesses in brain bacterial meningitis, encephalitis and hemorrhage

- ✓ lymphocytosis is observed in uremia, toxemia, chronic viral and fungal infection.

❖ **Bacteriological examination**

- ✓ It is carried out when the CSF cell count and protein contents are high
- ✓ The organisms are isolated in CSF and identified by cultural methods

❑ **General consideration**

- location to use for CSF Selection is based on the neurologic localization of the lesion.
- The atlanto occipital space (cerebello medullary cistern) is used to collect CSF
- when the lesions are above the **foramen magnum** or
- for lesions of the **extreme craniocervical spinal cord.**
- The lumbar subarachnoid space,L5-6, is used as the site of CSF collection for lesions **below** the **craniocervical spinal cord.**
- CSF collection is contraindicated when anesthesia is contraindicated and
- in animals with increased intracranial pressure (ICP) **because of the risk of brain herniation**
- Cell counts and cytology smears should be done within 30 to 60 minutes of CSF collection
- The low protein concentration of CSF causes the cells to lyse quickly
- If the CSF sample cannot be processed within 30 minutes:
- CSF cytology is being evaluated in most practitioners make concentrated smears by sedimentation techniques.

➢ **List some causes of a neutrophilic pleocytosis**

- Necrotizing vasculitis
- Infection
- Intervertebral disk disease
- Spinal trauma
- Necrosis
- Neoplasia (primarily meningioma)

➢ **List some causes of a mononuclear pleocytosis.**

- Canine distemper virus
- Granulomatous meningoencephalitis (GME)
- Necrotizing meningoencephalitis

- *Ehrlichia canis*
- Toxoplasmosis
- Neosporosis
- Bacterial meningitis after treatment with antibiotics

➢ **causes of a mixed pleocytosis?**

- granulomatous meningoencephalitis (GME) and
- many of the same diseases that cause a neutrophilic pleocytosis

Inflammatory conditions: invariably significant increase in **total cell count** and **protein**

❖ In bacterial infections
  - neutrophils are the predominant cell, CSF glucose **concentration is extremely low** and the fluid is usually **turbid**.

❖ In viral infections
  - mononuclear cells predominate, glucose is normal and CSF **AST** may be elevated.

❖ Toxoplasmosis and cryptococcosis are both usually associated with xanthochromic CSF.

Neoplastic conditions

❖ Total protein is elevated but **normal cell count** (so-called `albuminocytological dissociation') because tumour cells rarely escape into the CSF.

❖ slightly turbid or xanthochromic CSF(rarely).

- **Traumatic damage**

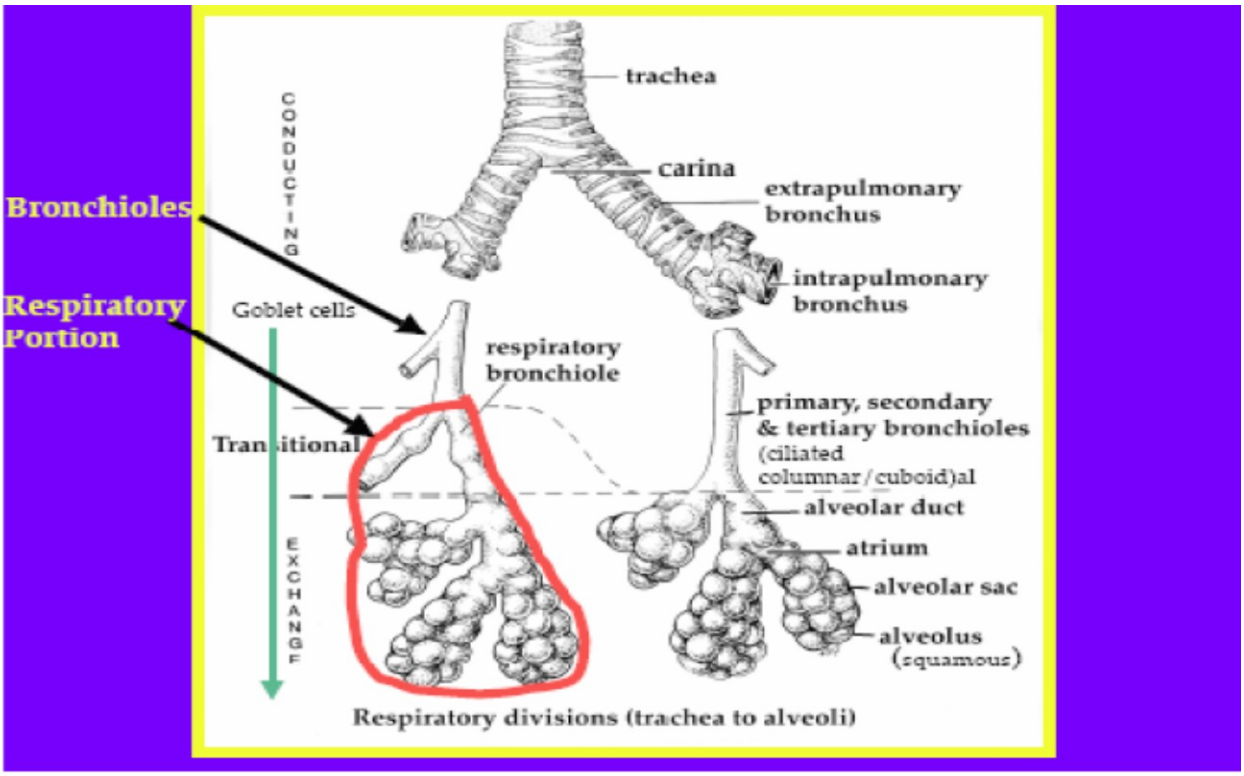
❖ Sub-arachnoid haemorrhage is associated with **increased protein** and moderately increased cell count (mostly neutrophils), and particularly with markedly xanthochromic CSF.

- However, in very recent haemorrhage (< 48 hrs old), **whole blood** may be evident.

Hydrocephalus

- Associated with increased CSF CK and LDH activities.

CHAPTER 4: RESPIRATORY SYSTEM FUNCTION TESTS



Respiratory failure

- The **respiratory failure** that occurs in animals with pneumonia, pulmonary edema and upper respiratory tract obstruction is caused by
- combinations of hypoventilation, ventilation/perfusion mismatch and diffusion impairment, which leads to ***hypercapnia*** *and* ***hypoxemia*****.**
- Characteristics of respiratory disease that lead to **dyspnea** or **lack of exercise tolerance** include:
- Flooding of alveoli with inflammatory cells and/or protein-rich fluid - **pneumonia and pulmonary edema**
- **Atelectasis** (collapsed alveoli and small airways) - pleural effusion, hemothorax, hydrothorax, pneumothorax, chylothorax, pyothorax, prolonged recumbency of large animals and diaphragmatic hernia.
- **Airway obstruction** -nasal obstruction, pharyngeal/ laryngeal obstruction, tracheal, bronchial obstruction, bronchoconstriction and bronchiolar obstruction.

Pulmonary edema

- Pulmonary edema as a sequel to pulmonary capillary hypertension or pulmonary microvascular damage occurs.
- e.g. in: Acute pneumonia (*Pasteurella haemolytica)*; Congestive heart failure.
- In large animals, pulmonary edema is usually limited to **acute heart failure**;
- hence bronchial capillary congestion and edema result in encroachment on airways and a decrease in ventilator efficiency.

➢ **Left sided heart failure** causes

✓ an increase in left ventricular end diastolic pressure, mean left atrial pressure and

✓ pulmonary venous pressure, interstitial edema in the lungs and,

✓ **if hydrostatic pressure is severe** enough,

✓ the net force for filtration of fluid across the pulmonary capillary bed is greatly increased and

✓ result in pulmonary edema, with the presence of fluid around the septal vessels and in the alveolar spaces

✓ accompanied by marked impairment of **gas exchange**, **dyspnea** and **death.**

Causes of Pulmonary edema

❖ *Hemodynamic edema (Transudate)*

--Increased hydrostatic pressure

- Congestive heart failure (cardiogenic edema)
- Impaired lymphatic drainage (tumor invasion of lymph vessel)

❖ Decreased osmotic pressure in blood (hypoalbuminemia)

- Hepatic disease/Nephritic syndrome/protein loss enteropathy

Permeability edema (exudate)

❖ Damage to the blood air barrier (type I and endothelium)

- Pneumotraphic viruses (influenza, BRSV)/Toxicants (NO2, SO2, H2S)/Free radicals
- Bacterial toxins (endotoxin)

❖ Excessive opening of endothelial gap

- During inflammatory response (inflammatory mediators)

- **Clinical Pathology of oedema**

- Laboratory examinations are of value only in differentiating the causes of the congestion or edema.
- Bacteriological examination of nasal swabs and a complete hematological examination (particularly for the presence of eosinophilia) are the standard examinations.

EMPHYSEMA

- Pulmonary emphysema is distension of the lung caused by over-distension of alveoli with **rupture of alveolar walls** with or without escape of air into the interstitial spaces.
- It is a common clinicopathological finding in **many diseases** of the lungs of **all species** and
- is characterized clinically by **dyspnea**, **hyperpnea**, poor exercise tolerance and forced expiration.
- The dyspnea of pulmonary emphysema is characteristically **expiratory in form** and is caused by anoxic anoxia and the need for forced expiration to achieve successful expulsion of the tidal air.
- It is commonly accompanied by an **audible expiratory grunt** in ruminants but less so in pigs and almost never in horses.
- **Clinical Pathology of Emphysema:**
    - Compensatory polycythemia may develop.
    - There are no characteristic hematological findings but, a leukocytosis and left shift may be evident if there is a significant **secondary bronchopneumonia**
    - An examination of feces for lungworm larvae may be desirable.
- In cases suspected of having an **allergic origin**, swabs of nasal secretion may reveal a high proportion of **eosinophils** and a hematological examination may show **eosinophilia.**

Atelectasis (incomplete expansion of lung or portion of lung)

- Atelectasis is collapse of the alveoli due to failure of the alveoli to inflate or because of alveolar compression.
- It is classified as obstruction (resorption), compression or contraction.
- ***Obstruction atelectasis*** occurs secondary to obstruction of the airways, with subsequent resorption of alveolar gases and collapse of the alveoli by fluid and exudates

- eg. pneumonia or aspiration of a foreign body.

❖ Airway obstruction such as exudate, parasites, food particles

- ***Compression atelectasis*** occurs when intrathoracic (intrapleural) pressure exceeds alveolar pressure, thereby deflating alveoli.

E.g. excessive pleural fluid or pneumothorax & in anesthetized horses.

❖ Large intra-thoracic masses such as **abscesses**, **tumors**, **pericarditis**, and **external trauma**.

❖ Collapsed alveoli, loss of alveolar spaces

- ***Contraction atelectasis*** occurs due to compression of parts of the lung by fibrotic changes in the pleura.

CHAPTER 5: ENDOCRINE SYSTEM FUNCTION TEST

The adrenal cortex

- The main hormone of diagnostic value is **cortisol,**
- secreted by the adrenal cortex in response to adrenocorticotrophic hormone (ACTH) produced by the pituitary gland.
- Overproduction of cortisol (**Cushing's disease**) can be due to
- tumour of the adrenal cortex itself (**20% of canine cases**) or to an ACTH-producing pituitary tumour (**80% of canine cases).**
- Idiopathic Cushing's disease is rare in the cat and usually **pituitary in origin.**
- In the horse, Cushing's is a disease of **elderly's** (usually ponies) and is invariably pituitary in origin.
- Underproduction of cortisol (**Addison's disease**) is confined to the dog, and is a result of **autoimmune destruction of the adrenal cortex.**

Hyperadrenocorticism, Cushing's disease

- chronic condition

Clinical Signs / Lesions

- due to combined gluconeogenic, lipolytic, protein catabolic & anti inflammatory / immunosuppressive effects.
- polyuria / polydipsia/ polyphagia

- hepatomegaly/ pendulous abdomen
- skin lesions
- susceptibility to bacterial infections
- **Others:** hypercoagulability, eosinopenia, lymphopenia / lymphoid involution
- It may involve three or four investigatory steps.
- The first step is based on the combined pattern of clinical and routine biochemistry and haematology results.
- **Measuring a single cortisol level** for diagnosis, does not suffice.
- Thus **dynamic cortisol testing** (ie. stimulation or suppression with exogenous hormones) is necessary to confirm or deny the diagnosis.
- In diagnosing suspected Cushing's case, assess all the results of

✓ clinical findings,
✓ routine biochemistry and
✓ haematology and
✓ Finally ACTH response and Treatment response evidence is necessary.
✓ If the ACTH stimulation results are inconclusive, **a low dose dexamethasone test** should be carried out.

ACTH stimulation test

- quicker and cheaper than the dexamethasone screening test, rare false positive result (which is dangerous)
- used to monitor Cushing's cases on treatment (dexamethasone response is unhelpful for that purpose)

➢ **ACTH stimulation test protocol**

(1) Collect baseline blood sample

(2) Inject one vial (250 mg) of tetracosactide (1/2 for cat & dog <5kg), IV

(3) Wait for the peak in cortisol which is closer to 90 minutes and Collect the 2nd blood sample.

Interpretation

- Normal dog the baseline result is usually around **100±300 nmol/l**, rising to <u>less than 500 nmol/l</u> post-ACTH.

- An increase to **over 1000 nmol/l** is a conclusively positive result, consistent with **pituitary-dependent Cushing's.**
- Between 500 and 1000 nmol/l post-ACTH the probability of Cushing's increases ± 500±600 nmol/l is **still unlikely** and requires further investigation to confirm;
- 600±700 nmol/l is possible but depends very much on the **clinical context**;
- 700±800 nmol/l is **quite likely**, but again one would consider it in relation to other findings;
- anything over 800 nmol/l can **reasonably be considered diagnostic** in an **unstressed patient**, in the presence of typical **clinical** and **routine biochemistry** and **haematology results.**
- **Adrenal Cushing's** is often associated with a **different pattern** of ACTH response.
- The baseline result may be elevated **above 400 nmol/l**, while there is much **less of an increase post-ACTH**.
- Unless the values are **quite high**, however, it is usually preferable to confirm these cases
- by the **dexamethasone suppression test** or **by Abdominal ultrasound**.
- A small proportion of Cushing's cases (**usually adrenal tumors**) show **a normal** ACTH response;
- thus in a very suspicious clinical situation a negative result from an ACTH stimulation test should be confirmed by **dexamethasone screening test.**

The dexamethasone screening test (low dose)

- This is the **next shot** if further investigation is necessary.
- In a normal animal a very low dose of **dexamethasone** can depress cortisol secretion effectively and **keep it depressed for over 8 hours**.
- In Cushing's disease the cortisol either doesn't suppress at all (esp. **adrenal tumors**) or bounces back in less than 8 hours (p**ituitary-dependent**).
- **Interpretation**: An 8-hour result of greater than 40 nmol/l is consistent with Cushing's.

High-dose dexamethasone suppression test protocol

- This differentiates the causes **(**specifically as **pituitary** or **adernal).**
- It is identical to low-dose screening test, but the dose of dexamethasone is **ten times** higher (0.1 mg/kg).

- This exploits the fact that the **higher dose** will **feed back** and **suppress ACTH secretion** from a pituitary lesion at least to some extent, but will have **little** or **no effect** on secretion from an adrenal lesion.
- **Steroid-induced alkaline phosphatase**
- Prolonged exposure to **high levels of corticosteroids** induces the canine liver to produce an isoenzyme of ALP which is different from the usual isoenzymes found in the serum.
- A method exists to detect this particular isoenzyme, and helpful in investigating possible Cushing's cases.
- But **not sensitive** or **specific** i.e. have no advantage over cortisol testing.

ACTH measurement

- This differentiates between **pituitary** and **adrenal Cushing's** cases
- **pituitary cushing's** have abnormally high ACTH, while **adrenal Cushing's** have abnormally low ACTH.
- **Limitation:** requires special transport of **frozen samples** makes it **impractical for routine use.**
- **Cushing's disease in the horse**
- Equine Cushing's disease is a **pituitary condition.**
- Most cases progress to diabetes mellitus, and Cushing's is practically the only cause of **diabetes mellitus** in horse.
- Therefore, in the absence of **recent exercise**, **stress**, **excitement** or a **concentrate feed**, **hyperglycaemia** is essentially **pathognomonic** for the condition.
- To investigate **the non-diabetic equine Cushing's,** the most satisfactory technique is probably the overnight (19-hour) ***dexamethasone suppression test.***

Overnight dexamethasone suppression test protocol

(1) Collect baseline blood sample.

(2) Inject soluble dexamethasone at a dose rate of **20 mg** for a 500 kg horse (0.04 mg/kg), either intravenously or intramuscularly.

(3) Collect second blood sample after 19 hours.

Interpretation

- In the normal horse, as in the dog, the baseline result is usu**ally around 100±300 nmol/l**, and this will fall to below 40 nmol/l by the second sample.

- A 19-hour result greater than **40 nmol/l** is considered to be consistent with Cushing's, if the patient **is unstressed** during the period of the test.

Hypoadrenocorticism, Addison's disease

- Lack of aldosterone causes massive renal losses of sodium and water, together with retention of potassium, with resulting **dehydration**, **hyponatraemia** and **hyperkalaemia.**
- The condition is essentially confined to the **dog**.
- The diagnosis is obvious from the **routine biochemistry** (Na and K) and h**aematology results.**
- A **normal ACTH response** will tend to rule out Addison's
- The finding of marked **hyponatraemia** and **hyperkalaemia** in the absence of severe renal dysfunction is virtually **pathognomonic** for Addison's disease and important to initiate treatment.
- Hormone investigations are required in less acute presentation or doubt.
- Although **the main clinical signs of Addison's disease** are due to **hypoaldosteronism** rather than low **glucocorticoid** levels, for diagnostic purposes it is **cortisol** which is measured.
- A **single random cortisol** is conclusive in ruling out Addison's if the result is **normal**, > 150 nmol/l.
- A very low cortisol (< **20 nmol/l)** from a sample taken before any corticosteroids are very suggestive of **Addison's.**
- For the most reliable confirmation of the diagnosis,
- an **ACTH stimulation** test is preferable if cortisol is still undetectable there is really no argument

The thyroid gland

- Secretes thyroxine (T_4) and tri-iodo-thyronine (T_3).
- Secretion is controlled by thyroid stimulating hormone (TSH) and thyrotrophin releasing hormone (TRH).
- T_3 is the more physiologically active hormone; however, it is T_4 (in different forms) which is the usual routine diagnostic measurement.

Hyperthyroidism

- due to **adenoma of the thyroid gland** (often bilateral).
- Carcinomas are uncommon.
- Most hyperthyroid are cats over the age of 10 years, sporadically in younger /5 years old cats &
- much less common in the older animals, dog.
- Thyroid carcinomas (malignant) are the more common cause of **canine hyperthyroidism**.

❖ **Presenting problem**

❖ Weight loss, polyphagia, polyuria, alopecia, GIT & nerve signs ***(T3/T4 → ↑metabolic rate, O2 consumption, heart rate, catabolism of muscle/adipocytes, erythropoiesis)***

Laboratory data

❖ Erythrocytosis; ↑ALT, ALP, CK; azotemia, hyperglycemia

❖ **1. Confirmation of hyperthyroidism**, especially in the cat, is done with a single elevated **total T_4measurement.**

❖ In the old cat, > **50 nmol/l is conclusive**, with values between 40 and 50 nmol/l regarded as highly **suspicious** in conjunction with a typical hyperthyroid pattern on routine biochemistry results.

❖ NB. T_4levels of up to **70 nmol/l** have been recorded in normal young cats, and that affects interpreting results from this test.

2. **Free T_4 measurement** can also be used as a diagnostic test for hyperthyroidism (if > 20 nmol/l).

- **Total T_4** easy to interpret and is regarded as the test of choice.

3. **T_3 suppression test** may be employed.

- In the **Euthyroid** (normal) animal, exogenous thyroid hormone will suppress **secretion of endogenous hormones** by negative feedback to the pituitary, while in the h**yperthyroid** animal hormone secretion continues regardless.
- Tri-iodothy-ronine (Tertroxin: Goldshield) is employed because there is no back-conversion in vivo of T_3 to T_4, and T_3does not interfere with T_4measurement.
- In the **dog**, while again a high T_4concentration is **diagnostic** in conjunction with **the right clinical signs**, **measurement of T_3** is can improve diagnostic accuracy.

- However, since the vast majority of cases have **palpable lumps** in the neck, **histopathology** of a biopsy is the best.

Hypothyroidism

- This is primarily a condition of the **dog** (>4years), and it is rare in other **species**.
- Iodine deficiency causing **goitre** and **hypothyroidism** has been recorded in horses.
- An **autoimmune aetiology** is postulated.
- The preferred **initial screening test is free T_4** in conjunction with **cholesterol**.
- Failing that, **total T_4**, again plus **cholesterol**, may be used.
- while **free $T_4$is** preferred if available, a properly validated **total T_4 method** is preferable to a free T_4method which is not validated for dogs.
- The cholesterol is included as an aid to decision-making in the inconclusive range.
- T_4 results **> 25 nmol/l** suggest, dog is almost certainly euthyroid, especially if the cholesterol is normal,
- but about **30%** of hypothyroid dogs have normal or only **slightly elevated cholesterol concentrations**, so a normal cholesterol result never excludes hypothyroidism.
- **Dynamic testing** (**TSH or TRH stimulation tests**) is considered the most **definitive approach**, but other methods are available and may be helpful in certain circumstances.
- **Measurement of endogenous TSH** is the test of choice for the diagnosis of hypothyroidism in man.

Thyroglobulin autoantibodies

- canine hypothyroidism is almost invariably of **autoimmune aetiology,** and it might be expected that a test for the appropriate antibodies would provide a definite answer.
- However, the usefulness of this approach depends very much on the **age of the patient** due to decay of humoral antibody with time.
- A **positive result** is an extremely strong pointer to hypothyroidism, but as `**false' negatives** can occur at any age,
- THUS, a **negative result** is of little value in excluding the condition.

Sex hormones

- Measurement of sex hormones is generally of benefit in **a few specific situations**

➢ **Oestradiol**

- This is the main biologically active **oestrogen**, and the one measured in clinical situations.
- The two situations where this measurement is helpful are **suspected Sertoli cell tumour in the male dog** and suspected residual **ovarian tissue in a supposedly spayed bitch or cat.**
- The Sertoli cell of the testis secretes oestradiol, and abnormally high levels (over about 200 nmol/l) are seen in most cases of Sertoli cell tumour where feminization is evident.

Receptal stimulation test

- If spayed bitch is showing signs of possible oestrus.
- However, if ovarian tissue is pre-stimulated by buserelin (a synthetic FSH/LH analogue, Receptal: Intervet), it will produce measurable oestradiol in circulation within 60±90 minutes after i/v injection.
- The dose rate given is `0.32 mg, based on a body weight of 12 to 15 kg
- **Limitation:** while a high level is **positive proof** that ovarian tissue is present, a low level does not conclusively rule it out.
- Recently, hCG (human chorionic gonadotrophin, Chorulon: Intervet, also an FSH/LH preparation) may be substituted for buserelin, at a dose rate of 20 iu/kg.
- A single blood sample is collected 60±90 minutes after injection, and
- an oestradiol measurement of greater than **20** pmol/l is indicative of the presence of ovarian tissue.
- **Entire bitches** generally show levels greater than 50 pmol/l.

➢ **Progesterone**

- The only routine **indication** for progesterone measurement is when it is necessary to demonstrate the presence of a **functional corpus luteum**.

Ovucheck Premate' test

- This is a **semiquantitative ELISA kit test** for progesterone in the serum of bitches, which is useful for detecting the time of **ovulation** and so helps achieve the optimum timing of mating.
- The kit produces a colour change which can be read by eye.

Testosterone

- This test is primarily useful in determining whether a male is **castrate** or **cryptorchid.**

- a single random sample is inappropriate, as a **low level** does not conclusively rule out the presence of testicular tissue, and a stimulation test is necessary.
- **The procedure (in all species)** is to inject human chorionic gonadotrophin (hCG) intravenously at a dose rate of 20 iu/kg, and sample 90 minutes later.
- In castrated males the testosterone level will remain very low (less than about 0.5 nmol/l),
- while males with one or more retained testes generally show much higher levels, certainly over 1 nmol/l and usually considerably higher than this.
- Elevated testosterone levels are also found in **mares** with granulosa cell tumours of the ovary.

7. **METABOLIC DISORDERS (CALCIUM AND PHOSPHATE)**

- Calcium and phosphate are also important **structural elements**, especially in bone.
- **correct dietary levels** are very important in maintaining correct mineral balance and
- **dietary deficiencies** are very commonly the underlying cause of mineral problems.
- The most important regulation of calcium/phosphate metabolism is by **parathyroid hormone (PTH)** and **vitamin D**, and **calcitonin.**

6.1. Calcium

- Calcium is of major importance in transmission at the **neuromuscular junction** and in the propagation of the contraction impulse within the muscle.
- It is also a major component of bone.
- About half of the plasma calcium is **free**, and this is the **active** proportion, while the other half is inactive, bound to albumin.

Increase (hypercalcaemia)

- This is **much less common** than hypocalcaemia.
- Probably the commonest cause of marked hypercalcaemia seen in veterinary practice is the **over-enthusiastic treatment** of hypocalcaemic patients with calcium borogluconate.
- genuine hypercalcaemia is almost always due to some type of **hyperparathyroidism**
- **Primary hyperparathyroidism**, usually due to an **adenoma** of the parathyroid gland, and
- primary pseudohyperparathyroidism, due to production of PTH by a tumour of a completely different tissue, are clinically very similar.

- The commonest cause of primary pseudohyperparathyroidism is a **perianal adenocarcinoma.**
- In addition, **a tumour** (such as a mammary carcinoma) extensively invading the skeleton with widespread bone lysis can lead to hypercalcaemia.
- Rarely, hypercalcaemia is also seen in cases of **severe thyrotoxicosis.**
- Once hypercalcaemia has been established, **radiographic search for the tumour** is usually the most productive investigation.
- Main signs are polydipsia/polyuria
- **Constipation** and **abdominal pain** (due to depressed neuromuscular excitability), ECG changes and in extreme cases cardiac arrest.

Decrease (hypocalcaemia)

- This is the **commoner encountered calcium abnormality**, and may have several causes.
- **Hypoalbuminaemia**
- When plasma albumin is low, the protein-bound calcium fraction decreases.
- Hypocalcaemia is ***only moderate***, and when the **free active** calcium concentration is unaffected this is **asymptomatic.**
- However, where the hypoalbuminaemia is due to ***albumin loss***, loss of calcium bound to this albumin may lead to development of a genuine, **symptomatic hypocalcaemia** over a period of time.

Parturient paresis

- Milk fever' in dairy cattle is extremely common, presenting as hypocalcaemic tetany shortly after calving
- dietary factors, hormonal factors, and the excessively high demand for calcium imposed on dairy cows in early lactation.

Oxalate poisoning

- This occurs in herbivores due to consuming large amounts of a plant high in **potassium oxalate ±** a number of botanical specimens have been implicated.
- Oxalate combines with calcium *in vivo* to produce insoluble calcium oxalate, which precipitates out in the renal tubules and **causes acute renal failure**.

Chronic renal failure

- Particularly in small animals.

- In these cases there is a failure to excrete phosphate, hyperphosphataemia and a consequent tendency for plasma calcium to fall.
- This in turn stimulates parathyroid hormone which increases release of calcium (and phosphate) from bone
- the hypocalcaemia is corrected, but the hyperphosphataemia is aggravated.
- As a result this condition (secondary renal hyperparathyroidism) manifests primarily as **hyperphosphataemia** rather than hypocalcaemia.
- The most effective treatment is **synthetic vitamin D (dihydroxytachysterol, AT-10)**, as the problem is one of calcium metabolism, not calcium deficiency, and oral calcium supplements on their own have relatively little effect.
- Patients on dihydroxytachysterol treatment should be monitored regularly, as it is quite easy to go a little too far and **produce iatrogenic hypercalcaemia.**
- **Note:** that many anticoagulants (e.g. EDTA, oxalate and citrate) act by removing calcium from solution.
- Calcium is **undetectable in samples** collected into any of these anticoagulants, and even contamination with a trace can lead to **erroneously low results.**

6.2. Phosphate

- Inorganic **phosphate** is of major importance in many metabolic pathways and, like calcium, it is a major component of bone.
- Plasma concentrations are again controlled by parathyroid hormone and vitamin D,
- but while parathyroid hormone unreservedly acts to raise plasma calcium concentrations, its **two modes of action** tend to have opposite effects on plasma phosphate concentration.

(1) It acts directly on **osteoclast**s to release bone salts into circulation, which increases both calcium and phosphate in plasma.

(2) It acts on **renal tubular cells** to increase phosphate excretion; this tends to lower plasma phosphate concentration, which in turn (by a mass action effect) increases release of phosphate (and calcium) from bone.

Increase (hyperphosphataemia)

- chronic renal failure, usually in small animals
- Failure to excrete phosphate
- increased parathyroid hormone secretion

- bone demineralization develops, known as secondary renal hyperparathyroidism, and
- patients develop gross skeletal abnormalities
- The phosphate assay is particularly sensitive to haemolysis, and erroneously high results are inevitable if the sample is not clear.

Decrease (hypophosphataemia)

- The classic case of hypophosphataemia in veterinary practice is the **`downer cow',** defined as a cow which remains recumbent post-partum in spite of adequate treatment for hypocalcaemia.
- These cows are typically bright, eating, drinking, ruminating, urinating and defaecating normally, have no other medical or surgical problem (e.g. mastitis, metritis or fractures), but simply won't get up.
- too low a dietary Ca/PO_4ratio, i.e. a calcium deficiency or a relative phosphate excess, will lead to secondary nutritional hyperparathyroidism.
- This usually results eventually in a normal plasma calcium concentration, but the effect on plasma phosphate concentration is variable depending on which of the **two effects of parathyroid hormone** on phosphate metabolism predominates.
- This varies with species, age of animal, and stage of the disease.
- If the bone mobilization effect predominates, hyperphosphataemia will be seen;
- if the renal effect (promotion of phosphate excretion) predominates then hypophosphataemia may paradoxically occur.
- **Other tests such as estimation** of plasma parathyroid hormone may also prove helpful.

CHAPTER 6: WATER, ELECTROLYTE AND ACID-BASE BALANCE

6.1 Introduction

Knowledge of the normal state is essential for formulation of a logical and accurate plan of therapy for the patient with abnormalities in water, electrolyte and/or acid-base balance. This fundamental knowledge coupled with an understanding of the basic compensatory mechanisms utilized by the body to correct these alterations is the basis for effective fluid therapy. The goal of this fluid therapy is to re-establish and/or maintain in the body certain basic conditions which favor normal cellular metabolism. These basic conditions are not those required for normal

functioning of one biochemical reaction, or one body organ, but are basic conditions required for all biochemical processes and all organs if normal function is to be restored. Specifically, the goals of this kind of fluid therapy are to insure that normal conditions prevail with respect to body water, tonicity of body fluids, specific body electrolytes and acid-base status.

6.2 GENERAL INFORMATION

6.2.1 Body Water (Body Fluid)

- 1. The proportion of water in an animal's body ranges from **45** to **70%** of the total body weight and is inversely proportional to the body fat content.
- 2. Body Fluid is divided into two compartments which vary in their electrolyte make-up.
    - a. Intracellular fluid **(ICF)**
        - 1) 65 to 75% of the total body water
    - b. Extracellular fluid **(ECF)**
        - 1) Approximately **25%** of the total body water
        - 2) Found in three locations:
            - a) Intravascularly **(plasma)**
            - b) Interstitially **(including lymph)**
            - c) Transcellularly **(CSF, Joint fluid, Intestinal contents)**
        - 3) Interstitial fluid is essentially an ultrafiltrate of plasma and water and electrolytes move freely within this compartment and between it and the intravascular fluid.
        - 4) Intravascular fluid has almost the same composition as interstitial fluid except for its higher protein level.
- 3. Sources of Water
    - a. Preformed water - water taken into the body as liquids and contained in solid foods.
    - b. Water of oxidation - water derived from oxidation of foods to carbon dioxide and water
        - 1) Continues to be formed from endogenous sources even though no exogenous water might be available.

2) Produces about 12 ml of water from the metabolism of each 100 calories.

4. Water Loss

a. Normal water loss is through the kidneys, lungs, skin **(perspiration)** and gastrointestinal tract.

1) urine accounts for slightly more than half the total fluid output of the body.

2) Water loss through the kidney is controlled by antidiuretic hormone (**ADH)** in response to plasma osmotic pressure and by aldosterone, which controls renal sodium excretion.

3) A decrease in body water, plasma sodium, or both is compensated for by an equivalent decrease in water, sodium excretion, or both.

b. Dehydration occurs when loss of body water exceeds intake.

1) This may result from excessive water loss without compensatory increased intake or from decreased intake with normal water loss.

2) Excessive loss of body fluid may occur in:

a) diarrhea

b) prolonged vomiting

c) sequestration of fluids in the digestive tract

d) prolonged fever

e) sweating

f) exudating burns or open wounds

g) excessive blood loss

h) uncontrolled polyuria without adequate compensatory water intake

6.2.2 Electrolytes

Substances that become ionized when placed in water

1. Electrolyte composition of body fluids

a. As previously stated, the body fluid compartments differ in their electrolyte concentrations. ***Table V.1*** indicates the typical electrolyte concentrations in the various body fluid compartments.

REPRESENTATIVE ELECTROLYTE CONCENTRATIONS IN THE BODY FLUID COMPARTMENTS (mEg/L)

Electrolytes	Intracellular Fluid	Extracellular Fluid	Interstitial	ntravascular
Cations	-	-	-	-
Sodium	**15**	**147**	**142**	-

Potassium	**155**	**4**	**5**	**5**
Calcium	**2**	**2.5**	**-**	**-**
Magnesium	**27**	**1**	**2**	**-**
Anions	**-**	**-**	**-**	**-**
Bicarbonate	**10**	**30**	**27**	**-**
Chloride	**1**	**114**	**103**	**-**
Phosphate	**100**	**2**	**2**	**-**
Sulfate	**20**	**1**	**1**	**-**
Organic acids	**1**	**7.5**	**-**	**5**
Protein	**62**	**0**	**16**	**-**

b. The total concentration of cations always equals that of the anions in all body fluids.

2. The unit of measurement for electrolytes is milli-equivalents per liter of fluid **(mEq/L).**

a. Milliequivalent values are measures of combining power and replace older methods of measuring electrolyte concentrations by weight (**e.g., mg/dl and mg%)** and volume **(e.g., vol %).**

b. A milliequivalent is the number of grams of solute contained in 1cc of a normal solution, and thus can combine equally with a similar portion of another normal solution.

c. Values expressed as ***mg%, mg/100 ml***, or **mg/dl** can be converted to ***mEq/L*** by using the formula:

$$\frac{\text{mg/100 ml} \times 100}{\text{atomic weight} \times \text{valence}} = \text{mEq/L}$$

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3. Specific Electrolytes

a. Sodium

1) Approximately 1/2 of the total body concentration of sodium is found in ECF.

2) The quantity of sodium in the body is controlled by dietary intake and loss.

3) The most important route for sodium excretion is through the kidney. Most sodium presented to renal tubules is reabsorbed in a process controlled by aldosterone.

a) Renal reabsorption of sodium requires an equivalent passage of hydrogen or potassium ions in the opposite direction.

4) Sodium is also lost in sweat and in digestive tract secretions.

a) In carnivores and most herbivores, sodium is reabsorbed in the lower intestinal tract.

b) In herbivores with large quantities of fluid in the feces, such as the cow and the horse, there may be considerable fecal loss of sodium.

5) A decrease in plasma sodium concentration (hyponatremia) occurs most frequently because of excessive sodium loss.

a) from the gastrointestinal tract through diarrhea or vomition

b) in renal disease in which the sodium conservation mechanism is operating deficiently because of tubular damage

c) Hyponatremia may occur with hyperglycemia due to increased sodium excretion to prevent hyperosmolarity.

6) An increase in plasma sodium concentration (hypernatremia) is rare and can occur when there is restricted water intake with excessive sodium intake, in advanced chronic renal failure with a low glomerular filtration rate, and with primary hyperaldosteronism.

b. Potassium

1) Potassium concentration is low in **ECF** and high in most cells of the body.

2) Most potassium is excreted by the kidneys through glomerular filtration and tubular secretion.

3) Aldosterone facilitates excretion of potassium since it causes increased sodium reabsorption by promoting the exchange of sodium in tubular fluid for potassium in the tubular cell.

4) Potassium excretion by the kidneys is also controlled by competition between potassium and hydrogen ions for reabsorption.

5) Alterations in serum potassium levels occur when there is a disturbance in the equilibrium between potassium in the **ICF** and potassium in the **ECF**.

a) In alkalosis, potassium moves into the cell in exchange for hydrogen ions and may cause hypokalemia.

b) In acidosis, potassium moves out of the cell in exchange for hydrogen ions and may cause hyperkalemia.

6) Plasma potassium increases about **0.6 mEq/L** for each **0.1** unit decrease in blood pH. Therefore, if an acidotic animal has a normal plasma potassium level, it should be considered hypokalemic and corrective therapy should be initiated.

7) In addition to its role in maintaining the tonicity of the **ICF**, potassium is of great importance in the mechanism of neuromuscular transmission.

a) Low concentrations of **K+** in the **ECF** result in profound muscular weakness and **ECG** abnormalities.

b) High concentration of **K+** in the **ECF (10-12 mEq/L)** result in severe myocardial disturbances and death due to cardiac arrest.

c. Chloride

1) Chloride concentration is low in **ICF** and high in **ECF**.

2) Excretion, absorption and distribution of chloride are passive processes in association with active sodium transport.

3) Unusual reduction in chloride concentration in the absence of comparable change in sodium, usually reflects sequestration of gastric juice in the stomach or vomiting.

d. Bicarbonate

1) Bicarbonate is mostly of endogenous origin in that it comes from the hydration of carbon dioxide to carbonic acid which then dissociates to bicarbonate and hydrogen ions.

2) Bicarbonate is lost through secretions to the digestive tract and in the urine.

3) Bicarbonate levels are regulated by respiratory and metabolic **(kidney)** processes.

6.2.3 Distribution of fluid among the two body fluid compartments

1. The distribution of fluid among the body fluid spaces is determined by osmotic pressures.

a. The homeostatic mechanisms of the body which maintain this function are designed to maintain the osmotic pressure of the extracellular fluid.

b. If ECF osmotic pressure can be maintained, it will also serve to maintain intracellular osmolarity.

c. If the osmotic pressure of the **ECF** is increased, water is removed from cells **(ICF)**, producing cellular dehydration and a new state of equilibrium between ECF and ICF at a new and different osmolarity.

2. The plasma proteins are confined to the vascular space and, in that location, exert an osmotic force that holds fluid in the vessels in opposition to the tendency of the blood pressure to force fluid out of the vessels.

3. The tonicity **(osmotic pressure)** of the **ICF** and **ECF** is determined by the total number of particles (electrolyte ions) dissolved in each of these fluids.

a. The clinical unit of measurement for osmolality is milliosmoles (mOsm) per kilogram of water.

b. The normal osmolality of plasma in domestic animals is about 300 mOsm/kg of plasma water.

c. Since sodium is the most abundant ion in the **ECF**, the osmotic pressure of the **ECF** is largely determined by the sodium concentration.

d. Potassium is the most abundant ion in the **ICF** and has a comparable role in maintaining normal intracellular hydration.

4. Normal hydration of the body depends, therefore, not only on optimum water in the body, but on optimum protein, sodium, and potassium in the appropriate fluid compartments to hold the proper amount of water in each compartment.

6.5 ACID-BASE REGULATION IN THE BODY FLUIDS

Normal metabolic processes in an animal body result in the production of relatively large quantities of acids. These acids are transported to the excretory organs, i.e., the lungs and the kidneys, without causing marked alterations in blood **pH.** This sensitive control of blood pH in the normal range of **7.3 to 7.5** is accomplished by the combined effects of the blood buffer system, the respiratory system, and the renal system. The body responds quickly to alterations in blood pH. Correction in these alterations occurs in steps, with buffer systems providing the immediate response to any pH alteration, followed quickly by the respiratory response. Later, the kidney mechanism is initiated and sustains the corrective activity for a longer time span.

A buffer is a mixture of a weakly dissociated acid and a salt of that acid. The blood buffers that play a role in control of blood pH are:

1. Bicarbonate/carbonic acid system
2. Oxyhemoglobin:reduced hemoglobin system
3. Monopotassium phosphate:dipotassium phosphate system
4. Plasma protein system

5. Monosodium phosphate:disodium phosphate system

The bicarbonate/carbonic acid buffer system is the single most important buffer system in the body fluids and our discussion will be restricted to this system.

6.5.1 The Bicarbonate/Carbonic Acid Buffer System

1. The Henderson-Hasselbalch equation is useful in understanding pH control of body fluids. This formula is as follows:

$$pH = pK + \log \frac{salt}{acid}$$

The equation for the bicarbonate/carbonic system is:

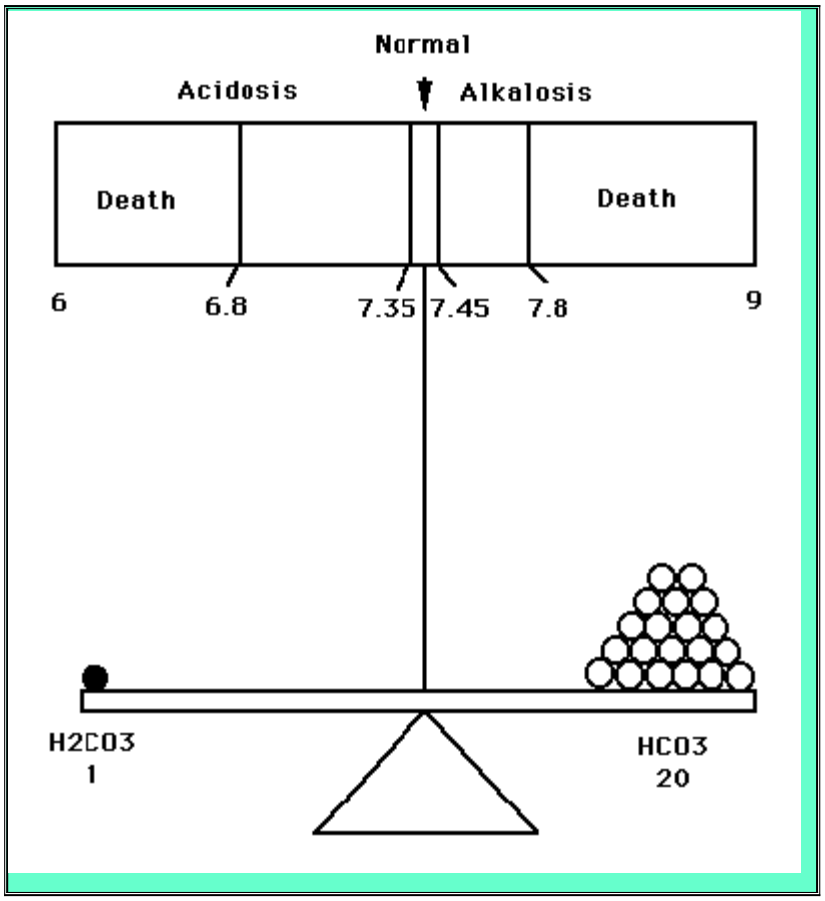
$$pH = 6.1 + \log \frac{[bicarbonate]}{[carbonic\ acid]}$$

Therefore, the pH of plasma is dependent upon the ratio of **HCO3- to H2CO3**. As can be seen in ***Figure V.1***, the normal ratio between these substances is 20:1. when one changes without a comparable change in the other, the pH becomes abnormal.

2. Carbonic acid is formed when hydrogen from cell metabolism combines with bicarbonate or when carbon dioxide produced by cell metabolism is combined with water inside the erythrocyte to form carbonic acid under the influence of cellular carbonic anhydrase. Blood carbonic acid is in equilibrium with dissolved

Figure V.1

The Biocarbonate/Carbonic Acid Buffer system



CO2 which is in turn controlled by ventilation. Abnormalities in carbonic acid or carbon dioxide, therefore, always result from abnormalities in ventilation.

a. When ventilation is decreased, carbon dioxide accumulates, carbonic acid is increased, and the condition is called respiratory acidosis ***(seen in pneumonia, pulmonary edema, etc.)***

b. When hyperventilation occurs, carbon dioxide is reduced, carbonic acid is decreased below normal and the condition is respiratory alkalosis.

3. Bicarbonate concentration is influenced by non-respiratory mechanisms such as renal function, digestive tract function, and tissue metabolism.

a. Increased bicarbonate or a decrease in acid **(HCl)** would reflect metabolic alkalosis ***(seen in vomiting)***.

b. Decreased bicarbonate results in metabolic acidosis and is very common in cases of diarrhea.

4. The above acid-base disturbances are summarized in **Table V.2.**

TABLE V.2

LABORATORY FINDINGS IN CLASSI UNCOMPENSATED ACID-BASE IMBALANCES(ACUTE)

	pH	P_{CO_2}	HCO_3	BE*
Respiratory acidosis	↓	↑	N	N
Metabolic acidosis	↓	N	↓	↓
Respiratory alkalosis	↑	↓	N	N
Metabolic alkalosis	↑	N	↑	↑

*** BE = Base excess**

N = Normal

6.5.2 Respiratory Control of Acid-Base Balance

1. The respiratory center found in the medulla oblongata is sensitive to blood levels of **pCO2 and pH.**

a. When blood **pCO2** increases above the normal value, the respiratory rate increases.

b. When blood **pH** drops, the respiratory rate increases.

c. When blood **pCO**2 is low, respiratory rate decreases.

d. When blood **pH** is high, respiratory rate decreases.

2. This regulation of the rate of pulmonary ventilation in response to changes in **pCO2** and **pH** serves as the basis for pulmonary compensation in alkalosis and acidosis.

6.5.3 Renal Control of Acid-Base Balance

1. Accumulation of nonvolatile acids and the subsequent depleting effect on bicarbonate ion content can be offset only by the renal ability to exchange sodium ions for hydrogen ions and the production of an acid urine.

a. As nonvolatile acid anions are filtered through the glomerulus, they are accompanied by an equivalent number of cations **(e.g., Na+)** in order to maintain electrical neutrality.

b. Through the activity of carbonic anhydrase, renal tubule cells combine carbon dioxide from their own metabolic activities with water to make carbonic acid which dissociates to hydrogen and bicarbonate ions.

c. The hydrogen ions pass into the tubule and an equivalent amount of sodium is returned accompanied by an equivalent amount of bicarbonate; thus, bicarbonate ions are replaced, hydrogen ions and nonvolatile acid anions are excreted and acid urine is produced.

d. The overall effect is restoration of the blood bicarbonate ion:carbonic acid ratio with a resultant correction of **pH.**

NOTE: In compensated acidosis or alkalosis, absolute concentrations of bicarbonate ions and carbonic acid may be changed, but as long as the ratio remains in the range of approximately 20:1, the pH may be in the normal range.

6.6 ACID-BASE DISTURBANCES

6.6.1 Respiratory Acidosis - carbonic acid excess (hypoventi-lation)

1. Causes

a. Pneumonia

b. Emphysema

c. Pulmonary edema

d. Pneumothorax

e. Paralysis of respiratory muscles

f. Morphine poisoning

g. Barbiturate poisoning

h. Occlusion of breathing passages

i. In closed gas anesthesia when oxygen is adequate, but carbon dioxide removal is insufficient.

2. Clinical Signs

a. Respiratory embarrassment

b. Depression of central nervous system **(disorientation, coma)**

3. Laboratory findings

a. Uncompensated

1) Urine **pH** - more acid

2) Blood **pH** - below **7.35**

3) Plasma bicarbonate - normal

4) Elevated **pCO2**

b. Partially Compensated

1) Elevated **pCO2**

2) Plasma bicarbonate - increased

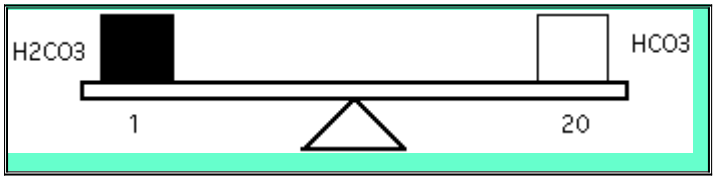
3) Plasma chloride - low; increased excretion of chloride by kidney to make more sodium available for bicarbonate.

4) Blood pH - decreased, but higher than uncompensated

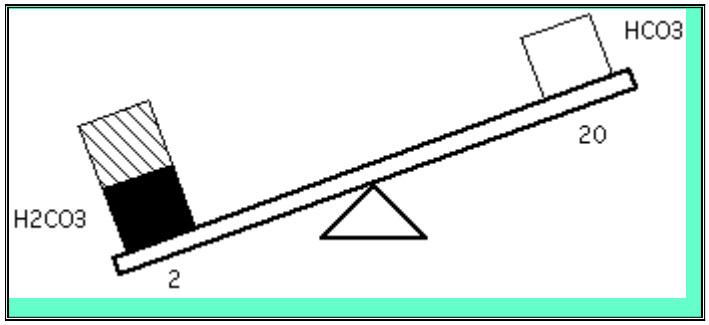
5) Urine **pH - acid**

4. Pathogenesis

a. Normal balance



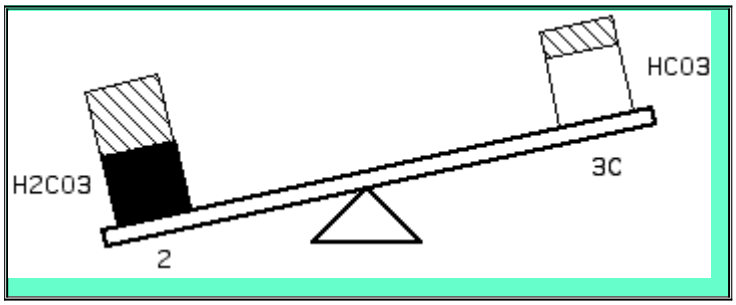
b. Respiratory acidosis - carbonic acid excess due to hypoventilation



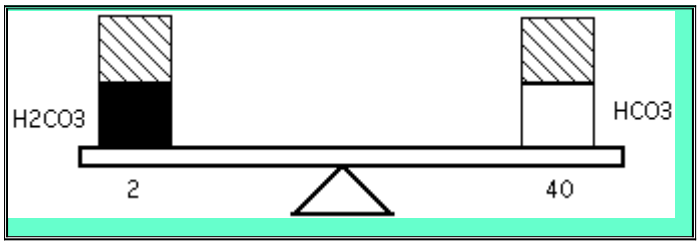
c. Body compensatory action

1) If the primary cause is not in the respiratory center **(e.g., CNS problem)**, the center will cause an increased pulmonary rate with a resultant decrease in **pCO2.**

2) The renal compensatory mechanism will conserve bicarbonate ions and excrete hydrogen ions and nonbicarbonate anions to produce more acid urine. There is also increased reabsorption of bicarbonate.



Partial Compensation



Complete Compensation

6.6.2 Respiratory Alkalosis - carbonic acid deficit (hyperventilation)

1. Causes - increased rate and depth of breathing

a. Fever

b. Oxygen lack

c. Respiratory center stimulation **(encephalitis, drugs such as salicylates)**

d. Hysteria and anxiety

2. Clinical signs

a. Deep, rapid breathing

b. Tetany, progressing to convulsions

3. Laboratory Findings

a. Uncompensated

1) Urine pH - more alkaline

2) Blood pH - over 7.45

3) Plasma bicarbonate - normal

4) Decreased pCO2

b. Partially Compensated

1) Decreased pCO2

2) Plasma bicarbonate - decreased

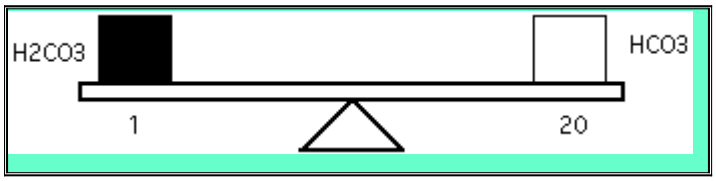
3) Plasma chloride - normal to high

4) Blood pH - increased, but lower than uncompensated.

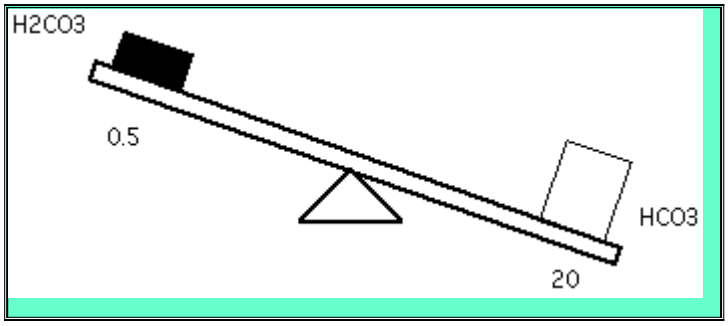
5) Urine pH - alkaline

4. Pathogenesis

a. Normal balance

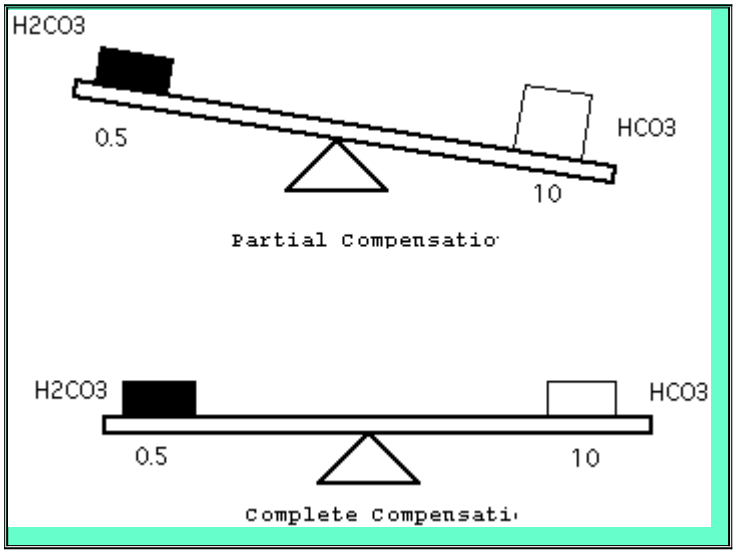


b. Respiratory alkalosis - carbonic acid deficit due to hyperactive breathing which results in an increased loss of carbon dioxide from the lungs.



c. Body compensatory action

1) The compensatory mechanisms are principally renal. Renal control is manifested by a decrease in ammonia formation, a decrease in bicarbonate reabsorption, retention of hydrogen ions **(exchanged for sodium)** and an increase in excretion of bicarbonate instead of chloride.



6.6.3 Metabolic (Nonrespiratory) Acidosis - bicarbonate deficit

1. Causes

a. Extreme diarrhea - the digestive juices in the small intestine contain large amounts of sodium bicarbonate, and when these are lost in the feces as a result of diarrhea, the body is depleted of sodium ion.

b. Renal insufficiency - causes retention of organic acid ions, inability to reabsorb bicarbonate and excrete hydrogen ions.

c. Ketosis in which large amounts of ketonic acids are produced and accumulate in the tissues **(diabetes mellitus, starvation).**

d. Lactic acid accumulation - heat stroke, excessive muscular activity, conditions of cellular hypoxia.

e. Sequestration of intestinal contents.

f. Excessive loss of saliva.

2. Clinical Signs

a. Hyperpnea - rapid rate and increased depth of respiration

b. Depression of central nervous system **(disorientation, stupor, coma)**

3. Laboratory Findings

a. Uncompensated

1) Urine pH - more acid

2) Blood pH - below 7.35

3) Plasma bicarbonate - decreased

4) Normal pCO2

b. Partially Compensated

1) Decreased pCO2

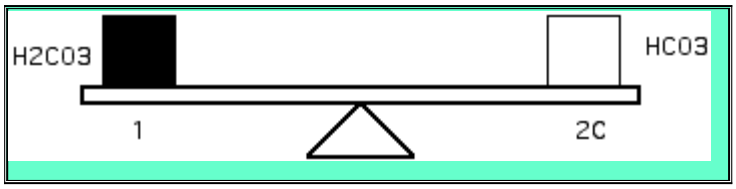
2) Plasma bicarbonate - decreased

3) Blood pH - decreased, but higher than uncompensated

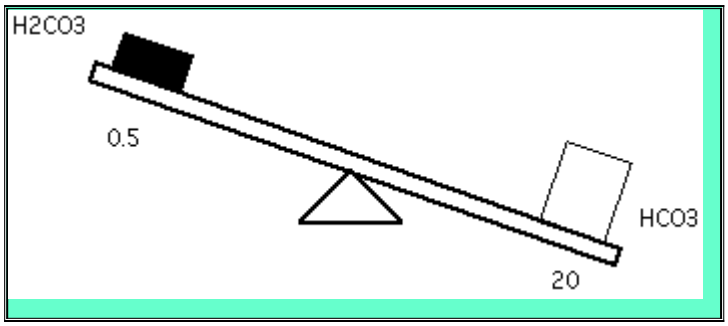
4) Urine pH - acid

4. Pathogenesis

a. Normal balance



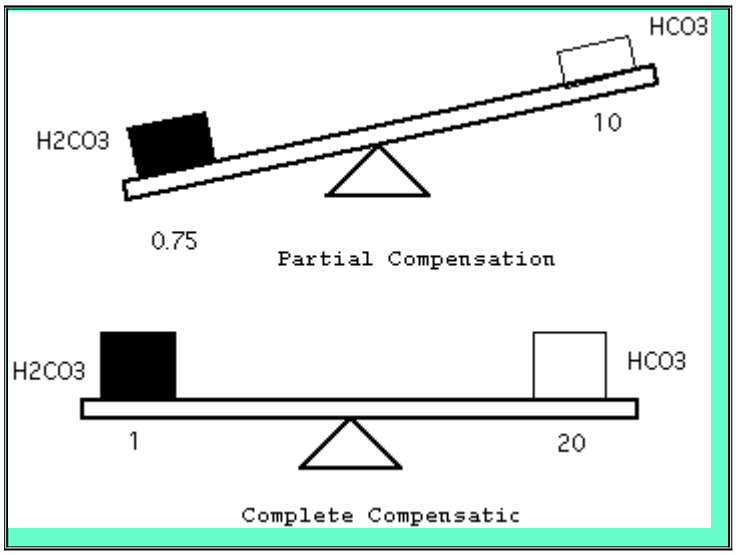
b. Metabolic acidosis - ketone and/or excess chloride ions replace bicarbonate ions.



c. Body compensatory action

1) The respiratory compensatory mechanism decreases **pCO2** by increased respiratory rate.

2) The renal compensatory mechanism will conserve bicarbonate ions and excrete hydrogen ions and nonbicarbonate anions to produce more acid urine. There is also increased reabsorption of bicarbonate.



6.6.4 Metabolic Alkalosis - bicarbonate excess

1. Causes

Accumulation of bicarbonate in extracellular fluid as a result of excessive acid loss.

a. Vomition - loss of chloride causes a compensatory increase in bicarbonate to maintain electrical neutrality

b. Sequestration of abomasal juices in ruminants with high GI tract obstructions

c. Potassium depletion with resultant movement of hydrogen ions into the intracellular fluid to replace lost potassium

d. Hyperadrenocorticism

e. Alkaline therapy

2. Clinical signs

a. Depressed breathing - slow and shallow

b. Tetany, progressing to convulsions

3. Laboratory Findings

a. Uncompensated

1) Urine pH - more alkaline

2) Blood pH - over **7.45**

3) Plasma bicarbonate - increased

4) Normal **pCO2**

5) Plasma chloride - low

6) Plasma potassium - may be low

b. Partially Compensated

1) Increased pCO2

2) Plasma bicarbonate - increased

3) Plasma chloride - low

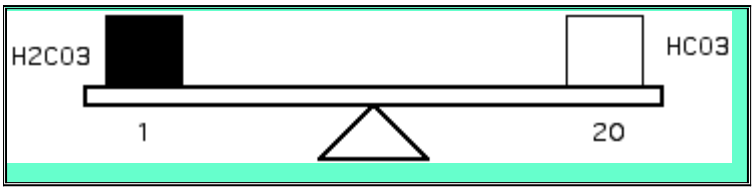
4) Plasma potassium - may be low

5) Blood pH - increased, but lower than uncompensated

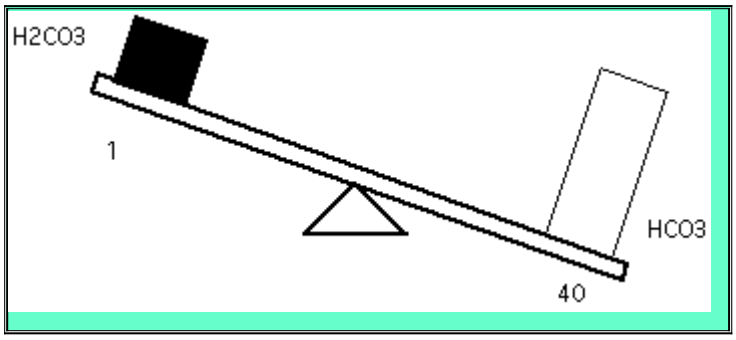
6) Urine pH - alkaline **(Paradoxical aciduria can occur)**

4. Pathogenesis

a. Normal balance



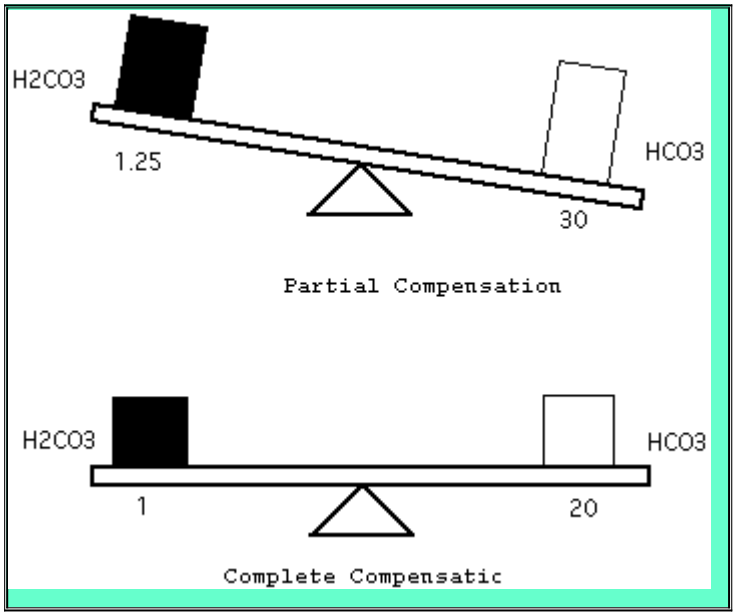
b. Metabolic alkalosis - bicarbonate ion increased due to loss of chloride ion or to excess ingestion of bicarbonate.



c. Body compensatory action

1) The respiratory response is a decrease in respiration in order to increase pCO2.

2) The compensatory renal mechanism is decreased sodium-hydrogen exchange, decreased ammonia formation, and increased excretion of bicarbonate.



6.7 EVALUATION OF CLINICAL PATIENTS

6.7.1 Some basic questions to be answered.

1. Is dehydration present?

2. Is the extracellular fluid hypertonic or hypotonic?

3. Is there an acid-base abnormality?

4. Is there an abnormality in the concentration of any specific important electrolytes?

6.7.2 Patient evaluation without laboratory tests.

1. The Clinical Examination

a. Clinical signs of dehydration.

1) Loss of skin turgor.

2) Dryness of mucous membranes.

3) Sunken eyes.

4) Listlessness or depression.

b. Clinical signs of acid-base abnormality.

1) Abnormal respirations.

c. Clinical signs of potassium disturbances.

1) Muscular weakness.

2) The electrocardiogram.

d. Clinical signs of sodium disturbances - no specific signs.

e. The value of the history and **physical examination.**

1) Is dehydration the result of being off feed and not drinking?

2) Has there been diarrhea?

3) Could there be sequestration of fluid in the digestive tract?

4) Has there been any other peculiar fluid loss?

2. The shortcomings of patient evaluation without laboratory tests.

a. There are no objective facts.

b. The guesses could be wrong.

c. The patient may be unusual.

d. There is no objective means of monitoring response to treatment.

3. More objective evaluation of the patient is indicated when:

a. The patient is especially valuable.

b. The clinical signs of a fluid balance disorder are unusually severe.

c. Large quantities of fluid have been given which may have induced an electrolyte disorder.

4. Patient Therapy

When patient needs are poorly defined, therapy must be general and conservative. When patient needs are well defined, therapy can be more specific, more radical and more successful.

6.7.3 Patient evaluation by laboratory tests.

1. The only way to determine objectively what, if any, specific derangements are present in the patient.

2. Some areas of importance include dehydration per se, **ECF** tonicity, acid base disorders, and derangements of specific important electrolytes.

3. Evaluating the severity of dehydration.

a. Clinical signs can be misleading.

b. PCV or hemoglobin is very helpful as an indicator of hemoconcentration.

1) Can be misled by preexisting anemia.

2) Can be misled by normal horse contracting spleen and elevating values in blood.

c. Total serum or plasma protein is a useful double check for hemoconcentration.

1) Easily done with a refractometer.

2) Not subject to change with splenic contraction.

3) May be misleading with preexisting protein abnormalities or concurrent protein losses.

d. Urea nitrogen

1) Severe dehydration results in diminished renal function.

2) High BUN is most often an indication of severe dehydration or shock.

e. The consensus of several of these determinations provides a much more reliable estimate of the presence and severity of dehydration than any one alone.

f. The effectiveness of treatment can be monitored objectively by one or more of these laboratory determinations.

4. Evaluating abnormal tonicity of the extracellular fluid.

a. No clinical signs indicate hypertonicity or hypotonicity.

b. ECF tonicity is related to the concentration of sodium since its related anions represent more than **90%** of the electrolytes in the **ECF.**

c. High sodium indicates hypertonic **ECF;** low sodium indicates hypotonic **ECF**.

5. Evaluating acid-base balance in the patient.

a. Clinical signs of abnormal respiration may suggest an acid-base problem but it is difficult to determine if primary respiratory disorders exist or if disturbances seen are normal compensatory reactions to a metabolic disorder.

b. Respiratory disorders can only be identified accurately by estimation of carbonic acid in the blood. This is called **pCO2.** It is a measure of the partial pressure of dissolved gaseous **CO2** in the blood. The **pCO2** is elevated due to hypoventilation **(respiratory acidosis)** and is abnormally low due to hyperventilation **(respiratory alkalosis)**.

c. Metabolic disorders are reflected in the plasma bicarbonate concentration. Low values indicate acidosis; high values indicate alkalosis.

d. The blood pH indicates the severity of the actual derangement in the body. Since it can be disturbed in respiratory disorders as well as in metabolic disorders, it is necessary to know the **pCO2** and the bicarbonate to understand how an acid-base disorder developed and how it should be corrected.

e. When primary disorders of ventilation can be ruled out with confidence, the plasma bicarbonate value alone is a reliable indication of the presence, severity and character of metabolic acid-base disturbances.

f. Most commercial laboratories do a test called **"CO2"** or **"total CO2."** This is essentially a measure of bicarbonate, the metabolic factor, and is not, as the term implies, a measure of **pCO2**, the respiratory component.

6. Evaluating potassium status of the patient.

a. There are no clinical signs which are a reliable reflection of potassium abnormalities in the patient.

b. The electrocardiogram may be helpful but is is not often readily available.

c. Even accurate determinations of plasma potassium concentration are sometimes misleading because the vast majority of body potassium is in the **ICF**, not the **ECF**, and the **ICF** cannot be sampled.

d. Low serum or plasma potassium usually indicates serious depletion of body potassium.

e. Elevated potassium values are usually the result of severe acidosis causing intracellular **K+** to more into the **ECF.**

7. A battery of tests is necessary to answer the four important questions regarding fluid balance in a clinical patient.

a. A complete evaluation can be obtained best by a battery of nine tests:

1) **PCV**, Total Protein, **BUN, Na+, K+, Cl+, pH, pCO2, HCO3-.**

2) This can be done with a single 5 ml blood sample if it is anticoagulated with heparin. But, it must be drawn anaerobically and the tests must be done promptly.

b. An abbreviated battery of tests is usually more practical:

1) Total Protein, **Na+, K+, HCO3- (or "total CO2")**

2) This battery can be run on serum or heparinized plasma. The sample need not be anaerobic and the tests need not be run promptly if the serum or plasma is removed from the cells soon after collection.

3) The elimination of **pH** and **pCO2** from this battery presupposes that no primary respiratory disorder is present.

6.8 FLUID THERAPY PRODUCTS

A. General

1. We must know the composition of each product we use in order to use it correctly.

2. Composition must be in terms that are comparable with plasma values, i.e., milliequivalents per liter.

3. One can predict, from the composition of the fluid, what effect it will have on the patient.

B.Common Fluids

Common fluids can be **"spiked"** with additional quantities of electrolytes especially needed, e.g., **potassium or bicarbonate.**

1.Sterile, concentrated solutions of potassium chloride and sodium bicarbonate are commercially available for this purpose.

a.The concentrates contain approximately **1 mEq/ml.**

b.Use of these products permits maintenance of sterile conditions.

2.Pure potassium chloride and sodium bicarbonate can be obtained and appropriate quantities added to other fluids when additional amounts are needed.

a.**1 gram** of sodium bicarbonate provides **12 mEq.** of bicarbonate.

b.**1 gram** of potassium chloride provides **14 mEq.** of potassium.

c.Sodium bicarbonate cannot be autoclaved. When bicarbonate powder has been added to a sterile solution it must be used without further sterilization. This represents a possible break in sterility.

3.When one is **"spiking"** fluids in this way, the fluid solution is usually quite abnormal in its composition; therefore, it is essential that the final composition of the fluid be known precisely if the administration of dangerous solutions is to be avoided.

C. Increase of Bicarbonate

C.Since bicarbonate cannot be autoclaved, most solutions do not contain this substance. Instead, they contain lactate or acetate ions which can be metabolized by the body. When this happens, endogenous bicarbonate replaces the acetate or lactate given. In this way, stable sterile solutions are available which, indirectly, make it possible to increase the bicarbonate in the body.

6.9 SELECTION AND USE OF FLUIDS AND ELECTROLYTES

6.9.1 Estimating the quantity of fluid required.

1.Slight dehydration represents a body fluid deficit of **6-8%** body weight.

2.Severe dehydration represents a fluid deficit of **10-12%** body weight.

3.Calculations are simplified if body weight is expressed in kilograms since a kilogram of fluid is one liter.

a.Example of severe dehydration:

500 kg horse with a fluid deficit of **10%** body **weight = 10% x 500 kg = 50 kg (50 liters)** of fluid needed.

b.Example of slight dehydration:

500 kg horse with a fluid deficit of **6%** body **weight = 6% x 500 kg = 30 kg (30 liters)** of fluid needed.

4.When unusual losses are continuing, e.g., diarrhea, the deficit is increasing even while replacement has been initiated.

5.The estimated requirement for fluid replacement need not be given in a short period of time. Except in critical cases, the calculated requirements can be replaced over a 24 to 36 hour period.

6.9.2 The kind of fluid to give.

1.The most conservative fluid therapy consists of using a **"balanced**" electrolyte solution, i.e., one having the same concentrations of the major electrolytes as normal plasma.

a.Such a fluid should not induce abnormalities in the patient.

b.It can be given by any route since it is isotonic.

c.Since it contains no toxic concentrations of any electrolyte, there is no danger in rapid administration except the danger of volume overload of the vascular system.

d.Such a fluid is lactated **Ringer's** solution.

2.This conservative therapy is indicated when there are no peculiar electrolyte disturbances or when there is no way to determine what, if any, electrolyte problems exist. It is not an effective means of correcting severe acidosis, hyponatremia, or hypokalemia.

3.When metabolic acidosis is likely, as in severe diarrhea, shock, and intestinal obstruction, and especially when it has been shown by laboratory tests to be a serious problem:

a.An unphysiologically high concentration of bicarbonate must be given to raise the low value in the patient to normal.

b.When additional sodium is also needed, as indicated by a low sodium value or as is predictable in diarrhea, a very useful solution is obtained by adding 3-5 grams of sodium bicarbonate to each liter of lactated Ringer's solution. The result is a high sodium-high bicarbonate solution ideally suited to the animal's condition.

c.If the sodium value is not low the fluid given should be high in bicarbonate but not abnormally high in sodium. such a solution is isotonic sodium bicarbonate or isotonic sodium lactate.

e.Correction of acidosis can be accomplished by the administration of very hypertonic solutions of sodium bicarbonate **(5% NaHCO3 in saline)** in relatively small quantities **(2-3 L/500 kg)**. This must be accompanied by the administration of large quantities of balanced solutions such as lactated **Ringer's.**

f.When it is desirable to estimate the needs of the patient for bicarbonate, the following formula is useful:

1)**HCO3- deficit/L x ECF (liters) = mEq.** needed.

2)**HCO3- deficit/L = Normal HCO3- - Patient HCO3- (This value is approximately 15 mEq/L in severe acidosis)**

3)**ECF = Body weight (kg) x 0.3. (The factor 0.3 is somewhat greater than the actual figure for ECF to insure adequate replacement).**

4)**Example:** A 450 kg horse is severely dehydrated and severely acidotic. ***What are its fluid needs?***

a)**fluid volume requirement = 10% body weight = 10% X 450 kg = 40 - 50 liters of fluid.**

b)Severe acidosis requires treatment with bicarbonate - ***how much is needed?***

Patient HCO3- = 12 mEq/L

Normal HCO3- = 24 mEq/L

HCO3- deficit = 12 mEq/L

ECF est. - 0.3 x 450 kg = 135 L

HCO3- needed = 12 mEq/L x 135 L = 1620 mEq.

c)Patient needs are estimated as **40-50 liters** of fluid and **1500-2000 mEq** of bicarbonate.

d)**Treatment Method #1: 3 liters of 5% NaHCO3 (1800 mEq) + 37 liters** of balanced electrolyte solution.

e)**Treatment Method #2: 40 liters** of balanced electrolyte solution supplemented with **3-4 grams of NaHCO3/L = 1500-2000 mEq NaHCO3.**

4.Severe hypokalemia, potassium values less than **2.0 mEq/L**, may require treatment with high potassium solutions.

a.Specific treatment is indicated if marked weakness or depression is seen.

b.It is probably not necessary if the animal has begun to eat since hay is high in potassium.

c.High concentrations of potassium should be administered with caution since the heart is so sensitive to slight elevations in serum potassium levels.

d.Supplementation of lactated Ringer's solution with **10 to 50 mEq/L** of potassium chloride **(1-3 grams/L)** may be useful. The solutions containing 20-50 mEq/L should be given very slowly.

e.Potassium supplementation can also be done, with much greater safety, by oral administration of potassium chloride. Thirty grams in 8 liters of water, 2-3 times per day has been recommended.

f. Hyperkalemia is usually associated with acidosis and prompt correction of the acidosis usually corrects the hyperkalemia without the administration of potassium supplements.

5.When peculiar electrolyte derangements have developed, peculiar fluids must be used to correct the problem promptly.

a.Such fluids are very unphysiological; while especially suited for their intended purpose, they are apt to induce severe abnormalities when used carelessly.

b.The more unusual a fluid is, the more essential it is to be certain it is indicated.

c.When objective patient evaluation by laboratory tests is not available, a more conservative **(possibly less effective)** fluid must be chosen.

6.9.3 The route for fluid administration

In severe dehydration, a major proportion of the fluid should be given intravenously.

Additional large quantities can be provided per os. This fluid is usually beneficial except in patients with intestinal obstruction.

Subcutaneous and intraperitoneal injections of fluid are not widely used in the horse but can be beneficial in smaller animals.

6.9.4 Miscellaneous Topics

An average daily intake of hay contains more potassium than 100 liters of lactated Ringer's solutions. Consumption of normal feed is the best way to replace potassium deficits. This also shows why potassium depletion is frequent in animals that are not eating.

Natural feeds contain very little sodium. Supplemental salt must always be available to herbivores. This is especially true when increased needs are present.

Water should always be available to dehydrated animals. Sometimes they will consume far more fluid voluntarily than can be given by any other means.

Electrolytes can be added to water for voluntary consumption, or administered by stomach tube. Do not make hypertonic solutions for oral use. Do not fail to provide pure water at all times as an alternative to electrolyte-containing water. It would be harmful to reduce water intake by the addition of repelling substances to the water.

CHAPTER 7: DIAGNOSTIC CYTOLOGY

Diagnostic cytology involves the examination of cells which have either naturally exfoliated or been artificially removed from a body cavity or a tissue mass. The cytological interpretation is

often valuable in establishing a diagnosis, identifying the disease process, directing therapy, forming a prognosis, and/or determining what diagnostic procedure should next be performed.

Some of the major advantages of cytological procedures are that they require minimal equipment, have rapid turnaround time and most are minimally invasive and minimally stressful for the patient. However, since cytologic interpretation is based upon the distinct characteristics of individual cells on a smear that lacks the source tissue's architectural patterns; it should be mainly utilized as a rapid screening procedure. Histopathology is usually more diagnostic and definitive in that it allows study of both structure and form of a tissue or organ. Cytology should be considered a tentative diagnosis requiring a histologic confirmation, particularly in lesions with suspected neoplastic characteristics.

7.1 METHODS OF SPECIMEN ATTAINMENT

A. Solid Tissue Cytology

The particular method used for obtaining cytological specimens is determined by the characteristics of the lesion to be sampled. The major goal is to obtain a significant number of well-stained intact cells that reflect the composition of the lesion.

1. Touch impressions or imprints

Imprints can be prepared from excised external lesions on the living animal or from tissues removed during surgery or necropsy. The imprints are easy to obtain but they collect fewer cells than scrapings and contain greater contamination **(bacterial and cellular)** than aspirates. Therefore, imprints from superficial lesions often only reflect a secondary bacterial infection and/or inflammation-induced tissue dysplasia. This markedly hinders their use in diagnosis of neoplasia. To reduce contamination and to obtain a more representative sample, touch imprints are made on freshly excised surfaces. If the excised surface is very bloody or moist, it should be blotted with absorbent paper. Touch the slide gently to the specimen, remove and quickly air dry. Dragging of the slide across the specimen will distort the cells. Tissue rich in connective tissue imprints poorly.

2. Scrapings

Scraping has the advantage of collecting many cells from the tissue, and therefore, is advantageous when the lesion is firm and yields few cells. Disadvantages are similar to those described for tissue imprints. The surface of the lesion is freshly excised and scraped with a

scalpel blade until material appears on the blade's surface. This material is then smeared thinly across a slide.

3. Swabs

Swab smears are collected only when imprints, scrapings and aspirates cannot be made; e.g., fistulous tracts, nasal and vaginal collections. The lesion or tissue is swabbed with a moist, sterile cotton swab. Sterile isotonic fluid, such as **0.9% NaCl**, should be used to moisten the swab. This helps minimize cell damage during sample collection and smear preparation. After sample collection, the swab is gently rolled, not rubbed, along the flat surface of a clean glass microscope slide.

4. Fine Needle Aspirations

Fine needle aspiration biopsies can be collected from masses such as lymph nodes, nodular lesions and internal organs. Aspiration of cutaneous masses avoids the superficial contamination common with imprints or scrapings, but collects fewer cells than scrapings. The tissue to be aspirated is immobilized as close to the skin surface as possible. **A 21-25** gauge needle attached to a 3-20 ml syringe is used **(a 12-ml syringe is a good all-around size)**. The softer the tissue being aspirated, the smaller the needle and syringe used. When needles larger than 21 gauge are used, tissue cores tend to be aspirated, resulting in a poor yield of free cells suitable for cytological preparation. Also, larger needles tend to increase the incidence of blood contamination.

The needle with syringe attached is introduced into the center of the mass and strong negative pressure is applied The cell population retrieved from a mass is highly dependent upon the site aspirated. In order to obtain a representative sample, the needle is redirected and moved to several areas in the mass, taking precautions to prevent the needle from leaving the mass. Negative pressure is maintained during the redirection. However, when the mass is not large enough for the needle to be redirected and moved without danger of the needle's leaving the mass, negative pressure is relieved during redirection and movement of the needle. In this situation, negative pressure is applied only when the needle is static. In both cases, aspirate may or may not appear in the syringe, but the needle will contain sufficient tissue for smears. Before removing the needle from the mass, the negative pressure is relieved. The needle is removed from the mass and skin and the needle contents are expressed onto a slide and a smear prepared.

B. Fluid Cytology

1. Centesis

Centesis is the process of perforation or tapping as with an aspirator, trocar, or needle, to obtain abdominal, thoracic, synovial or cerebrospinal fluid. The size of the trocar or needle used is dependent upon the species and centesis site. Whenever possible, at least 3 ml of fluid should be aseptically obtained to provide an amount sufficient for determination of specific and concentration for microscopic examination. Since exudates coagulate and modified transudates may coagulate, a portion of the specimen should be collected in an **EDTA** tube. A sterile tube should be used for one portion to assure a satisfactory specimen in the event that culture is indicated. Direct and sediment smears should be made for cytological examination. Sediment smears are prepared by centrifu-gation of the fluid for 5 minutes at 165-360 G to concentrate the cells. In addition to cytological examination, other properties **(e.g., specific gravity, total protein, glucose)** should be determined in order to pin-point the pathogenesis of the occurrence.

2. Catheterization

Catheterization procedures are used for transtracheal/ bronchial and prostatic washes. Once obtained, the fluid specimens are handled in a manner similar to centesis fluids.

7.2 PREPARATION OF SMEARS

7.2.1 Preparation of smears from aspirates of solid masses

Several methods can be used to prepare smears for cytologic evaluation of solid masses, including lymph nodes. The experience of the person preparing the smears and characteristics of the sample influence the choice of smear preparation technique. No matter what the technique used, the resultant smear must have a thin area where cells may settle flatly and expose a large surface area to view. If the smear is thick, the cells are supported more upright on the slide and have a smaller diameter. Cells in thick areas are taller and thus stain darker. In addition, a thick layer of proteinaceous and necrotic debris in the dried fluid surrounding cells interferes with staining. Some common cytological preparation techniques include:

1. Combination Technique

This method makes a squash prep of 1/3 of the aspirate, leaves the middle 1/3 untouched and thus concentrated, and gently spreads the remaining 1/3 of the aspirate. A portion of the aspirate is expelled onto a glass microscope slide (prep slide). Another glass microscope slide is placed over about one-third of the preparation. If additional spreading of the aspirate is needed, gentle digital pressure can be used. Excessive pressure should be avoided. The spreader slide is

smoothly slid forward. This makes a squash prep of about one-third of the aspirate. The spreader slide also contains a squash prep. Next, the edge of a tilted glass microscope slide **(second spreader slide)** is slid backward from the end opposite the squash prep until it contacts about one-third of the expelled aspirate. Then, the second spreader slide is slid rapidly and smoothly forward. This produces an area that is spread with mechanical forces like those of a blood smear preparation. The middle area is left untouched and contains a high concentration of cells.

2. Squash Preparation

This method is used for spreading viscous samples and samples with flecks of particulate material. A portion of the aspirate is expelled onto a glass microscope slide and another slide is placed over the sample to spread it. If the sample does not spread well, gentle digital pressure can be applied to the top slide; however, care must be taken not to place excessive pressure on the slide causing the cells to rupture. The slides are smoothly slid apart. This usually produces well-spread smears but, even when care is take, may result in excessive cell rupture.

3. Modified Squash Preparation

This method is also used for spreading viscous samples and has less tendency to rupture the cells. A portion of the aspirate is expelled onto a glass microscope slide and another slide is placed over the sample. This causes the sample to spread. If necessary, gentle digital pressure can be applied to the top slide to spread the sample more. Care must be taken not to use excessive pressure and cause cell rupture. The top slide is rotated about 45 degrees and lifted directly upward, producing a spread preparation with subtle ridges and valley of cells.

4. Needle Spread or "Starfish" Preparation

This technique tends not to damage fragile cells, but allows a thick layer of tissue fluid to remain around the cells which may interfere with staining. A portion of the aspirate is expelled onto a glass microscope slide. The tip of a needle is placed in the aspirate and moved peripherally, pulling a trail of the sample with it. This procedure is repeated in several directions, resulting in a preparation with multiple projections.

7.2.2 Preparation of smears from fluids

Smears should be made immediately after fluid collection. Smears can be made directly from fresh, well-mixed fluid or from the resuspended sediment of a centrifuged sample. The cellularity, viscosity and homogeneity of the fluid determine the selection of smear technique used. The most common techniques include:

1. Blood Smear Technique

This procedure is identical to that used for preparing smears of blood and will result in a smear with a feathered edge. This method is generally not appropriate for viscous samples. A drop of fluid sample is placed on a glass microscope slide close to one end, then another slide is slid backward to contact the front of the drop. When the drop is contacted, it rapidly spreads along the juncture between the 2 slides. The spreader slide is then smoothly and rapidly slid forward the length of the slide, producing a smear with a feathered edge.

2. Line Smear Concentration Technique

When the fluid cannot be concentrated by centrifugation or the centrifuged sample is of low cellularity, the line smear technique can be used to concentrate cells in the smear. Unfortunately, an excessive amount of fluid may also remain in the **"line"** and prevent the cells from spreading well.

A drop of fluid sample is placed onto a glass microscope slide close to one end, and another slide is slid backward to contact the front of the drop. When the drop is contacted, it rapidly spreads along the juncture between the 2 slides. The spreader slide is then smoothly and rapidly slid forward. After the spreader slide has been advanced about two-thirds to three-fourths the distance required to make a smear with a feathered edge, the spreader slide is raised directly upward. This produces a smear with a line of concentrated cells at its end, instead of a feathered edge.

3. Squash Preparation (Same as described above)

Smears of viscous fluids such as synovial fluid can be prepared using this technique.

7.3 CYTOLOGICAL STAINS

The two general types of stains most commonly used are the Romanowsky- type stains **(Wright's stain, Giemsa stain, Diff-Quik**) and Papanicolaou stain.

10.3.1 Romanowsky-type Stains

These are permanent stains which stain organisms and the cytoplasm of cells excellently. The stains tend to have a **"smudging"** effect on the nucleus, thus, nuclear and nucleolar detail cannot be perceived as well as with the Papanicolaou-type stains. However, the detail is usually sufficient for differentiating neoplasia and inflammation and for evaluating neoplastic cells for cytologic evidence of malignant potential. Smears to be stained with Romanowsky-type stains

must first be air dried to fix the cells and prevent them from falling off the slide during the staining procedure. These stains tend to dissolve lipids from cells leaving vacuoles. RNA stains blue and DNA stains purple with variations to red or pink. Mast cell granules may not stain with Diff-Quik.

New Methylene Blue Stain **(NMB)** is a useful adjunct to Romanowsky-type stains. It stains cytoplasm weakly, if at all, but gives excellent nuclear and nucleolar detail. NMB does not stain RBC hemoglobin and is thus useful in interpretation of blood contaminated samples. Fungi are stained and lipid droplets are outlined. This stain is a wet mount of a dried smear and is not permanent; however, the smear can be counter stained with Wright's stain to provide a permanent preparation. A combination of a drop of NMB and Sudan's or other oil stain will selectively stain lipid and give adequate cell detail. This stain combination is useful for fatty livers, chylothorax, aspiration pneumonia or lipid granulomas.

10.3.2 Papanicolaou Stains

These stains use wet fixed smears **(i.e., the smear must be fixed before the cells have dried)** and clearly illustrate cell structure and nuclear characteristics. They do not demonstrate bacteria and other organisms as well as Romanowsky stains. Papanicolaou-type staining requires multiple steps and considerable time.

7.4 MICROSCOPIC EVALUATION

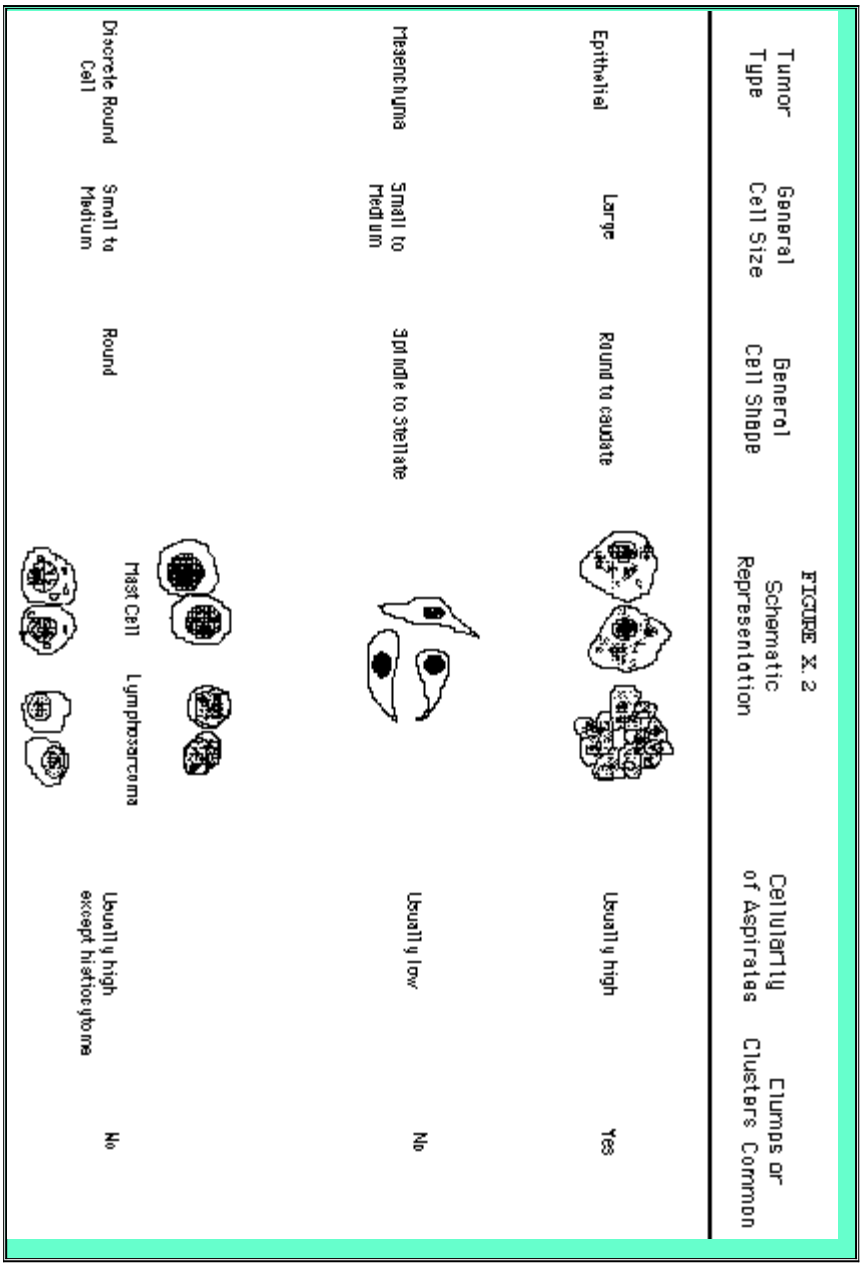
Once the smear has been prepared, stained and dried, it is scanned at low magnification **(4-10X objective)** to determine if all areas of the smear are stained properly and if there is adequate cellularity for evaluation. When proper staining is assured and all areas of increased and/or unique cellularity are recognized, magnification is increased to the 10X or 20X objective. An impression of the cellularity and cellular composition of the smear and of cell size are ascertained. Using the **40X** objective, nucleoli and chromatin pattern are discerned. Cell morphology is evaluated in detail with the 100X **(oil-immersion)** objective.

7.5 INTERPRETATION

An algorithm to aid in the general evaluation of all cytologic preparations is presented in **Figure X.1.** While a definitive diagnosis is not always achieved, the general process usually is recognized. Probably the most important judgment to be made when interpreting a cytologic sample is whether the lesion is inflammatory or neoplastic in nature. Samples containing only inflammatory cells or inflammatory cells and a few nondysplastic tissue cells indicate and

inflammatory lesion, whereas samples containing only tissue cells indicate a neoplastic or hyperplastic process. An admixture of inflammatory cells and tissue cells suggests neoplasia with secondary inflammation, or inflammation with secondary tissue cell dysplasia. Lesions determined to be inflammatory in nature can be classified into different categories of inflammation.

The inflammatory process can be classified as acute **when >70%** of the inflammatory cells are neutrophils, as subacute or chronic-active when **50-70%** of the inflammatory cells are neutrophils and **30-50%** are macrophages, and chronic when **>50%** of the inflammatory cells are macrophages. If **>85%** of the inflammatory cells are neutrophils, the process is classified as purulent or suppurative, granulomatous if inflammatory giant cells and/or numerious epitheli.



macrophages are present, and eosinophilic or allergic if eosinophils are numerous.

Often neoplastic lesions can be recognized as epithelial, mesenchymal **(spindle-cell)** or discrete round-cell tumors. Important cytological characteristics of these tumor types are illustrated in **Figure X.2**. Evaluation for malignant potential of tumors is estimated by evaluating tumor cells for indications of anaplasia and asynchronous development. The cytomorphologic criteria for this assessment are illustrated in **Figure X.3.** Descriptions for the various terms used to assess nuclear chromatin patterns are listed in **Figure X.4.**

Nuclear criteria of malignancy are more reliable than cytoplasmic criteria for estimating malignant potential. Recognition of more than 3 nuclear criteria of malignancy in a high percentage of tumor cells is strong evidence that the tumor is malignant. If malignant criteria are not recognized, the tumor is most likely benign. However, some tumors, such as canine thyroid tumors, may be malignant but show few, if any, criteria of malignancy. Therefore, if the cell type of a tumor cannot be recognized, caution must be exercised in classifying the tumor as benign.

FIGURE X.3

MALIGNANCY CRITERIA

CRITERIA	DESCRIPTION	SCHEMATIC REPRESENTATION
GENERAL CRITERIA		
Anisocytosis and macrocytosis	Variation in cell size, with some cells 1.5 times larger than normal.	
Hypercellularity	Increases cell exfoliation due to decreased cell adherence.	Not depicted
Pleomorphism (except in lymphoid tissue)	Variable size and shape in cells of the same type.	
NUCLEAR CRITERIA		
Macrokaryosis	Increased nuclear size. Cells with nuclei than 10 u in diameter suggest malignancy.	
Increased nucleus cytoplasm ratio (N:C)	Normal nonlymphoid cells usually have a N:C of 1:3 to 1:8, depending on the tissue. Ratios ≥ 1:2 suggest malignancy.	See "macrokaryosis"
Anisokaryosis	Variation in nuclear size. This is especially important if the nuclei of multinucleated cells vary in size.	
Multinucleation	Multiple nucleation in a cell. This is important if the nuclei vary in size.	

MALIGNANCY CRITERIA (CONT.)

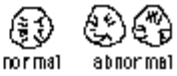
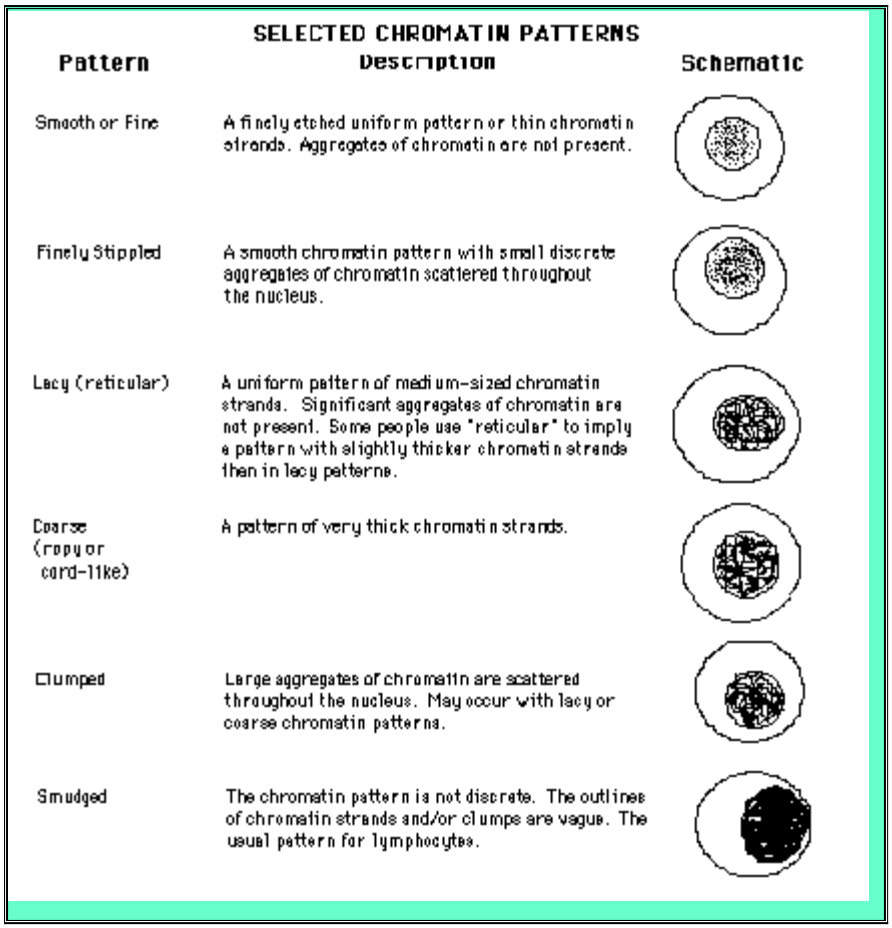
CRITERIA	DESCRIPTION	SCHEMATIC REPRESENTATION
Increased mitotic figures	Mitosis is rare in normal tissue.	 normal abnormal
Abnormal mitosis	Improper alignment of chromosomes	See "Increased mitotic figures"
Coarse chromatin pattern	The chromatin pattern is coarser than normal. It may appear ropy or cord-like.	
Nuclear Molding	Deformation of nuclei by other nuclei within the same cell or adjacent cells.	
Macronucleoli	Nucleoli are increased in size. Nucleoli > 5 strongly suggest malignancy. For reference, RBC are 6-7 u.	 RBC
Angular nucleoli	Nucleoli are fusiform or have other angular shapes. Instead of their normal round to slightly oval shape.	

FIGURE X.4



7.10 FLUID CYTOLOGY

Abnormal accumulations of fluid in body cavities and joints are relatively common occurrences in animals. Cytological examination of these fluids is utilized to reveal the cellular characteristics of the fluid so that the presence or absence of inflammation or neoplasia can be detected. Fluids to be discussed will include body cavity effusions (abdominal, thoracic and pericardial), synovial fluid, cerebrospinal fluid prostatic fluid, and trans-tracheal bronchoalveolar aspirations.

7.10.1 Body Cavity Effusions (Transudates and Exudates)

The body cavities of animals normally contain small quantities of fluid which is essentially an ultrafiltrate of blood. This fluid has few cells, chiefly of the mononuclear type. a process that impairs absorption, alters vascular pressure, or alters the concentration of albumin in the blood can result in accumulation of fluid in a body cavity, which is termed an effusion. Four basic mechanisms by which fluid may accumulate in abnormal quantities includes:

1. Increased capillary hydrostatic pressure (**e.g., venous stasis of the type seen with congestive heart failure).**

2. Decreased capillary oncotic pressure as a result of hypo-albuminemia and/or increased arterial pressure.

3. Increased capillary membrane permeability **(e.g., anoxia or inflammation).**

4. Lymphatic obstruction **(e.g., lymphadenitis, lymphangi-tis or neoplasia)**

Removal and examination of fluids from body cavities is indicated when there is excessive fluid accumulation or if the clinician suspects the presence of a lesion characterized by cellular exfoliation. An increased amount of fluid in a cavity is not a disease in itself but rather an indication of a pathologic process in the fluid production and/or removal system, or an accumulation from an ectopic source.

1. Fluid Collection

Most fluid transudates can be collected through a 16- to 20-gauge needle, whereas a 14- to 16-gauge needle may be required for exudates. A sample of the effusion should be collected in an EDTA tube to be used for a total nucleated cell count, total protein determination, and cytologic examination. Another sample should be collected in a serum tube if any biochemical analyses are to be performed.

a. Thoracentesis

Pleural effusions are typically abundant and bilateral but may be mild, unilateral and/or compartmentalized. Radiography is helpful in determining the extent and location of the effusion. Under most circumstances a 1- to 1 1/2-inch needle will suffice, except in some large domestic animals in which a 2- to 2 1/2- inch needle may be required. The site for thoracentesis varies, but the seventh or eighth intercostal space is chosen in most animals. The puncture should be made in the middle of the intercostal space to avoid damaging intercostal vessels found just caudal to the ribs. Thoracentesis is preferably performed with the animal in a standing position. In cases of accidental blood contamination, blood will be apparent either during the first or very last portion of the aspiration. Blood will be present throughout the aspiration if hemothorax is present

b. Abdominocentesis

In small animals, the ventral midline of the abdomen, 1-2 cm caudal to the umbilicus, is the usual site of needle insertion. In the horse, the preferred site is at the lowest portion of the abdomen

between the xiphisternum and umbilicus. A 1-inch needle is generally sufficient for small animals and a 2-inch needle or teat cannula for horses. Open-needle abdominocentesis is reportedly more sensitive than aspiration via syringe; therefore, a syringe is usually not attached. If a syringe is attached, only mild negative pressure should be applied because it is easy to aspirate omentum or other abdominal contents against the needle opening and inhibit fluid collection.

c. Pericardicentensis

Fluid is less frequently removed from the pericardium, but if there is an abnormal accumulation or pericardial fluid, percardicentesis may be of diagnostic and therapeutic value. An 18- to 20-gauge needle of sufficient length to reach the pericardium is required. The site of for puncture is at the cardiac apex. After the cardiac apex beat is located, the needle is carefully advanced toward the heart. A constant negative pressure is maintained in the syringe. When the needle enters the pericardial sac, fluid will appear in the syringe. If the needle contacts the cardiac wall, pulsation occurs and the needle will move. Should this occur, the needle location is slightly altered in order to prevent damage to the heart or its blood vessels. A small quantity **(0.5 to 1.0 ml)** of fluid is adequate for cytologic evaluation. If the fluid collected is bloody and clotted, this usually indicates that a blood vessel has been entered during collection. If a bloody fluid does not clot, this is an indication that defibrinated blood is present in the pericardial sac.

2. Physical Classification of Effusions

On the basis of nucleated cell counts and total protein concentrations, effusions may be classified as either transudates, modified transudates or exudates. A transudate is an excess accumulation of normal fluid **(filtrate of plasma)** in any body cavity or space. Transudate accumulations result primarily from obstructive phenomena and usually contain few cells and have low specific gravity and protein content. However, any transudate that remains in a body cavity for a period of time causes irritation to the lining of that cavity. This irritation generally results in increased cellularity, primarily due to proliferated mesothelial cells, and an increase in protein content that is generally due to fluid leakage from lymphatics. Fluids of this type are termed modified transudates. An exudate is an excess accumulation of abnormal fluid in any body cavity or space. It is usually the result of both obstructive and productive phenomena. The cellular and protein content are generally much higher than that of transudates. Since most exudates are inflammatory, this increased cellularity is usually due to accumulations of white blood cells.

The general parameters evaluated in effusion cytology include:

a. Physical and Chemical Characteristics

1) Color

a) serum or plasma - light or dark yellow

b) Erythrocytes - red, dark red, or reddish brown

c.) Leukocytes - white, cream, yellow, gray or green

d) Chyle - milky white (**after centrifugation)**, peach or pink

e) Bilirubin - yellow, orange, red or brown

2) Transparency or Turbidity

a) Erythrocytes - grayish-red turbidity

b) Leukocytes - from light turbidity to thick, creamy pus

c) Fat droplets - milky turbidity

3) Coagulation - depends upon the amount of fibrin present.

a) Transudates - rarely show spontaneous complete or partial coagulation

b) Exudates - usually coagulate very quickly after removal from the serous cavity.

4) Protein Concentration - Refractometer

a) Transudates less than 3 g/dl

b) Exudates more than 3 g/dl

5) Specific Gravity - Refractometer

a) Transudates under 1.017

b) Exudates over 1.017

6) Bilirubin**, BUN**, Creatinine, globulin, amylase, cholesterol, triglycerides and other determinations may facilitate interpretation.

b. Cellular Elements

The approach to cytologic evaluation of fluids is essentially the same as the approach to the cytologic evaluation of solid masses. The populations of cells present is determined and compared to those found in the normal fluid of the given cavity. It must then be decided if the cells in the fluid accumulation are normal, the result of inflammation, or the result of neoplasia.

Normally, fluid content of the body cavities is low and contains few cells. Occasional white blood cells with the same features as those in the peripheral blood will be seen. Additionally,

mesothelial lining cells are seen. The following is a list and description of those cells which may be present in variable numbers in effusions:

1) Mesothelial Cell

These cells line the serous surfaces of the pleural, pericardial and peritoneal cavities. With Romanowsky stain, the normal exfoliated cell is a large cell **(12-30 microns)**, with extreme cytoplasmic basophilia which may obscure the nucleus. The cytoplasmic margin may or may not present reddish hairlike processes **(eosinophilic brush border).** Nuclei are of uniform size and are hyperchromic. Binucleation may be observed.

Pale-staining mesothelial cells may also be observed and represent ***in situ*** cells removed during the centesis procedure. The cells are usually in clusters. The nuclei are of uniform shape and size. The density of RNA and DNA is low, giving a pale-staining overall appearance to the cells.

The accumulation of large quantities of transudative fluid causes mesothelial cells to exfoliate in relatively large numbers. The transudate fluid is biologically a perfect cell-culture medium in which the mesothelial cells may replicate and transform into so-called blast-transformed, reactive or activated mesothelial cells. These are highly variable in cytologic appearance. The nuclei are generally less hyperchromic than those of non-transformed mesothelial cells. The chromatin generally has a finely reticular pattern and nucleoli may be present. Mitotic figures and multinucleate forms are common. The cytoplasm is slightly basophilic and may contain phagocytic debris, since activated mesothelial cells may become phagocytic. For this reason, they cannot be differentiated from the monocyte-derived macrophage.

Blast-transformed mesothelial cells often imbibe water and swell to unusually large size. The swollen cells have a clear cytoplasmic area with the nucleus displaced, giving a signet-ring appearance to the cell. The transformed cells may occur singly but more often , the cells suspended in the fluid do not divide completely and result in clusters of cells.

Since many of the morphologic criteria of malignancy are observed in blast-transformed mesothelial cells, it is important that the nuclear criteria be stringently applied when differentiating reactive mesothelial cells from neoplasia.

2) Macrophage

Mesothelial-cell reactions are accompanied by variable numbers of macrophages, depending on the amount of particulate detritus. Macrophages tend to be sticky and form cell clusters of a pale or light appearance. The cells are large **(15-50 microns)** and they generally have a single oval to

bean-shaped nucleus; however, multinucleation and variable nuclear shapes may be present. Their nuclear chromatin is lacy and their cytoplasm is frequently vacuolated and may contain phagocytic debris.

3) Neutrophil

Neutrophils are present to some degree in most effusions and tend to predominate in effusions associated with inflammation. The two general cytological classifications, non-degenerate and degenerate, and their significance have been discussed in a previous section of this syllabus.

4) Lymphocytes and Plasma Cells

Lymphocytes are present in small numbers in many effusions and may be predominant in chylous, pseudochylous and lymphosarcomatous effusions. Care must be taken not to confuse reactive lymphocytes in inflammatory conditions with neoplastic lymphocytes. Neoplastic lymphocytes are usually lymphoblasts and are characterized by a moderate amount of clear to blue cytoplasm, variably shaped nuclei, finely stippled nuclear chromatin and nucleoli of variable shape and size. These cells are larger than neutrophils.

5) Eosinophils

Moderate to large numbers of eosinophils may be seen in effusions secondary to mast-cell tumors, heartworms, allergic reactions and hypersensitivity. They are commonly seen in bovine aspirates, perhaps due to the high incidence of ***Setaria*** nematodes within the peritoneal cavity of cattle.

6) Mast Cells

Mast-cell tumors within body cavities may be associated with effusions and frequently exfoliate large numbers of mast cells into the effusion. However, mast cells are observed in small numbers in effusions from dogs and cats with many different inflammatory disorders.

7) Erythrocytes

Erythrocytes may be seen cytologically within effusions secondary to hemorrhage or contamination with peripheral blood. Erythrocytes are useful as a marker in measuring size of other cells. It is important to differentiate bloody taps from true intracavity hemorrhage. This differentiation is based upon clinical signs and the presence or absence of intact platelets and erythrophagocytosis. Macrophages containing hemoglobin pigment are indications of chronic hemorrhage. A hemorrhagic effusion has a **PCV a**nd **WBC** counts similar to pripheral blood. Modified transudates and exudates containing blood usually have a **PCV** of 5% or less.

8)Neoplastic cells

Neoplastic cells may be observed in effusions with many different types of neoplasia. Distinction of transformed mesothelial cells from malignant cells in effusions may be difficult by ctyologic study. Identification of the neoplastic cells depends upon recognition of the cell type and malignancy criteria. Various carcinomas, adenocarcinomas, lympho-sarcomas, mast-cell tumors, hemangiosarcomas and mesotheliomas may exfoliate neoplastic cells.

c.Type of Effusions and Their Causes

Attempts are made to classify effusions as transudates, modified transudates and exudates. This is often difficult because of the overlapping of many of the parameters. In attempts to simplify this procedure, the following discussion on classification is based solely on the total nucleated cell count **(TNCC)** and total protein **(TP)** concentration of the fluids.

1)Pure Transudate

Transudates are usually due to decreased plasma oncotic pressure,principally from hypoalbuminemia. Characteristics of a transudate are:

Colorless and clear fluid

Less than 3.0 g/dl protein in canine and feline thoracic and abdominal fluid.

Less than 2.0 g/dl for thoracic fluid in large animals

Less than 1.5 g/dl for horses and less than 3.5 g/dl for ruminant abdominal fluids

Less than 1000 nucleated cells/ul in canine and feline fluids

Less than 5000 nucleated cells/ul in large animal fluids

Cell population consists of nondegenerate neutrophils, macrophages and reactive or trans-formed mesothelial cells in low numbers

2)Modified Transudate

Most modified transudates occur as a result of fluid leakage from lymphatics or blood vessels. Such leakage is caused by increases in hydrostatic pressure or permeability. Both of these conditions allow high-protein ultrafiltrate fluid to pass into the cavity. Neither of these conditions results in chemotactants in the cavity; therefore, large numbers of inflammatory cells do not migrate into the fluid. This produces a high protein fluid with low to moderate nucleated cell counts.

Modified transudates are the least specific classification of effusions. Effusions in this classification can develop secondary to many nonspecific disorders. Most modified transudates

are transitory and may progress to nonseptic exudates. The effusion itself may initiate an inflammatory response. Some of the general characteristics of modified transudates are as follows:

Low to moderate cellularity with TNCC or between 1000 - 7000 cells/ul

Total protein concentrations of between 2.5-7.5 g/dl

Color variation from amber to white to red and frequently slightly turbid to turbid

Nondegenerate neutrophils, meosthelial/macro-phage cell types, small lymphocytes or neoplastic cells may predominate, depending on the cause of the effusion.

3)Exudates

Exudates occur most commonly due to chemotactants in the cavity as a consequence of an inflammatory process. Occasionally, an exudate may develop due to abundant exfoliation of cells from a tumor or secondary to a chylous effusion. Any modified transudate may develop into an exudate. The general characteristics of exudates are:

High protein concentrations of greater than **3.0 g/dl**

High T**NCC or >7000 cells/ul**

Color variation from amber to white to red

Turbid to cloudy

The predominant cell type is dependent upon the cause of the exudate. In inflammatory exudates, the neutrophil generally predominates with variable numbers of macrophages and lymphocytes.

Inflammatory exudates are generally divided into two main categories, non-septic and septic.

a)Nonseptic Exudates

This type of effusion is the product of inflammation caused by irritants that are not toxic to neutrophils.

Causes include gallbladder or urinary bladder rupture and sterile foreign bodies.

It may develop from any of the causes of modified transudates.

Characteristics are:

(1)White or pink and cloudy fluid

(2)3.0-5.0 g/dl of protein

(3)3000-50,000 or more cells/ul

(4)Nondegenerate neutrophils and variable numbers of macrophages, erythrocytes and lymphocytes. Small numbers of cells may have pyknotic and karyorrhectic nuclei.

b)Septic Exudates

They are caused by a wide variety of microorganisms that are toxic to neutrophils

Septic exudates are characterized by:

(1)White, red, or yellowish, cloudy fluid

(2)3.0-5.5 g/dl of protein

(3)300-100,000 or more cells/ul

(4)Degenerate neutrophils and variable numbers of macrophages

(5)Intracellular and/or extracellular bacteria

Exudates caused by Nocardia sp. or Actinomyces sp. in the dog have special features:

(1)Microcolonies composed of pleomorphic, filamentous rods are observed. Some organisms occur in branching chains with a beaded appearance.

(2)Neutrophils around the microcolony are degenerate, whereas neutrophils distant from the microcolony or in aspirates not containing microcolonies are nondegen-erate.

d.Effusions in Selected Disorders

1)Infectious Peritonitis and Pleuritis

Peritonitis and pleuritis are associated with release of chemotactants and vasoactive substances within the respective cavities. This results in an exudative effusion due to increased capillary permeability with a massive outpouring of peripheral blood neutrophils and high-protein plasma filtrate. Degenerate neutrophils generally predominate in bacterial infections and intracellular and/or extracellular organisms may be seen. Spirochetes occasionally are seen in association with bacterial peritonitis and pleuritis, especially secondary to bite wounds. Mycotic, protozoal and rickettsial organisms may also be etiological agents in infectious peritonitis/pleuritis.

2)Tissue Inflammation

Intracavity organ inflammation (**e.g., heart, liver, spleen, lymph nodes, lungs, etc.)** or a walled-off abscess may result in an associated body cavity effusion. The mechanisms are similar to those described for infectious peritonitis/pleuritis in that inflammation causes the release of chemotactic and vasoactive substances. These in turn result in the influx of inflammatory cells and high protein fluid into the involved area. When this process extends into the associated cavity, effusive inflammation results. **TNCC** and **TP** levels will determine if this effusion is classified as a modified transudate or exudate. In this type of effusion, nondegenerate neutrophils

generally predominate, but macrophages, mesothelial cells and lymphocytes are also present. The mononuclear cell component will increase with chronicity.

Cytological evaluation of these effusions is typically nondiagnostic as to etiology or to the site of original tissue involvement and must be correlated with physical findings, history and other laboratory test results to pinpoint the exact problem.

3)Feline Infectious Peritonitis (FIP)

An effusion associated with **FIP** is one cause of a nonseptic exudate **(pleural and/or peritoneal)** in cats. This effusion is an odorless, straw-colored to golden, tenacious fluid that may contain flecks or fibrin strands. The fluid is generally cloudy and has an extremely high protein concentration (**3.5 to 8.0 g/dl)** consisting predominantly of gamma globulin. This high protein concentration and fine particles of cell detritus give Wright's stained smears of the fluid a precipitous eosinophilic background. NMB-stained fluids may show a background of fibrin in needle-like meshwork form. There is a mild to marked increase in the **TNCC (can vary from less than 1000 to 23,000 or more cells/ul)** consisting primarily of nondegenerate neutrophils **(60-80%)** and lesser numbers of macrophages, lymphocytes and occasionally plasma cells. In chronic effusions, the percentage of neutrophils may decrease as the number of mononuclear cells increases. Blast-transformed mesothelial cells and associated cell clusters are seldom found in FIP fluids.

While the cytological findings are not diagnostic alone, when associated with history and clinical findings, a presumptive diagnosis of FIP can be made.

4)Bile Peritonitis

Rupture of the gall bladder or bile duct can produce a nonseptic exudative effusion. The effusion is discolored by bile pigments giving it a typical yellow-orange color. Bile is an irritant and results in increased cell counts, particularly neutrophils and macrophages. The fluid will give a positive icotest and is high in cholesterol. In Wright's stained smears, bile peritonitis is characterized by phagocytized yellow green to brown green pigment within macrophages.

5)Uroperitoneum

Rupture of the kidney, urinary bladder, urinary tract or patent urachus that results in release of urine in the abdominal cavity can also produce a nonseptic exudative effusion. Urine is an irritant and results in increased in mononuclear cell count. Very acute rupture causes an elevated urea nitrogen in the abdominal fluid **(compared to blood urea nitrogen levels);** however, this is

rapidly reabsorbed by the lining tissues and levels soon equilibrate with blood levels **(generally within 24 hours).** Creatinine is more poorly absorbed by the lining tissues and will remain elevated above blood levels for longer periods. Therefore, measurement of the creatinine level is more reliable than measurement of the urea nitrogen level.

6)Chylous/Pseudochylous Effusions

Chylous effusions can occur secondary to many disease processes, including trauma, heart disease, inflammation and neoplasia. These effusions in dogs and cats seem to occur most frequently in the thorax and are generally bilateral. Chylous effusions occur as a result of lymphatic duct leakage or rupture. These effusions are odorless and vary in color from milky white to yellow or pink, depending on the animal's diet and the number of **RBCs** in the fluid. Overnight refrigeration of the chylous fluid will typically produce a **"cream layer"**. Tests for protein levels give inaccurate values because of interference by lipid droplets. **TNCC** are usually less than **10,000/ul.** While small lymphocytes are typically thought of as the predominant cell type, chylous effusions occur in which neutrophil and/or macrophages predominate. Lysed cells are common in effusions rich in lymphoctyes. Bacterial infection is uncommon due to the bacteriostatic effect of the fatty acids in chyle.

Most milky white effusions are chylous, but occasionally they are pseudochylous. These effusions most commonly occur with neoplastic of inflammatory disorders, but may be associated with any chronic effusion. Unlike chylous effusions, the white color of pseudochylous effusions is not due to fat **(chyle)** but to cellular debris, protein, lecithin globulin complexes and/or cholesterol granules.

Chylous and pseudochylous effusions have been differentiated by staining smears with fat stains such as Sudan III. A positive stain confirms the presence of a chylous effusion; however, since cytologically identifiable fat may be absent from some chylous effusions, a negative result does not confirm pseudochylous effusion. The use of ether to clear the chylous effusion is also not a reliable differentiation test.

The best available diagnostic test for differentiation between chylous and pseudochylous effusion is measurement of the triglyceride and cholesterol concentrations in both the effusion and peripheral blood. With chylous effusions, the triglyceride concentration is higher in the effusion than in the serum and the cholesterol concentration is higher in the serum than in the

effusion. The reverse is true of pseudochylous effusions. Also, the cholesterol/ triglyceride ratio of the effusion fluid is **<1.0** in chylous effusions.

7)Heart Disease Characterized by Heart Failure

A common sequelae to heart failure or liver congestion is the development of a modified transudate effusion termed ascites. This effusion develops secondary to increased intrahepatic pressure and leakage of high-protein hepatic lymph. Some specific characteristics of this fluid are:

-pink to red and cloudy fluid

-2.5-5.0 g/dl protein

-300-5500 cells/ul

-Variable numbers of erythrocytes, nondegenerate neutrophils, mesothelial cells, macrophages , and occasionally eosinophils and lymphocytes

There is no cytologic finding in these effusions that is pathognomonic for heart failure and other clinical examinations are necessary to establish the diagnosis.

Cats may develop a pleural effusion secondary to feline chronic congestive heart failure **(cardio-myopathy).** These effusions are yellow to milky white and typically consist of **>50%** small mature lymphocytes. While the color of the fluid may be similar to that of a chylous effusion, the triglyceride concentration may or may not be increased.

8)Hemorrhagic Effusion

Hemorrhagic effusions are usually caused by trauma, surgery, infarction of the intestine, neoplasia and hemostatic defects. Hemorrhagic effusion must be differentiated from blood contamination due to bloody taps and organ taps **(e.g., liver and spleen).** Unfortunately, differentiating blood contaimination from acute hemorrhage can be difficult. However, if acute hemorrhage is severe, clinical signs of blood loss should be evident. When blood enters a body cavity, the platelets quickly aggregate, degranulate and will disappear within 45 minutes. In conjunction with these platelet changes, the blood will coagulate rapidly and then undergo mechanical defibrination. Therefore, hemorrhagic effusions do not clot. Also, within hours of release into the cavity, the **RBC**s are phagocytized and digested by macrophages. These and other factors can be used to confirm the existence of hemorrhage and to estimate its duration.

Recent hemorrhage (**within several hours)** is characterized by:

Clear supernatant fluid and red sediment

Protein and cell count values similar to those of peripheral blood.

Intact erythrocytes

Leukocytes of similar morphology and distribution as those in peripheral blood

Platelet aggregates

More longstanding or resolving hemorrhagic effusions are characterized by:

Erythrocytes and leukocytes that appear older and distorted.

Supernatant fluid may be pink due to hemolysis

Absence of platelets

Macrophages with phagocytized erythrocytes and hemosiderin and/or hematoidin crystals.

In summary, the absence of erythrophagocytosis and presence of platelets suggest either peracute hemorrhage or a bloody tap. The presence of erythrophagocytosis and platelets suggests either chronic persistent hemorrhage or previous hemorrhage and a bloody tap. The presence of erythrophagocytosis and absence of platelets suggest chronic or previous hemorrhage.

9)Neoplasia

The characteristics of an effusion associated with neoplasm will be variable depending upon the tumor involved. Neoplastic effusions may have characteris-tics of obstructive, inflammatory, or hemorrhagic effusions. Most effusions caused by tumors not exfoliating neoplastic cells are modified transudates. Effusions caused by tumors that are exfoliating cells into the cavity and/or are secondarily inflamed will be exudative in nature. In all cases, particularly in inflammatory exudates, care must be taken not to confuse dysplastic cells and/or normal or reactive mesothelial cells with neoplastic cells.

The most common cause of feline neoplastic pleural effusion is mediastinal Lymphosarcoma. In horses, lymphosarcoma is also the most commonly identified neoplasm in pleural and peritoneal effusions. These fluids are generally characterized by:

Whitish to pink and cloudy fluid

3.0-6.0 g/dl of protein

4500-45,000 cells/ul

Immature lymphocytes, usually lymphoblasts

Other neoplasms which may exfoliate into the body cavities include mast-cell tumors, mesothelioma and various carcinomas, adenocarcinomas and sarcomas. Malignant mesothelioma, a primary pleural or peritoneal tumor, is a rare cause of effusion in animals. They

are extremely difficult to diagnose cytologically because of the variability of normal mesothelial cells. Diagnosis generally requires a biopsy and it is difficult even on histopathologic examination to make a definitive diagnosis.

Since many tumors do not exfoliate neoplastic cells, the absence of neoplastic cells within effusions does not rule out neoplasia. As in many instances, a positive finding is positive but a negative finding necessitates further diagnostic evaluation.

7.10.2 Synovial Fluid

Evaluation of synovial fluid is a valuable adjunct to assessment of many lameness cases. In addition to cytologic evaluation, the fluid should be assessed for volume obtained, color, turbidity, viscosity, mucin **(hyaluronic acid)** quality/concentration, and protein concentration.

Based on laboratory tests and cytological findings, arthropathies can usually be categorized as hemorrhagic, degenerative **(non-inflammatory, non-exudative)** or inflammatory **(exudative)** in origin. Unfortunately, all synovial fluid specimens will not fall neatly into one of these categories. This is especially true of longer-standing problems which tend to develop characteristics of more than one category, thus making classification and interpretation more difficult.

Degenerative or non-inflammatory joint diseases usually result from trauma to a joint and/or degenerative changes of the articular surfaces. The latter may be caused by acute or chronic trauma as a result of accidental injury or congenital or acquired anatomic disorders that predispose articular surfaces to abnormal stresses. Additional causes include metabolic and nutritional abnormalities and neoplasia.

Inflammatory joint diseases include those caused by both infectious and noninfectious causes. Both are usually associated with an effusive exudate showing a moderate to marked increase in neutrophil numbers and variable increase in large mononuclear cell numbers. Etiological agents associated with septic arthritides in the various species include bacteria, fungus, Mycoplasma, Protozoa, Rickettsia and Virus. The noninfectious inflammatory joint diseases can be subgrouped into immune-mediated and nonimmune-mediated causes.

1.Types of Abnormalities

a.Non-exudative joint disease

1)Osteoarthritis

2)Osteochondrosis and other degenerative joint diseases

3)Hip dysplasia

4)Ruptured cruciate

5)Traumatic joint disease

6)Tarsal hydrarthrosis

b.Exudative joint disease

1) Nonseptic arthritis and autoimmune conditions

2)Septic arthritis

2.Routine Analysis and Differential Diagnosis

Normal synovial fluid is essentially a dialysate of plasma. Hence, concentrations of electrolytes and nonelectrolytes **(such as glucose and urea)** are similar to those in blood. It is essentially without fibrinogen and most other clotting factors and therefore does not clot, even in vitro. With blood contaimination, intraarticular hemorrhage or protein exudation in various inflammatory diseases, samples may clot unless processed immediately or added to an anticoagulant. **EDTA** is preferred for cytological examination. Heparin is recommended for the mucin clot test because **EDTA** interferes with the test by degrading hyaluronic acid. Both anticoagu-lants are suitable for other routine tests.

a.Volume

1)The volume is nearly normal in degenerative and traumatic joint disease

2)Increased fluid occurs in inflammatory processes and hydrarthrosis

b.Color and Turbidity

1)Normal synovial fluid is transparent and colorless in most species and transparent and yellow in the horse.

2)Blood is red if fresh and amber if old. If the fluid is blood tinged, hemarthrosis must be distinguished from iatrogenic contamination at collection. Figure 15 outlines the procedures for differentiating between these conditions.

3)Abnormal samples with increased cellularity exhibit variable discoloration .

4)Turbidity indicates cells or fibrin.

c.Viscosity

1)Normal synovial fluid is very viscous because of its high concentration of hyaluronic acid.

2)Normal fluid should not separate from the tip of the hypodermic needle until it has dropped 1-2 inches.

3)Decreased viscosity is caused by degradation of synovial mucin by bacterial hyaluronidase or by dilution of synovial mucin by serous effusion into the joint

d.Mucin Clot Test

1)Following centrifugation, undiluted synovial fluid supernatant is added to 2-5% glacial acetic acid at a **ratio of about 1:4**. This causes mucin to agglutinate.

a)A normal or good mucin clot will be tight and ropy

b)A poor clot indicates degradation of synovial mucin,usually by bacterial enzymes, or dilution by excessive effusion into the joint.

c)Poor clots are associated with septic arthritis while good clots usually occur with traumatic and degenerative joint disease.

e.Fibrin Clot

1)Normal fluid contains no fibrinogen and should not clot.

2)Clotting indicates blood contamination or inflammation.

f.Chemical Constituents

1)Total protein **(A/G ratio)** - In arthritis there is often decreased albumin and increased globulins.

2)Glucose - Serum/synovial ratio **(1:1)**. Decreased in infectious arthritis due to the glycolytic activity of bacteria.

g.Immunologic Evaluation

1)Rhemuatoid factor

a)An IgM with specificity for IgG

b)Positive in 60% of the dogs with RA and 10% of the dogs with SLE.

2)ANA (Antinuclear antibody)

a)Positive in 10% of dogs with RA and 90% of dogs with SLE.

h.Cell Evaluation

Cytololgical examination of synovial fluid requires properly made smears. Since normal synovial fluid is viscous, direct smears must be made by slowly advancing the spreader slide to create thin smears. The high viscosity of the fluid will result in cells evenly dispersed in the smear. Cells will not accumulate at the feathered edge unless the viscosity of the fluid has been altered by dilution with exudative effusion.

The background material is mucin and the density reflects the thickness of the smear. In Wright's stained specimens, the background is granular. Clefts in the background may result from fibrin formation and separation of the mucin.

Synovial fluid has a limited number of ways in which it can respond in disease. Therefore, the major questions in examination of synovial fluid are, Is there inflammation? and is there sepsis? Normal synovial fluid contains low numbers of mononuclear cells. Lymphocytes predominate and monocytes-macrophages are variable. Occasionally, synovial lining cells may be present. Neutrophils and RBCs are rare in normal synovial fluid, and when found, are usually the result of blood contamination from the sampling procedure. In small animals, inflammation is determined by finding more than **3000 WBCs/mm3** or greater than 12% of the cells are neutrophils. In large animals, nucleated cell counts greater than 1000 cells/ul **(500 cells/ul in the horse)** with similar increases in the percentage of neutrophils is indicative of inflammation. In all species, sepsis is determined by finding degenerated WBCs or bacterial **(especially if engulfed by WBCs).** For unknown reasons, it is often much more difficult to detect etiologic agents, particularly bacteria, in synovial fluid than in aspirates from other body sites. Culture of the fluid is generally required to detect or confirm infectious agents.

RBC counts increase with blood contamination, traumatic conditions and with certain inflammatory conditions. The highest elevation of **WBC** cell counts occur in septic and autoimmune arthritis. Mild increases occur in traumatic and degenerative disease. Lymphocytes and monocytes comprise 90% of the cells in normal fluid and predominate in tarsal hydrarthrosis and traumatic and degenerative joint diseases. Neutrophils predominate in bacterial and certain nonseptic inflammatory diseases; e.g., lupus erythematosus **(SLE)** and rheumatoid arthritis **(RA)**. Occasionally, osteoclasts, multinucleated giant cells, may be found in smears and are indicative of cartilage erosion and exposed bone.

7.10.3 Cerebrospinal Fluid

Removal and laboratory examination of **CSF** are indicated whenever there is clinical evidence suggesting CNS disease. Lesions involving the CNS do not consistently or uniformly cause changes in the CSF. The results of CSF analysis may be within normal limits in many instances of neurologic disease, and even when CSF cytologic abnormalities are present, they are often nonspecific. Alterations of CSF probably depend more on the location and extent of the CNS lesion than on cellular abnormalities. Thus, disease of the meninges produces greater alterations

in CSF than do most diseases of CNS parenchymal tissue. Septic meningitis may cause suppuration within the CSF; inflammatory cells may be numerous and levels of exudative protein markedly increased. In contrast, viral disease affecting the CNS, such as canine distemper, usually cause only a mild increase in CSF nucleated cell numbers.

Collected CSF should be placed in **EDTA** anticoagulant at the same concentration as used for blood samples. Refrigeration at 4o C also aids cell preservation. Because cells in CSF sometimes degenerate rapidly, cell counts and cytologic examination should be performed within 30 minutes of collection.

1.Physical Examination

a.Color

1)The normal CSF is crystal clear and colorless and resembles distilled water. In viral encephalitis or meningitis, the CSF may remain clear.

2)Hazy pink CSF may be associated with a traumatic tap or intracerebral or subarachnoid hemorrhage.

3)Bright red CSF indicates fresh blood from iatrogenic hemorrhage. Centrifugation of the fluid will produce a clear, colorless supernatant. Blood contamination will invalidate the results of CSF analysis but a crude correction can be made by discarding 1 WBC and **1 mg%** protein for every **1000 RBCs** present.

4)Dull red or brown fluid indicates previous hemorrhage.

5)Xanthochromia **(pale orange or yellow)** is due to bilirubin from degenerating **RBCs.** It indicates hemorrhage of at least 2-4 days and can persist for up to 40 days. It may be associated with tumors, trauma, spinal cord compression and abscesses and can have protein concentrations greater than **400 mg%.** Xanthochromia may also be due to altered permeability of the blood brain barrier allowing influx of pigments from blood plasma **(e.g., unconjugated bilirubin).**

b.Turbidity

Turbidity will be apparent when the CSF contains 300-500 cells/ul or more. Bacteria or mycotic agents can contribute to turbidity. Bacterial meningitis may be only slightly turbid to almost pure pus which may clot. Viral encephalitis, trauma, tumor, abscess may show turbidity due to large amounts of protein, fibrin and/or cells.

c.Coagulation

Normal CSF does not coagulate. Coagulation may occur if damage to the blood brain barrier permits fibrinogen to enter the CSF or if CSF collection results in hemorrhage.

2.Chemical Constituents

a.Protein

1)**CSF** protein levels are normally very small and consist mostly of albumin.

2)Globulin protein is of interest in that normal CSF is relatively free of globulins and their concentrations are increased in pathological conditions. The Pandy test is a qualitative test for high molecular weight protein (**globulin)**. Add 1-2 drops of **CSF** to 1 ml of Pandy reagent **(10 g phenol crystals/100 ml of distilled water)** and observe for white turbidity. Normal CSF produces only faint turbidity. With abnormally large amounts of globulins, the solution becomes cloudy.

3)Urine protein reagent strips may be used to grossly detect protein. An elevated protein level would usually be represented on reagent strips as >100 mg/dl .

4)Quantitative tests are colorimetric or turbidometric. In contrast to cell evaluation, protein quantitation need not be done immediately. Fluid may be frozen and evaluated at a later date. The Coomassie brilliant-blue technique is the more commonly used spectrophotometric assay. Normal values are <48 mg/dl for dogs and cats, **<70 mg/dl** for horses and **<60 mg/dl** for ruminants.

5)Quantitation of protein fractions in both serum and CSF may be necessary to differentiate leakage of plasma protein across the blood-CSF barrier from increased synthesis of immuno-globulins **(IgG)** within the **CNS**. Correlation between CSF albumin and CSF globulin levels indicates a blood-CSF barrier dysfunction with both albumin and globulin entering the CSF with equal facility, but at a greater than normal rate. In contrast, low serum IgG concentrations, high **CSF IgG** levels and normal CSF albumin levels indicates a local CNS IgG production and not leakage across the blood-CSF barrier.

6)Increased total protein or globulin occurs in:

a)Encephalitis or meningitis which increases the permeability of the blood-brain barrier to plasma proteins. Levels are generally markedly increased by bacterial meningitis and somewhat less altered by viral meningitis or encephalitis. Toxoplasma infections may also greatly increase CSF protein levels.

b)Brain or spinal cord abscess

c)Hemorrhage or blood contamination during a traumatic tap.

d)Tissue destruction

e)Neoplasm - total protein greater than **100 mg%**

f)convulsive states

g)From high intracranial pressure due to a brain tumor or intracerebral hemorrhage.

7)The pathologic mechanisms altering CSF protein levels are complex and it must be remembered that CSF protein content may increase without a concomitant increase in cell numbers.

b.Glucose

1)Normally averages **75 mg% and** is dependent on blood glucose concentrations **(should be measured concurrently)**. Normal CSF glucose values are **60-80%** of the blood glucose level.

2)Decreased levels are seen in hypoglycemia **(normal CSF/blood glucose ratio)** or in the presence of pyogenic organisms or rapidly growing tumors which utilize glucose. **(decreased CSF/blood glucose ratio)**

3)Increased levels are seen in hyperglycemic states **(normal CS/blood glucose ratio)**

c.Sodium

1)CSF sodium levels are slightly less than that of blood.

2)The levels are increased in CSF **(more than 160-200 mEq/L)** in water deprivation **(Salt poisoning syndrome of swine).**

d.Enzymes

1)Increased CSF creatine kinase activity has been reported in a wide variety of neurologic disorders. Elevated **CSF CK** levels in dogs **(normal values = 3 IU/L)** appears to be a nonspecific but sensitive index of CNS disease.

2)**SGOT (AST)** is found to be elevated in canine distemper, purulent meningitis, or damage to brain tissue.

3)SGPT **(ALT)** is found to be elevated in canine distemper.

3.Cytologic examination

a.Total Nucleated Counts

Total cell counts of CSF can be determined by using a hemocytometer with a Neubauer ruling. A capillary pipette is used to place CSF on one chamber of the hemocytometer. The cells in all 9 squares are counted and the total multiplied by **1.1** to determine the total cell count/ul.

Differentiation of RBC and nucleated cells is done using the high-dry objective of the microscope. Nucleated cells are larger than RBC and have a granular appearance.

Normal CSF is free of **RBCs** and contains **<8 nucleated cells/ul** in dogs and cats and **<5/ul** in all large animal species. Total nucleated cells counts in the CSF are mildly elevated (pleocytosis) in a variety of disorders, and, though cytologic findings may suggest that inflammation is chronic **(primarily macrophages)** or chronic-active **(nearly equal numbers of macrophages and neutrophils)**, no specific etiology may be apparent. Nucleated cell counts and cytologic findings of CSF examination can be difficult to fit into classifications conforming to specific disease. All classifications appear to overlap. Pleocytosis of CSF indicates abnormality, but CNS disease may exist in which there is no pleocytosis and the cells which are present are normal.

b.Differential Cell counts

The nucleated cell population of normal CSF is predominantly mononuclear consisting of small lymphocytes and a few macrophages, endothelial cells and histiocytes. Neutrophils are not normally found in CSF unless the tap is traumatic. They should not constitute **>10%** of the total cell count.

Neutrophilic pleocytosis usually indicates pyogenic or bacterial infection, an abscess, bacterial encephalitis or meningitis. Under these conditions, counts may vary from 50 to over a 1000 cells/ul. Elevated neutrophil numbers may also occur in noninfectious inflammatory conditions. Attempts should be made to identify bacteria and the fluid should be cultured. In ruminants, pigment granules are common and must be distinguished from bacteria.

The CSF of animals with mycotic and protozoal encephalitides will have varied pleocytosis, consisting of neutrophils, mononuclear cells or eosinophils. An increase in TNCC consisting primarily of lymphocytic cells may indicate viral infections, uremia, fungal infections, postvaccinal inflammation, chronic infection and toxic conditions. Monocytes/macrophages are often associated with lymphocytes and will increase in conjunction with lymphocytes. They may predominate in the CSF in FIP.

Pleocytosis is rarely observed in noninflammatory conditions involving the CNS. In animals with spinal trauma, a slight increase in cell numbers may occur. Animals with congenital malformations of the CNS generally have normal CSF. Animals with cerebral infarcts may have increased numbers of RBC in the CSF and erythrophagocytosis by macrophages is a diagnostic feature of this condition.

Neoplastic cells may be evident if the meninges are involved. Filtration techniques are usually needed to demonstrate these cells. In most CNS tumors, CSF samples will have a normal or only slightly increased nucleated cell count consisting predominantly of mononuclear cells.

7.10.4 Prostatic Fluid

Evaluation of an enlarged prostate may be greatly enhanced by cytological evaluation. Clinical signs suggestive of prostatic enlargement include difficult defecation or difficult micturition. Rectal palpation of the prostate may reveal symmetric enlargement, unilateral enlargement or focal irregularities.

1.Collection and Preparation of Specimens

There are several ways in which cytological specimens may be obtained from the prostate gland. Prostatic fluid may be obtained by digital massage while aspirating with a syringe through a urinary catheter passed to the level of the gland. In this procedure, the tip of the catheter can be palpated per rectum as it approaches the base of the prostate. Care should be taken to properly lubricate the catheter and cleanse the tip of the penis to assure clean technique and to avoid introducing infection. This technique may cause fluid to be drawn into the syringe or only a few cells may be drawn into the tip of the catheter. Following massage and aspiration, negative pressure is released and the catheter withdrawn. Cells collected at the tip of the catheter are placed on a slide, spread by the **"squash-prep"** technique and evaluated. Fluid should be kept sterile for bacterial culture if warranted.

More prostatic material can be obtained by prostatic washing. In this procedure, a small amount of saline is used to wash the urethral lumen in the area of the prostate. Gentle injection and aspiration is used while gently massaging the prostate. Fluid with low cellularity should be centrifuged to concentrate the cells for slide preparation.

In addition, direct fine-needle aspiration techniques can be used to obtain prostatic material for evaluation, especially if prostatic cysts are suspected.

Another less desirable method is to obtain material by inducing ejaculation. This procedure produces samples that contain contaminant substances from other parts of the reproductive tract.

2.Cytological Examination

The prostate consists of numerous crypts lined by cuboidal epithelium. In cytological preparations, sheets of cuboidal epithelium are commonly seen. In normal smears, these epithelial cells will be uniform with round to oval nuclei. The nuclei are central in location and

have a homogeneous to fine reticular chromatin pattern. The cytoplasm may be granular and slightly acidophilic. Aspiration and wash specimens frequently contain cells of nonprostatic origin including spermatozoa, squamous cells from the distal urethra or external genitalia and urothelial cells.

A condition commonly seen in older dogs and believed to be caused by sex hormone imbalances is prostatic hyperplasia. Cytologically, the hyperplastic prostatic epithelial cells resemble normal cells but are usually greater in number. Large sheets and clusters may exfoliate. In these cell clusters, cytoplasmic boundaries are often indistinct. The cytoplasm is basophilic and slightly granular. Nuclei are round to oval and may be eccentric in location. Chromatin patterns are fine and nucleoli are generally not discernible. The nuclear/cytoplasmic ratio is increased as compared to normal prostatic epithelial cells. Inflammatory cells may occasionally be seen and suggest that the hyperplasia may be a result of inflammation.

Aspiration specimens of prostatic cysts are highly variable cytologically. Some cysts may contain poorly cellular fluid containing only a few epithelial cells and cellular debris. Sometimes moderate numbers of normal or slightly hyperplastic epithelial cells are found.

Squamous metaplasia of the prostate may occur under the influence of estrogen-like hormone activity, such as occurs in Sertoli-cell tumors, or as a sequel to chronic prostatic irritation or inflammation. Under these influences, the prostatic epithelium undergoes metaplasia to a squamous-like epithelium. Similar changes may also occur to some minor degree in normal older animals. Aspirates are moderately cellular with clusters of slightly basophilic to slightly acidophilic pale-staining cells. These cells are very large and have a flattened appearance. An occasional cell may contain a pyknotic or karyorrhectic nucleus. Occasional inflammatory cells and hyperplastic epithelial cells may be present.

Cytological specimens from an inflamed prostate will contain large numbers of inflammatory cells. Neutrophils are usually most numerous, but variable numbers of macrophages may also be present. Bacteria are frequently found and may represent an etiologic agent or a contaminant from some other region of the genital or urinary tract. Phagocytized bacteria are considered to be involved in the etiology of the prostatitis.

Specimens from prostatic abscesses will contain large numbers of degenerated neutrophils with a background of cellular debris. Single or small clusters of prostatic epithelial cells may be present and may exhibit hyperplastic to metaplastic changes.

Prostatic neoplasia generally results in prostatomegaly. The prostate may be very large, irregular and asymmetric. Aspirates of prostatic adenocarcinoma are moderately to markedly cellular. The neoplastic cells have the cytologic features common to adenocarcinoma of other organs. Anisokaryosis, nuclear enlargement, nuclear irregularity and an increased nuclear/cytoplasmic ratio are often evident. Nucleoli are present and are usually small, single and uniform, but large and sometimes irregular forms may occur. Cell membranes are usually distinct in well differentiated tumors but are indistinct in more poorly differentiated tumors. Cohesion of cells is often apparent and acinus formation may occur. Prostatic adenocarcinomas frequently metastasize to the iliac lymph nodes and cytological examination of these nodes should be performed in suspected adenocarcinoma cases. In addition to adenocarcinoma, transitional cell carcinomas may sometimes involve the prostate.

7.10.5 Tracheal Washes and Transtracheal Bronchoalveolar Aspiration Biopsy

Diagnostic cytology of samples from the respiratory tract has been used extensively in dogs, cats and horses and to a lesser degree in ruminants. These procedures are used routinely in the evaluation of pulmonary disease. Examination of secretions of the tracheobronchial tree and associated structures can yield useful information on developing or established lower respiratory tract disease including hypersensitivity reactions, inflammation, infectious agents and neoplasia. Depending upon the method of collection used, the specimens obtained are of variable volume and concentration techniques may be used in conjunction with direct smears.

1.Methods of Collection

The most common procedures for obtaining specimens from the tracheal/bronchial tree include percutaneous or endoscopic tracheobronchial aspiration **(TT/B)** and bronchoalveolar lavage **(BAL)**. The fluid used in these procedures tends to damage and/or distort cells and induces changes in inflammatory cells. Brush or punch biopsy of bronchial lesions is a more direct and satisfactory method for obtaining specimens when lesions are visible during bronchoscopy.

2.Cytological Evaluation of Tracheal/Bronchial Washes and Lavages

Oropharyngeal contamination is indicated by the presence of superficial squamous cells and Simonsiella bacteria. Superficial squamous cells are large epithelial cells with abundant, angular cytoplasm and a small round nucleus. Simonsiella bacteria divide lengthwise and line up in parallel rows. This gives the impression of a single large bacterium. These organisms are nonpathogenic and are often seen free in the smear or adhered to the surface of superficial

squamous cells. Specimens from animals with aspiration pneumonia may also contain squamous epithelial cells; however, in these cases, there should also be an accompanying increase in degenerated neutrophils and a mixed intracellular bacterial population. Plant material and fungal hyphae or spores are occasionally observed in normal herbivores and do not necessarily indicate oral contamination in these species.

a.Mucus

Mucus is seen in virtually all wash specimens, including those from normal animals. Mucus appears as blue to pink homogeneous strands. These may frequently be twisted or whorled. Mucus in inflammatory conditions stains eosinophilic due to incorporation of inflammatory proteins and material from lysed cells. Specimens from animals with chronic respiratory conditions causing excessive production of mucus, such as chronic obstructive pulmonary disease, may have mucus casts of small bronchioles. These are called Curschmann's spirals and appear as spirally twisted masses of mucus that may have perpendicular radiations that impart a test-tube-brush-like appearance.

b.Cellular Elements

Total cells counts on **TT/B** wash fluids are generally not performed because of the mucus content and the dilution of the specimen with saline. Total cell counts are usually performed on BAL specimens but care must be taken in their interpretation because of the dilution factor.

Erythrocytes may be seen in **TT/B and BAL** fluids with disorders causing vascular damage or RBC diapedesis into the lung. Intrapulmonary hemorrhage may be of pulmonary origin (**e.g., trauma, infarct, foreign bodies, infectious diseases, neoplasia)** or also may occur with disorders of non-pulmonary origin **(e.g., heart failure, pulmonary embolism, hemostatic disorders)**. RBCs and/or RBC breakdown produces **(hemosiderin, hematoidin)** within alveolar macrophages is an indication of chronic hemorrhage. These cells are often referred to as heart failure cells; however, they may occur as a result of any disorder which causes intrapulmonary hemorrhage. Hemosiderin-laden macrophages are common in high-speed performance horses that have experienced pulmonary hemorrhage at least 72 hours previously. The presence of hemosiderin can be confirmed with special stains **(e.g., Prussian blue)** which stain iron-containing compounds, such as hemosiderin, blue.

The predominant cell types observed in TT/B wash specimens from normal animals include respiratory epithelial cells and alveolar macrophages with low numbers of nondegenerate

neutrophils and small lymphocytes. In pathologic conditions, increased numbers of inflammatory cells **(neutrophils, eosinophils, lymphocytes and macrophages)**, RBCs, mast cells, and/or dysplastic and neoplastic cells may be present.

a.Epithelial cells

Types of epithelial cells that may be observed in **TT/B wash and BAL** specimens include ciliated and nonciliated columnar and cuboidal cells and mucus-secretory cells.

Generally, the most abundant cell type is the ciliated columnar epithelial cell. These cells are elongated or cone shaped with apically located cilia and a basally located nucleus. The basal cytoplasm frequently terminates in a tail. The nucleus is round to oval with a finely granular chromatin pattern. The non-ciliated columnar cell is identical to these cells but does not have cilia. Ciliated and nonciliated cuboidal epithelial cells resemble their columnar counterparts except that they are as wide as they are tall. These latter cells are generally from the bronchial regions and are, therefore, more common in BAL specimens.

Goblet cells are not commonly seen in specimens from normal animals; however, any chronic pulmonary irritant may result in increases in their numbers. These cells appear as an elongated or columnar cell with a basally placed nucleus. They secrete mucus which appears as coarse metachromatic cytoplasmic granules which frequently distend the cytoplasm. Occasionally, the cytoplasm may be so distended as to give a rounded shape to the cell. Granules from ruptured goblet cells may be seen free in the smear.

b.Macrophages

Alveolar macrophages are readily observed in TT/B washings from normal animals and may be the predominant cell type. Depending upon the pathologic state, macrophages vary considerably in size and activity level. Unactivated alveolar macrophages are rounded mononuclear cells 15 to 20 microns in diameter with abundant blue-gray, granular cytoplasm. The nucleus is round to bean shaped and eccentrically positioned. Activated macrophages will have more abundant cytoplasm that will be vacuolated or foamy and may contain phagocytized material. These activated cells may form huge, multinucleated phagocytic cells that may exceed 80 microns in diameter. These multinucleated forms are common in chronic lung diseases such as chronic obstructive pulmonary disease.

Increased numbers of macrophages are seen in many subacute and chronic lung disorders such as congestive heart failure, granulomatous pneumonia, lipid pneumonia and various chronic

persistent inflammatory processes. Various pigments from RBC breakdown **(hematoidin, hemosiderin)** or inhalation of polluted air **(anthracotic pigment)** may be found in the cytoplasm of phagocytic macrophages.

c.Neutrophils

In specimens from normal animals, low numbers **(generally <5%)** of nondegenerate neutrophils may be present. These cells will resemble those of peripheral blood. Neutrophil numbers will be increased in nearly all conditions that cause inflammation, including both infectious and noninfectious conditions. Infectious disorders include bacterial, mycotic, viral and protozoal diseases. Noninfectious disorders include tissue irritation or necrosis secondary to inhalation of toxic substances and tissue necrosis resulting from neoplasia. In clinical cases of chronic obstructive pulmonary disease, tracheobronchial aspirates often show a rise in the numbers of nondegenerate neutrophils along with activated macrophages, particularly multinucleated giant cells.

As with other cytological specimens, assessment of neutrophil preservation is critical and the cells must be examined for degenerative changes indicative of infectious or toxic processes. Care must be taken in making this assessment in that smudging and/or rupture of neutrophils may result during collection and preparation of the specimen.

d.Eosinophils

Eosinophils are present only in very small numbers **(<5%)** in **TT/B and BAL** fluids from normal animals. Eosinophils may be seen in increased numbers **(>10%)** with type I hypersensitivity reactions such as allergic processes **(e.g., allergic bronchitis/ pneumonitis, feline asthma)** and parasitic migration. Eosinophlic granuloma may demonstrate eosinophilic lung infiltrates in affected cats. Increased numbers are commonly seen in specimens from dogs infected with heartworm, dogs and cats infected with lungworm, foals infected with Parascaris equorum **(during the pulmonary migration stage of the parasite),** horses with Dictyocaulus arnfieldi infection and in cattle infected with Dictyocaulus viviparus. In addition to increased eosinophil numbers, parasite ova and/or larvae may occasionally be identified.

Rupture of eosinophils results in free granules present in smears and can be confused with bacteria. Eosinophil granules may coalesce into a large crystal known as a Charcot-Leyden crystal. These are elongated double pyramids and may occur in any condition that results in accumulation of large numbers of eosinophils. Increased numbers of neutrophils and

macrophages may be seen along with increased numbers of eosinophils if tissue irritation is sufficient to induce an inflammatory response.

e.Miscellaneous Cell Types

Increased numbers of small lymphocytes in **TT/B and BAL** specimens generally denote nonspecific inflammation and are of limited diagnostic value. Mild increases in their numbers may occur with airway hyperreactivity, viral disease of the tracheobronchial tree and in chronic infections. Immune stimulated lymphocytes have abundant cytoplasm which stains deep-blue because of increased protein synthesis. Transformation into plasma cells with the characteristic nuclear halo or negative golgi apparatus may occur.

Increased numbers of mast cells may occur in specimens from animals with various inflammatory lung disorders. The presence is generally of little diagnostic significance.

Various atypical cells may be seen with pulmonary metaplasia, dysplasia or primary and metastatic neoplasia. Epithelial cells adapt to chronic irritation by undergoing metaplasia. The normal respiratory epithelium is replaced by stratified squamous epithelium. The metaplastic cells mimic maturing squamous epithelium and must not be confused with neoplastic cells. Epithelial cells will also react to irritation by becoming dysplastic. Dysplastic changes include variation in cell size and shape, increased nucleus/cytoplasm ratio, increased cell basophilia and increased numbers of immature cells. Care must be taken not to confuse inflammation induced dysplastic changes with neoplasia.

Unless the neoplasm has invaded the tracheobronchial tree and invaded bronchioles are not blocked by mucus plugs, neoplastic cells are rarely found in **TT/B and BA**L fluids. When neoplastic cells are observed, they are generally from adenocarcinomas. These large epithelial cells may be present as single cells or as clusters. They generally have basophilic, vacuolated cytoplasm and high nucleus/ cytoplasm ratios, coarse nuclear chromatin and prominent nucleoli.

7.11 SOLID TISSUE CYTOLOGY

Cytological procedures can be used to evaluate tissue masses and organ parenchyma for the purposes of detection and/or classification of pathological conditions. One of the most widely used applications of cytological evaluation is the identification and classification of tissue masses.

The usual composition of a mass is a proliferation of tissue cells, an accumulation of inflammatory cells, or both. The algorithm presented in **Figure X.1** indicates the procedures for evaluating a cytological specimen from a mass. If it is determined that the cell population is primarily inflammatory, attempts should be made to classify the reaction and to identify an etiologic agent.

Tissue cells found on cytologic smears may arise from normal tissue, hyperplastic and/or dysplastic tissue, or neoplastic tissue. The observed cells indicate a proliferative tissue mass if they are non inflammatory tissue cells of one type; i.e., a uniform population. This conclusion is often made difficult by concurrent inflammation and necrosis in many neoplasms. The higher the percentage of inflammatory cells in the population, the lower one's confidence that the mass is not primarily inflammatory. The presence of inflammation can induce dysplastic changes in surrounding tissue cells which can be confused with neoplastic processes. Cells undergoing dysplasia may show mild to moderate variation in cell, nuclear and nucleolar size and shape, increased nucleus:cytoplasm ratio, and coarse chromatin. Dysplasia generally does not cause the bizarre nuclear and nucleolar morphology characteristic of neoplasia. In any case, care must be taken in the interpretation of these lesions. They should be biopsied or treated and re-evaluated cytologically when the inflammatory process has subsided.

7.11.1 Cytologic Classification of Tumor Types

The shape of the cells, their association with other cells, especially in tissue fragments, and cytoplasmic features are used to indicate the tissue of origin of a tumor (**See Figure X.2).** Once the cell type of a neoplasm is ascertained, the mass is evaluated for malignancy based upon the criteria listed in **Figure X.3.**

1. Epithelial Cell Tumors

a.Epithelial in origin

b.Often exfoliated in clusters, clumps or sheets of round to oval cells

c.In some cases, may appear arranged in ductular or acinar patterns around a central lumen **(adenoma or adeno-carcinoma).**

d.Cytoplasm may also appear distended by a secretory product.

2.Sarcomas

a.Connective tissue **(mesothelial)** in origin

b.Do not exfoliate regularly and scrapings are often necessary.

c.Usually found as individual cells

d.Elongated, fusiform or spindle shaped cells.

3.Discrete Cell Neoplasms

a.Consist of cells which are round to oval in shape and exfoliate readily.

b.Cells are not intimately associated with each other.

7.11.2 Cytologic Features of Selected Tissue Masses

1.Masses of Epithelial Origin

a.Basal Cell Tumor

Basal cell tumors are neoplasms of the basilar layer of the epidermis. They are common in cats and dogs. They can be pigmented, especially in cats, and often contain cystic spaces. The tumors are generally benign but can be locally invasive or malignant. Their malignancy potential is difficult to predict cytologically; however, it is reported that those exhibiting solid or basosquamous tendencies tend to behave malignantly.

The cell type found in these tumors is a primitive epithelial germ cell that does not exhibit differentiation toward squamous cells or adnexal structures. Histologically, these cells are arranged as palisaded cords or ribbons embedded in fibrous stroma. This pattern is caused by the tendency of basal cells to line up along basement membranes within the tumor.

The tumor is readily imprinted and aspirated and yields large numbers of cells arranged singly or in groups. Sometimes a row or ribbon of several cells may be found. Individual cells are uniformly small, approximately the size of a RBC, and dark staining. The **N:C ratio** is generally **1:1; therefore,** only a scant amount of pale blue cytoplasm can be visualized in most of the cells. Their nuclei are variably-sized but regular in shape and have finely etched, lacy to finely stippled chromatin and contain multiple indistinct nucleoli. Mitotic figures may be common.

b.Squamous Cell Carcinoma

Squamous cell carcinoma originates in surface stratified squamous epithelium. It is among the most common of all dermal and oral neoplasms and is found in all domestic species. Due to its tendency to become ulcerated and inflamed, deep aspiration is the preferred method for obtaining cells for cytological examination. The cells generally exfoliate in clusters with variable numbers of individual cells.

Tumor cells from well-differentiated squamous cell carcinomas show slight cellular atypia and little evidence of neoplasia. These tumors are difficult to distinguish from dysplastic conditions

of the skin resulting from chronic irritation or infection. The degree of differentiation of a squamous cell carcinoma is directly proportional to the degree of cellular anaplasia and indirectly proportional to the degree of keratinization. In poorly differentiated tumors, the cells exfoliate in clusters typical of other epithelial neoplasm; however, a few individual keratinizing cells usually are present which confirm an interpretation of squamous cell carcinoma.

In moderately to poorly differentiated tumors, marked variation in cell, nuclear and nucleolar size, nuclear and nucleolar number and shape, nucleus:cytoplasm ratio **(1:3 to 1:5)** and cytoplasmic basophilia occurs. Individual cellular morphology varies from normal large, mature squamous cells to small or medium-sized round cells, with a small amount of very basophilic cytoplasm and large, round nuclei that may have a very coarse, ropy chromatin pattern and contain multiple, prominent, irregularly shaped and sized nucleoli. Some cells may contain small clear vacuoles which occasionally aggregate around the nucleus and appear to coalesce, forming a clear ring around the nucleus **(perinuclear halo).**

c.Circumanal Gland Adenoma or Perianal Adenoma/ Adenocarcinoma

The perianal glands encircle the canine anus, and a few cells can be located in the skin of the tail, the prepuce, the thigh, and over the dorsum of the back. Perianal adenomas and adenocarcinomas can occur at any of these locations. These tumors are most commonly seen in the male. Perianal adenomas and adenocarcinomas often ulcerate because of pressure necrosis of the overlying epidermis and mechanical irritation of the tumor.

Cytologically and histopathologically, it is difficult to differentiate between perianal gland hyperplasia and benign neoplasia. Generally, large numbers of cells exfoliate and occur in clusters with a few scattered individual cells. The cells are uniform and medium in size and shape. They have a moderate amount of pink to tan-staining foamy cytoplasm. At high magnification, the cytoplasm may appear granular. The cells contain uniform rounded nuclei with 1-2 small round nucleoli. Because the cells resemble hepatocytes, the tumors are sometimes referred to as hepatoid gland tumors. Keratinization may be a feature in cells which have undergone squamous metaplasia. In some aspirates, a row of flattened reserve cells may be seen surrounding the clusters of larger cells. These cells are more basophilic and have **N:C ratios** of from **1:1 to 1:2.**

Perianal gland adenocarcinomas may demonstrate numerous criteria of malignancy or they may be well-differentiated and difficult to distinguish from the benign tumor. The most common malignancy criteria demonstrated by these tumors are variation in nuclear and nucleolar size and variation in nucleolar **number/cell.**

d.Sebaceous Gland Adenomas/Adenocarcinomas

Tumors of the sebaceous glands are more common in older dogs and usually appear as wart-like growths. They generally exfoliate cells in groups and clusters and acinar patterns may be seen.

The predominant cell type in the sebaceous gland adenoma is the large mature secretory cell which has foamy cytoplasm and a small, central to slightly eccentric nucleus. The nucleus has dark staining slightly coarse chromatin and an indistinct or indiscernible nucleolus. The nucleus may be totally obscured by secretory material. In addition to these mature cells, adenomas may also contain basilar reserve cells. These cells are immature and contain little or no secretory material. Their cytoplasm is basophilic and the **N:C ratio** is approximately **1:2.** Care must be taken not to interpret these cells as malignant.and contain little or no secretory material. Their cytoplasm is basophilic and the N:C ratio is approximately **1:2.** Care must be taken not to interpret these cells as malignant.

Sebaceous gland adenocarcinomas are relatively uncommon. Cytologic preparations usually consist of groups of extremely basophilic reserve cells showing numerous criteria of malignancy. Only a few cells will contain secretory material. An occasional cell will produce a large amount of secretory material which presses the nucleus to the cell margin resulting in a signet-ring appearance.

e.Transitional Cell Carcinoma

Transitional cell carcinoma may have papillary, nonpapillary or invasive growth patterns. The most common site is the bladder trigone, but they may also occur in other areas of the bladder, the renal pelvis and the tubular system. Direct tumor aspiration and urine sediment evaluation reveal cells with similar cytologic features. Transitional cell carcinomas exfoliate isolated cells and cell clusters, frequently including very large clusters of cells with marked pleomorphism. The cytoplasm of these exfoliated cells is often deeply basophilic. In urine specimens, the cytoplasm may contain relatively light-staining vacuoles, indicating that the cells are undergoing hydropic degeneration. This degeneration may be a result of the toxic effects of urine upon the cells. Within the cellular clusters, cytoplasmic borders are usually indistinct and cell crowding is

evidenced by cellular and nuclear molding. The nucleus: cytoplasm ratio is high in most cells; however, a few very large cells with abundant cytoplasm and a low nucleus:cytoplasm ratio are generally present. Nuclei are large, circular to ovoid, with finely to coarsely reticulated chromatin. Binucleate forms are common. Nucleoli may be indistinct to large and prominent.

2.Masses of Spindle Cell Origin

Inflammation and other irritation can cause mesenchymal cells to become dysplastic and exhibit changes similar to neoplastic manifestations. In many cases, histopathologic examination is necessary to definitively diagnose these tumors. Since many of the spindle cell tumors are embedded in a dense ground substance, imprints generally contain few neoplastic cells and the ones obtained are discrete and individual. Scrapings generally results in a greater population of cells. As with imprints, cells obtained by scraping are generally not intimately associated.

a.Granulation Tissue

Granulation tissue is composed of young proliferating fibroblasts and vascular tissue. Since these young fibroblasts are plump spindle cells with anaplastic characteristics, it is difficult cytologically to differentiate granulation tissue from fibrous tissue neoplasia. Like neoplastic fibroblasts, hyperplastic fibroblasts are active proliferating cells and exhibit large nuclei with multiple nucleoli and abundant cytoplasm with increased basophilia. However, unlike neoplasia, hyperplastic cells are proliferating under control mechanisms and therefore maintain a constant nuclear/cytoplasmic ratio. Additionally, nuclei of proliferating fibroblasts retain the normal finely granular chromatin pattern of the more mature normal fibrocyte. The differentiation between fibrous hyperplasia and fibrous neoplasia is particularly important in the equine where both sarcoids and excessive granulation tissue are common. Histopathological evaluation should be performed to make this definitive assessment.

b.Fibroma/Fibrosarcoma

Fibromas can occur in the dermis or subcutaneous tissue. Unlike fibrosarcomas, they seldom ulcerate. Scrapings yield a small number of cells arranged individually or in small groups. The cells are relatively uniform in size and shape and tend to have very elongated spindle shapes with a moderate amount of light blue cytoplasm. The nuclei are round to oval with a smooth to lacy chromatin pattern and may contain 1-2 small, round, indistinct nucleoli.

Fibrosarcomas can arise from cutaneous or subcutaneous tissues and may ulcerate and become secondarily infected. Fibrosarcomas of the dermis do not occur with great frequency in the dog

but oral canine fibrosarcomas are relatively common. Sarcoids in horses resemble fibrosarcomas cytologically; however, they generally do not demonstrate malignant neoplastic behavior.

On cytological preparations, cells taken from fibrosarcomas are generally less spindle shaped than cells from fibromas. Many of the cells may be plump and/or oval shaped. Others may be stellate or have only a single indistinct tail of cytoplasm. The cytoplasm is often coarse and basophilic with Wright's stain or Diff Quik. Often small vacuoles are seen in the cytoplasm. Nucleoli may be singular and very large or multiple. With increased malignancy potential, large nucleoli are present and are often very irregular and angular. Chromatin is frequently clumped, particularly along the nuclear margin.

c.Lipoma/Liposarcoma

Mature lipocytes are large and distended with fat. They usually rupture during the aspiration procedure; therefore, aspirates of lipomas usually yield abundant free fat with a few lipocytes giving the smears an oily appearance which will not dry. Staining of the aspirate in an alcohol-based stain will dissolve the fat leaving clear areas and a few intact lipocytes on the smear. Lipocytes have pyknotic nuclei that are pressed against the side of the cell membrane by huge fat globules. Fat stains, such as Sudan IV and oil red O, may be used on fresh smears before alcohol fixation to verify the presence of these cells. New methylene blue stain may also be used. Lipomas are benign tumors but they can occasionally infiltrate between muscle masses. These infiltrative lipomas are difficult or impossible to remove and can cause death of the animal. In horses, pedunculated lipomas may be life threatening because of their potential for causing strangulation of the gut.

Unlike lipomas, cytologic preparations from liposarcomas may contain numerous cells. Aspirates, imprints and scrapings from liposarcomas may contain free fat, some mature lipocytes and lipoblasts, and appear greasy. Or they may contain very little free fat, few mature lipocytes, many lipoblasts and don't appear greasy. Histopathologic examination demonstrates that the cells contain abundant cytoplasm. This feature is not noted in most cytologic preparations because the cell membrane is visualized poorly. Cytologically, the cells have very light cytoplasm containing vacuoles of varying size and number. Some of the larger vacuoles may cause indentation of the cell nucleus. In general, the more immature and anaplastic cells have fewer and smaller fat globules. The lipoblast nucleus is typically round and variation in nuclear size and multinucleation is usually present in the preparation.

d.Melanoma/Melanosarcoma

Dermal melanomas are common in dogs and horses but rare in cats. Although generally benign in dogs, cutaneous melanomas of the oral cavity, lips and digits have a high incidence of malignancy. Aspirates generally contain a large to moderate amount of cells but, occasionally, only scattered cells mixed with blood are aspirated. In all instances, melanin pigment almost always will be found in the background. The tumor aspirate contains predominantly individual cells, but sheets or rare clusters may be found. Cell morphology varies from round, oval, stellate or spindle shaped.

The cells from both benign and malignant tumors have a moderate to abundant amount of cytoplasm with a low **N:C ratio.** Melanomas are often extremely malignant and bizarre forms are common. Tumor giant cells and cells with giant nuclei are often seen. Melanin pigment appears as brown-black to green-black cytoplasmic granules of irregular size and shape. This pigment might appear as a salt-and-pepper sprinkling in occasional cells; or might be densely packed and obscure the nucleus. Even in tumors apparently devoid of pigment, a thorough examination of the smear will generally reveal a few cells containing a few small pigment granules interspersed throughout their cytoplasm. Anisokaryosis and numerous mitotic figures are usually additional features of these **"amelanotic"** neoplasms. In general, the less pigmented the melanoma, the more malignant the neoplasm.

e.Hemangioma/Hemangiosarcoma

Hemangiomas and hemangiosarcomas are tumors of blood vessel endothelium and are contiguous with the blood vascular system. The spindle cell tumor origin of hemangiomas and hemangiosarcomas may be difficult to recognize on cytologic preparations because of the massive blood contamination that may occur.

The cells of hemangiomas are difficult to differentiate from non-neoplastic endothelial cells. They tend to be oval, spindle or stellate in shape, have moderate to abundant amounts of light to medium blue cytoplasm, and contain a medium-sized round to slightly oval nucleus. The nucleus has a smooth to fine lacy chromatin pattern and may have 1-2 small, round, indistinct nucleoli.

Neoplastic endothelial cells aspirated from hemangio-sarcomas range in morphology from apparently normal endothelial cells to medium or large cells with marked cytologic criteria of malignancy **(e.g., marked variation in cell, nuclear and nucleolar size; increased N:C ratios, nucleolar prominence and angularity, cytoplasmic basophilia.**

f.Osteosarcoma

Osteosarcoma is the most common tumor involving bone. A striking feature is that some of these malignant osteoblasts can be seen embedded in and apparently secreting an osteoid matrix which appears as fibrillar bright-pink material on Wright's stained slides. Osteoid is not found in most aspirates from osteosarcomas; however, when found, its presence provides strong evidence that a tumor is of bone origin.

The principal cell is the malignant osteoblast which may appear singly or in clusters. The cells are polygonal to fusiform with abundant foamy basophilic cytoplasm. Several clear cytoplasmic vacuoles and/or scattered pink cytoplasmic granules may be present. A Golgi area is often apparent. Nuclei of malignant osteoblasts are highly variable in size, exhibiting coarse chromatin patterns and multiple nucleoli. The nuclei may be eccentrically located and appear as if they are being "spit out" by the cell. Multinucleate forms may be present due to incomplete cellular division. These cells resemble osteoclasts; however, they are not osteoclasts but tumor giant cells and will exhibit nuclear criteria of malignancy.

g.Chondrosarcoma

Chondrosarcomas are the second most common tumor of bone and are difficult to differentiate from osteosarcomas cytologically. The ribs, turbinates and pelvis are the most common sites for chondrosarcomas of dogs, while the scapulae, vertebrae and ribs are most common sites in cats. One useful but inconsistent cytologic feature on low-power examination of aspirates is lakes of bright pink, smooth or slightly granular material in which cells may be embedded. This material is the intercellular matrix of cartilage sometimes called chrondroid.

Individual neoplastic chondroblasts have cytologic features similar to those of osteosarcoma cells. They vary from round to fusiform, with large nuclei and dark-blue cytoplasm. Anisokaryosis is prominent, and multinucleate tumor cells may be found. The cytoplasm Often contain several small clear vacuoles, and occasional cells may contain fine pink cytoplasmic granules. If the tumor is causing bone lysis, osteoclasts may also be found in cytologic specimens.

3.Discrete Round Cell Tumors

The round cell tumors consist of three sarcomas **(transmissible venereal tumor, mast cell tumor and lymphosarcoma)** and the histiocytoma, a benign tumor or questionable origin. The round cell tumors typically exfoliate well. Like epithelial cells, the cells are round and have

distinct cell membranes. Like mesenchymal tumors, however, the cells from round cell tumors exfoliate individually rather than in clusters.

a.Mast Cell Tumor

Smears made from Mast cell tumors contain few to many round to oval cells containing a moderate amount of cytoplasm with variable numbers of small, red-purple granules **(when stained with Wright's stain).** The number of granules seen within a given neoplastic mast cell may vary from tumor to tumor and even within a tumor. Generally, mast cell neoplasms containing relatively few granules are considered to be formed by less differentiated, more primitive cells than are highly granulated neoplasms.

The nuclei of neoplastic mast cells are round to oval, variably sized and generally eccentric. They appear to stain palely because of the intense staining of the cytoplasmic granules. Nuclear criteria of malignancy are present although nuclei may be so obscured by granules that these features are indistinguishable. Mitotic figures may be present.

Mast cells contain heparin and its anticoagulant effect may result in local hemorrhage. Because of the local histamine release that occurs when mast cells degranulate, inflammatory cells are commonly noted in aspirates. Some tumors have a marked infiltrate of eosinophils which may be a distinct cytologic feature.

b.Transmissible Venereal Tumor (TVT)

The TVT is a discrete cell tumor of dogs most commonly found on mucous membrane surfaces, such as the penis, the vagina, and the oral or nasal cavities. The neoplasm is believed to have a viral etiology, currently unclassified. The TVT meets both cytologic and histologic criteria for malignancy but reports of metastasis are rare. In many cases the neoplasm will regress spontaneously.

Cells from TVTs exfoliate extremely well giving numerous cells in aspiration, impression or scraping preparations. Most of the cells are uniformly round; however, an occasional large or multinucleated cell may be observed. The cells have a moderate amount of light gray to blue cytoplasm with distinct boundaries. Numerous cytoplasmic vacuoles may be present. The nuclei are usually round and eccentrically placed and contain coarse, cord-like chromatin and 1-2 large prominent nucleoli. Variation in N:C ratio is a common feature. TVTs generally exhibit a high mitotic index. Tumor infiltration by plasma cells and and macrophages is common.

c.Histiocytoma

Histiocytomas are benign dermal neoplasms that occur most commonly as solitary growths on the head or the extremities of young dogs. The overlying epidermis is often ulcerated and accompanied by secondary inflammation of the neoplasm. Histiocytomas usually yield only a few cells on cytological preparations. The tumor cells are pleomorphic and resemble monocytes and epitheliod cells. They have a moderate amount of pale blue cytoplasm that often has ill-defined boundaries and may contain a few small vacuoles. Nuclei of histiocytoma cells are variable in both size and shape and a few multinucleated cells may be present. The nuclei may be indented like those of monocytes. Although the mitotic index may be quite high, nuclei of most cells appear benign and do not exhibit nuclear criteria of malignancy. The nuclei have finely etched, lacy to finely stippled chromatin patterns and contain multiple indistinct nucleoli. Lymphocytic infiltration of the tumor is a common feature.

d.Lymphosarcoma

Cutaneous lymphosarcoma can occur as a part of systemic lymphosarcoma or, rarely, as a disease localized to the skin only. It is usually manifested as multiple plaque-like lesions, but may occasionally occur as solitary nodules. Aspirates of these lesions usually yield highly cellular cytological preparations. Although numerous histo-pathologic classification schemes based on tumor architecture have been proposed for lymphosarcoma, the cytologist is limited to describing the tumor based on the predominant cell type. These cytologic classifications are lymphoblastic, lymphocytic and histiocytic lympho-sarcoma.

In canine multicentric lymphosarcoma, the lymphoblastic form of the disease is much more common than the lymphocytic or histiocytic forms. The neoplastic lymphoblasts are large, often several times the size of erythrocytes, and have a small to moderate amount of light to medium blue-staining cytoplasm. There is often displacement of this cytoplasm to one side of the nucleus. The nuclei are indented to irregular with smudged to stippled chromatin patterns and contain one or more nucleoli. Mitotic figures can be frequent. Because these immature cells are fragile, it is common for them to rupture during preparation of the smear. Naked, smeared nuclei and cytoplasmic fragments frequently are observed scattered among the intact cells.

Smears from cytologic preparations of lymphocytic lymphosarcoma consist of small lymphocytes that cannot be readily differentiated from normal lymphocytes. These tumors require histopathologic examination for definitive diagnosis.

7.11.3 Cytologic Features of Miscellaneous Tissues

1.Mammary Gland Abnormalities

Aspirates from normal mammary glands are generally acellular or contain only blood. If mammary tissue is present, it consists of acini of secretory cells. These cells have a moderate amount of basophilic cytoplasm and round, dark uniform nuclei. Active cells will appear foamy due to the accumulation of secretory material. Sheets or fragments of simple cuboidal duct epithelium may be present and appears as small cells with scanty cytoplasm and basally located ovoid nuclei. A third cellular component, the myoepithelial cell, may also be present and appears as a dark staining, naked, oval nucleus or as a spindle shaped cell.

Mammary cysts result when a dysplastic process causes dilatation of the duct system with the formation of large cavitations. They are common in middle-aged and older bitches, but may occasionally occur in younger dogs. Though considered benign, mammary cysts may be precancerous. Aspirated fluid is yellow, brown, green or blood-tinged and is of low cellularity consisting primarily of **"foam cells"** and pigment-laden macrophages. Dense clusters of cyst epithelial lining cells exhibiting minor degrees of nuclear pleomorphism may also be present.

Dysplastic and benign neoplastic lesions include lobular hyperplasia, adenosis, adenomas and papillomas. Aspirates from these all appear similar cytologically and contain numerous epithelial cells occurring singly or in sheets and clusters. The cells exhibit little or no pleomorphism. The nuclei have evenly dispersed chromatin patterns and contain single, small, round nucleoli. Pigment-laden macrophages may be present.

Complex adenomas, fibroadenomas and benign mixed tumors involve both epithelial and stromal elements. Smears of aspirates from these lesions contain spindle-shaped connective tissue cells and clusters of epithelial cells similar to those described in the preceding paragraph.

Numerous classifications of malignant neoplasms may involve the mammary gland, including various carcinomas, sarcomas and mixed origin tumors. Histologically, areas of cartilagenous/osseous metaplasia may be found in these various tumor types. Nests of these metaplastic connective tissue cells may also be seen in cytologic preparations.

Adenocarcinomas are the most common malignant tumor and have the classic cytological criteria previously described for this tumor type as do the less common mammary squamous cell carcinomas. Aspirates of anaplastic carcinomas contain very large, very pleomorphic epithelial

cells occurring individually or in small clusters. These cells have a high N:C ratio, bizarre nuclear and nucleolar forms and exhibit a high mitotic index. Multinucleate forms are common.

2.Lymph Node Cytology

Three general processes can cause lymph node enlargement, hyperplasia, inflammation and neoplasia. Cytological examination can be used to differentiate between these conditions.

a. Normal Lymph Nodes

Cytologic examination of a normal size lymph node is warranted to ascertain the presence of certain microbial agents and to determine whether neoplastic metastasis has occurred.

Small **(9 micron)** mature lymphocytes are the predominant cell type in aspirates of normal lymph nodes and make up **85 to 90%** of the cells observed. These cells have condensed nuclei, only slightly larger than a red blood cell, and scant cytoplasm consisting of a narrow rim around the nucleus.

The medium lymphocyte, or prolymphocyte, is somewhat larger than the small lymphocyte and contains more abundant pale cytoplasm. It has a nucleus with less densely packed nuclear chromatin and a nuclear diameter approximately equal to two red blood cells **(i.e., 10-15 microns)**.

Large lymphocytes, or lymphoblasts, are the less common than either small or medium lymphocytes in normal nodes. They are large cells **(15-25 microns)** with basophilic cytoplasm which may appear as a broad or narrow rim around the nucleus. The nucleus is vesicular with a fine, diffuse chromatin pattern and may contain multiple distinct nucleoli.

Plasma cells and plasmablasts are derived from antigen stimulated B-lymphocytes and may be present in small numbers in normal lymph nodes. Plasma cells have small, round, eccentric nuclei with condensed chromatin. They have abundant deep blue cytoplasm with a prominent clear Golgi zone. Plasmablasts are more immature and are larger with less aggregated chromatin and a higher nucleus:cytoplasm ratio. Their very blue cytoplasm often contains vacuoles.

Cytoplasmic fragments called lymphoglandular bodies are a characteristic feature of lymphoid tissue. They are round, homogeneous, basophilic structures similar in size to platelets. They should not be confused with organisms.

b. Reactive Hyperplasia

Lymph nodes become reactive or hyperplastic when they are antigenically stimulated, such as occurs when high concentrations of antigens reach the draining nodes. A reactive hyperplastic

lymph node resembles a normal lymph node cytologically; however, the size of the lymph node aids in differentiation since a normal node should not be enlarged. As with normal lymph nodes, specimens from reactive hyperplastic nodes contain a heterogeneous cell population. The small lymphocyte predominates, composing approximately **60-70%** of the cells. The numbers of medium and large lymphocytes are increased and may make up to **15%** of the total cell population. Mitotic figures may be prominent. Mature and immature plasma cells are generally increased in number and make up to **5-10%** of the cell population in some areas of the smear. Plasma cells that contain many discrete vacuoles (**Russell bodies**) are called Mott cells or Russell body cells and are commonly seen in reactive lymph nodes. Macrophages may be increased in number, particularly when hyperplasia of sinus macrophages occurs. Increased numbers of lymphoglandular bodies are generally present.

c. Lymphadenitis

The cytology of an inflamed lymph node will vary depending upon the etiology. Neutrophils **(degenerate or nondegenerate)**, eosinophils, or macrophages can predominate. Inflammation is probably present when the total cell population of the lymph node is **>5%** neutrophils or **>3%** eosinophils. Macrophage numbers can increase in inflammation, hyperplasia and sometimes in neoplasia. Macrophages may also appear as epithelioid cells and multinucleated giant cells in granulomatous inflammation.

d. Lymphosarcoma

Lymphosarcoma is characterized by a uniform population of large, intensely basophilic immature cells **(usually prolymphocytes or lymphoblasts)** which give the smear a homogeneous cellular appearance. When these immature cells comprise **>50%** of the cell population, a diagnosis of lymphosarcoma can be reliably made, but smaller numbers may be present in early stages. Mitoses may be more numerous than in hyperplasia. Macrophages, called **"tingible-body"** macrophages, containing bluish cytoplasmic globules may be present and indicate intense lymphopoiesis and cell turnover. Lymphoglandular bodies are more numerous than in hyperplasia. Unlike lymphoid hyperplasia, plasma cells are usually few in number. Occasionally, lymphosarcoma is manifested as the small, well-differentiated lymphocyte type. This form is very difficult to distinguish from hyperplasia because the small lymphocyte predominates in each.

e. Metastatic Neoplasia

Malignant tumors frequently metastasize via lymphatics. This can result in the proliferation of neoplastic tissue in the draining lymph node. The presence of cells not normally found in lymph nodes or an increase in numbers of certain cell types normally present may suggest metastatic neoplasia.

3. Testis

Unilateral or bilateral enlargement is the major indication for fine-needle aspiration biopsy and cytologic evaluation of the testis. Fine needle aspiration may also be used to evaluate an enlarged epididymis. Enlargement of these organs can be due to inflammatory or neoplastic conditions.

The three major neoplasms of importance in the testis include seminomas, Sertoli-cell tumors and interstitial-cell tumors. These tumors are difficult to differentiate by cytologic examination alone and interpretation must be accompanied by a thorough history and clinical examination.

a. Seminoma

Aspirate from seminomas are usually highly to moderately cellular. The cells are of variable size with high nuclear:cytoplasm ratios. Distinct cells with intact cytoplasmic membranes may be sparse and ruptured cells are common. The nuclei are variable in size with homogeneous to finely reticular chromatin. Bi- and multiple nucleation is common. Nucleoli are prominent, relatively large and frequently multiple and irregular. Mitotic figures are common. Small lymphocytes are frequently scattered throughout the stroma.

b. Sertoli-Cell Tumor

Aspirates from Sertoli-cell tumors are highly cellular. The tumor cells usually have abundant light-staining cytoplasm which contains numerous distinct variable sized vacuoles. Nuclear chromatin patterns are coarsely reticular and small to large nucleoli may be present. Mitotic figures may be found. Rarely, spindle-shaped cells with abundant cytoplasm may be present.

4. The Vagina

The vaginal epithelium is a target tissue for ovarian hormones, changing from 2-4 layers thick to a multilayered epithelium under their influences. These changes result in exfoliation of large numbers of superficial epithelium cells. Examination of these cells from the vagina is a simple technique that can be used to accurately monitor the progression of proestrus and estrus in dogs and cats. Cytologic examination of vaginal smears is also useful in detecting inflammation and neoplasia in the female reproductive tract.

a. Classification of Vaginal Epithelium

Vaginal epithelial cells will vary in age and morphology during the various stages of the estrous cycle. The four main classifications include basal, parabasal, intermediate and superficial epithelial cells.

Basal cells are from the deepest layer of the epithelium lining, near the basement membrane. These cells give rise to the other epithelial cell types in the vaginal lining. Basal cells are small with a small amount of cytoplasm and a relatively high nuclear: cytoplasm ratio. They are rarely seen in vaginal smears.

Parabasal cells are located just above the basal cell layer of the epithelium. They are small round cells with round nuclei and a small amount of cytoplasm; however, they are considerably larger than basal cells. Large numbers of parabasal cells may exfoliate when the vagina of a prepubertal animal is swabbed. These cells are usually quite uniform in size and shape.

Intermediate cells vary in size depending on the amount of cytoplasm present but are generally twice the size of parabasal cells. Their nuclei are approximately the same size as those of parabasal cells, no matter how large the intermediate cell is. As intermediate cells increase in size, their cytoplasm becomes irregular, folded or angular, similar to the cytoplasm of more superficial epithelial cells.

Superficial cells are the largest epithelial cells seen in vaginal smears. As they age and degenerate, their nuclei become pyknotic and then faded, and occasionally they disappear. Their cytoplasm is abundant, angular and folded. As the cells degenerate, they undergo cornification.

b. Characteristics of the Stages of the Canine Estrous Cycle

Depending upon the reference used, the canine estrous cycle is divided into four or five stages. These stages are anestrus, proestrus, estrus and metestrus. The fifth stage, diestrus, is described as occurring after metestrus and before anestrus. In some references, diestrus and metestrus are used synoymously.

1) Proestrus

Smears obtained in early and mid-proestrus contain a mixture of epithelial cells, including parabasal, small and large intermediate and superficial cells. **RBCs** may be numerous and a few neutrophils may be present. As proestrus progresses, there is a steady increase in the number of cornified epithelial cells so that by late proestrus, large intermediate and superficial cells predominate. Parabasal and small intermediate cells are no longer seen on smears about 4 days

before the LH peak of estrus. As estrus approaches, neutrophils totally disappear and **RBC** numbers decrease substantially.

2) Estrus

Cytologically, early estrus is characterized by a marked decrease in red blood cells, an absence of neutrophils and a continuing increase in the number of cornified epithelial cells. The time of maximum cornification is variable and may range from as early as 6 days before to 3 days following the **LH** peak. Large numbers of bacteria are commonly observed on and around superficial epithelial cells. Neutrophils may reappear during tthe last day or two of estrus and indicate its end.

3) Metestrus

Cytologically, metestrus is characterized by an abrupt change in relative numbers of superficial epithelial cells with a marked increase in the proportion of parabasal and intermediate cells. Neutrophils appear in variable numbers and usually coincide with increased numbers of parabasal and intermediate cells. **"Metestral cells",** which are parabasal epithelial cells containing a neutrophil in the cytoplasm, are often present. Individual cytologic preparations made during the transition period from late estrus to early metestrus, without benefit of prior preparations, can appear very similar to smears made in early or mid-proestrus. At both times there can be a similar mixture of superficial and nonsuperficial cells.

4) Diestrus and Anestrus

Diestrus follows metestrus and is characterized by increasing numbers of parabasal and intermediate cells and a few of the more superficial cells. If present, neutrophils and bacteria are few in number. This stage will blend into the quiescent phase of the heat cycle, anestrus. during normal anestrus, the vaginal mucosa is dry and secretions are absent. Parabasal and intermediate cells predominate.

c. Vaginitis and Metritis

Vaginitis and metritis can be suspected in animals with a history of a lack of sequential estrus cycles and when mild vulvar swelling is observed with a white to pink vulvar discharge. The vaginal smear will demonstrate a large number of neutrophils, some in clumps. When these occur with noncornified epithelium, it may be difficult to differentiate inflammation from proestrus or metestrus. Neutrophils associated with cornified epithelium indicate inflammation because the two cell types do not normally occur together. Degenerate neutrophils indicate septic

inflammation. Vaginal smears from animals with pyometra or metritis usually contain large numbers of very degenerate neutrophils. Clusters of foamy secretory endometrial lining cells may also be seen in endometritis. Endometrial hyperplasia is characterized by the presence of endometrial lining cells without associated inflammation.

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