MODULE-1: INTRODUCTION AND SCOPE OF VETERINARY PATHOLOGY

Learning objectives

- In this module, the viewer will learn on the fundamental things involved in disease processes (Pathology), diagnosis, prognosis, branches of pathology and scope of veterinary pathology in diagnosis and treatment of ailing animals.

INTRODUCTION AND SCOPE OF VETERINARY PATHOLOGY

The aim of study of veterinary and animal sciences is to produce a competent veterinarian. The veterinarian is involved in the diagnosis of diseases of livestock and poultry. The study of diseases is essential component of pathology.

- Pathology (Gr. Path(o) - Disease; logos: science, treatise, sum of knowledge in a particular subject)
- Pathology is the subject linking basic subjects of anatomy, histology, embryology, physiology and biochemistry to clinical subjects of medicine, surgery and obstetrics and gynaecology.
- Pathology is subject of terminology.
- It is important to learn different terminologies used in describing disease conditions. The definition of each terminology is the gateway to understanding the particular disease process.

VETERINARY PATHOLOGY - DEFINITIONS

- Pathology literally means study of disease or discourse of disease
- Pathology is that branch of medicine treating of the essential nature of disease especially of the changes in body tissues and organs which cause or caused by disease (Dorland).
- Pathology is study of the molecular, biochemical, functional and morphological aspects of diseases in the fluids, cells, tissues and organs of the body. Summary: Pathology is study of the functional and morphological alterations in tissues and fluids of the body during the disease (Thomson, 1984)

Health

- Normal condition of the body and mind i.e. with all the parts functioning normally.

Disease (Dis – Negative ease)

- Any deviation from or interruption of normal structure or function of any body part, organ, or system that is manifested by a characteristic set of symptoms and signs whose aetiology, pathology, and prognosis may be known or unknown.
- Disease is any departure from healthy state.
- The following are the essential components in the study of diseases :

Aetiology

- The science dealing with causes of disease
- Study of causation of disease

Incubation period

- Incubation period is the time that lapses between the action of a cause and manifestation of disease.

Pathogenesis

- Pathogenesis is development of morbid conditions or of disease or mechanism by which the causes produce diseases. e.g. In traumatic reticuloperitonitis in cattle sharp objects like nails ingested are usually trapped in the reticulum.
- Movement of reticulum and pressure from pregnant uterus in cows favours the piercing of foreign body through the wall of the reticulum, setting up reticulitis and then into peritoneal cavity causing injury causing peritonitis.
- If the foreign body is carrying pyogenic organisms purulent inflammation is found.

Clinical signs

- Clinical signs are outward manifestations of the patient's suffering from diseases while alive.

Lesions

- Lesions are macroscopical (visible to naked eye) or microscopical changes in tissue structure.

Pathognomonic lesion(s)

- Pathognomonic lesion(s) is/are characteristic for a particular disease e.g. Blue tongue in sheep- Haemorrhages in the base of the pulmonary artery and base of the aorta.

Course of the disease

- Course of the disease is the duration of time through which the series of changes characteristic of disease pass through to their ultimate end.

Termination of disease

- Termination of disease is recovery or death or may prolong for a considerable length of time as in chronic disease.

Diagnosis

- Diagnosis is the art of determination of the nature of disease, its causes, symptoms, lesions etc.

Morphological diagnosis

- Where diagnosis is based on the alterations observed in a tissue or organ. i.e. naming the lesion e.g. Pneumonia (Inflammation of lungs), enteritis (Inflammation of intestine). This provides information to clinician on the extent, duration, distribution and type of lesion.

Aetiological diagnosis

- Where specific cause of the disease can be identified i.e. naming the cause. e.g. Cause of pneumonia-Bacteria, virus, fungus, foreign body

Specific or definitive diagnosis

- Where the pathognomonic lesions are characteristic of the disease can be observed. i.e. naming the specific entity involved e.g. Corrugated appearance of intestine in Johne's disease in cattle, haemorrhages in the base of the aorta and pulmonary artery in blue tongue in sheep

Differential diagnosis

- Differential diagnosis is aimed at diagnosing a disease by differentiating from different diseases based on clinical and pathological findings. This is the first step in diagnosis.

Prognosis

- Prognosis is pronouncing probable/expected outcome of the disease
- Prognosis of a disease is the estimate by a clinician of probable severity and outcome of the disease.

Sequelae

- Final end result of the disease

Morbidity

- Morbidity is the percentage of affected animals that get exposed
- Number of animals exposed is 100
- Number of animals affected is 50
- Morbidity 50%

Mortality rate

- Mortality rate of a disease is the percentage of deaths among animals affected by that disease.
- Number of animals exposed is 100
- Number of animals affected is 60

- Morbidity 60%
- Number of deaths among affected animals 30
- Mortality rate 50%

Autopsy

- Autopsy is seeing with one's own eyes (Used in human medicine)
- The pathologist cuts open a corpse to see the lesions in diseases.

Necropsy

- Necropsy is seeing a carcass (Used in veterinary medicine)

Biopsy

- Biopsy is examination of biological samples like fluid, tissue, etc., collected from living animals.

Scope of Pathology

- Pathology is aiding in the diagnosis of diseases.
- Helps in understanding the disease process.
- Hence, prognosis, control and rationale treatment and prevention are possible.

BRANCHES OF PATHOLOGY

- **General pathology** deals with fundamental processes that are common to more than one tissue or organ
- **Systemic pathology** is study of diseases peculiar to certain systems or organs
- **Special pathology** is study of diseases caused by specific microbial pathogens
- **Clinical pathology** is that branch of pathology used in the diagnosis of the diseases in the hospital at the patient's bedside. Pathology applied to find the solution to clinical problems especially the use of laboratory methods in clinical diagnosis.
- **Comparative pathology** is the study of diseases of animals and comparing them to those occurring in man.
- **Nutritional pathology** is the study of disease processes resulting from deficiency or excess of essential foods
- **Experimental pathology** means the study of disease artificially produced in animals
- **Chemical pathology** deals with alterations in biochemical processes in diseases
- **Toxicopathology** means the study of diseases caused by toxic substances
- **Oncology** (Gk. Onco-Tumour) is study of tumours.

The purpose of study of pathology is to diagnose, treat, control and prevent the diseases from the knowledge gained through the cause, pathogenesis and effects. These are achieved through examination of tissues from living animals (Biopsy) and dead animals/carcass (Necropsy) or by experimentation.

Thus pathology deals with disease processes involving aetiology, pathogenesis and clinical effects of diseases in animals and tries to explain what went wrong. It is linking the basic

knowledge gained in anatomy, histology, physiology and biochemistry and clinical subjects in making diagnosis of diseases and helps in treatment, prevention and control of diseases.

MODULE-2: AETIOLOGY

Learning objectives

- Through this module, the viewer will learn the causes of diseases, their classification and developmental defects (Anomalies and monsters).

AETIOLOGY
DEFINITION AND CLASSIFICATION

Definition

- Aetiology is defined as study of causation of disease.

Classification

There are several agents or factors that can produce disease in animals and which originate from within the body or outside the body. Thus, the causes are broadly classified into two categories.

- Predisposing causes (Intrinsic factors)
- Definitive causes (Extrinsic factors)
    - Predisposing causes make the animal susceptible to definitive causes. These include
    - Heredity-Inherited-Glycogen storage diseases e.g. Pompe's disease (Lysosmal acid maltase deficiency) in cattle, dog
    - Species-Rinderpest is found in cattle not in other animals.
    - Breed-Certain breeds are more susceptible to some diseases than others. Heavier breeds of dogs suffer from bone diseases
    - Age-Young animals are comparatively more prone for disease than adults.
    - Sex-Diseases of reproductive organs in respective sex.

Colour (Pigmentation)-Sunburns in melanin deficiency, eye cancer in Hereford cattle

DEFINITIVE CAUSES

Definitive causes are actual agents that produce diseases. These are

Physical causes

- Trauma-Mechanical, Accidents
- Excess heat-Burns
- Excess cold-Frost bite, cold shock
- Radiation-UV irradiation, x-radiation

Chemical causes

- Acids-HCl, H 2 SO 4
- Alkalis-NaOH
- Inorganic chemicals-HgCl 2 is nephrotoxic
- Organic chemicals-CCl 4 -Hepatotoxic

Viable/biological causes

- Bacteria-Anthrax bacilli
- Viruses-Foot and mouth disease virus (Picorna virus)
- Mycoplasma-Respiratory disease in chicken
- Rickettsia-Ehrlichiosis
- Fungus-Dermatomycosis, aspergillosis (e.g. Brooder pneumonia in chicks)
- Parasites-Haemonchosis in ruminants, ascaridiasis (Pups, buffalo calves)

Nutritional causes

- Excess-Hypervitaminosis A, D
- Deficiency-Hypoproteinaemia, hypovitamonosis (e.g. Xerophthalmia in vitamin A deficiency, star gazing in chicken in vitamin B 1 deficiency), Hypocalcaemia (Milk fever in high yielding cows)

Immunological diseases-Hypersensitivities-Anaphylaxis

Toxins-Phytotoxins, Zootoxins (Snake venom), Pesticides (Organochlorines, organophosphorus compounds)

Physical causes-Traumatic injuries

- Perforation is a wound caused by a bullet or nail.
- Laceration is a wound in which there is tearing of tissues. e.g: Automobile accidents.
- Concussion is a violent shock caused by an injury and is usually applied to injuries of the head. There may or may not be loss of consciousness.
- Sprain is an injury of joint in which there may be stretching or rupture of ligaments, muscles or tendons. In this anatomical relationship of the structures is maintained.
- Luxation or dislocation is deviation from its original position in which the anatomical relationship are not maintained and the ligaments may be torn.
- Fracture is discontinuity of a bone.

Miscellaneous causes

- Atrogenic disease is a condition produced by the physician by over or needless medication.
- Idiosyncrasy is different reaction of animals to drugs.

DISTURBANCES IN DEVEOPMENT (ANOMALIES & MONSTER)

- Anomaly is developmental defect affecting an organ or part of the body.
- Anomaly is the disturbance of development that involves an organ or a portion of an organ.
- Monster is an animal in which extensive abnormal developments are present.
- A Congenital disease is one in which the patient is born with the disease whereas an inherited disease is one which is due to factors in the genetic materials received from the parents.

CLASSIFICATION OF ANOMALIES

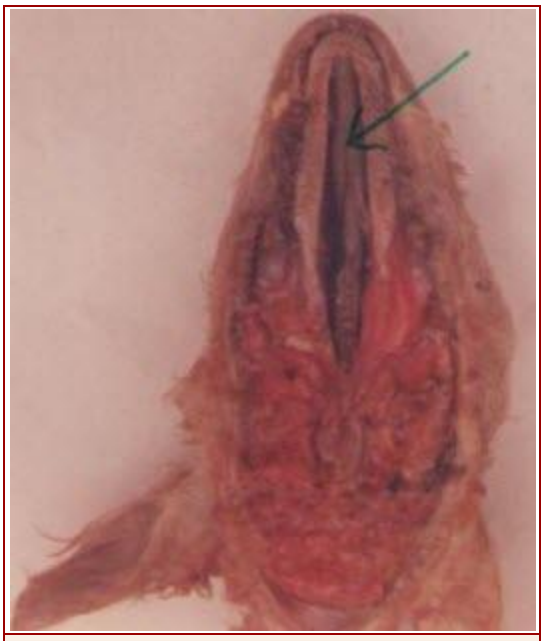
A. Arrest of Development

1. Agenesia is an incomplete and imperfect development of an organ or part and aplasia is absence of an organ or part.

- Acrania is absence of most or all of the bones of the cranium.
- Amelia is absence of one or more limbs.
- Anencephalia is absence of the brain.
- Hypocephalia is incomplete development of the brain.
- Hemicrania is absence of half of the head.
- Exencephalia is defective skull with brain exposed or extruded. If the protruding brain contains a ventricle which is filled with excessive amount of fluid, the malformation is a hydrencephalocele.
- Arhinencephalia is absence or rudimentary development of the olfactory lobe with corresponding lack of development of the external olfactory organs.
- Agnathia is absence of the lower jaw.
- Anophthalmia is absence of one or both eyes.
- Abrachia is absence of the forelimbs.
- Abrachiocephalia is absence of forelimbs and head.
- Adactylia is absence of digits.

2. Fissures on the median line of the head, thorax, and abdomen.

- Craniooschisis (skull)
- Cheiloschisis (lip), often referred to as harelip.
- Palatoschisis (oral) cavity, often called cleft palate. Harelip and cleft palate result from faulty development of the maxillary process derived from the first visceral arch.
- Rachischisis (spinal column).
- Schistorrachis or spina bifida (spinal column)
- Schistothorax (thoraz or sternum).
- Schistosomus (abdomen).
- Schistocormus (thorax, neck or abdominal wall). Results from arrested development of the amnion.



Palatoschisis

3. Fusion of paired organs

- Cyclopia (eyes)
- Ren arcuatus (kidneys), often referred to as horseshoe kidney.

B. Excess of Development

1. Congenital hypertrophy

- Hemi hypertrophy (partial)

2. Increase in the number of a part

- Polyotia (ears)
- Polyodontia(teeth)
- Polymelia (limbs)
- Polydactylia(digits)
- Polymastia (mammary gland)
- Polythelia(teats)

DISPLACEMENTS DURING DEVEOPMENT

A. Displacements of organs

- Dextrocardia is transposition of the heart to the right side.
- Ectopia cordis cervicalis is displacement of the heart into the neck.

B. Displacements of tissues

- Teratoma is inclusion of multiple displaced and also neoplastic tissue within an individual.
- Dermoid is inclusion within an individual of a mass containing skin, hair, feathers, or teeth depending on the species and often arranged as an epidermal cyst (Dermoid cyst).
- Odontoid cyst is inclusion within an individual of a mass of dental enamel and cement.
- Dentigerous cyst is inclusion within an individual of one or more imperfectly formed teeth.
- Fusion of Sexual Characters
- Hermaphrodite is an individual having both testicular and ovarian tissue. Pseudohermaphrodite is an animal having unisexual development of the sex glands (either testicular or ovarian tissue), but having also either a unisexual or bisexual development of the other parts of the genitalia.
- Freemartin is a female calf having arrested development of the sex organs and being the twin of perfect male.

MONSTERS

A monster or monstrosity is a disturbance of development that involves several organs and causes great distortion of the individual. For the most part monsters possess a duplication of all or most of the organs and other parts of the body. They develop from a single ovum. They are therefore the product of incomplete twinning.

Classification of the Monsters

- Twins Entirely Separate
  - Although separate, these twins are in a single chorion. One twin as a rule is well developed; the other is malformed (acardius). In the malformed foetus there is arrested development of the heart, lungs, and trunk. Such monsters may lack a head (acephalus), limbs and other recognizable features (amorphous), or the trunk (acormus).
- Twins United
  - These twins are more or less completely united and are of symmetrical development.

TWINS UNITED

A. Anterior Twinning: The anterior part of the individual is double, the posterior single.

- Pygopagus – united in the pelvic region with the bodies side by side.
- Ischiopagus – united in the pelvic region with the bodies at an obtuse (not pointed) angle.
- Dicephalus – two separate heads; doubling may also affect the neck, thorax and trunk.
- Diproosopus – doubling in the cephalic region without complete separation of heads; only the face doubled.

B. Posterior Twinning: The posterior part is double, the anterior single.

- Craniopagus – brains usually separated; bodies as a rule at an acute angle.

- Cephalothoracopagus – union of head and thorax.
- Dipygus – doubling of posterior extremities and posterior part of body.

C. Twinning Almost Complete: Duplication of the whole trunk or the anterior or posterior extremities with parallel, ventral arrangement of the foetuses. The pair is joined in the region of the thorax, and also often in the abdominal region.

- Thoracopagus – united only by the thorax.
- Prosopothoracopagus – besides the union the thorax the abdomen, the head and neck are united.
- Rachipagus – thorax and lumbar portion of the spinal column united.

MODULE-3: HAEMODYNAMIC DERANGEMENTS-1

Learning objectives

- In the first part of this module, the viewer will be taught on changes in cardiovascular system in disease processes like various conditions leading to congestion, bleeding (haemorrhage) and leakage of fluids from blood vessels and its accumulation in various sites of the body (Oedema).

HYPERAEMIA AND CONGESTION

Definition

- Hyperaemia is increased volume of blood in affected tissue or part.

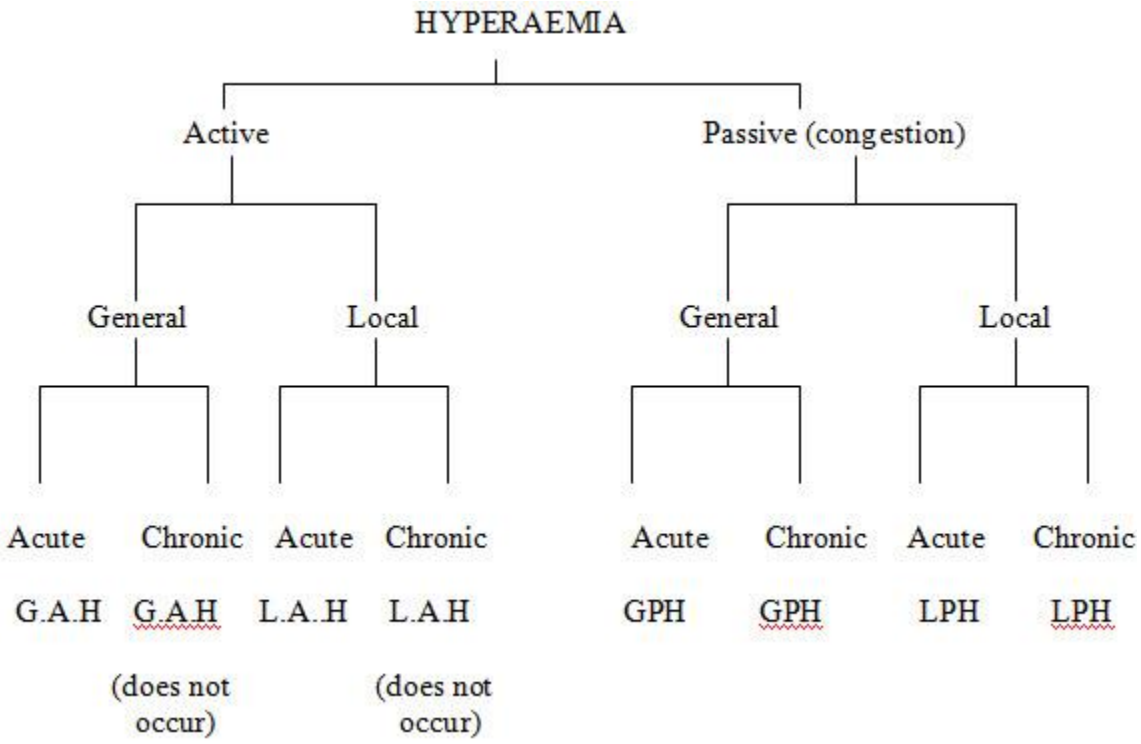
Hyperaemia (Active hyperaemia)

- Occurs in arterioles or arteries
- Increased blood flow in capillaries

Congestion (Passive hyperaemia)

- Occurs due to impaired venous drainage
- Stasis of blood in veins

CLASSIFICATION OF HYPERAEMIA



ACTIVE HYPERAEMIA

- Increased blood in arterial side
- Usually due to inflammation
- All active hyperaemia are acute
- Chronic active hyperaemia does not occur
- Occurs when there is a demand for oxygen and nutrients - increase metabolism
- It is beneficial.

ACUTE GENERAL ACTIVE HYPERAEMIA

Increased blood throughout the body

Causes

- Various systemic diseases. E.g. Pasteurellosis, erysipelas
    - Rapidly beating heart → increased blood supply
- Renal diseases - due to retention of fluids

Macroscopically

- Bright red color or organs

Microscopically

- Arteries and capillaries dilated with blood

Result

- Disappears if cause is removed

ACUTE LOCAL ACTIVE HYPERAEMIA

- Increased amount of blood in arterial system within a local area (leg, Stomach, lung)
- Most common type of hyperaemia

Causes

- Physiological
  - Occurs in stomach and intestine following a meal
  - Lactating mammary gland
  - Muscles during exercise
  - Genital tract during oestrus

Blushing

- Acute inflammation

Macroscopically

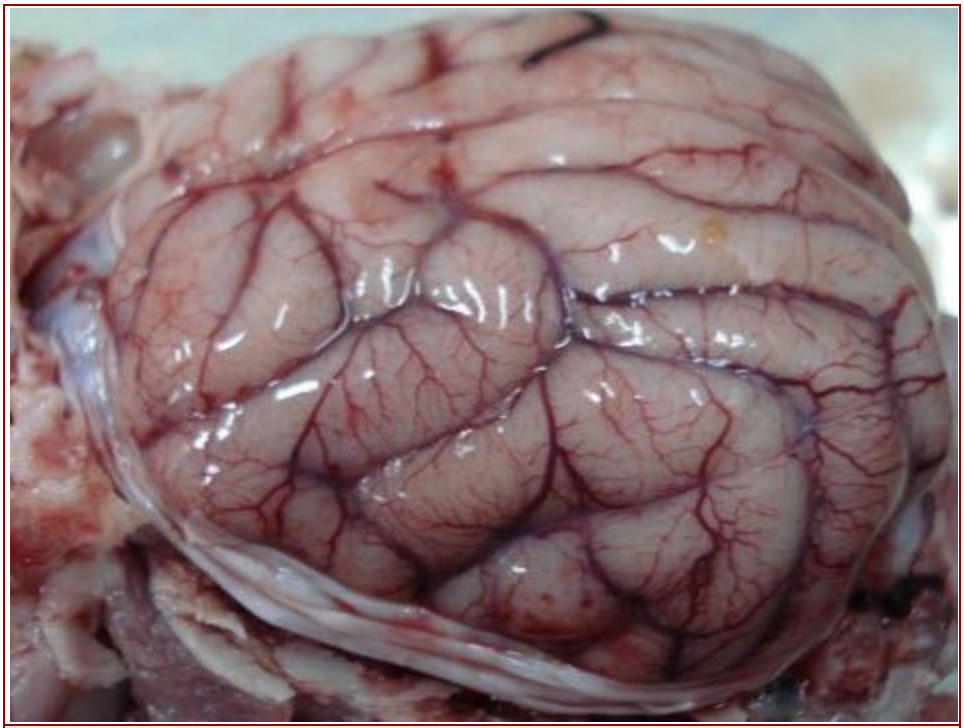
- Enlarged, swollen, heary
- ↑ warmth in Skin

Microscopically

- In live animals, arteries, arterioles and capillaries are distended with blood
- Difficult to detect in dead animals

PASSIVE HYPERAEMIA OR CONGESTION

- Increased blood in the venous end due to improper drainage.
- GRNERAL - if interference is central (i.e.) lungs, heart
- LOCAL - if vein of an organ or body
- It can be acute or chronic



Brain congestion

- Chonic venous congestion is more common

ACUTE GENERAL PASSIVE HYPERAEMIA

- Increase in the amount of blood on the venous side of circulatory system
- Due to sudden obstruction to the flow of blood in heart and lungs.

Causes

- Heart failure
  - Degeneration and necrosis of myocardium
  - Myocardial infarction
- Pneumonia
- Pulmonary thrombosis or embolism
- Hydropericardium, Haemopericadium, etc.
- Hydrothorax, Haemothorax, etc.

Macroscopically

- Organs are blue in color (Unoxygenated blood)
- Veins distended with blood
- Organs enlarged, heavy
- Upon incision, blood oozes out

Result

- Causes are mild → Recovery
- Causes are severe → Death

CHRONIC GENERAL PASSIVE HYPERAEMIA

Increased blood on venous end persisting for long period of time causes Permanent changes (fibrosis, atrophy).

Causes due to central lesions in heart and lungs

- Heart lesions
  - Stenosis of valvular openings
- Valvular insufficiency
  - Failure of cusps of valves to close property
  - Inflammatory tissue
  - Thrombus
- Myocardial failure
  - Degeneration and necrosis of muscles
  - Degeneration and necrosis of muscles

Contraction of muscles

↓

Blood pushed in arteries

↓

But accumulates in venous side

- Anomalies of heart
  - Persistent foramen orale
  - Interventricular septal defects

Blood moves from one chamber to another

↓

Arterial blood pressure maintained

↓

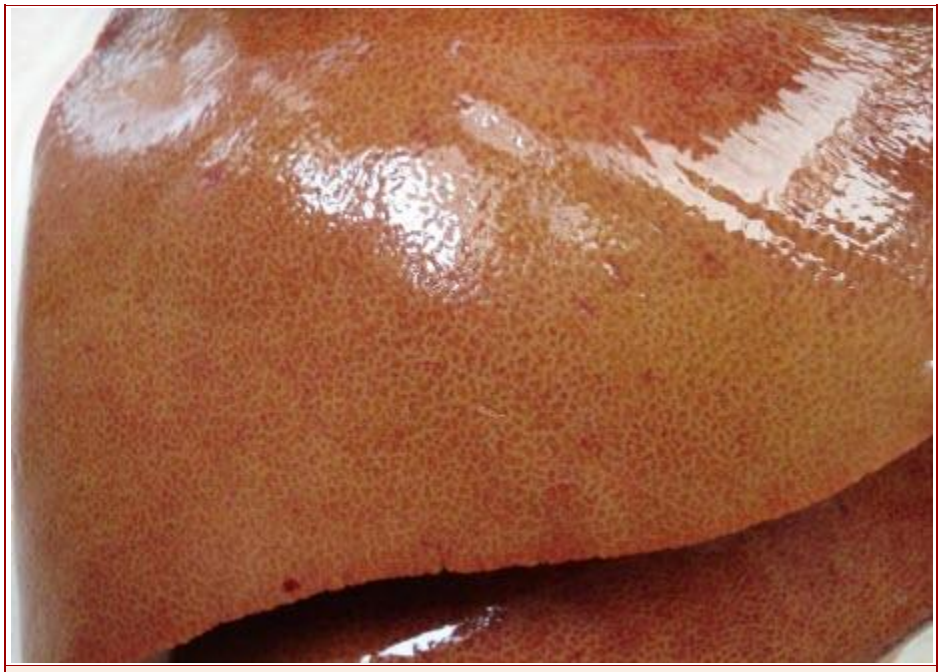
Blood accumulates in venous end.

- Constrictive lesons in pericardium
  - Traumatic pericarditis in cattle
- Lesions of lungs
  - Obliteration of capillary bed in lungs
  - Prevents free flow of blood through the lungs
  - Retards flow through right side of heart
  - Blood back flows into Liver
  - Causes
    - Chronic alveolar pulmonary emphysema in horses (BROKEN WIND)
    - Pneumonia
    - Hydrothorax, haemothorax
  - Compression of major pulmonary vessels
    - Tumours
    - Cysts
    - Abscesses

Lesions in CVC

Liver

- Lesions in Rt A-V valve or lungs
- Increase in size and weight on section,
- "Nutmeg pattern"



Liver - CVC

- Central veins are prominent
- Area surrounding central vein is congested
- Congested area is surrounded by hypoxic areas

Morphologic features of CVC

- Veins all over the body engorged with blood
- Blood is bluish red in color
- Oedema of tissues
- Atrophy of organs
- Degeneration and Necrosis or organs

Microscopically

Mitral valve diseases

↓

Affected in Left – sided heart failure

↓

Alveolar capillaries distended with blood

↓

Rupture of capillaries

↓

Minute intra – alveolar haemorrhages

↓

Haemosiderin release from RBCs

↓

Phagocytosed by macrophages

↓

Heart failure cells (Macrophages)

↓

Fibrosis (induration) of alveolar septa

↓

Brown induration of lungs

↓

(due to haemosiderin)

Spleen

- Enlarged and cyanotic
- Due to congestion of Liver
- Occurs in vegetative endocarditis (swine) and
- Traumatic pericarditis (cattle)
- Hard and indurated - **Cyanotic induration**

Kidneys

- Pressure on renal veins by
  - Tumours of adrenals
  - Abscesses
- Grossly, enlarged and dark purple
- Cortico-medullary junction – dark red in color

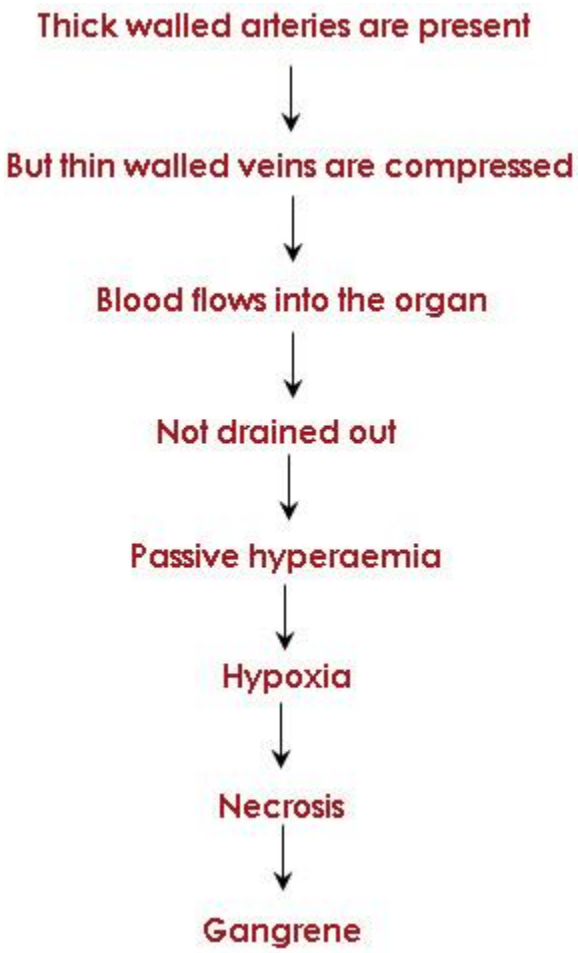
ACUTE LOCAL PASSIVE HYPERAEMIA

Increase in blood in the veins of a portion (foot, tail, kidney etc) Due to sudden obstruction to blood flow

Causes

- Malposition of viscera
  - Volvulus, intussusception, torsion
- External pressure
  - Ligatures, tourniquets, bandages

Pathogenesis



HYPOSTATIC CONGESTION

Accumulation of blood in ventral portions of the body due to gravity.

Causes

- Occurs in heart diseases
- Recumbency
- Inactive animals
- Large animals
- Heart failure - **Agonal congestion**

Appearances

- Veins in ventral portion or organs distended with blood
- Lungs - increase capillary bed
- Intestine & kidneys – necrosis and gangrene
- Causes pneumonia and gangrene of intestine

Significance

- Indicates
  - the side of animals which was ventral at the time of death
  - Heart was not able to pump properly
  - Location of body in medico–legal cases

Grossly and microscopically

Veins are engorged with blood

↓

Necrosis of endothelial cells

↓

Haemorrhage

CHRONIC LOCAL PASSIVE HYPERAEMIA

- Increase in amount of blood for a long time in veins
- Permanent tissue changes (atrophy, fibrosis)

Causes

- External pressure
  - Tumors, abscesses
- Obstruction from within
  - Thrombus (blood clot)

Gross and microscopic appearance

- Enlarged initially later undergoes atrophy
- Veins - bluish blood
- Oedema due to increase permeability of capillaries
- Fibrosis

HAEMORRHAGE

Definition

- It is the escape of blood from a vessel.

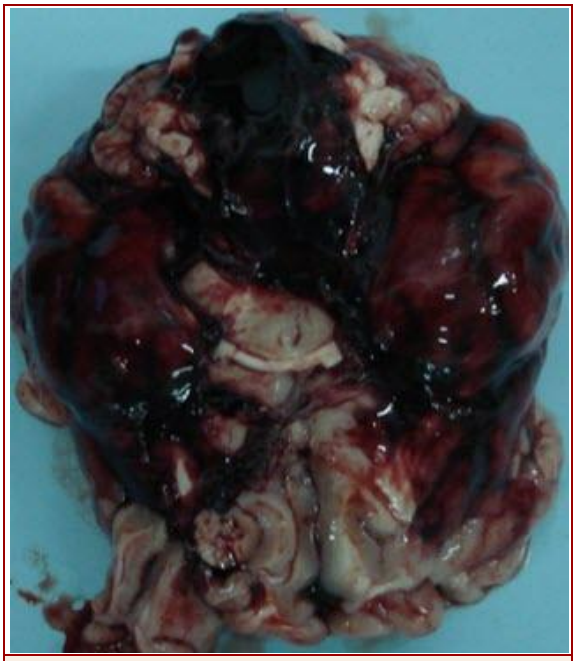
Two types

- Haemorrhage by rhexis : When there is rupture of a blood vessel

- Haemorrhage by diapedesis : When blood leaves through intact blood vessels

Site of haemorrhage

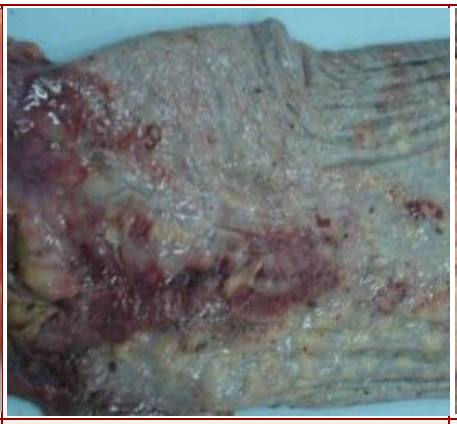
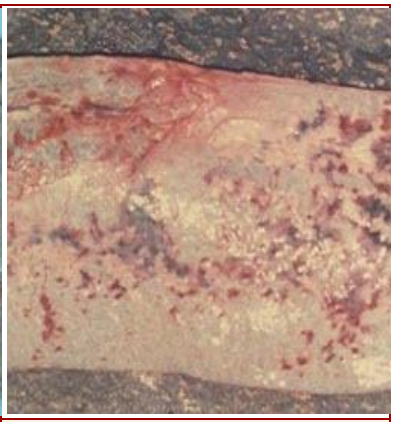
Epistaxis	Bleeding from nose
Haematemesis	Blood in vomit
Haemoptysis	Blood in sputum
Metrorrhagia	Bleeding from uterus
Enterrohagia	Bleeding from intestine
Melena	Blood in stools
Haematuria	Blood in urine
Haemothorax	Blood in thoracic cavity
Haematocoel	Bleeding into tunica vaginalis
Hemosalphinx	Bleeding in oviducts
Hematoma	Tumour-like accumulation of blood
Apoplexy	Haemorrhage into brain

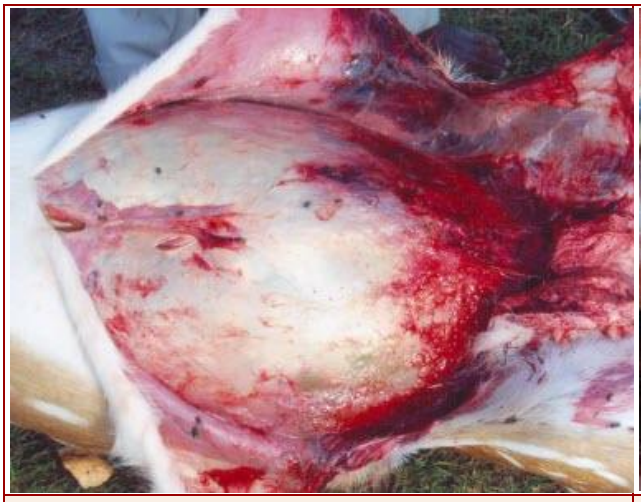
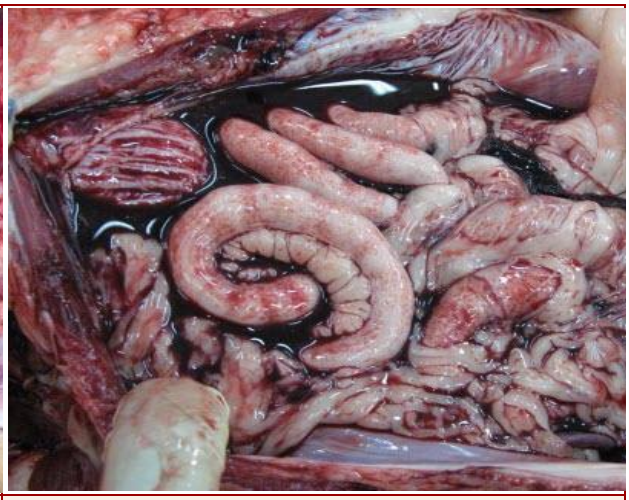


Apoplexy

Size of haemorrhage

- Petechiae: minute; pinpoint
- Purpura: approximately 1cm in size
- Ecchymoses: 1 – 2 cm in size
- Extravasation: Larger area

		
Petechiae-Intestine	Purpura - Spleen	Ecchymoses - Spleen

	
Extravasation	Internal haemorrhages-Abdominal cavity

Source of haemorrhage

- Cardiac
- Arterial
- Venous
- Capillary

Causes

- Conditions affecting the blood vessels
- Conditions affecting the blood

Conditions affecting blood vessels

- Trauma: Lacerations, incisions, contusions
- Clumps of bacteria, swine erysipelas, anthrax, haemorrhagic septicaemia
- Necrosis of vessel wall

    - Ulcers in gastric mucosa
    - Neoplasms
- Diseass of vessel walls
    - Aneurysm - e.g. Strongylus vulgaris infection in horses
    - Atheroma
- Toxic injury to capillary endothelium
    - Bacterial : Anthrax, haemorrhagic septicaemia, black quarter
    - Viral : Hog cholera
    - Chemicals : Arsenic, phosphorus, chloroform, cyanide
    - Enterotoxins : Sheep & calves – Clostridium welchii - ASPHYXIA
- Increased blood pressure
    - Excessive exercise → increased blood pressure → Rupture of blood vessel - Seen in race horses
- Hypoxia and lack of nutrition
    - Passive venous congestion → damage to endothelium - Haemorrhage

Conditions affecting blood constituents

- Haemophillia: Hereditary sex linked disease; Delayed clotting
- Thrombocytopenic purpura: Decrease in platelets seen in toxaemias
- Nutition
    - Deficiency of vitamin K
        - Vitamin K which is required for prothrombin formation and in its absence clotting will not take place.
        - Increased use of sulpha drugs may not permit intestinal microflara to synthesis vitamin K
    - Deficiency of vitamin C
        - Vitamin C is required for formation of ground substance. Invitamin C defeciency capillary endothelium becomes more fragile leading to haemorrhage
- Heparinoid state
    - In anaphylactic shock and irradiation, excess of heparin is found which impairs clotting.
- Plant toxins
    - Brakern fern and sweet clover poisoning prevent prothrombin formation

Microscopical appearance

- Presence of erythrocytes outside blood vessels
- Recent haemorrhage stains deeply

Haemorrhage disintegrates due to action of tissue enzymes

↓

Haemoglobin → Haemosiderin (Iron) and hamatoidin (Non iron)

↓

Bilirubin

↓

Phagocytosed by macrophages

- Prussian blue reaction reaction to demonstrate iron

Significance and result

- Depends on volume, rate, site. Sudden loss of about 30% of blood volume or slow losses of large volume of blood will have no clinical significance. e.g. Stomach worm infection
- Site of haemorrhage is very important. Small haemorrhage in brain is fatal whereas small haemorrhage in skeletal muscle or subcutaneous tissue is NOT FATAL. Haemorrhage in pericardial sac (CARDIAC TAMPONADE) is fatal.
- Iron deiciency anaemia is due to repeated and chronic loss of blood from external surface
- When erythrocytes are retained in body cavities, joints, tissues, iron is recaptured and haemoglobin is synthesized.

Fate of haemorrhage

- In small haemorrhage fluid portion is reabsorbed, WBCs move into blood vessels and RBCs are phagocytosed
- In larg haemorrhage, RBCs are haemolysed and haemoglobin is split into haeme (Haemosiderin which is iron containing portion of haeme and haematoidin is iron free portion) and globin.

Arrest of haemorrhage

Vascular contraction	Small blood vessels
Platelet aggregation	White clot
Clot formation	Red clot → When blood flow is slow
Tissue pressure	• Increased perivasular pressure in tissue • Decreased intra vascular pressure
Decreased blood pressure	Large harmorrhage ↓ Decreased BP ↓ No bleeding

OEDEMA

Definition

- Abnormal accumulation of fluid in the intercellular tissue spaces or body cavities
  - Localized : Due to obstruction of venous outflow – leg
  - Generalized : Chronic venous Congestion or heart failure

Terms used to describe oedema

- Anasarca: Generalized subcutaneous oedema
- Ascites: Fluid in peritoneal cavity
- Hydrothorax; Edematous fluid in thorax
- Hydropericardium: Edematous fluid in pericardium

Oedema is of two types

1. Inflammatory oedema
2. Non-inflammatory odema

Mechanism of oedema formation

Two forces called “STARLING’S FORCES “

- Filtration force: Expels fluid from the vessel
- Absorption force: Draws fluid into the vessel

Physiology of fluid balance

- At the arterial end of capillary hydrostatic pressure is 45mm of Hg and osmotic pressure of blood is 30mm of Hg (due to albumin / globulin). Therefore, fluid expelled into the intercellular space (filtration force) is 15mm Hg.
- At te venous end, hydrostatic pressure of blood falls to 15mm of Hg and osmotic pressure of blood is 30mm of Hg. Therefore, absorption force is 15mm of Hg

CAUSES OF OEDEMA

- Decreased plasma osmotic pressure
- Increased hydrostatic pressure
- Increased permeability of vascular endothelium
- Lymphatic obstruction
- Sodium retention

Decreased plasma osmotic pressure - Hypoproteinemia (Albuminemia)

- Decreased protein synthesis

- Excessive loss from blood - Low osmotic pressure in the blood - More fluid flows into intercellular space

Hydrostatic pressure at arterial end is 45mm Hg and osmotic pressure at arterial end is 20mm Hg. So, the rate of fluid flow into tissues is 25mm Hg. Osmotic pressure at venous end is 20mm Hg and hydrostatic pressure is 15mm Hg. Thereby, the rate of fluid flow in to vein is 5mm Hg. Because of the pressure diffeences (Hydrostatic and osmotic pressure) at the arterial and venous end, the rate of fluid accumulation in tissues is 20mm Hg

Decreased plasma osmotic pressure mostly results in **generalised and severe oedema**

- Malnutrition
- In advanced hepatic disease (Cirrhosis), protein synthesis will be affected leading to nutritional or cachetic oedema
- Loss of protein through intestine and stomach - stomach worms → Parasitic oedema
- Kidney or renal amyloidosis – blood lost in urine - Renal odema

Increased hydrostatic pressure

- General or passive hyperaemia → venous stasis
- Central lesion in heart or lungs or local obstruction in a vein

Hydrostatic pressure at arterial end is 45mm Hg, whereas osmotic pressure is 30mm Hg. So the rate of fluid flow into tissues is 15mm Hg. At the venous end, osmotic pressure is 30mm Hg and hydrostatic pressure is 25mm Hg. The rate of fluid flow into vein is 10mm Hg. So the rate of fluid accumulating in tissues is 5mm Hg.

- This type of oedema is mild. Mainly the cause is in the heart. Hence called **cardiac oedema**.

Increased permeability of capillary endothelium

- Due to venous stasis → increased hydrostatic pressure
- Inflammation

Lymphatic obstruction

Causes

- Tumours, cyst, abscess, bandages, thrombi
- Parasites (*Demodex canis*, mites)
- Filariasis – *Wucheria bancrofti* - humans
- Inflammatory conditions – farcy; ulcerative lymphangitis

In lymphatic obstruction, fluid and protein in intercellular space will not be drained leading to oedema (LYMPHOEDEMA)

Sodium retention

Causes

- Congestive heart failure
- Nephrosis/Nephritis
- Acute renal failure

Due to failure of excretion sodium in urine, water will be retained leading to generalized oedema

Differences between transudate and exudate

S. No.	Characters	Transudate	Exudate
1	Colour	Clear, water like pale yellow	Cloudy, white, yellow-red
2	Consistency	Thin, watery no tissue fragments	Thick, creamy, contains tissue fragments
3	Odour	None	Have odour
4	Ph	Alkaline	Acidic
5	Specific gravity	1.015 or less	1.018 or higher
6	Protein	Low, < 3%	High > 4%
7	Cell count	Low	High, RBCs, WBCs
8	Enzyme count	Low	High
9	Bacteria	None	Present
10	Inflammation	None	Present

Macroscopical appearance

- Swollen, increase in weight
- Cold due to decrease blood, flow and increase heat dissipation
- Less color
- No pain
- Incision results in flow of fluid from cut surface
- Pits on pressure
- Fibrosis

Microscopical appearance

- Space between adjacent cells widened
- During life space filled with fluid
- H&E stain - fine granular material - stains faintly pink - ↑ pink if ↑ protein

- Atrophy of parenchymatous cells
- Fibrosis - chronic cases

Significance and result

- Disappears if cause is removed
- Oedema in lung & brain are fatal
- Subcutaneous oedema impairs wound healing

TYPES OF OEDEMA

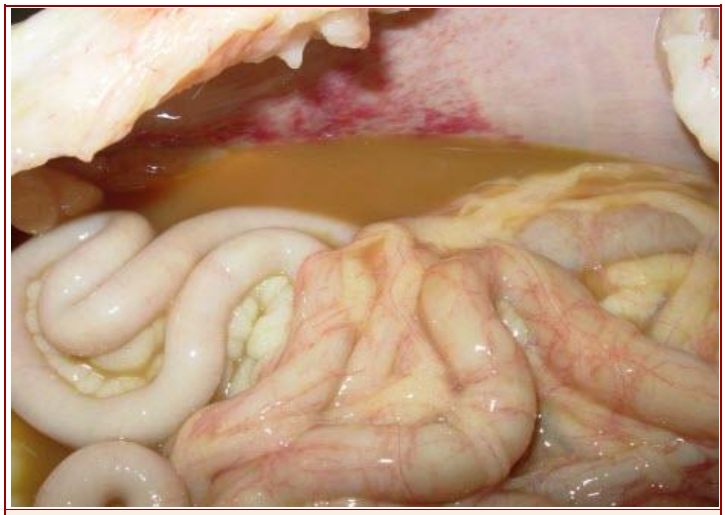
1. Inflammatory oedema
2. Cardiac oedema
3. Renal oedema
4. Hunger/Famine/War oedema
5. Pulmonary oedema
6. Cachetic oedema
7. Myxoedema
8. Parasitic oedema
9. Angioneurotic oedema
10. Brisket disease

1. Inflammtory oedma

- Toxins damage blood vessels - Increased permeability of endothelium - Fluid rich in protein pass out - “INFLAMMATORY EXUDATE”

2. Cardiac oedema

- Congestive heart failure leads to CVC which results in insufficient renal circulation ischaemia leading to oliguria with diminished chloride excetion. This results in sodium retention which raises tissue osmotic pressure aggrevating oedema



Oedema - Abdominal cavity - Ascites

- Causes for cardiac oedema
  - Increased hydrostatic pressure of blood
  - Increased vascular permeability
  - Sodium retention
- Symptoms
  - Oedema of dependant parts
  - Traumatic pericarditis in bovines

Cardiac oedema may develop in horses with chronic vesicular emphysema.

3. Renal oedema

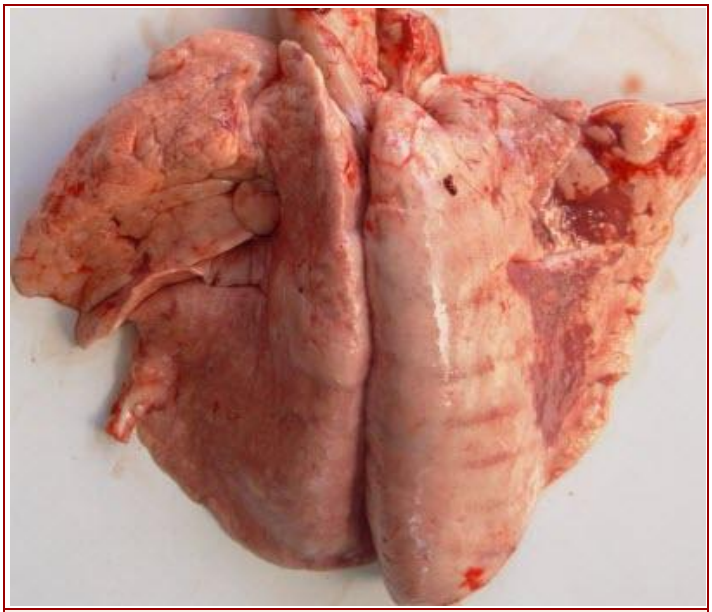
- In acute glomerulonephritis (in man), oedema in face and eyelids are usually seen.
  - Causes of acute glomerulonephritis are
    - Decreased osmotic pressure of blood
      - Toxins damage glomerular capillaries resulting in albuminuria and hypoproteinaemia.
    - Increased osmotic pressure of ECF
      - In acute nephritis, oliguria / Anuria results in sodium retention
    - Increased capillary permeability
    - Increased hydrostatic pressure in capillaries in venous side
      - Toxins damage kidney and heart causing cardiac failure and its outcome is CVC
- Subacue nephritis and nephrosis
  - Decreased colloidal osmotic pressure of blood
  - Increased sodium retention
  - Hypoalbuminaemia stimulates adrenal cortex to secrete increased amount of aldosterone which helps in reabsorption of sodium chloride. This retained salt increases osmotic pressure and cause oedema.
- Chronic glomerulonephrtis
  - Hypertension for long period throws great strain on heart resulting in heart failure and thereby causing CVC which increases blood pressure in capillaries. As a resut of this, oedema occurs.

4. Hunger / Famine / war oedema

- Hypoproteinaemia → Decreased plasma osmotic pressure
- War / famine → Decreased protein availability

5. Pulmonary oedema

- Causes
  - Cardiac failure - hypertension; valvular disease - pericarditis
  - Renal lesions
  - Pressure on pulmonary veins by neoplasm
  - Injury to brain
  - Rapid removal of effusion from pleural / peritoneal cavity
  - Poisons
  - Infections



Oedema - Lung - Rib markings

6. Cachetic oedema

- Anaemia
- Wasting diseases
- Malnutrition
- Cardiac illness

7. Myxoedma

- This occurs in chronic thyroid deficiency. In this condition there will be increased protein accumulation in tissue fluid which raises pressure of fluid locally and water is drawn into the site.

8. Parasitic oedema

- This type of oedema is most commonly seen in animals suffering with stomach worms, liver flukes, amphistomes. During migratory life of cercaria, haemorrhage & necrosis occurs in liver. Adult flukes inhabitats bileduct causing chronic irritation of lining mucosa of the duct resulting in cirrhosis. Affected liver cannot synthesis protein leading to oedema formation.
- Due to hypoproteinemia, there will be an accumulation of fluid in lower jaw called as “BOTTLE JAW” which is a characteristic feature of parasitic oedema.

9. Angioneurotic oedema

- In man, allergens like snake venom produces hypersensitivity reaction which increases capillary permeability resulting in oedema in lips, glottis, thorax
- In animals (cattle, horses), endogenous / exogenous allergens (plant, protein; fish meal) cause release of histamine which damage blood vessels and oedema results.

10. Brisket disease

- Cattle moved to high altitude 9000ft above sea level develop oedema in abdomen, brisket, neck and jowl.
- At high altitudes, partial pressure of oxygen is decreased. The resulting hypoxia develops polycythemia (Increased viscosity of blood) and polypnoea (Increased heart beat). Cardiac muscle becomes degenerated as it works in hypoxic condition and hence hypertrophied heart slowly dilates and which draws valves downwards resulting in valvuar incompetency and gives rise to chronic venous congestion.
- Reason for development of oedema in high altitude
    - Hypoxia
    - Chronic venous congestion - Develops due to increased capillary blood pressure and hypoxia

MODULE-4: HAEMODYNAMIC DERANGEMENTS-2

Learning objectives

- In this part of this module, the viewer will learn about other changes occurring in cardiovascular system in diseases viz. formation of blood clots within the CV system (Thrombosis), circulation of foreign bodies (Emboli), blockage of vessels leading to death of tissues (Infarction) for want of oxygen and nutrition and shock.

THROMBOSIS

- Formation of clotted mass of blood within the cardiovascular system
- Clotted mass – Thrombus (singular) and thrombi (plural)

Differences between thrombus and blood clot

Thrombus	Blood clot
Formation	
Blood vessels Platelets Blood clotting system	Blood clotting system
Composition	
Platelets Fibrin	Only fibrin
Prognosis	

Life threatening	Life saving

Causes for

- Injury to endothelium
  - Trauma : lacerations, contusion, rupture, i/r injection
  - Toxins : Streptococci, erysipelothrix (vegetations)
  - Degenerations : Atherosclerosis (damage to intima)
  - Viruses : Hog cholera virus
  - Parasites : Strongylus vulgaris in anterior mesenteric artery in horses
  - Tumours : Invading tumours

Mechanism of thrombus formation

- Active
  - Antithrombotic factors and prothrombotic factors are seen on surface of endothelium
- Passive
  - Endothelium is thromboresistant wheras subendothelial connective tissue is highly thrombogenic.
  - Subendothelial onnective tissue consists of collagen, elastic, fibrinonectin, laminin glycosoaminoglycans and thrombosporin.
  - Damage to endothelium exposes the subendothelial connective tissue and activates intrinsic blood clotting pathway and platelet adhesion.

Antithrombotic factors (present on endothelial cells) - Inhibit thrombosis

- Anticoagulant properties
  - Thrombo modulin - Protect against action of heparin and thrombin which converts fibrinogen to fibrin
- Anti platelet properties - Inhibit platelet aggregation
  - Prostacyclin (PGI2)
  - Nitric oxide (NO_2)
- Fibrinolytic properties
  - Tissue plasminogen activator (tPAs) - Promotes fibrinolytic activity in blood and reacts against blood clots

Thrombotic factors

- Tissue factor (Thromboplastin)
  - Present on endothelium in small amounts
  - Activate extrinsic clotting pathway
  - Stimulated by
    - Endotoxins
    - Cytokines (IL – 1)
    - Tumour necrosis factor (TNF)
- von Willebrand factor (vWF)
  - Protein helps in platelet adherence thrombus
- Platelet Activating Factor (PAF)
  - Helps in platelet aggregation thrombus

- Inhibitor of Plasminogen Activator
    - Prevents fibrinolysis thrombus

Normal homeostasis: There will be a balance between antithrombotic and prothrombotic factors in normal endothelium.

Thrombus formation

- Increase prothrombotic factors
- Decrease antithrombotic factors

Alterations in constituents of blood

- Increase in number of platelets
    - Parturition
    - surgery
- Increase in adhesiveness of platelets
    - Parturition
    - Surgery
- Decrease in heparin (anticoagulant) in diseases
- Increased plasma fibrinogen and prothrombin
    - Trauma
- Increased viscosity of blood
    - Dehydration
    - Polycythemia
- Sludging of blood
    - Clumping of cells
- Increased fragility of RBCs
- Increased cortisone therapy – Rheumatoid arthritis - Increase blood lipids - Increase platelet aggregation - Coronary thrombosis

Alterations in normal blood flow

- Slowing of blood flow results in platelet aggregation
- Turbulence damage endothelium

Types of thrombus

- Arterial thrombus
- Venous thrombus
- Cardiac thrombus

Causes for slowing of blood

- Chronic venous congestion venous stasis
- Old and debilitated animals
- Varicose veins

Comon sites for thrombosis

- Animals
    - Scrotal plexus - horses
    - Vascular sinuses – horse and cows (Nasal passage)
    - Large veins of Broad ligament of uterus – cow
    - Anterior mesenteric artery - horses
- Humans
    - Leg veins – Congestive heart failure, bed ridden patients

CLASSIFICATION OF THROMBI

I. Based on location within blood vascular system

1.Cardiac thrombi

- Mural thrombus: Seen on the wall of left auricle
    - Bovines - black quarter
    - Caused by Clostridium chauvoei
- Valvular thrombus
    - Pigs – *Streptococcus pyogenes, Erysipelothrix rhusiopathiae*
    - Cattle– *Cornybacterium pyogenes*
    - Horses– *Streptococcus equi*
- Ball thrombus: Seen in auricle - Unattached. If it is large,it causes valvular obstruction

2. Arterial thrombi

- Located within arteries
- Common in domestic animals
    - Horses : *Strongylus vulgaris* larvae in anterior mesenteric artery
    - Dogs : *Spirocerca lupi* in aorta
    - Cattle: *Onchocerca armillata* in aorta

3. Venous thrombi

- Phlebothrombosis
- Common in bed ridden patients
- Rare in animals
- Seen in recumbent calves

Leg veins collapse and press against hard surface. Endothelium gets damaged and thromboplastin is released resulting in thrombus formation. In general passive hyperaemia, veins will be distended and leads to slowing of blood which favours thrombus formation.

Locations

- Human - Femoral, popliteal, iliac veins
- Animals
- Nasal vascular sinuses – Cow, horses
- Veins of broad ligament – Cow
    - Scrotal plexus – Horses

4. Capillary thrombi

- Seen in inflammation
- Injury to endothelium

5. Lymphatic thrombi

- Seen in lymphatics draining inflammation area
- Beneficial

II. Classification based on location within heart or bood vessels

- Mural thrombi - Attached to wall of heart / blood vessel
- Valuvlar thrombi - Valves
- Lateral thrombi - Attached to one side of blood vessel
- Occlusive thrombi - Attached to entire circumference of vessel
- Saddle thrombi -Site of bifurcation of blood vessel
- Canalised thrombi - New blood channel is formed through clot

III. Classification based on infectious agent

- Septic thrombi - Bacteria
- Aseptic thrombi - Without bacteria / parasites
- Parasitic thrombi - *Strongylus vulgaris* (Horses) and *Dirofilaria immitis* (Dogs)

IV. Classification based on colour of thrombi

- Pale / White Thrombi - composed of platelets and are seen in heart / aorta
- Red Thrombi – composed of platelets / fibrin, RBCs and WBCs and are seen in veins (Commonly seen)
- Mixed Thrombi –Mixture of White and red thrombi (White - Formed during fast flow of blood; Red - Formed during sluggish flow)
- Laminated thrombi
    - Type of mixed thrombi
        - Excessive exercise - increase blood flow to legs – White Thrombus
        - Rest - increase blood flow to legs - Red Thrombus

Fate of thrombus

- Propagation : Enlargement - obstruction of vessel
- Contraction : Shrinkage of thrombus may occur due to contraction of fibrin
- Embolus : Carried to other sites; and cause dangerous infarction
    - Enzymes from WBCs / platelets digest thrombi and emboli are formed
- Abscessation : Pyogenic bacteria in thrombus may gives rise to bacterial emboli
- Resolution : Fibrinolysis
    - Fresh thrombus – Complete digestion
    - Old thrombus – incomplete digestion
- Organization & Canalisation

Significance and results

- Negligible effects - Jugular vein; carotid arteries
- Beneficial effects - Control of haemorrhages
- Harmful effects - Vessel without collateral circulation
    - Infarction
    - Embolism
    - Passive hyperemia
    - Lymphoedema
    - Aneurysm – Strongylus vulgaris
    - Gangrene – intestinal thrombus
    - Colic, lameness
    - Septicaemia / Death

Character	Thrombus	Postmortem clot
Size	Fills vessels	Small
Consistency	Dry & friable	Smooth / glistening
Color	White, red, mixed	Red / yellow
Attachment	Yes	No
Endothelium	Damaged	Undamaged
Composition	Platelets	Fibrin
Rapidity of blood flow	Formed in flowing stream	Stagnant stream
Animal	Living	Dead
Organization	Yes	No
Structure	Laminated (Line of Zahn)	Homogenous

EMBOLISM

- An embolus is any foreign body floating in blood. The process is called embolism.

Location of embolism

- Artery / venous / capillaries / lymphatics
- In domestic animals emboli always occurs in arteries
- In human, venous embolism is common
- Thrombus in leg vein may form emboli to reach large blood vessel, right side heart and pulmonary artery embolism

Types / Causes of emboli

- Thrombotic emboli : Thrombo embolism – arteries (Thrombi detach to form emboli)
    - Heart – vegetations
    - Parasitic; atherosclerotic; bacteria
- Bacterial emboli : Septicaemia
- Parasitic emboli : *Dirofilaria immitis* - Pulmonary artery – dog
    - Schistosomes – Portal; mesenteric; Nasal blood vessels
    - Trypanasomes – If tartar emetic is given rapidly, it kills large number of organism and forms emboli on coronary vessels which is fatal.
    - Filarial - Lymphatics emboli in brain
- Neoplastic emboli : Clumps of tumour cells in circulation producing metastatic tumours.
- Fibrin - In blood transfusion, when blood is improperly defibrinated / inadequate anticoagulants
- Fat emboli - In fracture of long bones, fat in the marrow cavity gets dislodged and forms emboli. These are lodged in lungs and leads to death.
    - "**Fat embolism syndrome**" (Acute respiratory symptoms, tachycardia neurological symptoms)
- Air or gas emboli
    - Incision of large neck veins (surgery / suicide)
    - Air sucked into veins Embolism
    - In criminal abortion Pumping air into uterus
    - Air in large uterine vein
    - Emboli
    - Enters circulation
    - Heart
    - Foamy blood
    - Acute heart failure – **Sudden death**
- Caission's disease
    - Humans
    - Sudden change in atmospheric pressure
    - Under water construction workers
    - Deep sea / scuba divers
    - Unpressurised aircrafts ascends rapidly
    - Under water construction workers
    - Increase air pressure within under water compartment to compensate water pressure
    - Breathing
    - Increase air dissolve in blood, tissue fluid and fat
    - If the worker surfaces suddenly i.e decompresses
    - Dissolved gases come out as bubbles (O_2, CO_2, N_2)
    - O_2 and CO_2 are soluble and cause no harm; N_2 which is insoluble form emboli
    - AIR embolism (Brain, heart etc)
    - "**Caission**" means - water tight chamber used underwater
    - Also called "BENDS" – severe cramping pain
- Clumps of normal of normal body cells
    - Occurs when tissue / organ is damaged
- Amniotic fluid embolism
    - Complication of labour
    - Infusion of amniotic fluid (Epithelial cells, fat, mucin, meconium)
    - Maternal circulation

    - Due to tear in placental membrane or rupture of uterine veins
    - Maternal mortality (Respiratory distress; cyanosis, shock, convulsions, coma, death)
    - Rare in domestic animals - due to anatomical differences in placental / uterine structures

Pradoxical emboli

- Emboli that pass directly from the right auricle into the left auricle through patent foramen ovale thereby emboli originating from vein will be lodged in systemic vessels instead of being in pulmonary vessels.

Significance / Result of embolism

- Character of emboli
    - size - large emboli → large blood vessel blocked
    - septic / aseptic – new foci of infection
    - neoplasms – metastasis
- Number of emboli
    - Increased sites of obstruction
- Organs involved
    - Liver / Lung / muscle - Large blood supply
    - Very little effect
    - Heart / Kidney / Spleen - no collateral circulation - Infarction

INFARCTION

- An infarct is an area of coagulative necrosis results due to sudden blockage of an end artery which has no collateral circulation.

Causes

Thrombus / embolus

- Pressure on the vessel wall causing ischaemia
    - Ligatures
    - Decubitus ulcers
    - Torniquets
    - Tumours
    - Cysts / abscess
    - Volvulus / intussusception of intestine
- Contraction of vessel wall

Ergot poisoning

↓

Smooth muscle contraction

↓

Narrowing of blood vessel

↓

Ischaemia

↓

Seen in extremities (legs, ears, tail, wattles)

- Hypotension →□Shock →□Ischaemia →□Brain infarction

Pathogenesis

Thrombus (blocking of end artery)

↓

Ischaemia → capillaries dilate to increase blood supply

↓□ □ □ □ □ □ □ □ □ □ □ □↓□ □ □ □ □ □ □

Hypoxia Area red color [Red infarct]

↓

Damage to endothelium

↓

Haemorrhage

↓

>2 hours - Fusion of RBCs into homogenous mass

↓

Degeneration of cells

↓

24hours - Coagulation necrosis of cells

↓

72hours - Lysis of RBCs

↓

Release of haemoglobin

↓

Loss red color [Pale infarct]

↓

Inflammation

↓

Scar (yellow / brown due to haemosiderin

Macroscopical appearances

- Red or pale in color
- Cone shaped – apex of cone is at the point of obstruction of vessel - base towards periphery

Infarcts of kidneys

- Common in cows and pigs
- Yellow or pale
- Wedge shaped – seen in cortex
- Apex at arcuate arteries
- Base at capsular end of cortex
- No capsular necrosis
- Appears as healed, depressed areas
- Causes
    - Cardiac vegetations – Corynebacterium pyogenes, streptococci
    - Cows – very common – emboli of uterine vein after parturition

Infarcts of spleen

- Pale or red color
- Seen in borders
- But in dogs, it is band like
- Due to cardiac thrombi

Infarction of intestines

- Common in horses – anterior mesenteric artery (Strongyle worms)
- Whole surface of bowel is affected
- Red in color
- Causes

Volvulus, intussuception, strangulation

↓

CVC

↓

Necrosis

↓

Gangrene

- Sequelae
    - Fatal
    - Toxaemia
    - Shock
    - Peritonitis

Infartion of brain

- Common in man
- Due to arteriosclerosis
- Animals – Dogs – automobile accidents
- Cerebral infarction
- Softening → Myelin engulfed by microglia – " **Compound granular corpuscles**"
- Organization or cyst formation (neuroglial cells)
- Cyst with yellow fluid "**Apoplectic cysts**"

Infarcts of heart

- Common in man due to arteriosclerosis
- Not seen in animals
- Red or pale

- Healed infarcts – Scar
- Sequelae of cardiac infarcts – Myomalacia cardis

Infarcts of liver

- Causes
    - Tumours
    - Thrombus due to Clostridium hemolyticum in bovines
    - Red in colour

Infarcts of lungs

- Common
- Cone shaped
- Red color
- Causes
    - Emboli from
    - Cows – Uterine veins & posterior vena cava (Abscess)
    - Horses – Mesenteric veins
    - Pigs – Pulmonary veins (Hog cholera)
    - Hypostatic congestion
    - Chronic venous congestion
    - Cattle and sheep – Pasteurella infection → Pulmonary infarction (Haemorrhagic septicaemia)

Sequelae of infarcts

- Organization and scar formation
- Gangrene
- Death (Brain, heart, intestine) – SHOCK/ Toxaemia/ Septicaemia

SHOCK

- A common grave medical emergency characterized by a reduction in effective circulating blood volume and in the blood pressure.

Definition

- Shock (cardiovascular collapse) is a circulatory dishomeostasis associated with loss of circulating blood volume and reduced output and or inappropriate peripheral vascular resistance.
- Although causes of shock can be diverse the underlying cause of shock are relatively stereotyped i.e. hypoperfusion.

Causes of shock

- Trauma / burns
- Profuse haemorrhage
- Bacterial septicaemia

- Myocardial infarction (man)
- Pulmonary embolism (man)
- Psychic stimuli (man)
- Crushing injuries (automobile accidents) in dogs
- Cold, exhaustion, depression animals
- General anaesthesia

Classification of shock

- Primary shock
- Secondary shock

Primary shock (Syncope, fainting)

- Appears immediately after extensive injury
- Nervous stimuli in which widespread paralysis of capillaries occurs
- Animals
    - Rough handling of animals
    - Undue manipulation of intestine in abdominal surgery
- Humans
    - In psychic state like fear, excitement and apprehension, neurogenic impulses causes vasodilation and blood pressure decreases which leads to cerebral ischaemia and results in loss of consciousness (Pallid face, slow breathing, feeble pulse)
    - Transient / Patient recovers with rest.

Secondary shock

It is fatal

Disproportion in blood volume and volume of blood vascular space
↓
Decreased blood pressure
↓
Decreased cardiac output
↓
Reflex sympathetic vasoconstriction (Pallor of skin)
↓
Aldosterone, renin – Angiotensin activated
↓
Renal ischaemia → Death
↓
Increased cardiac action, decreased pulse rate
↓
Death

Causes of shock

1. Reduction in blood volume
    - Loss of blood from injuries (Haemorrhages)
    - Loss of fluid into injured tissues
        - Severe burns
        - Crushing injuries Oedema
        - Vomition
        - Diarrhoea Dehydration
        - Na deficiency
        - Addison's disease Dehydration
        - Diabetic coma
        - Poisons (Phosgene, mustard gas, ANTU)
2. Capillary bed dilation
    - Decreased cardiac output → decreased blood volume
        - Neurogenic Stimuli
            - Anxiety, fear, pain, bleeding wounds
        - Bacterial toxins
        - Burns, crushing injuries
        - Anoxia
3. Acute circulatory failure
    - Infarction, cardiac tamponade
    - Pulmonary embolism → No circulation → Shock

Classification based on fundamental underlying problem

- Cardiogenic shock
- Hypovolumic shock
- Blood maldistribution shock
    - Septic shock
    - Anaphylactic shock
    - Neurogenic shock

Cardiogenic shock results from failure of heart to adequately pump blood.

- This occurs due to
    - Myocardial infarction
    - Ventricular tachycardia
    - Fibrillation or other arrhythmia
    - Dilating and cardiac myopathy
    - Obstruction of blood flow from the heart
        - e.g. Pulmonary embolism and pulmonary or aortic stenosis
    - Other cardiac dysfunction
- Unsuccessful compensation leads to stagnation of blood and progressive tissue hypoperfusion.

Hypovolumic shock arises from reduced circulatory blood volume due to blood loss caused by haemorrhage of fluid loss secondary to vomiting, diarrhoea or burns. This leads to decreased vascular permeability and tissue hypoperfusion.

- Immediate compensatory mechanisms to increase vascular pressure
    - Vasoconstriction and fluid movement into plasma.
- Loss of about 10% blood volume can occurs without consequence, but when blood loss approaches 35-45% blood pressue and cardiac output can fall dramatically.

Blood maldistribution shock is characterised by decrease peripheral vascular resistance and pooling of blood in peripheral tissue.

The systemic vascular dilatation results may dramtically increase microvascular area and although the blood volume is normal. The effective circulating blood volume is decreased.

- Anaphylactic shock is generalised type I hypersensitivity.
    - Causes
        - Exposure to insect or plant allergen.
        - Drugs
        - Vaccine

Interaction of an inciting substance with Ig E and mast cell results in mast cell degranulation, release of histamine and systemic vascular dilatation, increased vascular permeability and tissue hypoperfusion.

- Neurogenic shock
    - Causes
        - Trauma (particularly nervous system)
        - Electrocution (Lightening stroke)
        - Fear
        - Emotional stress

Here autonomic discharge that results in peripheral dilatation followed by venous pooling of blood and tissue hypoperfusion. When compared to anaphylactic and endotoxic shock wherein cytotoxic plays a major role in intial peripheral vascular dilatation.

- Septic shock
    - Common type of shock associated with blood maldistribution.
    - Here components of bacteria or fungi (endotoxin, a lipopolysaccharide within the cell wall of gram negative bacteria) which are released from degenerating bacteria is potent stimulus and causes for septic shock.

Pathogenesis of shock

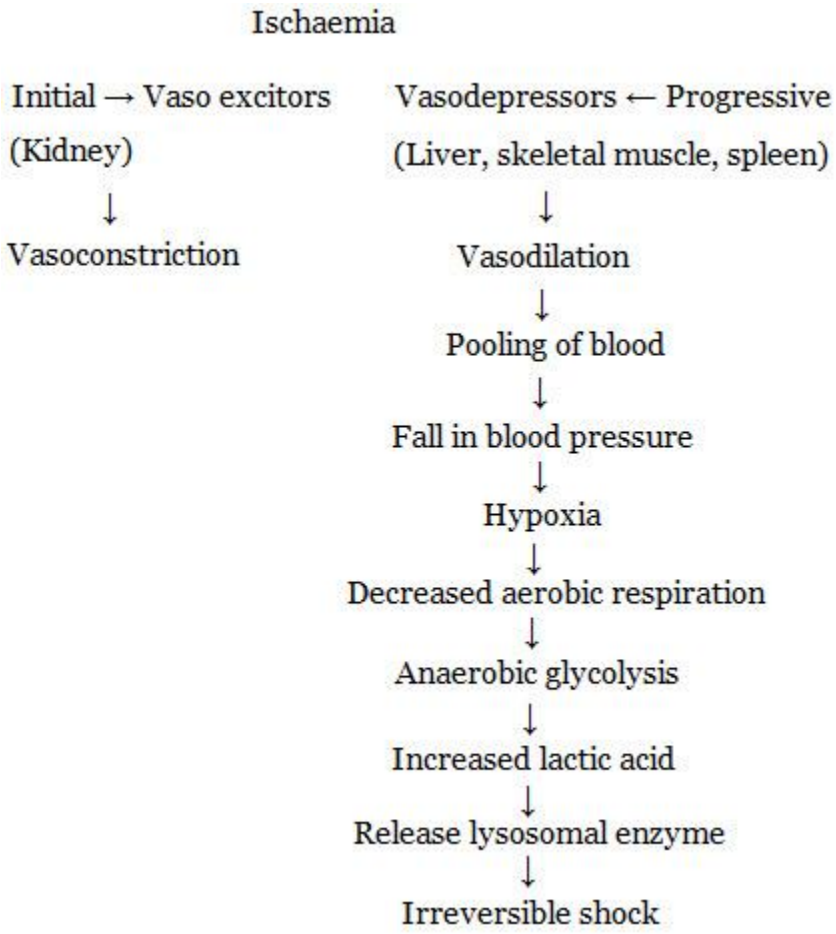
- Ischaemic shock

Haemorrhages / burns
↓
Reduce blood volume
↓
Reflex contraction of arterioles
↓
Ischaemia
↓
Degeneration / Necrosis

Septic shock

Bacterial toxins
↓
Absorbed from gut
↓
Increased vasomotor collapse (histamine, bradykinin, IL, PAF, Prostaglandin)
↓
Vasodilation
↓
Decreased blood volume
↓
Decreased cardiac output
↓
Decreased BP
↓
Hypoxia
↓
Death of cells

Vasoactive principles



Symptoms of shock

- Lethargy; recumbent; weak pulse rate
- Cold extremities
- Anxiousness
- Shallow breathing

Microscopical appearance

- Venules and capillaries engorged with blood
- Fat embolism in lungs – traumatic shock
- Fatty degeneration and necrosis in liver / heart
- Renal tubular necrosis – casts in tubules
- Adrenal cortex is foamy, due to depletion of cholesterol

Significance and results

- Recovery – on blood transfusion / supportive treatment
- Death – irreversible shock

- Renal insufficiency
    - Oliguria, anuria, uraemia
    - Pigment casts in tubules
    - Inflammatory oedema compresses renal parenchyma
    - Ischaemia – due to vascular collapse
    - Tubular degeneration and necrosis
- Cardiac failure
- Cerebral ischaemia - decreased BP → Anoxia → Neuronal degeneration

↓

Encephalomalacia

↓

Death

- Pulmonary infection – Pulmonary oedema → Bacterial growth

Morphology of shock

- Hypoxic cell injury
- Brain – Neurons – reversible cell injury
    - Irreversible cell injury (ischaemic encephalopathy)
- Heart – Subpericardial / Subendocardial haemorrhages and necrosis
- Kidneys - Acute tubular necrosis
- Lungs
    - Resistant to hypoxic cell injury
    - Not affected in hypo volumic shock
    - But changes seen in endotoxic or neurogenic shock
- GIT – patchy mucosal haemorrhages "Haemorrhagic enteropathy"
- Liver – Fatty changes / central necrosis

Macroscopical appearance

- Haemorrhages – Pale tissues
- Increased vascular permaeability – Oedematous tissues
- Passive congestion of liver, kidneys, lungs, intestines
- Petechiae on serous surfaces
- Fatty changes ← necrosis in liver, kidney, heart
- Pulmonary oedema and congestion
- Kidneys enlarged, pale cortex, red – blue pyramids
- Adrenal cortex – brilliant yellow – early stage reduced size / Pale – Later stage

MODULE-5: CELL SWELLINGS, GLYCOGENOSIS, FATTY CHANGES, HSP, LSD

Learning objective

- This module deals on cellular accumulations like fluid, glycogen and fat caused by different aetiological agents besides lysosomal storage disease. The role of heat shock proteins in cell injury (HSP) is also discussed

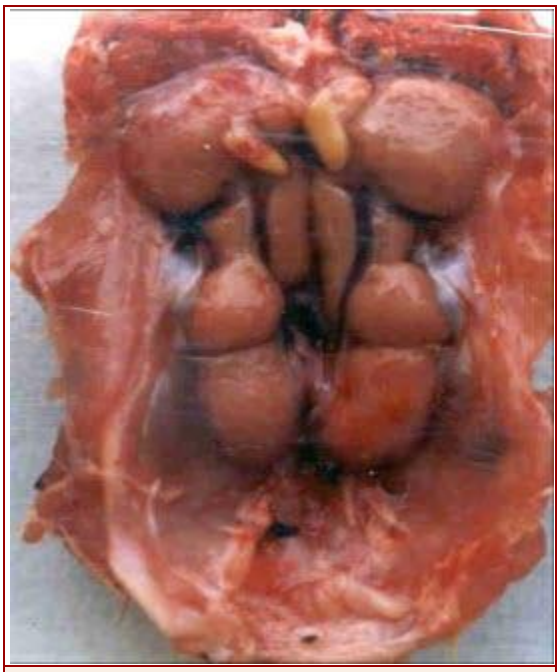
ACUTE CELL SWELLING

This occurs whenever the cells are incapable of maintaining ionic and fluid homeostasis. It is difficult to appreciate the change with light microscopy. It is the first change to all forms of injury to the cell. The organ is swollen.

Causes

- As discussed in the reversible cell injury

Grossly,organs appears pallor, increase in turgor, increase in weight when involves all cells in the organ.



Acute cell swelling - Swollen kidneys - Chicken

Microscopically, enlargement of cells is mostly observed in liver, convoluted tubules of the kidney,or in skeletal and cardiac muscle. Cytoplasm stains slightly more eosinophilic and more granular than normal. It is discernible by compression of microvaculature of organs. e.g. hepatic sinusoids and capillary network inrenal cortex.

Hydropic degeneration

A variant of cell swelling with excessive accumulation of fluid leading to even bursting of cells. It is caused by more severe irritant.

Causes

- Physical causes
  - Rubbing
  - Friction injury
  - Axe handling
  - Ill fitting shoes
- Thermal injuries
  - Fire accident
  - Hot substances – water and oil
  - Blisters are seen.
- Chemicals
  - Application of croton oil, rediodide of mercury
- Infectious agents
  - FMD in cattle – vesicles
  - Pox – blisters in stratified squamous epithelium
- Neoplasm
  - Cervical cancer

Grossly, blisters are seen onskin. Fluid escapes on incision and blister collapses consequent.

Microscopically, cells are swollen. Cytoplasm shows vacuoles which represent distended and sequestrated segments of endoplasmic reticulum. Cells may enlarge with coalition of fluid and may burst showing blisters and vesicles. Prickle cell layer is affected. Eosin stains pink depending on protein content. Using negative method of staining i.e. staining of fat or glycogen, vacuoles with water are identified.

Sequelae

- Healing occurs rapidly in uncomplicated cases without scar formation. Invasion of pyogenic bacteria like *Streptococci* and *Staphylococci* may cause abscesses or septicaemia.

Mucinous or mucous degeneration

Mucinous or mucous degeneration is the excessive accumulation of mucin in degenerating epithelium cell. Mucin is glassy, viscid, stringy, slimy glycoprotein normally produced by epithelium cell lining mucous membranes. Mucus is mucin mixed with water.

Causes

- It is caused by mild irritant.
- Mechanical or chemical injury (Disinfectant or soap).
- Thermal injury by heat or cold.

- Infectious diseases – Canine distemper, bovine viral diarrhoea

Grossly, mucous covering is seen as clear transparent material on mucous membrane which is stringy and slimy inconsistency. e.g. common cold. Mucosa is hyperaemic. In oestrus, large amount of mucous is normally produced which may be hanging from vulva of cattle.

Microscopically, cytoplasm shows small droplets of mucous which may coalesce forming large droplets displacing nucleus to side and compressing the nuclei. As the mucin accumulation continues the cell ruptures and desquamated. Haematoxylin stains the mucin blue. Mucicarmine and PAS stains the mucin red.

Sequelae

- On removal of the causative agent, epithelium lost is repaired by regeneration following stoppage of overproduction of mucin.

Mucoid or myxomatous degeneration

Mucoid is a glycoprotein similar to mucin in connective tissue found in foetus but not in adult tissue.

Causes

- Neoplasm of connective tissue e.g.Myxoma and myxosarcoma
- Thyroid deficiency in human –myxoedema
- Cachexia, starvation, parasitism or chronic disease

Grossly, adipose tissue shows the change. Affected tissue is shrunken, flabby, flaccid in consistency and has translucent jelly-like appearance.

Microscopically, degenerated tissue stains intensely blue with haematoxylin, nuclei are hyperchromatic and intercellular fluid takes slight bluish tinge.

Sequelae

- In cachexia, the fat becomes normal on correction of condition. Tumours indicate embryonal nature and it isunfavourable. Pseudomucin which resembles mucin degeneration is secreted by ovarian cystadenomas and parovarian cysts. Pseudomucin is not precipitated by acetic acid and stains pink with eosin whereas mucin is precipitated by aceticacid and stains blue with haematoxylin. Pseudomucin is not harmful and secretion of a normal cell.

Hyaline degeneration (Hyaline change)

(L.hyaline – glassy) It is the descriptive terminology of microscopical appearance. Affected tissue appears homogenous glassy and pink in H & E staining. It may be found in different conditions.

- Keratohyaline

- Cellular hyaline
- Connective tissue hyaline

Keratohyaline

- It is normally found in stratum corneum. Pathological amounts of keratohyaline
    - Mechanical injury - e.g. saddles andharness
    - Papillomas in dogs and cats
    - Chlorinated naphthalene poisoning in cattle causes hyperkeratosis
    - Hypovitaminosis A –keratinisation of epithelium of digestive and upper respiratory tract
- May be protective but the condition like corns may be very painful.
- Removal of the cause results in desquamation of excessive keratohyaline and epithelium becomes normal.

Cellular hyaline

- The dead cells are kneaded together forming homogenous mass resembling sand; since it stains with iodine it is called corpora amylacea (Starch-like). They are commonly seen in prostate.They are observed in lungs in pneumonia, pulmonary infarction, mammary glands of cows which are dried off quickly, in brain as brain sand, in islets of Langerhans in diabetes and in renal nephritis as the renal tubular epithelium gets desquamated and forms hyaline cast with albumin.

Connective tissue hyaline

- This is found in old scars, degenerating stroma of tumours, lymph nodes in chronic inflammation and arteriosclerosis. This is permanent change persisting for life.

Gout

- This is mainly observed in birds in which the end product of protein metabolism is uric acid (insoluble inwater) and is produced in liver. Mammals are ureotelic organisms i.e. urea is the end product of protein metabolism which is water soluble.
- The gout is defined as increase in the amount of deposition of uric acid and urates in tissue (viscera and joints).
- There are two types of gouts
    - Visceral gout
    - Articular gout

Causes

It is mainly due to

- Failure of urinary excretion of urates
    - Obstruction of ureters
    - Renal damage
    - Dehydration (common with water deprivation)
- Hypovitaminosis A
- Oosporin (mycotoxicosis)

- Sodium bicarbonate treatment

Hyperuricaemia is the result insodium bicarbonate toxicity. It is the sequel of alkalosis with protein breakdown. It is found only at necropsy.

Visceral gout

- The deposits of urates are found in kidney, serous surfaces of heart, mesentery, air sacs and peritoneum and in severe cases deposition are found in the synovial sheath, tendons, joints and muscular surfaces. It appears as white chalky coat.
- Microscopically, urate crystals found in clusters which are pale elongated and needle shaped. It is surrounded by inflammatory cells like neutrophils, lymphocytes and foreign body giant cells and fibroblasts. On H & E section, it appears as clefts.

Articular gout

- It is a sporadic problem.

Clinical signs

- Leg shifting, lameness and inability to bend the toes are observed. Tophi are characteristics of articular gout which are deposition of urates around joints particularly of the feet. The joints are enlarged with deformed feet. Deposits are seen as white semifluid substance.

GLYCOGEN STORAGE DISEASES (GLYCOGENOSES)

- The cells may accumulate abnormal amount of glycogen in the cytoplasm. The cells are swollen with foamy cytoplasm. The condition is not common in animals. It may be associated with prolonged hyperglycaemia and there may be lack of enzymes that metabolize carbohydrates. It is a group of disease in which two forms are recognized in animals.
- **Type II or Pompe's disease:** There is deficiency of lysosomal α-glucosidase with accumulation of glycogen in the lysosome of brain, muscle and liver. This condition is seen in cattle, dogs, cats and sheep.
- **Type III or Cori-Forbes' disease:** There is deficiency of amylo-1,6-glucosidase which converts glycogen to glucose. Hence, glycogen is stored in the cytoplasm of liver, heart, skeletal and smooth muscle and nerve cells. This condition is reported in dogs and cats.
- **Type Ia (von Gierke disease in humans):** There is deficiency of glucose-6-phosphatase that catalyses hydrolysis of glucose-6-phosphate to glucose and phosphate. Affected pups show tremors, weakness and neurological signs due to hypoglycaemia and growth retardation and progressive hepatomegaly is found as they develop. Massively enlarged hepatocytes show vacuolations with aggregation of glycogen rosettes.

GLYCOGEN OVERLOAD

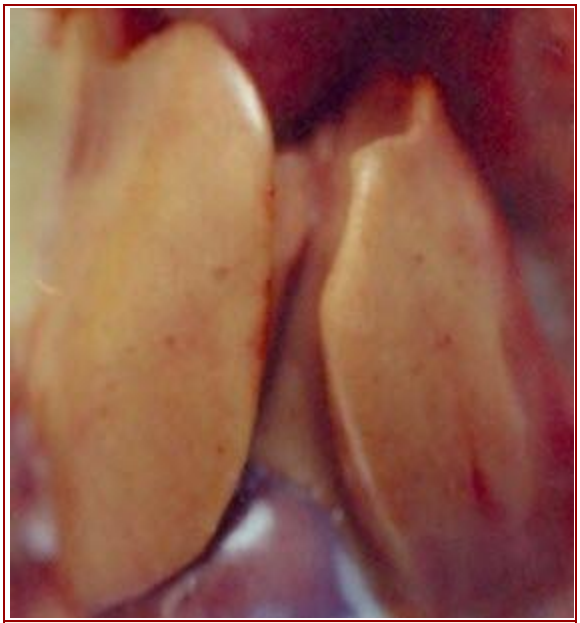
- It is excessive intracellular accumulation of glycogen with derangement in glucose or glycogen metabolism. The condition is not a significant entity in animals as compared to humans. Glycogen overload may be encountered in:

    - Neutrophils in inflammation
    - Fast growing neoplastic cells
    - Necrotic areas
    - Diabetes mellitus
    - Liver of young and growing animals
    - Well-fed animals
- **Grossly,** changes are not usually detected, but pale enlarged liver is observed in steroid induced hepatopathy.
- **Microscopically,** clear cytoplasmic vacuoles in the hepatocyte represent the glycogen. To demonstrate glycogen, sample should be collected immediately after death and tissue must be fixed in non-aqueous solution like alcohol to avoid loss of glycogen since the glycogen is water soluble and glycogen is converted to glucose after death. Glycogen stains red with PAS and Best's carmine staining.

MECHANISM OF HEPATIC LIPIDOSIS

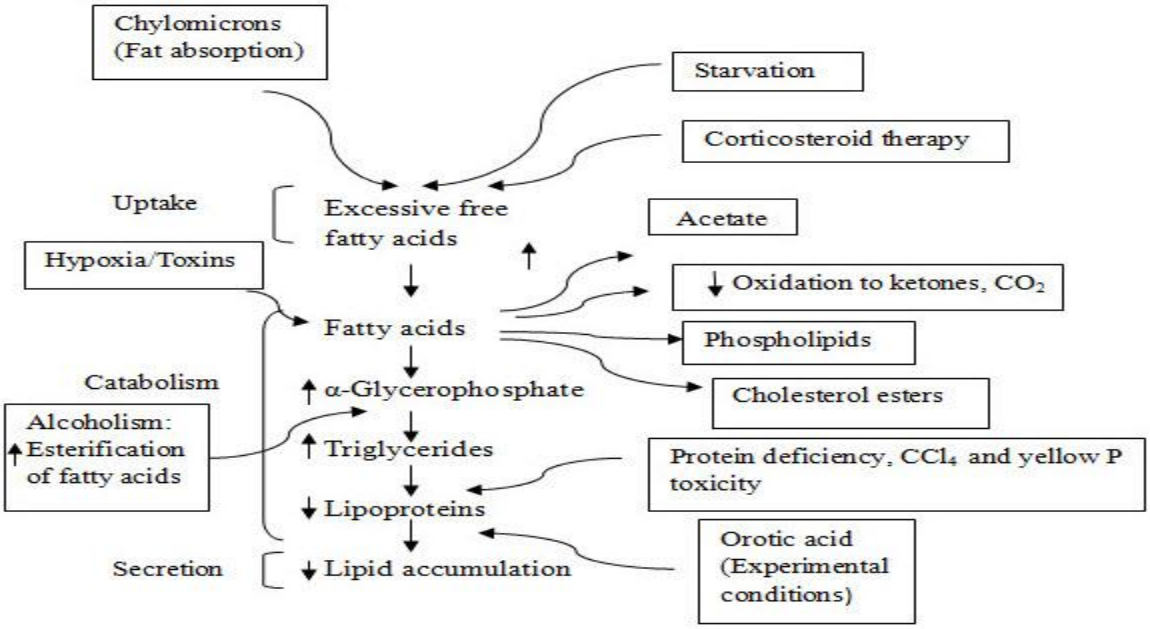
It is the accumulation of triglycerides or true fats and cholesterol in the cytoplasm of parenchymatous cells. Lipidosis is more common than other conditions.

- Mobilization of free fatty acids from the gut (Chylomicrons) or adipose tissue
- Mitochondrial injury leading to decreases in β-oxidation of fatty acids to ketones etc. (Hypoxia, toxins)
- Decreased apolipoprotein synthesis e.g. CCl_4 poisoning and aflatoxicosis
- Failure to form lipoproteins
- Failure to release lipoproteins from hepatocytes
    - The last two conditions are uncommon.
- Hepatic lipidosis can occur from one or more mechanisms. Fatty acid mobilisation from adipose tissue is common in animals following higher energy demand. Starvation increases triglyceride mobilisation. Protein malnutrition affects apolipoprotein synthesis. Chemicals like CCl_4 and yellow phosphorous can also induce hepatic steatosis.
- **Grossly** , enlarged, pale to yellow, soft and friable liver is found in moderate to higher grade fatty changes. Enlarged with rounded borders. Upon incision, fat droplets are seen on the blades of knife. Tissue may float in the fixatives.



Fatty liver - Chicken - Yellow

- **Microscopically,** hepatocytes show vacuolations which may be small, clear to variable sized and may also form a single large vacuole, pushing the nucleus to a side. During the processing of fat tissue with xylol clearing, the fat will be dissolved by the xylol and gives vacuolated appearance in the Haematoxylin and eosin stained sections. To differentiate from hepatic degeneration, fluid and glycogen accumulation, cryostat sections are used to stain fat. Special stans for fats are sudan III, sudan black, scarlech red and Oil Red O. Oil Red O stains fat red and while it is PAS negative, sudan III and sudan black imparts black colour and scarlech red imparts red colour
-



HEAT SHOCK PROTEINS

- Heat shock proteins (HSPs) are intracellular chaperones (Fr. an older woman who looks after a girl, today's context to look after proteins within the cell). There are about ten families. e.g. Hsp 60, Hsp 90 and Hsp 70.
- Hsps are not commonly present in blood and body fluids. Hence, their presence indicate physical damage.
- These are involved in protein folding, degradation of protein (Hsp 70) assembly of protein, thermotolerance, buffering and expression of mutations.
- Stress causes protein aggregation and degradation. e.g. heat, UV radiation, etc
- They also play a role as intracellular chaperones of antigenic peptides. Antigen presenting cells (APC) express receptors that ligate Hsp bound antigen peptides. Hsp alone or peptide alone is non-immunogenic. Combination elicited MHC class I restricted antigen specific CD8 cytotoxic T cell responses (immunity to cancers).

LYSOSOMAL STORAGE DISEASE

These are genetically determined diseases with reduced lysosomal enzyme synthesis and now understood that it may also be caused by other contributory factors like lack of enzyme and substrate activators. The lysosomal dysfunction leads to accumulation of normally degraded substrates leading to death of cells. The disease kills the developing foetus or may be manifested in neonatal or early life.

- Examples
  - Lipid storage disease
  - Mucopolysaccharidoses
  - Mucolipidoses
  - Glycogen storage diseases

Lipid storage disease

- Lysosomes which are lacking enzymes do not degrade fat substrate in te cytosol leading to accumulation of lipid materials in the brain, liver or other organs. In the central nervous system: a) deficiency of β-galactosidase in the lysosome results in GM_1gangliosidosis b) deficiency of β-hexosinamidase results in GM_2gangliosidosis.
- **Grossly,** atrophy of brain, and rubbery consistency of brain are seen. Globoid mucodystrophy may occur in later stages with loss of myelin.
- **Microscopically,** the neurons are enlarged; cytoplasm is foamy, finely granular and vacuolated with displacement of nuclei. In GM_1gangliosidosis, whorles and laminar arrangements of membranes are seen in the CNS and other visceral organs.

Mucopolysaccharidoses

- The defectively degraded glycosaminoglycans (GAG) are stored in the cells that normally degrade them. These are genetically determined group of diseases. There is lack of specific hydrolases in lysosomes which results in massive accumulations of GAG polymers and formation of giant lysosomes.
- **Mucopolysaccharidosis type I**, is caused by the absence of a-L-iduronidase in Siamese cats and Plott

Mucolipidoses

- There is genetic deficiency of ganglioside sialidase enzyme and consequent accumulations of glycolipids and GAGs in the form of granulofibrillar vacuoles in hounds. The affected animals show deformities of face and bones. Corneas are clouded. Abnormally large granules are seen in the leukocytes. Excess GAGs in urine of weaning animals confirms this disease

Naemann-Pick Type-C disease

- There is accumulation of sphingomyelin in lysosomes deficient in sphingomyelinase in dogs and cats. The cytoplasm of nerves, hepatocytes and mononuclear cells and phagocytes show vacuolations.

MODULE-6: CELL INJURY AND NECROSIS

Learning objective

- In this module, the viewer will learn about reversible and irreversible cell injuries with their pathogenetic mechanisms in hypoxic, chemical, free radical and virus induced injury models. Local death of cells (Necrosis) and programmed cell death (Apoptosis) will also be taught.

REVERSIBLE AND IRREVERSIBLE CELL INJURY - CAUSES AND MECHANISM

External causes

- Physical causes
    - Trauma by cutting objects and blunt objects
    - Electrical – Lightning, high frequency current
    - Heat - Sun stroke, burns, fever
    - Cold - Local tissue freezing, cold shock
    - Radiation - UV / X /cosmic radiations
    - Pressure - Increased or decreased pressure
- Chemicals
    - Nutritional - Excess / deficiency
        - Excess - HypervitaminosesA and D
        - Deficiency –protein, calorie, vitamins and minerals
    - Agrochemicals– nitrates
    - Environmental deficiency
        - Water –dehydration
        - Oxygen –asphyxia
        - Sunlight – forvitamin D formation (Hypovitaminosis D)
    - Biological toxins

        - Bacterial and fungal toxins
        - Arthropod and snake venom
        - Pesticides –organochlorine compounds
- Biological causes
    - Acellular – Viruses, prions
    - Prokaryotes – Bacteria, chlamydia, rickettsia,mycoplasma
    - Eukaryotes – Protozoa, fungi
    - Metazoan parasites – Trematodes, cestodes, nematodesand insects

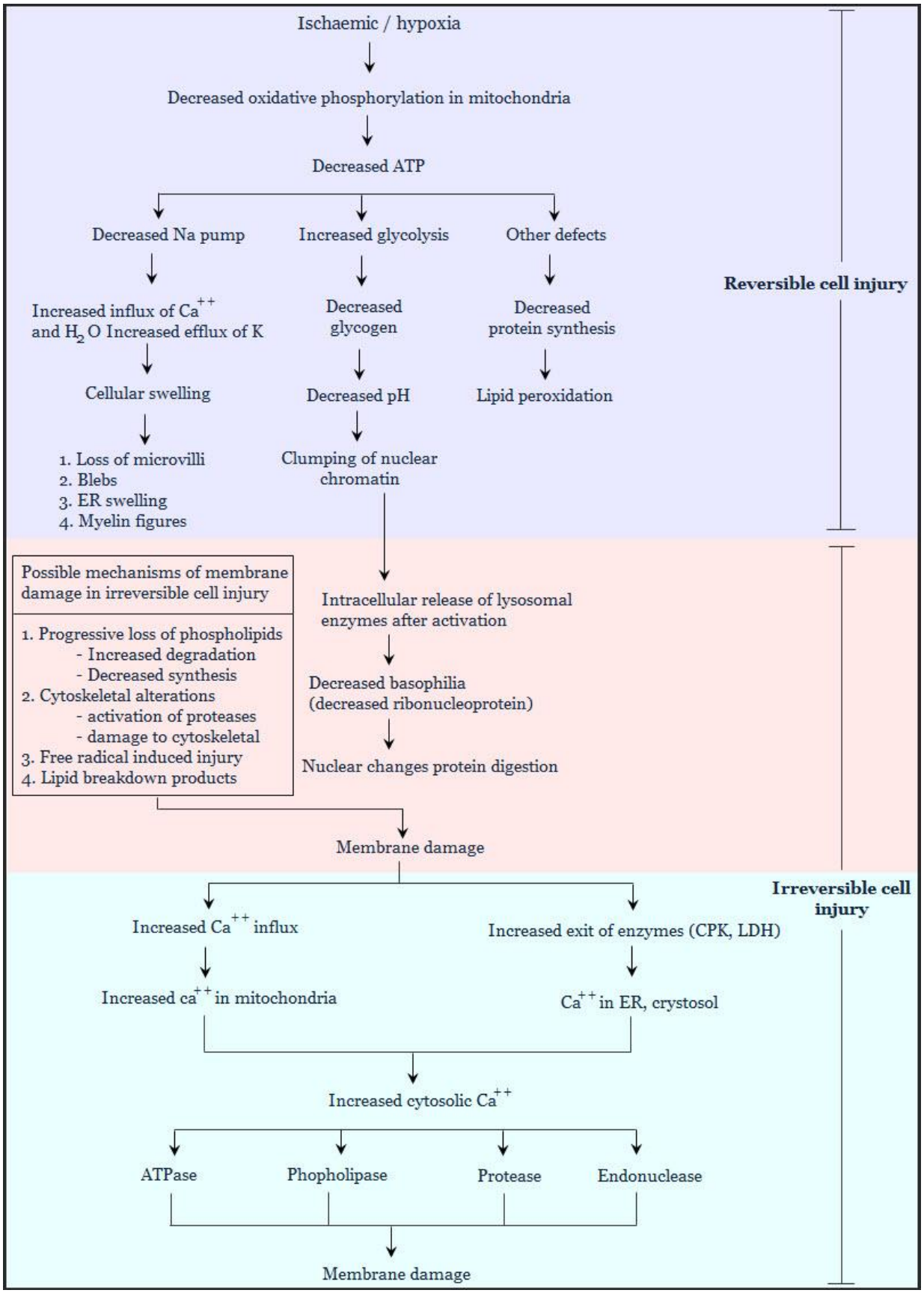
Internal causes

Genetic causes– mutation of genes to chromosomal defects

The common causes of cell injury

- Hypoxic injury
- Free radical injury
- Chemical injury
- Virus induced injury

Events in ischaemic cell injury


Ischaemic / hypoxia
Decreased oxidative phosphorylation in mitochondria
Decreased ATP
Decreased Na pump
Increased glycolysis
Other defects
Increased influx of Ca++ and H2O Increased efflux of K
Decreased glycogen
Decreased protein synthesis
Cellular swelling
Decreased pH
Lipid peroxidation
1. Loss of microvilli
2. Blebs
3. ER swelling
4. Myelin figures
Clumping of nuclear chromatin
Reversible cell injury
Possible mechanisms of membrane damage in irreversible cell injury
1. Progressive loss of phospholipids
- Increased degradation
- Decreased synthesis
2. Cytoskeletal alterations
- activation of proteases
- damage to cytoskeletal
3. Free radical induced injury
4. Lipid breakdown products
Intracellular release of lysosomal enzymes after activation
Decreased basophilia (decreased ribonucleoprotein)
Nuclear changes protein digestion
Membrane damage
Irreversible cell injury
Increased Ca++ influx
Increased exit of enzymes (CPK, LDH)
Increased ca++ in mitochondria
Ca++ in ER, crystosol
Increased cytosolic Ca++
ATPase
Phopholipase
Protease
Endonuclease
Membrane damage

Reversible cell injury is non-lethal and previously referred as degeneration. Irreversible injury causes necrosis or death of the cells. The two patterns found inreversible injury are

- Cellular swelling
- Fatty change

Free radical injury

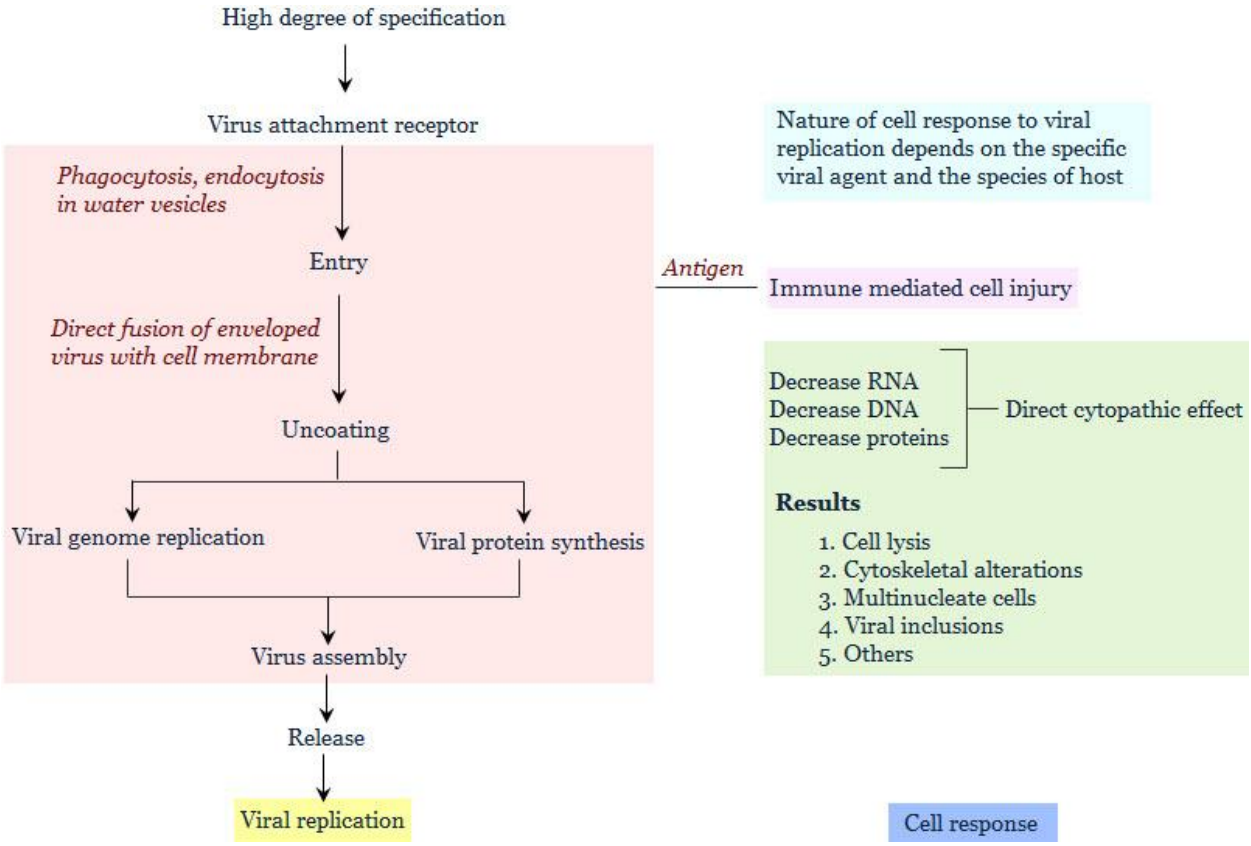
- Free radicalsare chemical species that have single unpaired electron in the outer orbit.Free radicals are extremely reactive and unstable and enter into reaction with inorganicand organic substances, proteins, lipids or carbohydrates, particularly free radicals react with membrane and nucleic acid. They initiate autocatalytic reactions. Free radicals may be initiated in cells with radiant energy (UV /Xrays), generation of endogenous oxidative reaction or enzymatic metabolism,exogenous chemicals or drugs e.g. Chloroform, carbon tetrachloride
- The oxygen-derived radicals are superoxide, hydrogen peroxide and hydroxide. Thesecause lipid peroxidation, protein damage and DNA damage. The antioxidants(endogenous or exogenous) are helpful in scavenging the free radicals e.g.Vitamin E, sulphur containing amino acids (cystine, methionine), glutathioneand ceruloplasmin.

Chemical injury

- Chemicals can induce cell injury directly by reacting with critical cellular molecules e.g. mercuricchloride poisoning. Mercury binds with sulphydryl group and other proteins andcause increased cell membrane permeability and inhibition of ATPase dependant transport or indirectly by converting chemicals which are not biologicallyactive into reactive toxic metabolite that attack target cells. Mostly reactive free radicals formed can induce membrane damage and can cause direct injury by covalent binding to membrane lipid and protein. e.g. Carbon tetrachloride (CCl_4)poisoning. Carbon tetrachloride is converted to CCl_3 in hepatocyteswhich acts on membrane and generate lipid peroxides. The autocatalytic reactionresults in membrane damage involving rough endoplasmic reticulum, detachment ofribosomes, reduced protein synthesis and fatty liver due to lack of lipidacceptor protein. Lipid peroxidation products can also damage plasma membraneto increase permeability to sodium and water resulting in cell swelling.

Virus induced cell injury

This may be through immune mediated reaction and direct cytopathic effect.



Viruses that induce cellular changes are of two types

- Cytolytic / cytopathic viruses which cause variousdegree of cell injury and cell death.
- Oncogenic viruses which stimulates host cellreplication may produce tumours.

NECROSIS

Necrosis (Gr. Nekrosis-Deadness) is defined as death of cells in a living vascularised tissue or local death of cells in a living animal.

Grossly, necrotic tissue is pale, grayish white, dull and depressed surrounded by hyperaemic zone.

Microscopically, nuclear changes are characteristic. These are

- Pyknosis: Shrinkage or condensation of nucleus which takes up deep blue colour. The chromatin condenses to a structureless mass
- Karyorrhexis (Gr. Karyo-Nucleus; rhexis-Fragmentation): Fragmentation of nucleus.
- Karyolysis (Gr. Karyo-Nucleus; Lysis-Dissolution): Dissolution or disappearance of nucleus.
- Cytoplasm is swollen, homogeneous and stained intensely pink due to decreased basophilia with loss of ribosomes.

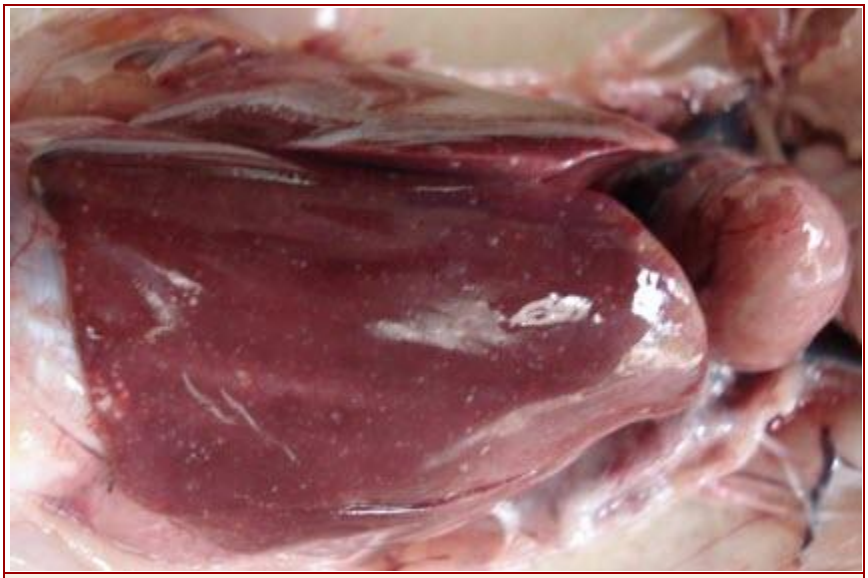
Depending on the gross and microscopic features necrosis is divided into following four types.

- Coagulative necrosis
- Caseation necrosis
- Liquefactive/suppurative necrosis
- Fat necrosis

Coagulative necrosis

- "While architectural details of tissue are retained, the structural details are lost due to necrosis"
- This type of necrosis occurs due to ischaemia, whit muscle disease in vitamin E and selenium deficiency, necrosis of liver in *Fusobacterium sphaerophorus* infection, mercuric toxicity in renal tubular epithelial cells and cutaneous or mucosal epithelium in contact poison with phenol.

Grossly, the necrotic tissue is dry, white or grayish white and homogeneous and slightly depressed from the surrounding healthy tissue.



Coagulative necrosis

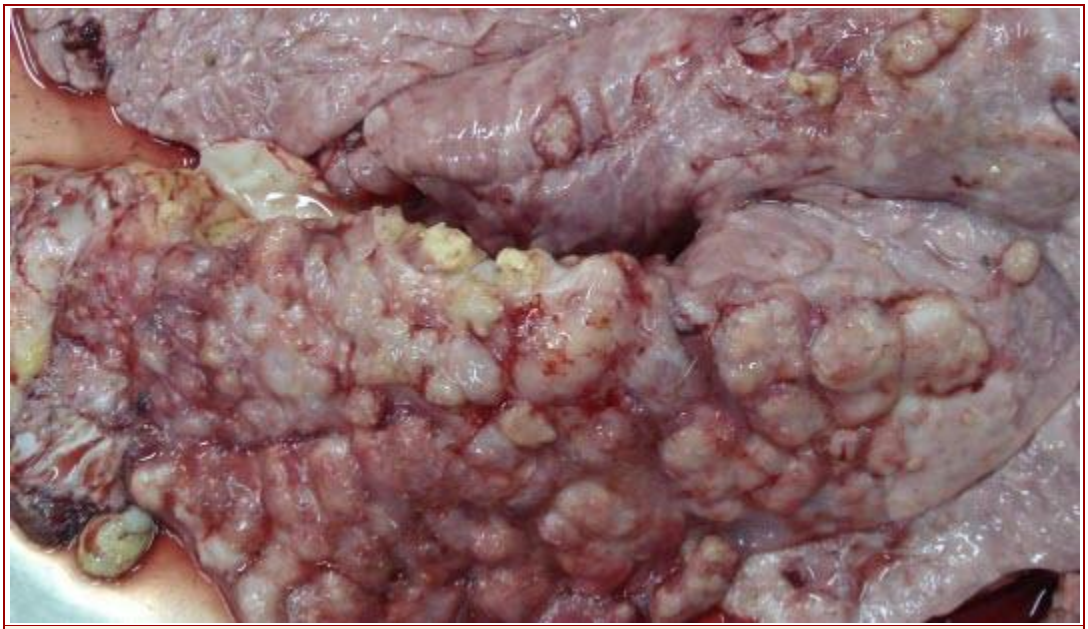
Microscopically, the architectural details of the area is maintained and cellular details are lost. This is due to blockage of proteolysis with denaturation of proteins including enzymatic proteins of the cell. The cellular shape is preserved and nuclear details are lost (Nuclei show pyknosis, karyorrhexis and karyolysis or absence). The cytoplasm appears homogeneous and eosinophilic due to coagulation of protein. It takes long time for the removal of dead materials because the autolytic enzymes are destroyed and no leukocytic responses. This type of necrosis is characteristically found in parenchymatous organs like kidney, liver and muscle except the brain.

Caseous necrosis (L. Caseous - Cheese)

- The architectural and cellular details are lost. This is more chronic type of lesion often associated with poorly degraded lipid materials of bacterial origin. This type of necrosis

occurs in *Mycobacterium tuberculosis* infection, oesophagostomosis and caseous lymphadenitis in sheep and tularaemia in primates.

Grossly, the necrotic tissue is converted into a homogeneous, soft, friable, grayish white cheesy granular mass. The dead tissue attracts calcium deposits and is enclosed within a connective tissue capsule.

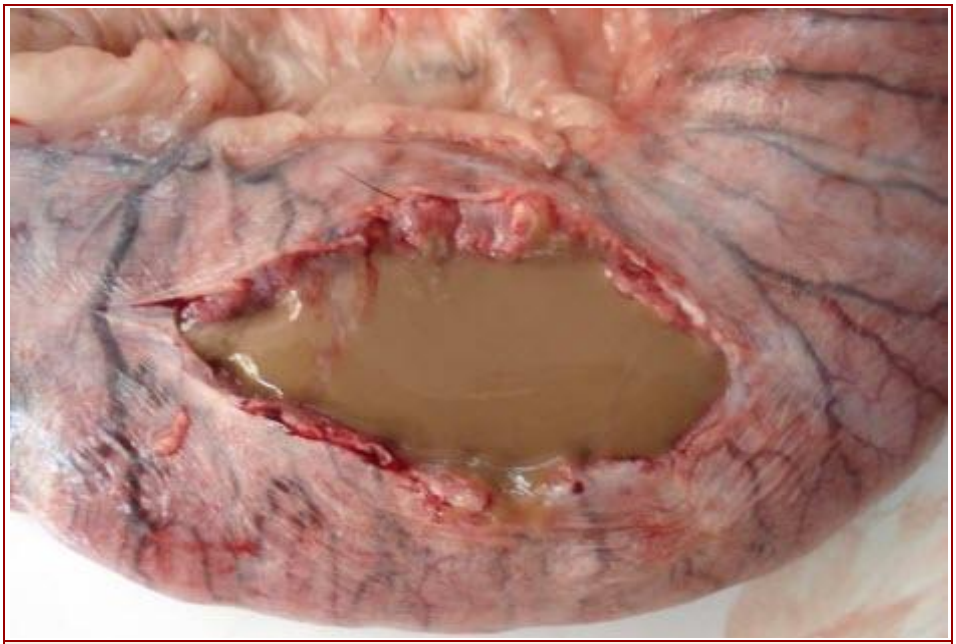


Caseous necrosis - Tuberclosis - Lung

Microscopically, structure less, amorphous necrotic area surrounded by epithelioid cells, giant cells, lymphocytes and plasma cells with central area of dystrophic calcification

Liquefactive necrosis

Necrotic tissue is liquid in consistency. It is especially seen in the central nervous system (malacia) and any infection with pyogenic bacteria leading to pus formation (Abscess). The former is due to severe hypoxic or toxic injury with focal dissolution of the neuropil. The later is due to autolysis or heterolysis from enzymes of neutrophils leading to collection of pus containing necrotic tissue, microorganisms and dead neutrophils (Suppuration). The pus becomes caseous and insipid if stands for longer time.



Liquefactive necrosis - Pyometra

Microscopically, the pus or the purulent area shows dark, contracted and agranular neutrophils with varying amounts of tissue debris, fibrin and plasma proteins. An abscess is a localised collection of pus (Liqefactive necrosis) caused by suppuration, deep in tissues. The process is designed to contain the pathogenic organisms and sequestering necrotic tissue from spreading in the animal. The pyogenic organisms cause localized necrosis and attract neutrophils to the necrotic areas. This is a part of inflammatory response.

Fat necrosis

It is death of adipose tissue in a living animal. There are different types of fat necrosis

Enzymatic fat necrosis

It is commonly found in steatitis (Inflammation of fat) and other inflammatory lesions affecting adipose tissue, e.g. Pancreatic fat

- **Pathogenesis -** In acute pancreatic necrosis and pancreatitis, the lipase released from acinar cells gets activated and saponification occurs by digestion of triglycerides into glycerol and fatty acids. Glycerol being water soluble, is absorbed. The released fatty acids when combine with calcium results in the presence of chalky white flakes.
- **Grossly**, hard, white, opaque masses resembling that of soap flakes are seen. The fat loses yellow translucent nature.
- **Microscopically**, necrotic adipocytes may show eosinophilic shadow outlines, become basophilic due to dystrophic calcification and surrounded by inflammatory reactions along the area due to acute to chronic injury. Fat solvents do not remove necrotic fat.

Traumatic fat necrosis

It results from mechanical injury to adipose tissue.

- **Causes** : working, biting, parturition (perivaginal fat in cattle, subcutaneous and intramuscular fat in recumbent cattle)
- **Grossly**, firm, opaque, chalky masses found in the area with acute to chronic inflammatory reaction.
- **Example:** Surgical injury to subcutaneous fat, injury to vagina during dystocia and abdominal fat necrosis in cattle

Mesenteric, omental and retroperitoneal fat show necrosis containing large masses. Stenosis of intestine may occur in extreme cases.

Nutritional fat necrosis

This is the result of necrotic alteration in fat associated with extreme emaciation. **e.g.** Tuberculosis and Johne's disease in cattle and sheep.

- **Grossly**, necrotic fat is opaque, foamy and chalky white and may be calcified.
- **Microscopically**, necrotic adipocytes are pale pink (eosinophilic) and show numerous clubs (fatty acids) and crystals. The derivatives of fat, glycerol dissolves in body fluids, and fatty acid crystals dissolve in fat solvents leaving clefts. Calcified area is basophilic, surrounded by chronic inflammatory cells.
- **Differential diagnosis:** Inflammatory reaction and calcification are lacking in autolytic fat.

APOPTOSIS

Programmed cell death in which there is death of individual cells without inciting inflmmatory processes. In embryogenesis and normal growth, physiologic cell death occurs which may be referred as programmed cell death or apoptosis. Apoptosis can also occur in pathologic diseases.

Mechanism of Apoptosis

There are two processes:

- Initiation phase mediated by caspases
- Execution phase in which enzymatic degradation leads to cell death

Initiation phase

There are two pathways of initiation of apoptosis:

- Extrinsic receptor initiated pathway
- Intrinsic mitochondrial pathway

These two pathways are interconnected and converge to activate caspases

- **Extrinsic pathway:** On cross linkage of Fas (Death domain) by its ligand three or more molecules come together and bind to cytoplasmic Fas-associated death domain (FADD) which in turn binds to inactive forms of caspase-8 via death domain. These activated caspases trigger a cascade of caspase activation and mediate execution phase of

apoptosis. FLIP protein inhibits apoptosis by binding to procaspase-8. This mechanism is used to protect infected normal cells from Fas mediated apoptosis.

- **Intrinsic mitochondrial pathway:** There are more than 20 antiapoptotic proteins. Of which Bcl-2 and Bcl-x are located on the mitochondrial membrane of cytoplasm. Bcl-2 and Bcl-x are replaced when cells are deprived of survival signals or stress by proapoptotic members like Bak, Bax and Bim. This leads to increased mitochondrial membrane permeability and release of several proteins which activate caspase cascade e.g. cytochrome C from mitochondria which binds to Apaf-1(Apoptosis activating factor-1protein). The complex activates caspase-9. Apoptosis activating factor from mitochondria also neutralizes various apoptotic inhibitors which block caspase activation.

Execution phase

- The final proteolytic cascade is mediated by the proteases (Caspase: 'c'- cystine protease that cleaves aspartic acid residues). There are more than 10 members in caspase family which are grouped into initiator and executioner groups depending on their order in which they are activated during apoptosis e.g. caspase-8 and 9 are initiator caspases and caspase-3 and 6 are executioner caspases.
- These caspases are hydrolysed autocatalytically following cleavage of initiator caspase to generate the active form. The enzymatic death programme sets in motion by rapid and sequential activation of other caspases. These caspases can act on many cellular components like cytoskeleton and nuclear matrix proteins. Cytoskeleton destruction and nuclear break down occurs. Caspase target proteins of transcription, DNA replication and DNA repair in the nucleus e.g. caspase-3 activates cytoplasmic DNAs.
- Not only gross changes, but microscopical changes are also not obvious since single cell death occurs.

Histopathologically,

- Shrinkage of individual cells: cells-size smaller, cytoplasm is dense and organelles are tightly packed.
- Condensation of chromatins: Most characteristic in apoptosis. Aggregation of chromatins under nuclear membrane with variable shape and size (Semilunar shape)
- Cytoplasmic fragmentation
- Cytoplasmic buds containing fragments of nucleus: Cytoplasm shows excessive surface budding and formation of membrane bound fragments (Apoptotic bodies) containing cytoplasm and tightly packed organelles with or without nuclear fragments. Nucleus itself may break up into two or more fragments
- Presence of apoptotic bodies in the adjacent cells and phagocytes
- Inflammation is absent.

MODULE-7: PM CHANGES AND GANGRENE

Learning objective

- What happens to the animal after death (Post-mortem changes-Autolysis) is answered in this module and how to differentiate the PM changes from ante-mortem (inflammation) changes will also be discussed. The viewer will also learn about the events in invasion of saprophytic organisms in dead tissue (Gangrene)

POST MORTEM AUTOLYSIS AND NECROSIS

S.No.	Post mortem autolysis	Necrosis
1.	Absence of inflammatory reaction	Presence of inflammatory reaction
2.	Autolytic changes are seen uniform throughout the tissue	Diffuse or focal adjacent living and dead tissues are seen.

POST - MORTEM CHANGES

Somatic death

- Somatic death is the death of the body as a whole.
- When respiration and cardiac action have stopped, the animal is said to have undergone somatic death. After death, the cells undergo certain changes (post mortem changes), which a pathologist must have knowledge of to distinguish them from lesions found in disease. By a careful study of a postmortem changes one can determine the probable time of death and this is of great importance in medicolegal cases.
- Factors influencing the rate of postmortem autolysis
- Species of animal: Pig-soft and moist muscle- rapid in onset, Horse-dry and firm muscle-slow in onset
- Organ involved: the degrees of the expression of postmortem changes vary from tissues to tissues. The presence of bacterial flora, enzyme secretions and the availability of moisture and substrates influence the rate of postmortem autolysis. Pancreas-high amount-rapid changes. Fibrous tissue-less amount-slow changes. Retina-most sensitive, separates from choroids. Adrenals, liver, testis-abdominal organs also show autolytic changes.

Putrefaction

Decomposition of tissues brought about by the protein splitting anaerobic saprophytic organisms, results in the formation of gas and variety of foul smelling substances- ammonia, hydrogen sulphide, indol, skatol and putrescent amines-like “putriscience and cadaverine”. The tissue turns black or dark-green as a result of formation of iron sulphide from break down haemoglobin. The common putrefactive organisms are *Clostridium spp*. normally present in faeces, leads to pronounced postmortem changes in the body like gaseous distension, softening etc. Bacterial flora present in GIT and respiratory tract bring about the post-mortem changes rapidly under favourable conditions.

Sequence of postmortem changes

1. Algor mortis
2. Rigor mortis
3. Livor mortis- hypostatic congestion
4. PM clotting of blood
5. Imbibition of hemoglobin
6. Imbibition of bile
7. PM desquamation
8. PM softening

9. PM discoloration
10. PM distention
11. PM displacement
12. PM rupture of organ and tissue

1. Algor mortis

- Algor mortis is cooling of the body. It commences at or before the stoppage of blood flow. The rate of cooling depends on the following factors:
- External atmospheric temperature
- Air currents
- The thickness of hair coat or wool
- Adiposity of the animal
- Amount of fermentable ingesta in the digestive tract
- Larger animals cool slowly; so also in sheep, with thick wool cooling occurs slowly. Limbs and other extremities cool more rapidly than the trunk. The rate at which post mortem changes takes place depends on the rate of cooling and other factors detailed below:

A. Surrounding atmospheric temperature

- Since the postmortem changes are brought about by enzymatic and bacterial activity, high temperature that accelerates this activity will naturally bring on the post mortem changes soon. So in summer, the carcass putrefies quickly. Cold on the other hand retards the enzymatic and bacterial activity. Freezing and deep freezing may stop the activity completely. Hence, carcasses are in perfect state of preservation under polar ice-caps for considerable length of time.

B. State of the body at the time of death

- Higher the temperature at death, sooner do postmortem changes commence.

C. State of muscular activity of animal prior to death

- In animals, that have been very active prior to death post mortem changes commence quicker. This is found in animals that die in chase. Similarly, animals that are killed or die of strychnine poisoning and in animals that die of tetanus, postmortem changes appear early.

The reasons are

- higher body temperature
- greater production of lactic acid in muscular contractions and exercise
- Size of animal

Since body cools slower and so heat is retained longer in larger animals, postmortem changes appear quicker in them

E. External coverings

- Since thick hair or wool retard heat, dissipation, postmortem changes are seen sooner in thick haired or coated animals
- Fatness of animals
- Fat is a poor conductor of heat and so heat loss in fat carcasses is slow, with resultant speedier onset of postmortem changes.
- Infection of animals
- Widespread bacterial infection, especially septiceamic in character, at the time of death begins on postmortem changes earlier.

The following are the changes noticed after death:

2. Rigor mortis

Rigor mortis is contraction of muscles after death.

This is a contraction of muscles after death so that the joints become stiff and body is rigid. Rigor mortis develops first in those muscles that are very active. e.g heart, palpebral muscles, muscles of the head and neck. Gradually other muscles of the forelimbs, the trunk and the hind limbs, are affected in that order. It passes of also in this order, starting first in the head. Usually, rigor mortis appears in 1 to 8 hrs after death and may disappear from 20-30 hours. The following factors hasten the onset of rigor mortis.

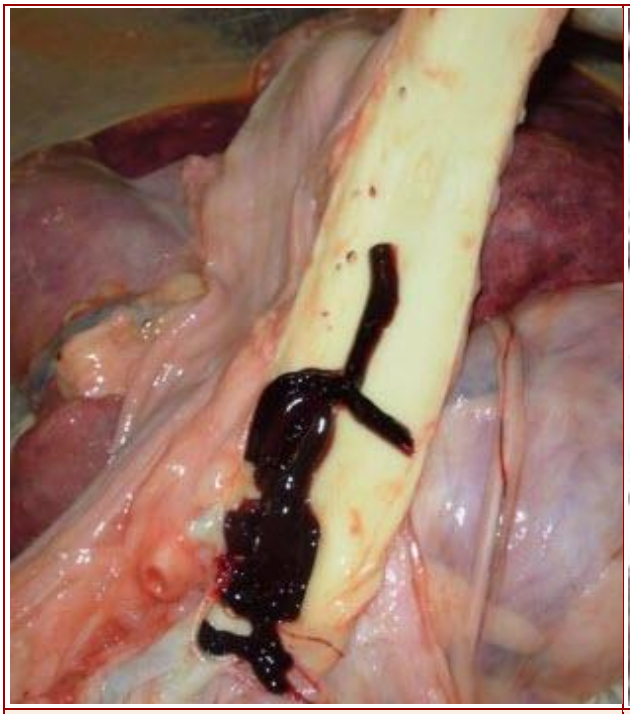
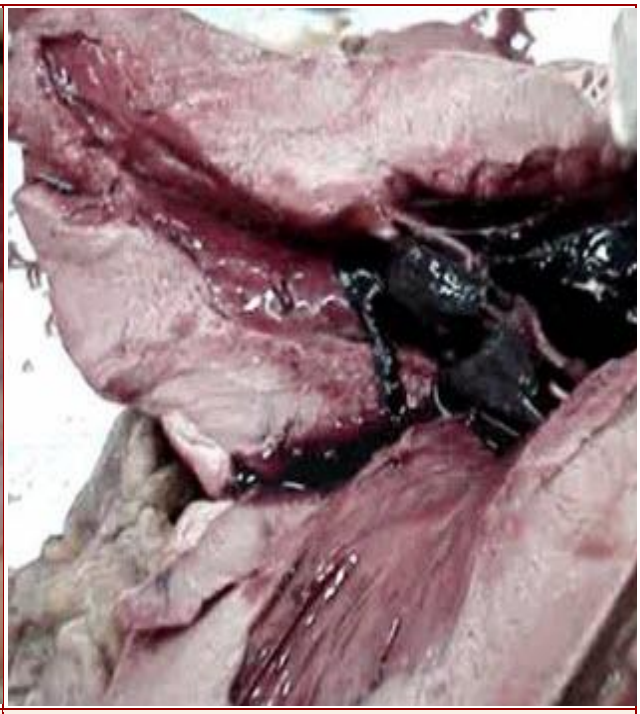
- High atmospheric temperature
- Active exercise- hunting, fighting, racing or struggling
- Strychnine poisoning
- FTetanus

Causes of rigor mortis

- The exact mechanism is not known. After death, there is a great overturn of high energy phosphate bonds in the muscle. Adenosine triphosphate (ATP) which breaks down is resynthesized by the energy derived from glycosis. So long as ATP is present, rigors do not occur. With the exhaustion of glycogen, all of ATP is degraded and rigor occurs, since in the absence of ATP relaxation of muscles cannot occur. For the relaxation of the muscles to occur, a considerable quantity of ATP must be absorbed to the muscle proteins. Hence onset of rigor is delayed in well fed animals with large quantities of stored muscle glycogen. But in starved animals, rigor naturally commences earlier. Subsequently when there is no longer any energy necessary for keeping up the chemical activity in the muscle fibres, rigor passes off.
- Onset of rigor mortis is slow in cold weather and in emaciated and cachectic animals. In the later, it is due to the complete exhaustion of chemical systems producing energy.

3. Livor mortis: Hypostatic congestion is, due to gravity, accumulation of blood in vessels of organs that are found on the lower side of the recumbent animal.

4. PM clot is the coagulation of blood in the vessels after death. Chicken fat is the white clot while current jelly clot is the red clot seen in the clot. PM clot is formed after death of animal.

	
PM clot	Cow - Heart - PM clot

5. Imbibition of hemoglobin: PM staining is pinkish discolouration of endothelium of larger vessels due to haemoglobin (liberated from lysed erythrocytes) after death.

6. PM imbibition of bile is the yellow pigmentation of the tissue occurring in the vicinity of gall bladder.

7. PM softening is softening of tissues, after death, by the action of autolytic enzymes of the cells and the proteolytic ferments of the saprophytes and infecting bacteria.

8. PM discoloration: Pseudomelanosis coli is staining (blackish / greenish discolouration) of intestines due to formation of iron sulphide (H_2s + Fe from Hb = Iron sulphide) after death of animals.

9. PM bloat / PM emphysema is accumulation of gas in the rumen and intestines due to fermentation of food after death.

10. PM displacement of organs: This may occur following handling of carcass by rolling etc.

11. PM rupture of organ and tissue: This may be attributed to softening and handling but devoid of any inflammatory reaction.

In equine practice, stud fee is payable only on the birth of a live foal. So, the veterinarian may be required to certify as to whether a foal was born alive or dead. The two criteria to be looked for are:

- Does the lung float in water? If it floats the foal was born alive since presence of air renders the lung buoyant. Air can be present in lung only if the animal had breathed and breathing can occur only if the foal was born alive.
- Did it suckle? Presence of milk or curds in the stomach is valid evident that the foal was alive at birth and had suckled.

GANGRENE

Definition

- Gangrene is a necrotic area invaded by saprophytic organisms leading to putrefaction.

Types of gangrene

- There are three types of gangrene
  - Dry gangrene
  - Moist gangrene
  - Gas gangrene

Dry gangrene

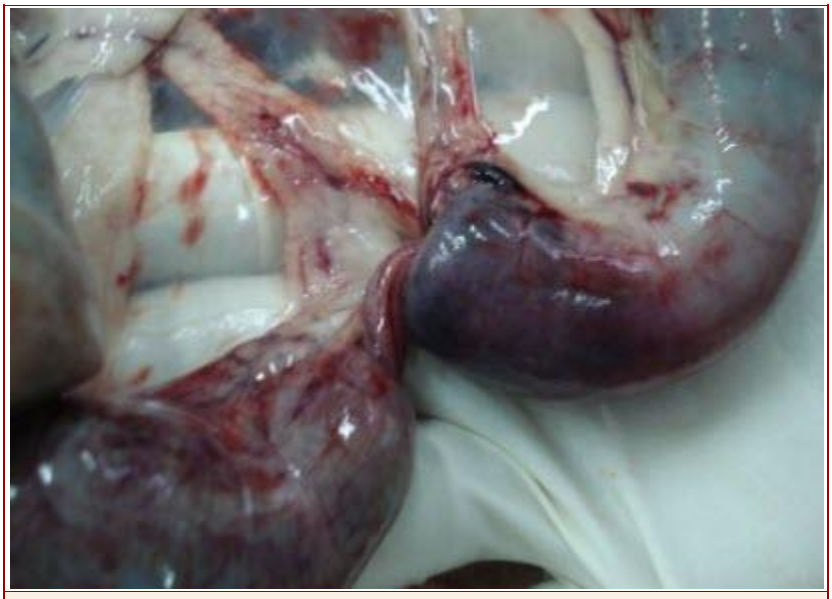
Dry gangrene represents an area of coagulation necrosis resulting from infarction followed by mummification. The extremities of the body like tail, ears, legs and udder are affected.

- Causes
  - Toxins (phytotoxins and ergotoxins): The toxins cause marked peripheral arteriolar vasoconstriction and damage to capillaries leading to thrombosis and infarction.
  - Fescue poisoning
  - Cold (Frost bite): Direct freezing and ice crystal formation leading to cellular damage, vascular damage and ischaemic necrosis.
- Gross pathology
  - Affected part is dry (dehydration due to exposure to environment), shrivel (dehydration) and brown to black (due to formation of iron sulphide: iron from haemoglobin degradation, sulphide from putrefaction), proliferation of bacteria due to unfavourable environment, temperature and moisture.
  - However, at the junction of living and dead tissue, there is a line of demarcation due to active inflammatory reaction.

Dry gangrene

Moist gangrene

- Causes: Intestine desplacements: Intususception, volvulus, incarceration
- Gross pathology
    - The affected parts are soft, moist and reddish brown to black, foul smelling or putrid odour due to hydrogen sulphide, ammonia and mercaptanes. The environment is conducive for rapid growth of bacteria. There is no line of demarcation between live and dead tissue.



Moist gangrene - Intussusception

- Histopathology
    - Initial coagulation necrosis with a few bacterial multiplications. Later liquified due to rapid proliferation of bacteria and infiltrating neutrophils.

Gas gangrene

Anaerobic bacterial proliferation producing toxin and damaging the tissues. **Examples:** *Clostridium perfringens*, *Clostridium septicum* introduced by penetrating wounds.The clostridia proliferate in necrotic tissue under anaerobic environment and produce toxins which cause tissue damage. The *Clostridia chauvoei* spreads haematogenously from the intestine and lodges in muscle which requires some injury and necrosis for the spores to germinate and bacteria to proliferate.

- Gross pathology
  - Affected parts are dark red to black, contain gas bubbles, serosanguineous exudates and foul smelling.
- Histopathology
  - Coagulative necrosis of muscle, bacteria, serosanguineous exudates and gas bubbles are seen

MODULE-8: PIGMENTATIONS

Learning objective

- This module takes the learner through pigmentation and its types (Exogenous and endogenous).

EXOGENOUS PIGMENTS - 1

Colouring agents

Colouring agents are called as pigments. Tissues may be discoloured (e.g. Jaundice, tattoo) or excessively coloured (e.g. Melanosis) in diseases.

- Origin
  - External or exogenous pigments
  - Internal or endogenous pigments

Exogenous pigmentations

In exogenous pigmentations colouring substances can enter the body by three different routes.

- Respiratory route by inhalation
- Alimentary route by ingestion
- Cutaneous route by injection

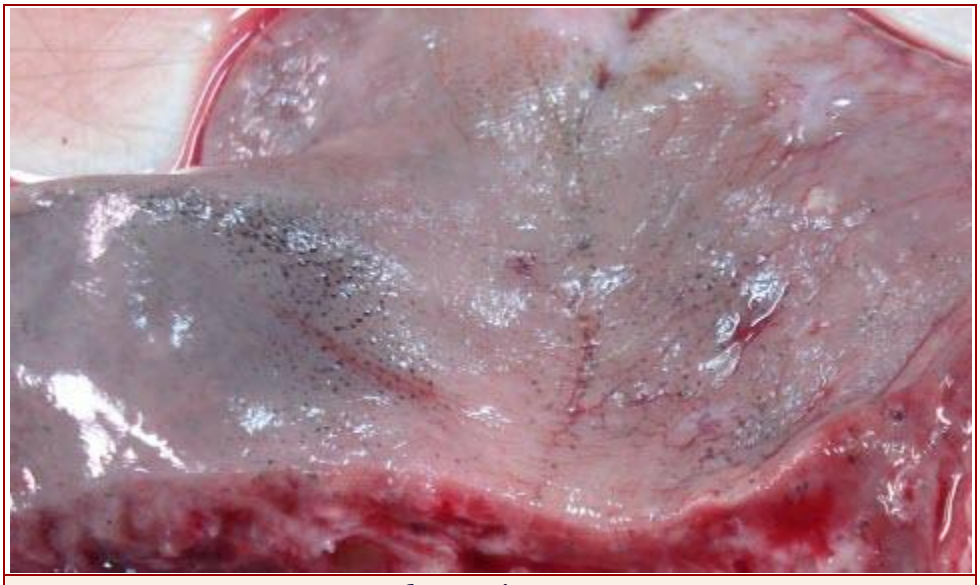
Of these three entries, entry through respiratory route is the most common pathway for exogenous pigmentations. This results in pneumoconiosis characterized by pigmentation and fibrosis. Pneumoconiosis is a general term applied for any permanent deposition of substantial amounts of particulate matter in lung disease by inhalation; Depending upon the type of exogenous pigment, the conditions are termed as follows

- Coal dust-Anthracosis
- Stone dust -Silicosis
- Iron dust -Siderosis

- Cotton dust –Byssinosis
- Asbestos dust -Asbestosis
- Cement - Chalicosis

Anthracosis

- **Sources:** Air pollution (Near busy high ways- zoo animals and dogs)
    - Coal mines (Horses and mules)
        - The carbon particles inhaled are phagocytised by alveolar macrophages and transported through regional tracheobronchial lymph nodes. The carbon particle being inert is not metabolised by the body and hence remains in the tissue permanently.
- **Grossly** , the lung shows **peppered** appearance. The carbon deposits in sub-pleural area are seen as black foci. Regional lymph nodes may show carbon deposits in the medulla because of concentration of sinus macrophages in that location.



Anthracosis - Lung

- **Microscopically** , fine black granules may be found within the macrophages or deposited extracellularly in the lungs (alveolar wall) or around the peribronchial areas. The pigments are resistant to solvents of bleaching agents and non-reactive. The carbon being mildly irritant elicits slight pulmonary fibrosis.

Silicosis

Silicosis is deposition of silica in the lung. The condition is more common in human beings than in animals as an occupational hazard who are working in mines and quarries. The crystalline form of silica is more harmful irritant than amorphous form. The silica is a powerful irritant and is insoluble in body fluids.

- **Grossly** , lung shows multiple, small discrete nodules in the parenchyma. Similar lesions may also be found in the regional lymph nodes and pleura. Extensive fibrosis may predispose to pulmonary tuberculosis.

- **Microscopically** , the nodular regions are formed by concentric layers of hyalinised collagen.

EXOGENOUS PIGMENTS - 2

Tattoo

- Tattooing is a method of identification of animals in which the carbon pigments used are deposited in the dermis.
- The carbon pigments may be found as phagocytised by macrophages or remains free in tissue.
- It evokes no inflammatory reaction.

Carotenoid pigments

Lipochrome pigments, not lipofuscin pigments.

Sources: β-carotene and fat soluble phyto-pigments

Grossly , the pigments are normally found in the cells like adrenal cortex, corpus luteum, Kupffer and testicular cells and in plasma/serum and fat of horses and Jersey cattle. The fat is discoloured to yellow to orange-yellow. Holstein cattle, sheep, goat and cats store little or no carotenoids in which fat is white and serum is clear. In starvation fat atrophy, the adipocytes become dark yellowish brown due to concentration of carotenoids.

Microscopically , pigments are not seen due to dissolution of pigments by alcohol and clearing agents (Fat soluble nature).

Tetracyclines

Deciduous teeth or developing teeth and bone may show yellow or brown deposits if the animals are treated with tetracycline antibiotics.

Plumbism

Plumbism is deposition of lead in the body in chronic poisoning.

Sources : Ingestion of lead containing grains, water, fodder, lead containing batteries, lead water pipes, etc.,

The lead sulphide (PbS) formed by hydrogen sulphide (H_2S) and lead imparts **"blue line"** in the gum, along the edges of teeth and gray colour to faeces. Hydrogen sulphide is derived from the putrefaction of food particles.

Argyria

- Argyria is deposition of silver as finely granular albuminate in tissue. There is grayish blue discolouration of skin and conjunctiva and internal organs.

- The pigment is extracellular and deposited in the cementic substances like dermis, arterioles and venules. This is a permanent blemish and not harmful.

Asbestosis

- Inhalation of asbestos particles leads to asbestosis and results in to interstitial fibrosis
- Asbestosis leads to mesothelioma in human beings
- Microscopicallly, ferruginous bodies were seen in the parenchyma.Ferruginous bodies are believed to be formed by macrophages that have phagocytized and attempted to digest the fibers

Siderosis

- Inhalation of oiron dust materilas can leads to siderosis and occurs commonly in horses, mules and dogs.
- Macroscopically, brown or rusty red pigemtation can be seen
- Microscopically, brown or black coloured irregularly shaped granules as spherical masses with in the macrophages

ENDOGENOUS PIGMENTS

- These include melanin, lipofuscin, ceroid and haematogenous pigments (Haemoglobin, haemosiderin, porphyrin).

Melanin

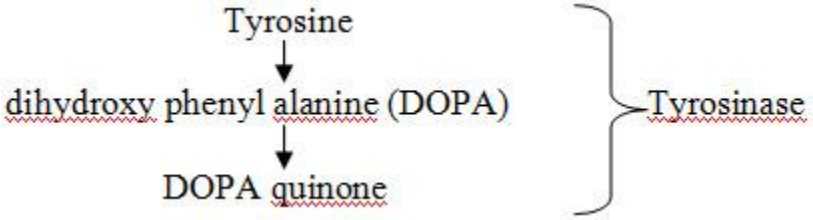
Melanin (G. Melas-Black) is a black pigment produced by oxidation of tyrosine to dihydroxy phenyl alanine by the copper containing enzyme tyrosinase in the melanocyte. The melanocytes are generally present in the basal layer of epidermis, retina, iris and pia-arachnoid of black animals and in the oral mucosa (Jersey cows). The melanin pigment protects from ultra ultraviolet radiation.

Pathologically, hypo/ hyper-pigmentation may occur in animals.

- Hypopigmentation
  - Copper deficiency in cattle and sheep results in loss of coat colour.
- Albinism: Melanin deficiencies due to lack of tyrosinase. The melanocytes appear normal. Lack of pigmentation in skin, hair, sclera or iris on exposure to sunlight may lead to development of skin cancer.
- Leukoderma is a condition in which local loss of skin pigments which is likely to be seen in the collar, saddle or harness.
- *Vitilogo* is partial or complete loss of melanocytes in the epidermis.
- Hyperpigmentation
  - The condition is found in melanomas and occasionally in malignant melanomas. Gray horses are susceptible to melanomas. Naevus (pigmented moles) is seen in human beings commonly. Focal accumulation of pigments occurs in mammary gland and surrounding fat in gilts and sows.
  - Melanosis is a hyperpigmented area sometimes found in the intestine, heart, lung, kidney etc. Melanosis of cornea may lead to blindness in some breeds of dogs e.g. Boxers, Western Terriers. The condition is bilateral and symmetrical.

Hyperpigmentation of skin (*Acanthosis nigricans*) may be associated with chronic injury and hyperadrenalism. The melanocytes contain melanosomes having the pigment. The macrophages laden with melanin are termed as melanophores.

- DOPA reaction



Tissue containing melanocytes convert DOPA to DOPA quinine are tested DOPA positive while melanophores give a DOPA negative test.

Lipofuscin-Ceroid

- **Lipofuscin** (L. Fuscus-Brown) is known as 'aging pigment' or 'wear and tear pigment' or 'biologic garbage'. They are brownish yellow pigments and are accumulated in post-mitotic cells like neurons, cardiomyocytes, skeletal myocytes and in slowly dividing cells like glial cells and hepatocytes. The pigment is intracellular.This pigment cannot be removed by lysosomal degradation or exocytosis. The pigment is a complex of lipid and protein derived from oxidation of polyunsaturated lipids derived from free radical injury and lipid peroxidation. They are referred to as residual bodies representing indigestible residues of autophagic vacuoles. The tissue discolouration is known as 'brown atrophy'.
- **Histochemistry:** Fat soluble dyes, acid fast, PAS-positive.
- **Ceroid** is a pathological pigment. Ceroid is an early form of lipofuscin containing partially oxidised polymerised unsaturated fatty acids. It has got similar chemical component to lipofuscin and occurs in response to severe malnutrition including hypovitaminosis E, cancer cachexia, irradiation and inherited neuronal ceroid lipofuscinosis.The pigment accumulates in Kupffer cells, hepatocytes, skeletal and smooth muscle myocytes. This pigment has a deleterious effect on the cell. Occasionally, the pigments are seen in the small intestine of dogs called intestinal lipofuscinosis and in nutritional panniculitis in cats, minks, foals and pigs (hypovitaminosis E). In cats it is also due to ingestion of fish products which contains highly concentrated unsaturated fatty acids. Hepatic ceroidosis: Salmons and cat fish fed with rancid diets.
- **Grossly,** lipofuscin pigment gives a brown discolouration to heart and skeletal muscle and thyroid. Lipofuscin in the presence of UV light produces brown fluorescence e.g. thyroid
- **Microscopically,** light golden brown to dark brown pigments are seen around the perinuclear areas of neurons and different myocytes. This pigment may also be extracellular (feline panniculitis) e.g. autosomal recessive- English Setter dogs

Haemosiderosis

- Haemosiderosis is deposition of haemosiderin in many tissues and organs.
- Haemosiderin is a golden yellow to brown granular crystalline pigments derived from hemoglobin and stored in cells. Normally Hemosiderin is present greatest amount in spleen of horse and least in spleen of dog.

- Systemic changes
- Localised changes

Causes

- Increased absorption of dietary iron
- Impaired iron utilisation
- Excess haemolysis
- Blood transfusion (Exogenous iron load)

These are occuring as systemic derangement in chronic passive hyperaemia involving lungs where haemorrhages are seen. This erythrocytes are lysed and the haemosiderin is phagocytosed and deposited in the lung. Haemosiderin laden macrophages are called heart failure cells. This along with increased fibrosis gives the lung hardness and brown discolouration. This is referred to as brown induration of lung.

Haemosiderin can also accumulate locally in haemorrhages known as localised haemosiderosis. e.g. Bruishes. A local haemorrhage impart different colours as the wound ages. First it appears red blue. Haemosiderin formed from lysed RBCs are taken by macrophages, red blue colour becomes green blue (Biliverdin formation). Then golden yellow colour haemosiderin deposits.

Microscopically

- Haemosiderin pigments is found in cellular cytoplasm appearing as coarse, granular yellow pigment. Histochemically, it appears blue from prussian blue reaction. It is an insoluble blue black ferric ferrocyanide.

Haemochromatosis

- This condition is due to extreme accumulation of iron in diseases. e.g. Human - Diabetes mellitus associated with hepatic fibrosis. In this iron overload disorder the iron content may reach 50 - 60g when compared to 2 - 3 times more than normal in adult .
- Animals - This may occur due to excessive dietary iron absorption and injection of iron

MODULE-9: CELL INJURY AND NECROSIS

Learning objective

- In this module, there are two parts. The first part explains about calcifications due to hypercalcaemic conditions and also dystrophic calcification. The second part deals with the jaundice, its types, causes, pathogenesis, diagnosis and differential diagnosis.

CALCIFICATION

Calcification is abnormal deposition of calcium salts in tissue other than bone.

- Calcium is normally present in blood and deposited in bones. Calcium if deposited in an abnormal tissue with normal or abnormal blood calcium level is considered as pathological condition.

- Pathological calcifications are
    - Metastatic calcification
    - Dystrophic calcification

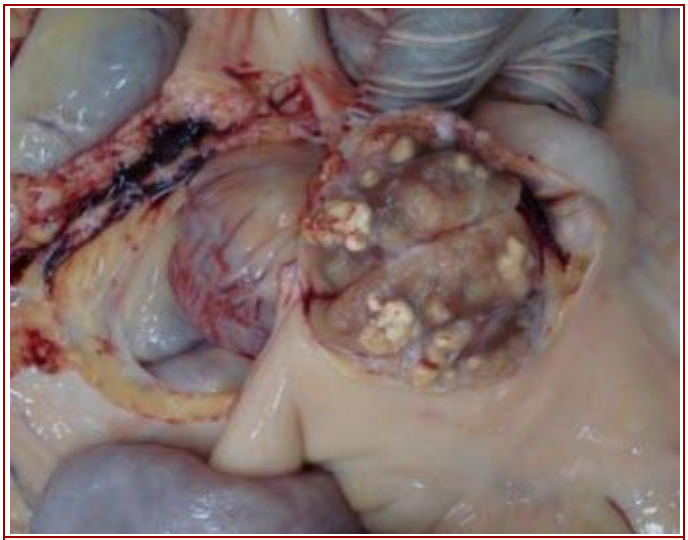
Metastatic calcification

- Deposition of calcium occurs in soft tissue following increase in the blood calcium (Hypercalcaemia i.e. .12 mg/dL). Hypercalcaemia may arise due to
    - Parathyroid tumour in which high levels of parathormone favours phosphate excretion through kidneys (hyperphosphaturia), hypophosphataemia and withdrawal of calcium from bones.
    - Primary and secondary bone tumours cause rarefaction of bone.
    - Nutritional cause with high vitamin D intake resulting in increased absorption of calcium.
    - Renal disease with retention of phosphate, hypophophosphaturia, depression of calcium, parathyroid stimulation and hypercalcaemia.
- Wherever acid is secreted calcium deposition occurs. e.g. Stomach-HCl; Kidneys-Hippuric acid; Lungs-CO_2.

Dystrophic calcification

Dystrophic calcification is calcification of abnormal tissue with normal blood calcium levels.e.g. Necrotic tissues (Tuberculous lesion, suppurative lesion, renal tubular epithelial cells in mercurial poisoning), scar tissue, dead parasites, atherosclerotic plaques, old thrombi

- Pathogenesis
    - Deposition of calcium occurs around the nidus. The phosphates from the dead tissues form the nidus. Further, the calcium combines with phosphates to form calcium soaps .
- Gross lesions
    - Hard, gritty mass and on section gritty sound is heard. Usually lesions are microscopical.

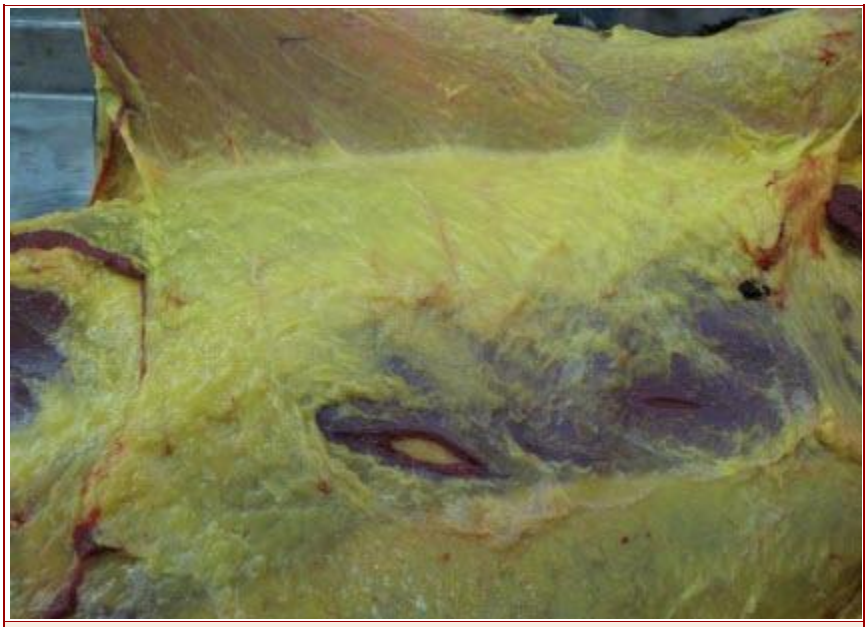


Calcifies nodule - TB - mesenteric lymph node

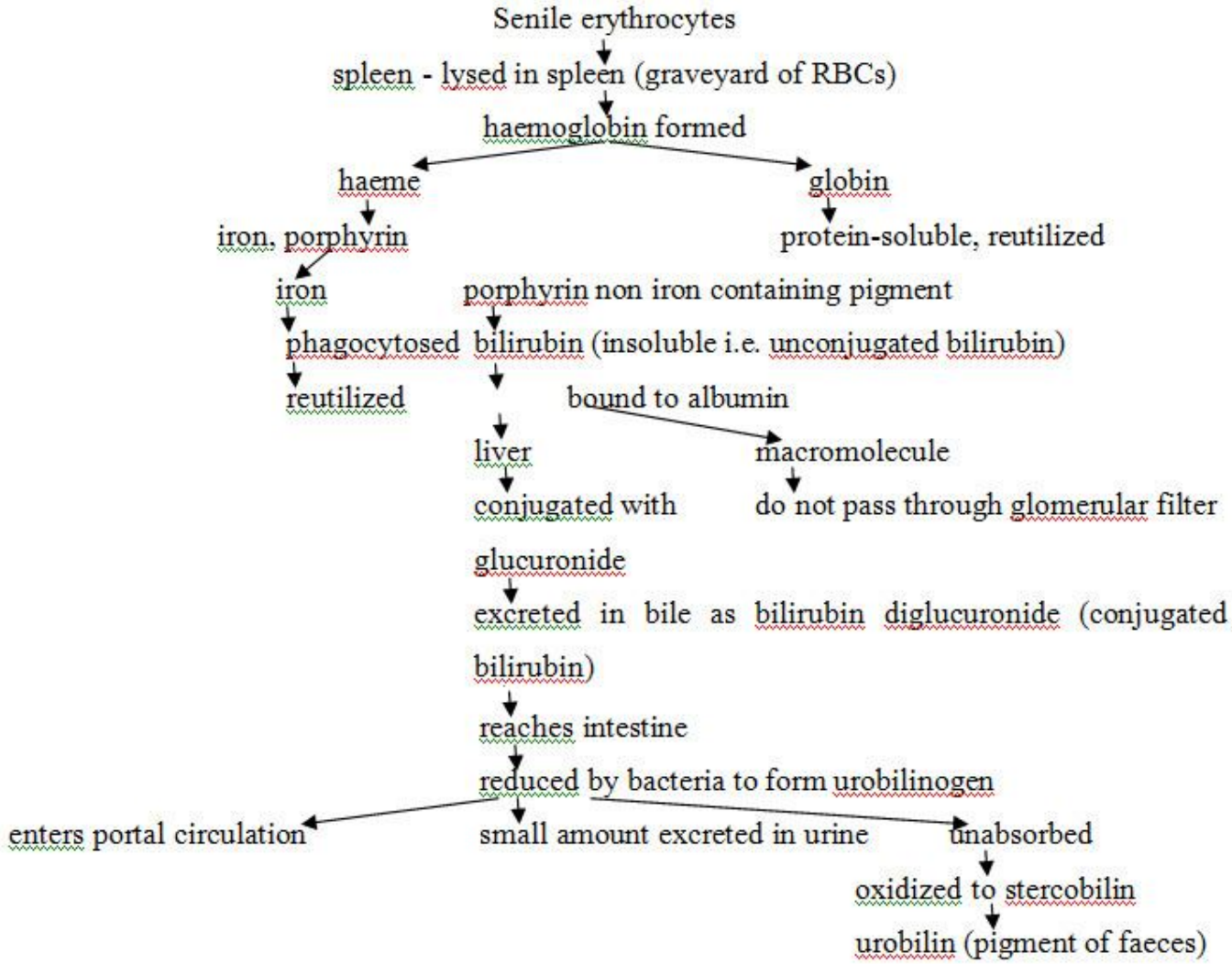
- Microscopic lesion
    - Calcified areas take up a blue colour with H&E stain and black with von Kossa stain

JAUNDICE

- **Jaundice** (French – yellow: icterus - Greek – jaundice). Jaundice is not a disease, it is a sign.
- Jaundice is defined as yellow discolouration of skin, sclerae, mucous membranes and internal organs caused by an increase in bilirubin concentration in tissues. To understand jaundice, it is essential to know the bilirubin production.



Jaundice - Icteric - Subcutis



Causes of jaundice

- Overproduction of bilirubin due to increased haemolysis.
- Reduced uptake in liver, impaired conjugation (lack of enzymes)
- Impaired intrahepatic secretion due to hepatic damage, intrahepatic cholestasis due to biliary obstruction
- Impaired extrahepatic secretion due to obstruction - Due to bileduct obstruction.

Jaundice is classified into

- Haemolytic or prehepatic jaundice
- Toxic or intrahepatic jaundice
- Obstructive or posthepatic jaundice

Haemolytic or prehepatic jaundice

- Causes
    - **Bacteria :** *Clostridium haemolyticum*, Leptospirosis

    - **Virus :** Equine infectious anaemia
    - **Protozoa :** Babesiosis, Anaplasmosis, Haemobartonellosis, Trypanosomosis
    - **Nutritional :** Phosphorus deficiency - Post parturient haemoglobinuria
    - **Phytotoxins :** Resin, Saponin
    - **Animal toxin :** Snake venom
    - **Chemicals :** Copper, selenium toxicity in sheep
    - Icterus neonatarum, incompatible blood supply
- Pathogenesis
    - Excessive haemolysis results in production of greater amount of unconjugated bilirubin. Since there is a rate limiting, all unconjugated bilirubin cannot be converted to conjugated bilirubin. Hence, some amount is left in the blood. Since large amount of conjugated bilirubin is formed, it stains faeces yellow. When excess quantity of urobilin is formed (faeces intense yellow colour) and is also responsible for abnormal intense yellow urine.

Toxic or intrahepatic jaundice

- Causes
    - **Bacteria :** Leptospirosis, Salmonellosis
    - **Virus :** Infectious canine hepatitis
    - **Phytotoxins :** Senecio, crotalaria
    - **Chemicals :** Phosphorus, chronic copper poisoning, chloroform, carbon tetrachloride.
- Pathogenesis
    - When heptocytes are necrosed, the liver is not able to convert normally formed unconjugated bilirubin. Since the degenerated cells are swollen and disorganised and biliary capillaries are blocked, conjugated bilirubin escapes into sinusoids and enters general circulation and excreted through urine. Hence, blood contains both conjugated and unconjugated bilirubin.

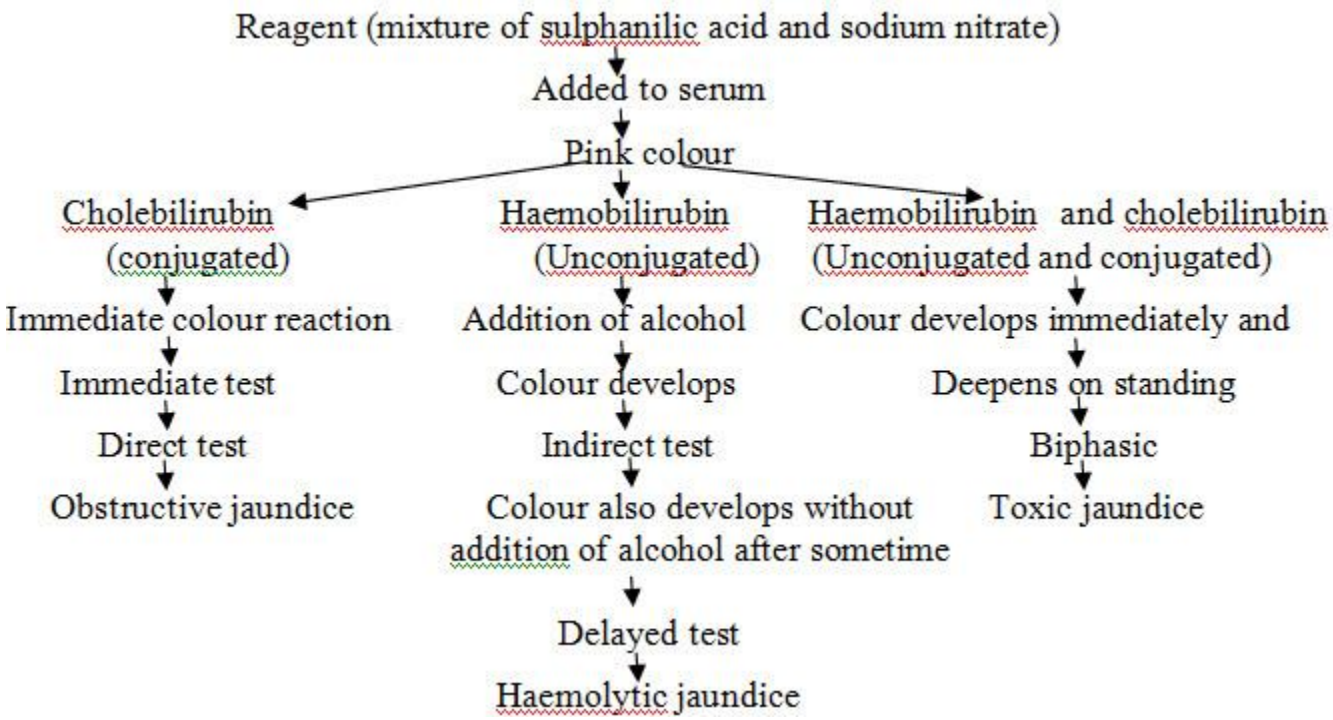
Obstructive or posthepatic jaundice

- Causes
    - **Blocking of bileduct from within**
        - *Ascaris lumbricoides* in swine
        - *Thysanosoma astiniodes* (fringed tape worm)
        - *Fasciola gigantica* in cattle
        - Gall stones
    - **Pressure on bile duts from outside**
        - Tumours, abscesses, granulomas, fibrosis, enlarged pancreas or lymph nodes
    - **Inflammatory processes in biliary system**
        - Cholangitis, cholecystitis – fascioliasis, *Dicrocoelium dendriticum*
    - **Closure of bile duct orifice in duodenum**
        - Duodenitis – thickening of mucosa
- Pathogenesis
    - The obstruction to normal flow of bile results in regurgitation of bile. In this case, the production of conjugated and unconjugated bilirubin is normal. Biliary stasis occurs due to (extra hepatic cholestasis) pressure, worms, inflammation and duodenitis. No urobilinogen is formed since bile is not entering to intestine. Faeces greasy and grey colour due to failure of fat emulsification and lack of

faecal pigment. Urine is not containing urobilin. Clotting defects will occur due to failure of obstruction of vitamin K which is required for prothrombin formation.

Chemical test for bilirubin

van den Bergh test



MODULE-10: PHOTOSENSITISATION AND GROWTH DISTURBANCES

Learning objective

- In this module, the learner will learn about the types and causes of photosensitisational skin lesions (Dermatitis) and various types of growth disturbances (Aplasia, hypoplasia, atrophy, hyperplasia, hypertrophy, metaplasia and dysplasia)

PHOTOSENSITISATIONAL DERMATITIS

Photosensitisation is activation of photodynamic chemicals on the skin by long wave length UV or occasionally by visible light. Necrosis and edema are produced in the exposed areas of skin of animals. The cellular damage by photosensitization is due to release of reactive oxygen species leading to mast cell degranulation and production of chemical mediators of inflammation.

Factors necessary for photosensitization in animals

- Oxygen
- Sunlight
- Photodynamic chemicals
- Skin devoid of hair or wool and lacking pigments

Types of photosensitization

- Type I: Primary photosensitization
- Type II: Abnormal porphyrin metabolism associated photosensitization
- Type III: Hepatogenous photosensitization

Type I: Primary Photosensitization

- Causes
    - Plants containing helianthrones (e.g. hypericine in *Hypericum perforatum*; fagopyrin in *Fagopyrum esculentum*) and furocoumarin pigments (e.g. *Cymopterus watsonii* and *Ammi majus*), tetracyclines and sulphonamides
- Examples
    - Phytotoxins from furocoumarin plants exposed to fungi or other injury may be absorbed into skin which reacts with UV light
    - Phenothiazine is converted into photoreactive compound when bypasses the liver, reaches the skin causing photodermatitis on exposure to sunlight

Type II: Abnormal porphyrin metabolism associated photosensitization

Due to inherited enzyme deficiency, abnormal porphyrin photodynamic metabolic products like uroporphyrin and protoporphyrin accumulate in blood and tissues. The uroporphyrin also causes discolouration of bone known as “osteohaemochromatosis” and teeth called “pink teeth”.

- Examples
    - Bovine congenital porphyria
    - Bovine haematopoetic protoporphyria

Type III: Hepatogenous photosensitization

- Hepatogenous photosensitization is caused by impaired hepatic capacity to excrete phylloerythrin derived from chlorophyll degradation in the alimentary tract, mainly affecting herbivores.
- Causes
    - Hepatocellular damage or injury (Toxic hepatitis due to *Lantana camara*, *Tribulus terrestris*, plants producing pyrrolizidine alkaloids, sporidesmins)
    - Inherited hepatic defects
    - Biliary obstruction
    - Infection: Leptospirosis
    - Chemicals: CCl_4 poisoning
- **Gross pathology,** hairless, non-pigmented skin exposed to sun light (**Horses:** face, nose, distal extremities; **Cattle:** teats, udder, perineum, nose; **Sheep:** pinnae, eyelids, face, nose, coronary band, facial eczema or “swollen head”), erythema, edema, blisters, exudation, necrosis and sloughing of necrotic tissue.

- **Histopathology,** coagulative necrosis of epidermis, subepidermal vesiculation, swelling of endothelial cells, fibrinoid degeneration and thrombosis of blood vessels leading to edema. Secondary bacterial infection culminate in sloughing of epidermis and adnexae.

GROWTH DISTURBANCES

- The disturbances in growth cover a broader spectrum of changes from no growth to uncontrolled growth. While uncontrolled growth (neoplasm) is dealt separately, the other forms of growth disturbances are considered in this chapter.
- Cells may fail to develop or adapt to changing environment or physiological or pathological stimuli.
    - Aplasia
    - Agenesis
    - Hypoplasia
    - Hyperplasia
    - Hypertrophy
    - Atrophy
    - Metaplasia
    - Dysplasia
- The cells respond to altered physiological or pathological stimuli by adapting themselves. These changes are reflected as atrophy, hyperplasia, hypertrophy, metaplasia and dysplasia besides aplasia and hypoplasia. Following an injurious stimulus or to stress, the normal cell's homeostatic state may respond with cellular injury resulting in either death or adaption. Hence, the cellular adaptation to the increased demand is a state in between normal and stressed.

APLASIA, HYPOPLASIA AND ATROPHY

Aplasia

- Aplasia (Gr. A: Without; not; Plasia: Development; formation) is the complete failure of an organ to develop. This developmental disturbance occurs in the embryo or foetus in utero. In the place of the organ, rudimentary tissue of fat and connective tissue are present. The condition is incompatible with life when it involves vital organs like heart, brain etc.

Hypoplasia

It is the failure of an organ or tissue to attain its full normal adult size.

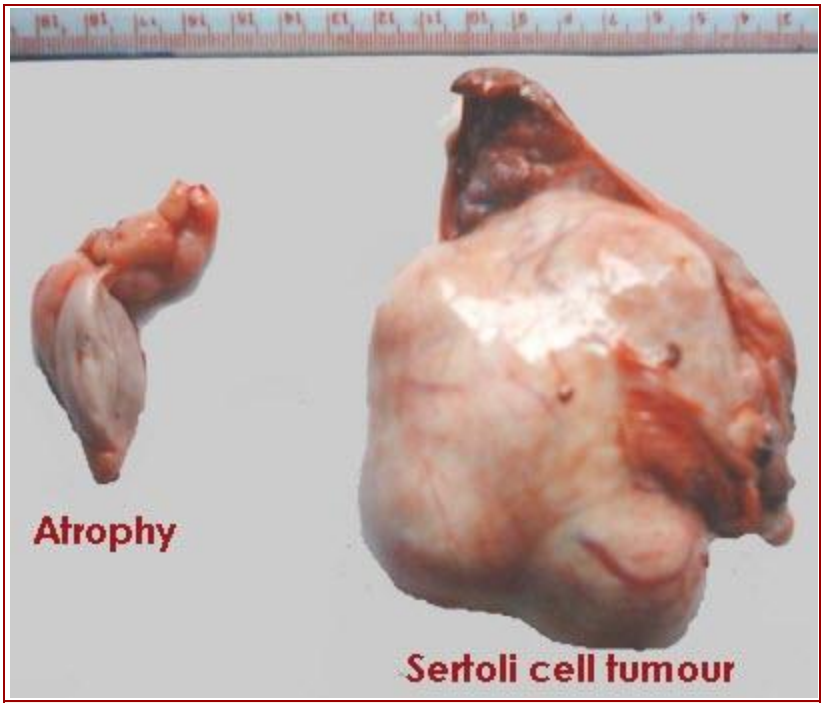
- Causes
    - Any injury occurring in late stages of development of fetus or neonates. e.g. Genetic mutation affects proper differentiation and migration of cells in embryo, virus causes hypoplastic changes; drug induced hypoplasia occurs through degeneration and necrotic changes.
    - Pathological changes: Organ will be smaller than adult size. The cells show alterations in lysosomes and inspissated protein in cytoplasm. The phagolysosomes increase in size with lipofuscin pigment.

Atrophy

- Atrophy is the decrease in the size (quantitative) or amount (numerical) of cells/tissues/organ after attaining full normal growth. Atrophy is representing adaptation to deficient nutrient supply, lack of stimulation and decreased work load. This may affect any organ or part of an organ.
- Atrophy can be broadly classified into physiological atrophy and pathological atrophy.

Physiological atrophy

- Involution of the organs can be observed as the age is advanced. Involution is the decrease in the size of the organ due to decrease in the number of cells, caused by apoptosis. e.g. Involution of thymus on attaining puberty, uterine involution after parturition (decrease in smooth muscle size and number)
- **Senile atrophy**: Atrophy of the organs occurs with ageing and reproductive organs like testis and ovaries are the first to show such changes. It is associated with loss of cells.



Left - Atrophy, Right - Sertoli cell tumour

Pathological atrophy

- **Nutritional atrophy:** This is due to starvation. Starvation of the tissue is caused by malnutrition, malabsorption, chronic infection, parasitism, neoplasia etc. Mismothering is also quoted in starvation atrophy in neonates. In starvation following depletion of glycogen and fat reserves, protein of the musculature and vital organs is lost, resulting in muscular wasting.

- **Angiotrophic atrophy:** Diminished blood supply (ischaemia, chronic passive congestion, anaemia) may lead to atrophic changes.
- e.g. Parasitic ischaemia caused by *Strongylus* larvae by the occlusion of femoral artery leads to atrophy of hind limb in horses. Hepatic atrophy can occur due to decreased portal venous blood flow. Chronic venous congestion results in centrilobular necrosis of liver due to inadequate oxygen and nutrition supplied to the hepatocytes.
- **Disuse atrophy**
    - Decreased work load: Decrease in the size of the body musculature due to inactivity as in the case of race horses.
    - Immobilization: Skeletal muscle atrophic changes can occur in plaster casted animals. In fracture, there will be decrease in the size of the myocytes.
- **Neurotrophic atrophy:** Decrease in the size of muscle fibres occurs if a nerve is severed or injured.
    - e.g. In horses, laryngeal muscle atrophy occurs due to the injury to left recurrent laryngeal nerve and shoulder muscle atrophy (sweening) occurs due to suprascapular nerve injury.
- **Pressure atrophy:** In space occupying lesions like tumours, abscesses etc., the neighboring tissues undergo atrophic changes mainly due to lack of nutrition from pressure ischemia.
- **Endocrine atrophy:** Prolonged steroid therapy leads to atrophy of zona fasciculata of the adrenal gland. Castration leads to atrophy of prostate. Hyperestrogenism associated with sertoli cell tumour results in seminiferous cell atrophy. Ovariectomy leads to uterine atrophy.

Pathogenesis

- In atrophy, the cells survive and are smaller in size with decreased function. There is imbalance between protein synthesis and degradation or loss of protein. That is excessive protein loss or degradation overproduction of proteins. Atrophy and atrophic changes can be attributed to autophagocytosis with destruction of cytoplasmic organelles like ribosomes, mitochondria and lysosomes and by ubiquitin-proteasome pathway wherein the proteins combine with ubiquitin, a cytosolic peptide and then it is destroyed (that is called proteasome).

Morbid/ gross changes

- Affected organs show decreased weight and volume, wrinkling of surface membrane and tortuous blood vessels too large for the volume of the tissue. Organs may be fibrosed and become firm. Fat shows serous atrophy (indicating starvation) i.e. clear/yellowish gelatinous material is seen in place of fat especially cardiac fat, renal fat etc.The organ may become soft and flabby and loss of tone and tissue colour.

Microscopic changes

- Cells are smaller than normal and decrease in number. Sometimes, complete disappearance of the cells is found. Adipocytes become smaller. Interstitial hyaluronic acid and mucopolisacharides are increased. Sometimes brown atrophy is

encountered. Brownish discolouration is due to the membrane bound, indigested residual bodies in the cytoplasm.

HYPERPLASIA

- Hyperplasia is the increase in the size of the tissue or an organ or a part of an organ due to quantitative increase in the number of cells.
- Hyperplasia is classified into physiological hyperplasia and pathological hyperplasia

Physiological hyperplasia

- **Physiological hyperplasia** may be the result of hormonal influence as in the case of increase in the size of mammary gland due to glandular epithelial cell proliferation in puberty and pregnancy.
- **Compensatory hyperplasia:** It occurs due to partial loss of hepatocytes in liver. Hepatic regeneration occurs following partial hepatectomy by the proliferation of surviving cells. These cells are primed from the matrix degradation products followed by proliferation under the influence of growth factors (HGF) and cytokines (TNF-α, IL-6 etc.) and aided by adjuvants like norepinephrine and growth inhibition influenced by TGF-β, with reduction in the growth factors and adjuvants. Compensatory hyperplasia can also be observed in abraded epidermis in which basal layer proliferates to form the superficial layers.

Pathological hyperplasia

This is most commonly caused by excessive hormonal stimulation. e.g. endometrial hyperplasia or effects of growth factors on target cells. In canine uterus, cystic endometrial hyperplasia occurs in prolonged progesterone secretion; in wound healing, hyperplasia of connective tissue (e.g. fibroblast and blood vessels) occurs under the influence of growth factors; hyperplasia also occurs in viral infections involving the epithelium i.e. epidermis or mucosal epithelium. e.g. papilloma virus infections. Pathological hyperplasia may also lead to cancerous growth.

- Pathological hyperplasia may be localized or generalized/diffused.
- Localized hyperplasia - e.g. Nodular hyperplasia in liver, spleen of aged dogs.
- Generalized/diffused hyperplasia- e.g. diffuse enlargement of an organ, prostatic hyperplasia in dogs and thyroid hyperplasia in case of goitre.
- Hyperplastic ability depends on different adult cell types. Accordingly three cell populations are identified:

1. Labile cells: These cells can proliferate normally. e.g. Epidermis, bone marrow cells

2. Stable cells: These cells proliferate when need arises. e.g. Liver, bone, cartilage, smooth muscle

3. Permanent cells: These cells have lost their ability to regenerate/ become hyperplastic. e.g. Neurons, cardiac and skeletal myocytes.

HYPERTROPHY

- It is the increase in the size of the cells or the organ. The number of the cells doesnot increase. The hypertrophic changes are seen in the permanent/stable cells. Striated muscles are most commonly affected.
- In microscopic view, the organ will be normal but the cells are bigger. The number and the size of the organelles will be increased due to the increase in the functional demand. e.g. smooth endoplasmic reticulum in hepatocytes are enlarged in chronic alcoholism and increase in the size of the rough endoplasmic reticulum and Golgi apparatus as a need for increased synthesis of proteins (e.g. collagen and immunoglobulin); the mitochondrial number varies with ATP requirements.

Types of hypertrophy

- **Physiologic hypertrophy:** It occurs following work or exercise/specific hormonal stimulus. e.g. Muscles in race and draft horses; in pregnancy with increased estrogen stimulation hypertrophy of uterus occurs and in lactation mammary gland development occurs under the influence of prolactin and estrogen.
- **Compensatory hypertrophy:** It occurs due to the loss of a part of the organ or loss of one of the paired organs (One kidney undergoes hypertrophy with the loss of the other) or due to the obstruction of the lumen in hollow muscular organ (Right ventricular hypertrophy in pulmonary stenosis).With the continued haemodynamic overload, the compensatory mechanisms fail, resulting in the decompensation and cardiac failure.

Mechanism of hypertrophy involves many signal transduction pathways with induction of a number of genes and synthesis of cellular protein. So there will be increase in growth factors, its receptors (TGF-β, fibroblast growth factor), transcriptional factor (C- fos) and vasoactive agents especially endothelin-1.

METAPLASIA AND DYSPLASIA

METAPLASIA

- Metaplasia is the reversible change in which one adult cell type is replaced by another adult cell type of the same germinal layer. It is also defined as the transformation of one cell type to another cell type within the embryological limits. Metaplasia may involve epithelial or mesenchymal tissue. In metaplasia, one type of epithelium may be converted into another, usually less special type or one type of mesenchymal tissue into another type. While metaplasia is reversible, it is considered as a double edged sword, as it may lead to cancer.

Mechanism

- Metaplasia may arise from reprogramming of stem cells (Reserve cells in epithelium) or from undifferentiated mesenchymal cells present in the connective tissue. The stem cells may differentiate following changes in signals through cytokines, growth factors and extracellular matrix. The tissue specific and differentiation genes involved are bone morphogenetic protein, TGF- β etc. that induce chondro-osteogenic expressions. Some transcription factors involved in the cellular differentiation are Myo-D for muscle, PPAR-γ for adipose tissue, CBFA-1for osteoblast differentiation.
- Metaplastic changes may be caused by chronic irritation, nutritional deficiency, neoplasm etc.

I.Epithelial metaplasia

Squamous metaplasia: - It may occur due to many reasons like chronic irritation, nutritional deficiency etc.

- Chronic irritation from chemicals, carcinogens or other chemicals.
    - Smoking: In lung of smokers, ciliated cuboidal and columnar epithelia of airways are converted into stratified squamous epithelium.
    - Estrogenism: Stratified squamous metaplasia of prostrate or urinary tract.
    - Calculi: Calculi of salivary gland, biliary calculi, pancreas etc.
- Nutritional deficiency: Vitamin A deficiency produces squamous metaplasia of esophageal mucous glands of chicken, transitional epithelium of urinary bladder, cuboid and columnar epithelial cells lining the eye and salivary gland ducts.

II Mesenchymal metaplasia

- Osseous metaplasia in injured soft tissue and metaplastic changes in mesenchymal tissue results in the formation of cartilage and bone in mixed mammary tumour of dogs and myeloid metaplasia leading to extramedullary haematopoesis in adult liver and spleen following injury to bone marrow.

DYSPLASIA

- The term dysplasia is applied to the tissue malformed during maturation. There will be alteration in size, shape and orientation of tissue. The condition is mainly affecting the epithelium. Dysplastic changes are commonly found in the eye, skin, brain and skeletal system. The developmental defect involved complex interactions among three germinal layers.
- In dysplasia, there will be loss of uniformity of cells and their architecture. It is characterized by pleomorphism (Change in the size and shape of cells), abnormally enlarged hyperchromic nuclei, increased mitosis and disorderly arranged cells.
- Dysplasia when marked and in which all layers of stratified squamous epithelium are involved, it is called '**preinvasive carcinoma**, or '**carcinoma in situ'**. The condition is mild to moderate and reversed if the stimulus is removed.

MODULE-11: INFLAMMATION-1

Learning objective

- In inflammation, in this module the learner will learn about the causes and cardinal signs, vascular changes and types of exudates in acute inflammation.

INFLAMMATION

Definition

Reaction of vascularised living tissue to local injury caused by microbes or necrotic tissue.

- Reaction of blood vessels

- Accumulation of fluid & leucocytes in extra vascular tissues
- Inflammation and repair always go hand in hand

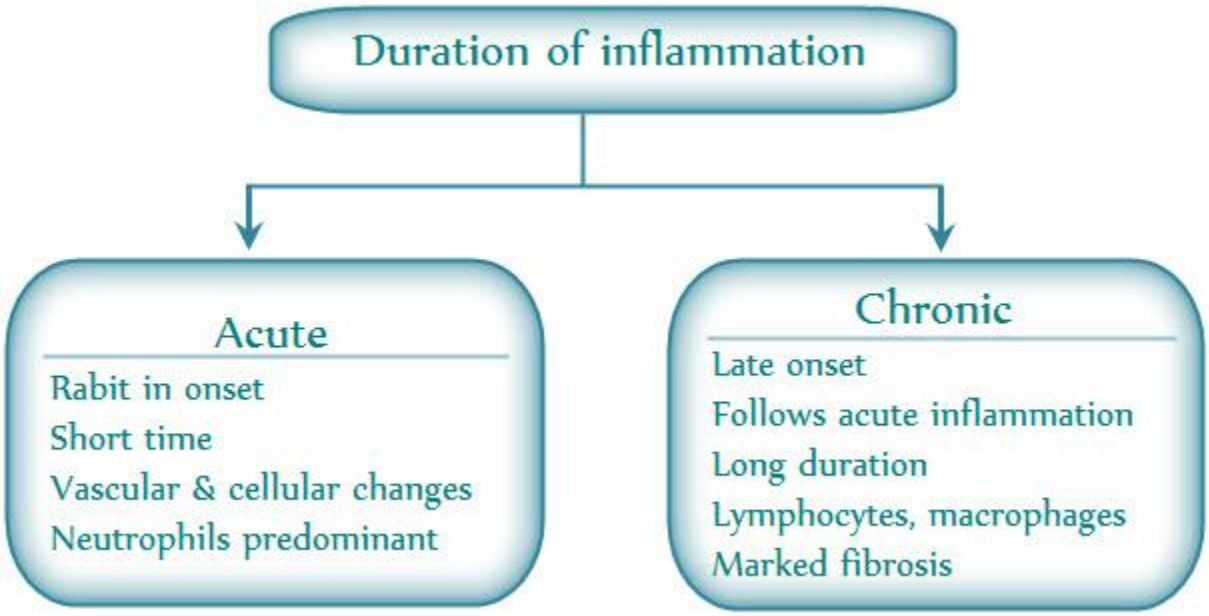
Beneficial Effects of Inflammation

- To destroy / dilute the injurious agent (microbes; toxins)
- Cell injury (necrosis)

Harmful Effects of Inflammation

- Chronic inflammatory reactions **e.g.** rheumatoid arthritis
- Atherosclerosis
- Pulmonary fibrosis
- Hypersensitivity reactions
- Insect bites
- drugs, toxins

Repair produces scars that causes mechanical obstruction and loss of functions



CARDINAL SIGNS OF INFLAMMATION

- There are five important local signs of inflammation. First four of them were described in first century (AD35) by the Italian scientist Cornelius Celsus. Rudolf Virchow (AD 858), the German pathologist added the fifth sign.
- Cardinal signs are mainly attributed to vascular changes at the site of inflammation.

The cardinal signs are:

- **Red (L. Rubor):** It is due to the increased supply of blood (hyperemia) to the area of inflammation.
- **Swelling (L. Tumour):** It is due to the increased blood flow, adding volume to the tissue and exudates into the inflammatory area.
- **Heat (L. Calor):** It is due to the increased blood supply to area of inflammation carrying warm blood from the interior of the body and increased rate of metabolism at the site of inflammation leading to increased production of heat.
- **Pain (L. Dolor):** Pain in the area of inflammation is due to the increased pressure on sensory nerve endings and stretching of tissue due to accumulation of exudates.
- **Loss of function (L. Functio laeso) :** The affected part looses its function due to swelling, pain and tissue destruction

ACUTE INFLAMMATION AND VASCULAR CHANGES

Aetiology (Causes) of inflammation

- Infectious agents – bacteria, fungi, virus etc.
- Chemical agents – acids, alkalies etc.
- Physical agents – burns, electricity, radiation, cold
- Immunological reactions – Ag – Ab reactions
- Nutritional imbalances – vitamins, minerals
- Necrotic tissue

Vascular changes in acute inflammation

Julius conheim(1839 – 1884)

- Changes in blood vessels following injury (tissue damage, microbial virulence factors, etc)
    - Momentary vasoconstriction
    - Vasodilation (arteriolar dilatation – nerve stimuli from axonal reflex also)
    - Increased blood flow
    - Opening of new capillary beds
    - Brought about by substances - Histamine – chemical mediators of inflammation

Changes in the rate of flow

- Increased vascular permeability (Vascular leakage)

Leakage of plasma proteins

↓

↓ Intravascular osmotic pressure

↓

↑ Osmotic pressure of interstitial fluid

↓

↑Outflow of fluid into interstitium

↓

Haemoconcentration

Essential for movement of leucocytes into ECF

- Haemoconcentration
- Endothelium becomes leaky
- Activated endothelial cells release ptostaglandin which causes vascular dilatation, cytokines (IL-1, TNF, TGF-β) which are chemotactic to leucocytes and procoagulants for coagulation. Besides perivascular mast cells degranulate and release histamine which increase post capillary permeability, heparin antagonizes coagulation and angiogenic and leukotrienes which induce pain. Substance P is released by the nerve.
    - By increasing the capillary bed in the area
    - Swelling of endothelial cells
    - Hemoconcentration
    - Margination of leucocytes

Slowing of blood flow – from capillary filing and endothelial swelling

- Margination
- Rolling – selectin – selectin receptors
- Pavementing- as surface ligands increase → Leucocytes
- Adhesion- integrin, ICAM
- Emigration

Diapedesis of erythrocytes

- Movement of erythrocytes outside the blood vessel during iinflammation.

Chemotaxis

- Unidirectional migration of cells towards a chemical attractant
- It is the force that attracts leucocytes into the inflamed tissue

Chemotactic agents

Exogenous	Endogenous
• Bacterial products	• Chemical mediators like C5a (complement) • Leukotriene B4 • Cytokines (interleukins)

Phagocytosis

- It is the process of taking particulate matter in the cytoplasm by cells

Pinocytosis

- Taking in fluid particles
- Discovered by ELLIE METCHNIKOFF in 1884

Steps in Phagocytosis

1. Recognition and Attachment
    - Micro-orgranisms are not recognized by neutrophils and macrophages until they are coated by naturally occurring serum proteins
2. Engulfment
    - Regurgitation during feeding
    - During degranulation leakage of hydrolytic enzymes, metabolic products (H_2O_2) and lysozymes from neutrophil into outside medium cause tissue damage. Kinins released cause vascular dilatation and nerve stimulation. Proteases liberated induce tissue damage, platelets aggregate and release PAF4 which is chemotactic to neutrophils and Coagulation factors causing polmerization of fibrin. PDGF stimulates fibrinogenesis andangiogenesis. Monocytes transform into macrophages to release collagenase, antimicrobial proteases, elastases, complements, IL-1 and TNF. Fever, myalgia and endothelial cell activation. Activation of systemic response leads to releaseb of acute phase proteins (complement, fibrinogen, etc) from the liver and leucocytes and increased haematopoiesis in bone marrow and lymphopoiesis in lymph node and spleen.
3. Killing and Degradation
    - Brought about by reactive oxygen species like hydrogen peroxide(H_2O_2)
    - Myeloperoxidase enzyme present in lysosome of neutrophils

$H_2O_2 \rightarrow$ HOCl (hypochlorous radical)

↓

Active antimicrobial (kills bacteria)

- Myeloperoxidase deficient neutrophils
    - superoxide, hydroxyl radicals $\rightarrow H_2O_2$

FATE AND CLASSIFICATION OF INFLAMMATION

Terminology of inflammation

Time	Extent	Exudate	Position	Anatomy	Suffix

Acute Chronic	Local Diffuse	Serous Fibrinous Catarrhal Purulent Haemorrhagic	Parenchy matous Epithelial Body cavities Glands	Nephr Hepat Rhin Ent Mast Perito	it is it is it is it is it is it is

- Sero – fibrinous
- Muco – purulent
- Fibrino – purulent

Fate of accumulation

- Complete resolution
- Healing by scar formation
- Abscess formation
- Progress to chronic inflammation

Killing of Bacteria by neutrophils

↓

Degradation of bacteria by acid hydrolases in granules of neutrophils

↓

TB bacilli aroid degradation by enzymes & present insidephagocytic vacuoles

↓

Spreads infection to other sites through lymphatics

↓

Tissue injury

Harmful effects of chemotaxis, phagocytosis

Release of products into the extracellular space

- Lysosomal enzymes
- Free radicals

- Arachidonic acid metabolites like prostaglandins

Classification of acute inflammation

- Based on the type of exudate

Catarrhal or mucous inflammation

- Exudates - Mucous
- Site - Occurs in cells capable of producing mucin
- Causes
    - Mild irritants
    - Chemicals (formalin, phenol, detergent)
    - Food poisons
    - Cold air, dust
    - Bacterial and viral infections
- Gross appearance
    - Clear, transparent, glistening
    - Slimy material containing
    - Water and mucous

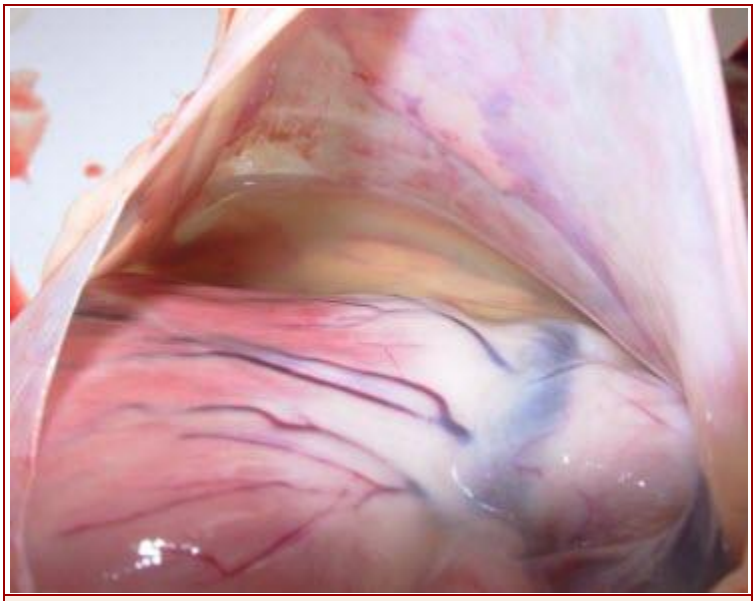
Catarrhal tracheitis - Broiler chicken

- Microscopical appearance
    - Proliferation of epithelial Cells
    - Desquamation into exudates
    - Neutrophils
    - Mucus stained blue with haematoxylin
- Sequelae
    - Recovery if cause is removed
    - If not progresses to chronic
    - On invasion with pyogenic organisms, it becomes mucopurulent
    - Fibrosis

Serous inflammation

- Exudate - Plasma or thin watery fluid
- Site - **Serous membranes** - Peritoneum, pleura, pericardium, joints

- Causes
  - Moderate - severe irritants
  - Chemical irritants applied on skin → "**BLISTERS**"
  - Traumatic injury
  - Burns
  - Viral infections – FMD, vesicular stomatitis
- Gross appearance
  - Blister formation
  - Clear, thin or watery fluid
  - Sometimes mixed with fibrin gives a frosty glass appearance



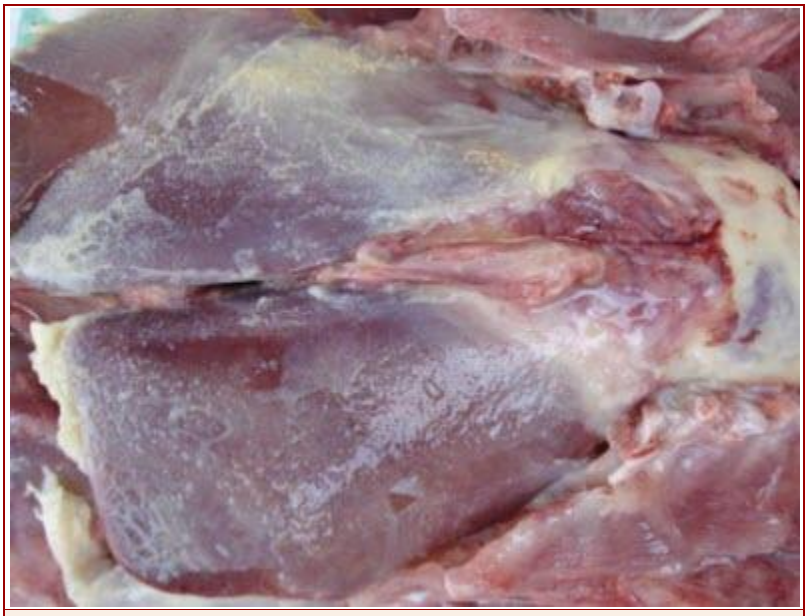
Serous inflammation

- Microscopically
  - Homogenous or finely granular exudates
  - Stains pink with eosin (intensity varies with amount of protein in the exudates)
- Sequelae
  - Fluid is resorbed if cause is removed
  - If not organized or fibrosed , adhesions with cavities will develop with increased in fibrin content.

Fibrinous inflammation

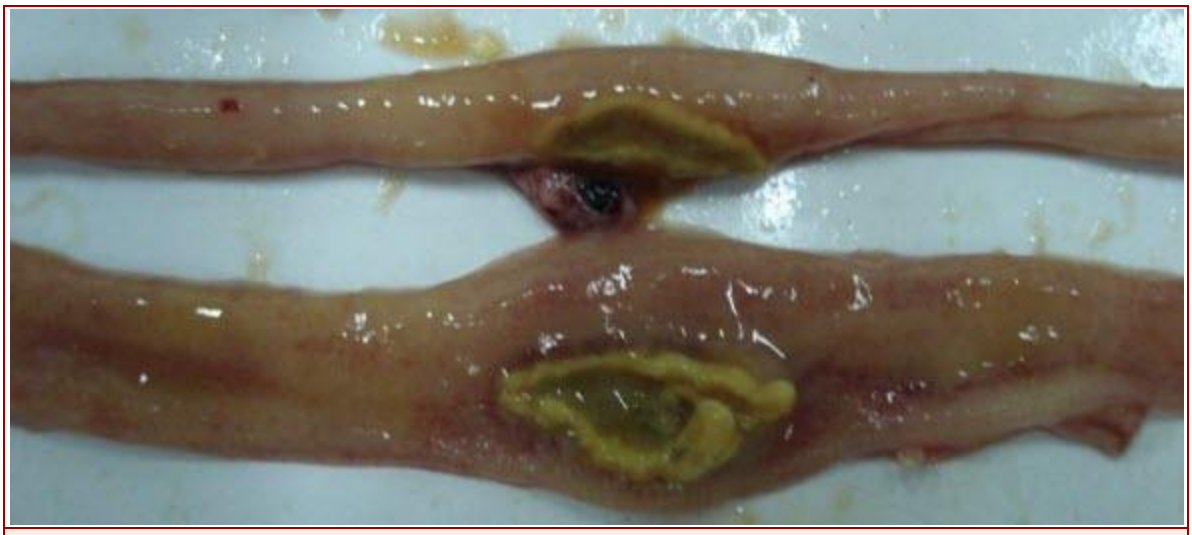
- Exudate – Fibrin
- Sites
  - Body cavities – Pleura, pericardial sac
  - Epithelial surfaces (mucous, serous, cutaneous)
  - Visceral organs (Lung, liver, kidneys)
- Causes – Severe irritant
  - Viral diseases - Feline enteritis, malignant catarrhal fever
  - Bacterial diseases – Salmonellosis, diphtheria

- Gross appearance
  - Organ are tenser or hard
  - Fibrin – stringy, yellowish net–like material



Fibrinous inflammation - Fowl liver

  - On mucosal surfaces
  - Casts – tubular organs
  - **Pseudomembrane formation** - Masses of fibrin not firmly attached to the mucous membrane or peeled off easily.
  - **Diphtheretic membrane -** Fibrin is firmly attached to the underlying tissue; the tissue undergoes coagulation necrosis
  - **Examples -** Diphtheria, swine fever



Diphtheritic inflammation-Chicken intestine

  - **True membrane** -Dead cells are included in exudates
  - **False membrane -** Without dead epithelial cells
  - "**Bread butter appearance" -** Fibrinous pericarditis
- Microscopical appearance
  - Fibrin appears as dirty pink, net–like
  - Entrapment of leucocytes and denuded cells found in the network
- Sequelae
  - Indicates severe injury
  - Not favorable – death supervenes
  - Desquamation of fibrin on epithelial surface
  - Reabsorption of from body cavities
  - Organization
  - Adhesions on serosal surfaces

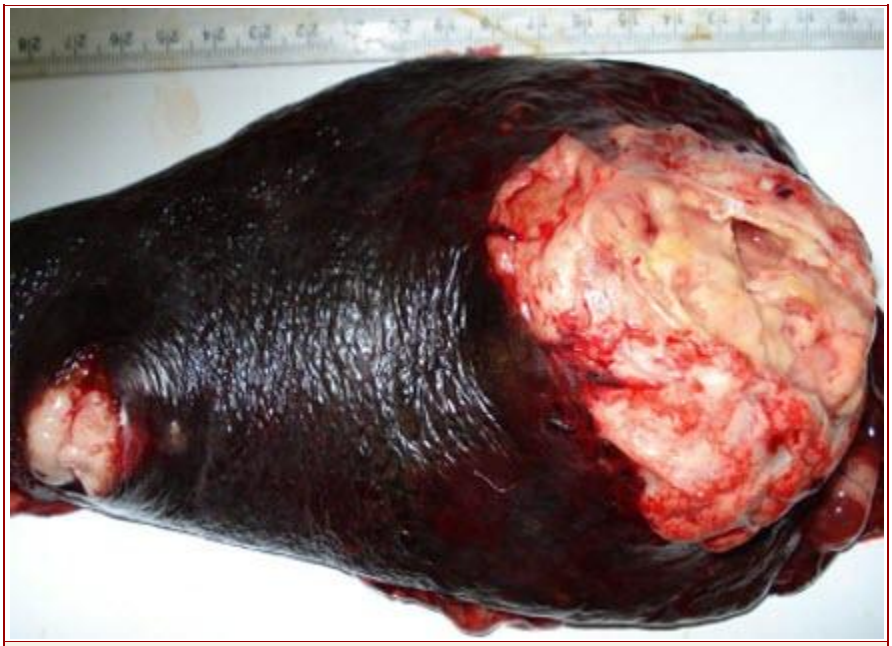
Suppurative inflammation (Purulent)

- Exudate – Pus
- Factors essential for pus fomation
  - Necrosis
  - Neutrophils
  - Digestion of necrotic tissue by protelytic enzymes
- Causes
  - Pyogenic bacteria – *Staphylococci*, *Streptococci*, *Escherichia coli*
  - *Cornybacterium pyogenes*, *Actinomyces bovis* etc
  - Chemicals – turpentine, $ZnCl_2$, Mercuric chloride

Suppuration / pus formation is not commonly seen in rabbits with tuberculosis due to the presence of antienzyme against proteases

- Characteristics of pus
  - Composed of - necrotic
  - Tissue cells
  - Serum

    - Alkaline – PH
    - Color – white, yellow, green, red or black, red
    - Consistency – thin, watery or creamy, thick
    - Pus serum – **liquor puris** - does not coagulate
- Definitions in suppurative inflammation
    - **Cellulitis** - Diffuse spreading suppurative inflammation of connective tissue
    - **Abscess -** Collection of pus locally within a closed cavity in an organ or tissue

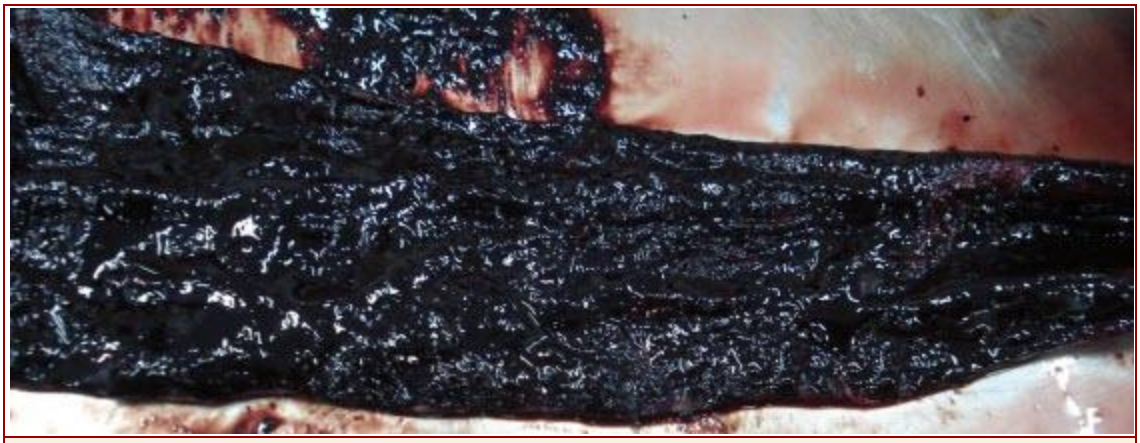


Suppurative inflammation - Splenic abscess

    - **Pyogenic membrane -** Limiting wall formed by partly damaged and partly living - where active warfare is going on to limit the spread of infection.
    - **Ulcer -** The discontinuity of skin or mucous membrane – resulting in opening of abscess
    - **Sinus -** Tract in the tissues communicating with an epithelial surface discharging pus from an abscess
    - **Boil / Furuncle -** Small suppurative inflammation on skin which involves hair follicle or sebaceous gland - *Staphyloccos aureus*
    - **Pustule -** Circumscribed cavity in the epidermis with pus

Haemorrhagic inflammation

- Exudate - Blood
- Cause - violent / severe irritant causes damage to blood vessels
    - Bacterial – Black quarter, anthrax, haemorrhagic septicaemia
    - Viral – Infectious laryngotracheitis in poultry
    - Protozoal – Coccidiosis
- Gross - presence of blood



Haemorrhagic inflammation - Tarry/black coloured digested blood

- Microscopical - RBC's in exudate
- Gangrenous inflammation

Thrombosis of blood vessels - ischaemia

↓

Necrosis

↓

Saprophytes e.g. **– Black quarter**

↓

Gangrene

MODULE-12: INFLAMMATION-II

Learning objectives

- In this module, the learner will learn about chemical mediators of inflammation and chronic inflammation.

CHEMICAL MEDIATRS OF INFLAMMATION

I.Cellular

- Preformed mediator in secretary granules

Mediators	Source
Histamine	Mast cell, basophils, platelets
Serotonin	Platelets
Lysosomal enzymes	Neutrophils

- Newly synthesised
    - Mediators Source
        - Prostaglandin All leukocytes, platelets, Endothelial cell
        - Leukotrienes All leucocytes
        - Platelet activating factor All leucocytes, endothelial cell
        - Activated oxygen species All leucocytes
        - Nitric oxide Macrophages
        - Cytokines Lymphocytes, macrophages

II. Liver – plasma

- Factor XII (Hageman factor) activation – kinin system (Bradykinin), Coagulation system
- Complement activation – C3a, C5a – Anaphylatoxins, C3b – phagocytosis of bacteria, C5b-9 – Membrane attack complex

Biologic activity

- Specific receptors on target cells
- Direct-enzymes
- Mediate oxidative damage

Chemical mediators

- Stimulate release and mediation of target cells themselves. The secondary mediators have similar or opposite effect.
- Chemical action – one or many target cells with different effects.
- Chemical mediators are short lived and scavenge oxygen species.
- Histamine and serotonin cause tissue damage.

Preformed mediators in secretary granules

Histamine

- Histamine is found in the granules of mast cells, basophils and platelets. It increases the vascular permeability of venules and dilates arterioles and induces endothelial junctional gap early response to inflammation.

Serotonin

- Serotonin (5-hydroxy tryptamine) - It is present in mast cell and platelets of rodents. It increases vascular permeability and involved in early inflammatory response.

Lysosomal components

- Lysosomal components leak during phagocytosis or regurgitation. Small granules contain lysosomes, collagen, alkaline phosphatase, histaminases and plasminogen activator. Large granules (azurophils) myeloperoxidases, bactericidal factor, acid hydrolases and neutral proteases are responsible for vascular permeability, chemotaxis and tissue damage.

Lysozyme

- Lysozyme (neuraminidase) is found in granulocytes, monocytes, macrophages and produced by epithelial cells of mucosa and glands of intestinal tract and secreted in milk, tear and saliva. Lysozyme catalyses the hydrolysis of peptidoglycans in bacterial cell wall.

Arachidonic acid metabolites

- The cell membrane phospholipids of neutrophils are acted upon by phospholipases. When arachidonic acid enters 5-lipoxygenase pathway, leukotrienes are produced e.g. LTc4, LTD4, LTE4. When acted through platelets lipoxins which are potent chemoattractants are produced.
- If acted by cyclooxgenase pathway, prostaglandins are produced.

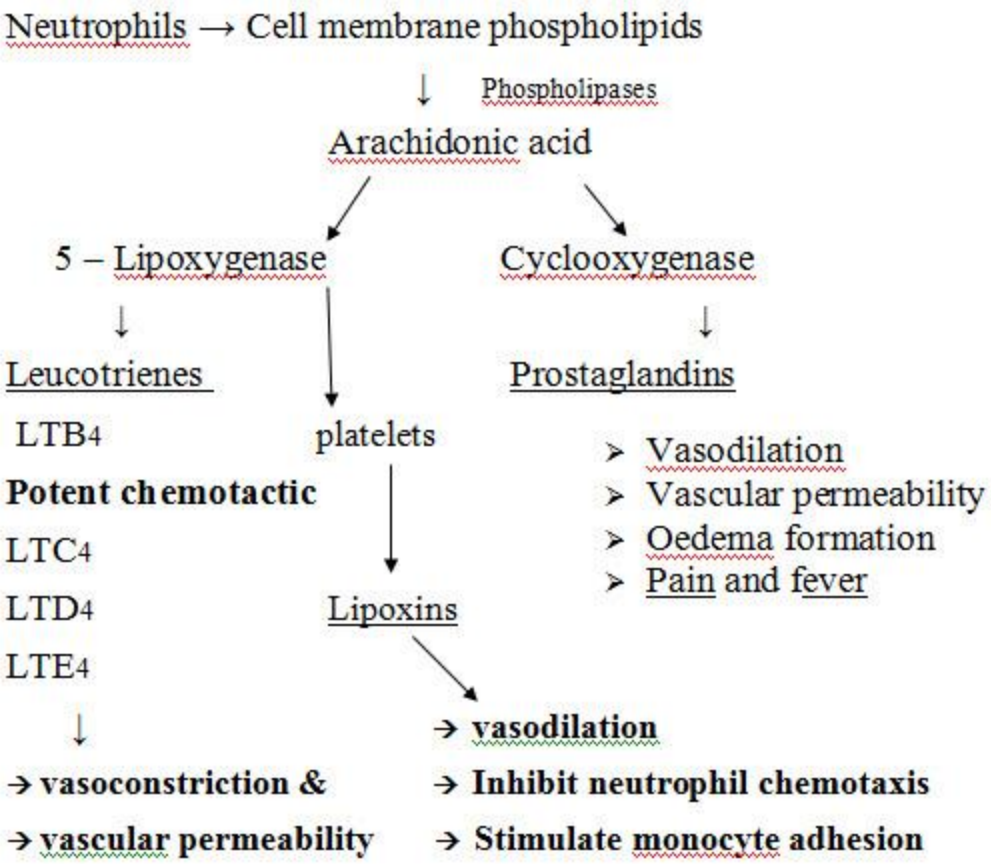
Newly synthesised mediators

Eicosanoids

- These are derived from arachidonic acid from injures cell membrane phospholipids. These are prostaglandins and leukotrienes. Prostaglandin derivatives PGI2, PGE2, PGD2 cause vasodilatation while thromboxaneA2, LTC4, LTD4, LTE4 cause vasoconstriction. LTC4, LTD4, LTE4 are also responsible for increased vascular permeability. LTD4 and HETE can induce chemotaxis and leucocytic adhesion

ARACHIDONIC ACID METABOLITES

(Prostaglandins, Leukotrienes, Lipoxins)



Note: Leukotrienes are 1000 times more potent than histamine

Cytokines

- These are derived from activated macrophages and lymphocytes and are proteins e.g. TNFα, IL-1 are produced by macrophages.
- γ-Interferon also induce acute phase response whereas interleukin 10 is a potent anti inflammatory cytokine.

Platelet activating factor (PAF)

- It is derived from degeneration of membrane phospholipids (platelets, neutrophils, endothelium). PAF causes increased vascular permeability and 100 to 10,000 times more potent than histamine higher concentration of platelet activating factor can stimulate platelets, enhance leucocyte adhesion to endothelium and stimulate vasoconstriction.
- IL-2 and TNF can produce endothelial activation adhesion of leucocytes production of arachidonic acid metabolite and nitric oxide.

Chemokines

- Chemokines are responsible for activation and migration of leucocytes in acute inflammation. alpha chemokines (C-H-C)- attract neutrophils, beta chemokines (C-C)

attract monocytes, lymphocytes, eosinophils and basophils, gamma chemokines (C) attracts lymphocytes and CX3C causes attraction and adhesion of monocytes and T-cells.
- Acute phase proteins - They are not normally present in plasma but markedly increase after injury. Hence of diagnostic value in inflammation. These are synthesized in liver in response to cytokines released by inflammation leucocytes (IL-1, TNF alpha)

Exmples function of acute phase protein

- Fibrinogen, fibrin Coagulation forming coagulant polymers
- C3 Backbone of complement cascade responsible for destruction of bacteria
- C-reactive protein Initiating complement dependent opsonisation
- Haptoglobulin Antioxidant by binding haemoglobin and saving iron

Interferons

- Interferons are produced by host cells in response to stimulation by virus, intracellular bacteria, foreign material and soluble protein. It is considered as first host defence against viral infection. Fibronectin, an alpha 2 glycoprotein on fibroblast surface and basement membrane. when in plasma helps in opsonisation of bacteria and promotes phagocytosis.

Oxygen derived free radicals

- Oxygen derived free radicals (superoxide, hydrogen peroxide and hydroxide radicals) derived from membrane damage like neutrophils can cause tissue injury and endothelial damage.

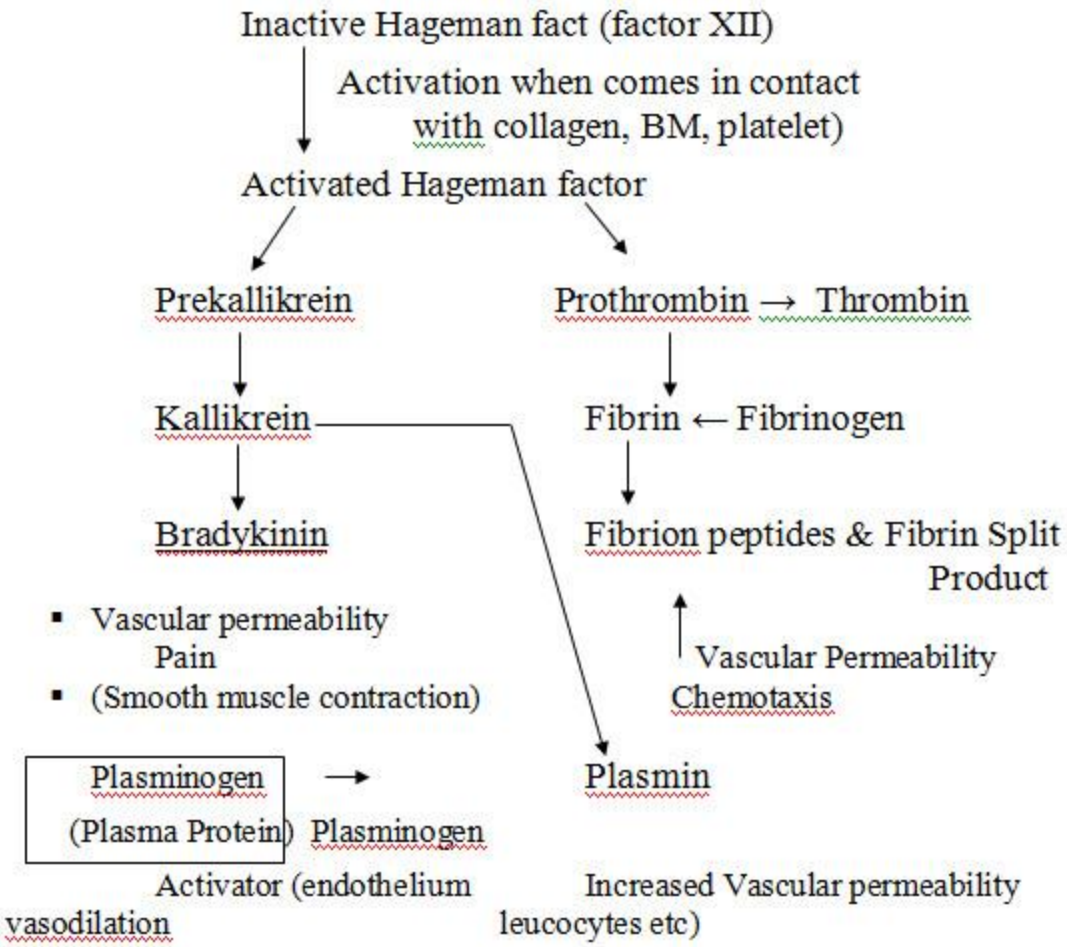
Nitric oxide

- Nitric oxide (NO) - Microbicidal agent in activated macrophages causes vascular dilatation. It is soluble and short lived free radical gas.
- Activation of Hageman factor results in cascade of reactions. The fragment which enhances inflammatory process and this stimulates complement system, kinin system, clotting system and fibrinolytic system.

PLASMA PROTEIN SYSTEM

Plasma proteases

(Kinin, Clothing, Fibrinolytic systems)



Kinin, clotting and fibrinolytic system

- The Hageman factor (factor XII) is activated on contact with collagen, basement membrane and platelets to produce prekallikrein. The prekallikrein is converted to kallikrein which will be converted to bradykinin that induces vascular permeability, pain and smooth muscle contraction.
- Kallikrein also mediates plasminogen, vascular permeability and vascular dilatation
- Activated Hageman factor is also involved in conversion of prothrombin to thrombin which in turn aids in conversion of fibrinogrn to fibrin. The fibrinolytic peptides and split products of fibrin can induces vascular permeability and chemotaxis.

Complement system

- Complement represented as C consists of 20 proteins in an inactive form in plasma and body fluids. Complement is mainly synthesised by liver. Complement system may be activated in one of the two ways, classic or alternate pathway. But both the pathways converge to produce a membrane attack complex (MAC) which is responsible for lysis of bacterial cell membrane and also results in mediating inflammation. e.g. chemotaxis, histamine release from mast cells (C3a) and procoagulant from platelets. C3b is a major opsonin protein which adhere to bacteria (opsonisation). It is recognised, phagocytosed and destroyed by neutrophils and monocytes.

Kinin system

- The vasoactive polypeptide (kinins) are derived from kininogen (plasma globulins). The kinins are potent mediator of vasodilatation, pain, increased capillary permeability. The bradykinin induces vascular leakage from post capillary venules. It is 10 times more active than histamine but short lived.

Clotting system

- Coagulation is seen following damage of endothelium in inflammation through fibrinolytic system the initiated by activated Hageman factor.

Actions of mediators in acute inflammation

Action	Mediators
Vasodilation	Histamine Prostaglandins Nitric oxide
Vascular permeability	Vasoactive amines C3a and C5a Bradykinin Leukotrienes C4, d4, E4 Platelet activating factor Substance P
Leukocyte chemotaxis and activation	C5a Leukotriene B4 Chemokines TNF and IL-1
Fever	TNF and IL-1 prostaglandins
Pain	Prostaglandins Bradykinin

CHRONIC INFLAMMATION

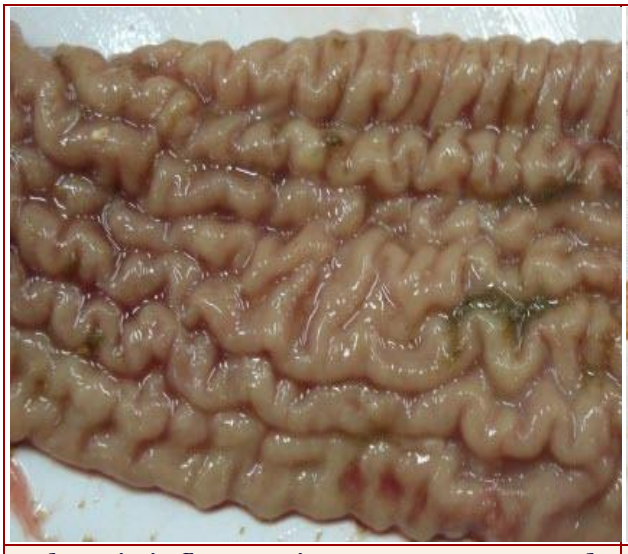
- Infiltration with mononuclear cells
- Tissue destruction and repair
- New blood vessels & fibrosis
- Long duration
    - Follow acute inflammation – persistence of causative agent
    - Chronic from the beginning – irritants of low intensity
    - Example – Tuberculosis , Johne's disease
    - Fungal diseases
- Prolonged exposure to toxic agents
    - Example – Asbestos, Silica particles

Causes of chronic inflammation

- Bacteria – Pasteurella aviseptica, Erysipelothrix rhusiopathiae
- Phytotoxins – Crotalaria, senecio
- Foreign bodies – sharp objects, dust, worms, inert objects
- Constant & repeated mechanical irritation
    - e.g.: kennel granuloma, calluses

Gross appearance

- Gray and firm, white, tough, hard (mature variety), nodules (granuloma) kidney – pitted appearance
- Smooth, dense, watery (newly formed)
- Yellowish, soft & easily cut



Chronic inflammation - JD - Corrugated intestine

Chronic inflammation - Liver - Shrunken - Multiple nodules

Microscopical appearance

- Vascular and cellular response is less
- Proliferation of fibrous connective tissue
- New blood vessel formation
- Mononuclear cells — predominant
    - Macrophages
    - Plasma cells
    - Lymphocytes
    - Giant cells
    - Neutrophils — in bacterial infection
- Encapsulation

Chronic inflammation is characterized by

- Tissue destruction and repair
- Infiltration of macrophages, lymphocytes and plasma cells
- Formation of new blood vessels and fibrosis
- Longer duration

Two types of chronic inflammation

- This may be sequel of persistent and resolved acute inflammation
- It may develop as a slowly evolving chronic process without an acute inflammatory phase

Persistent acute inflammation

- The lesions of persistent inflammation are progressively dominated by the presence of macrophages, fibrous tissue and blood vessels e.g. Acute fibrinous pericarditis - infiltrated with fibroblasts and collagen if not resolved.
- Chronic active inflammation includes apart from neutrophils, presence of macrophages and plasma cells which may be associated with osteomyelitis, metritis and epididymitis (Brucellosis). These are mostly caused by bacteria and fungi predisposed with deficient immunocompetency.

Evolving chronic inflammation

- Diseases like tuberculosis, actinobacillosis and osteoarthritis in which chronic inflammation begins as an asymptomatic process in which neutrophils are not present but infiltrated with macrophages which deals with persistent injury. It lacks cardinal signs of acute inflammation.

Granulomatous inflammation

- This is a form of chronic inflammatory process in which aggregates of large highly activated macrophages are present. When the macrophages take up bacteria, fungi, aberrant parasite (Toxocara larva) or inert substances (silica, asbestos) which cannot be killed or fully digested, monocytes are attracted to the site that are not ineffective in phagocytosis. So that the cells continue to infiltrate the lesion and are found in large numbers. The macrophages become larger and foamy because of accumulation of causative agents, debris from an injured tissue. So this foamy macrophages are referred to as an epithelioid cells which are the hall mark of granulomatous inflammation.

- The granulomatous lesions develop slowly over a period of several weeks or months before producing clinical signs of disease. The microorganisms involved do not cause endothelial damage and are not chemoattractive, so that acute inflammatory signs and neutrophils are not seen.

Other ways of classification of chronic inflammation

- **Chronic inflammation** – Simple type, predominantly cellular exudates predominantly containing lymphocytes. Macrophages and plasma cells are fewer. Sometimes both lymphocytes and macrophages may predominate (lympho-histiocytic) seen in early stages of chronic inflammation like viral infection.
- **Chronic active inflammation** - Besides cellular components of chronic inflammation, it also contains neutrophils, fibrin and plasma protein of acute inflammatory response.
- **Granulomatous inflammation** - Basic cellular exudate - Predominantly activated macrophages, also epithelioid macrophages, giant cells and lesser number of lymphocytes and plasma cells. e.g. deep seated mycoses, bacterial infection with Nocardia, Brucella, Mycobacteria and protozoa
- **Pyogranulomatous inflammation** - This type of inflammation contains similar cellular exudates like granulomatous inflammation that multifocal infiltration of neutrophils, fibrin and plasma proteins. A nodule like granulomatous areas with neutrophils is termed as pyogranuloma e.g. Common in blastomycosis.
- **Granuloma** - Distinct type with well defined macrophage infiltration. Usually it can be non-caseating or caseating. Non-caseating granulomas are round to oval containing numerous macrophages, variable epithelioid macrophages, some multinucleated giant cells, peripheral zone of fibroblast, lymphocyte and plasma cells. In caseating granuloma, the centre is having grey-white, yellow pasty necrotic debris resembling cheese (Latin caseous = cheese) e.g. tuberculosis

Result of chronic inflammation

- Delayed healing
- Permanent change or scar formation
- Distortion / Disfigurement of the organ / tissue (Inflammatory cells displace, replace or obliterate the tissue)
- Impairs mobility
- Epithelial surface – hyperplasia – Metaplasia – Neoplasia
- Increase intracranial pressure - destruction neurons and glia

Differences between acute and chronic inflammation

Acute	Chronic
Short duration	Long duration
Irritant – Sever	Low intensity
Marked vascular Changes	Less prominent
Profuse exudate	Scanty

Soft in consistency	Hard in consistency
No fibrosis	Proliferation of fibro vascular connective Tissue and epithelium

Granulomatous inflammation

- Chronic inflammation
- Circumscribed lesion
- No exudates or cellular changes

The histiocytes (macrophages) in the lesion have large amount of cytoplasm and resemble epithelial cells called “EPITHELOID CELLS” . Epitheloid cells fuse to form “Giant Cells” Foreign body giant cell .e.g. Langhan’s cell

Causes for granulomatous inflammation

- Bacteria – TB, JD, Actinomyces, Actinobacillus
- Fungus – *Aspergillus fumigatus*
- Foreign bodies – Silica, asbestos, inert material

Result

- Helps in localising the infection
- Allows inflammatory and immune mechanism to act for longer periods of time

Allergic inflammation

- Animal / person previously sensitized to foreign bodies
- Diagnosis of JD, TB, Glanders

Sensitized animal

↓

Injection of protein (Antigen)

↓

24hours – Neutrophils / oedema

↓

48hours – Number of neutrophils - ↓ neutrophils, ↑ eosinophils / macrophages

↓

72hours – Hot, painful diffuse swelling

↓

Subsides

Viral inflmmation

- Obligatory parasites
- Cannot survive outside the cells
- Once inside the cell, protected against antibodies
- “INCLUSION BODIES” – aggregates of virus
- Basophilic – replication is complete
- Acidophilic – ongoing replication
- Intracytoplasmic – i/c – Fowl pox, vaccinia, rabies
- Intranuclear – i/n – infectious canine hepatitis
- i/c and i/n – Small pox, Canine distemper

Reactions of cells to virus

- Hyperplasia – Shope Papilloma virus
- Hyperplasia and necrosis – Fowl pox

Hyperplasia

↓

↑ Keratinisation

↓

vacuolation of cytoplasm

↓

inclusion bodies

↓

Necrosis

- Proliferation quickly followed by necrosis. e.g Vaccinia
- Necrosis alone – FMD, Rabies – Cytocidal
- Inflammatory cells - Lymphocytes, Plasma cells, Macrophages
    - No neutrophils
    - No suppuration

Rickettsial inflammation

- *Anaplasma marginale, Ehrlichia canis, Chlamydia psittaci* (intermediate between bacteria & virus)
- Obligatory parasites
- Transmitted through arthropod vectors

MODULE-13: INFLAMMATION-III

Learning objective

- In this part of inflammation, the learner will be taught about role of different cells in inflammation and systemic effects of inflammation

CELLS IN INFLAMMATORY RESPONSE

Neutrophils

- Synonyms etc.
  - Microphages of Metchnikoff
  - Polymorpho nuclear cells
  - First line of cellular defense
  - Pus cells
- Morphology / Character
  - 10 – 20 µ diameter
  - Band shaped or segmented nucleus (3-5 segments) (PMN)
  - Cytoplasm eosinophilic
  - Granules in cytoplasm rich in lysosomal enzymes
  - Rapid amoeboid movement
  - Aggressive phagocytosis
- Origin
  - Myeloid tissue of bone marrow
  - Attracted to injured area by chemotaxis (C_3,C_5)
  - No reproduction at inflammatory site
- Condition encountered
  - First line of defense
  - Pygoenic organisms
  - Increased in early inflammatory response
- Functions
  - Phagocytosis
  - Killing and destruction of bacteria and dead cells through liposomes and proteolytic enzymes
  - Energy source for other cells (MNC)

Eosinopihls

- Morphology / Character
  -
  - Cytoplasmic granules are large and eosinophilic and contain basic protein (toxic to parasite)
  - Motile, sluggishly phagocytic, chemotactic

    - Nuclues - Bilobed
- Origin
    - Myeloid tissue of bone marrow
    - No reproduction at site of inflammation
- Condition
    - Appear late in inflammation
    - Most prominent in conditions where there is no immune response. e.g. hay fever, asthma in man, parasitic conditions)
    - Allergy
    - Parasitic infections
- Functions
    - Chemotactic
    - Phagocytic – killing parasite
    - Hypersensitivity reactions

Basophils

- Morphology /Character
    - 10 – 15 µ in diameter
    - Blue granules in cytoplasm
    - Motile
    - Non-phagocytic
    - Seen in small numbers in inflammation
    - Large lobulated nuclei
    - Granules contain heparin and histamine but no acid hydrolyses
- Mast cells
    - Connective tissue cells
    - Mononuclear nuclei
    - Larger in size
    - Abundant cytoplasm
    - Granules contain heparin, histamine and proteolytic enzymes
    - Some animals rich in serotonin
- Functions
    - Both basophils and mast cells release heparin / histamine in response to Ag – Ab complexes
    - The immunoglobulin IgE binds relectively to the surface of mast cells and basophils

↓

Triggers degranulation

↓

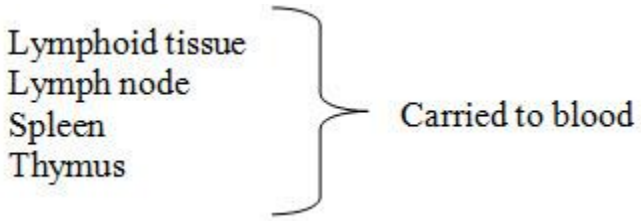
Release of histamine and other mediators, anaphylaxis (C3a and C5a anaphlyotoxins)

    - In acute inflammation
        - Trauma
        - UV light
        - Heat

- Cold

Lymphocytes

- Morphology / characters
  - 7 – 12 µ in diameter
  - Nucleus round
  - Heavy chromatic compact granules within the nucleus
  - Cytoplasm invisible
  - Cytoplasm, homogenous, pale blue and may contain a few azurophil granules
- Origin



  - Two lymphocytic population - T lymphocytes → Cell mediated immunity
  - B lymphocytes transform into PLASMA CELLS.
  - When they come in contact with antigen and produce antibodies – Humoral immunity
- Condition
  - Occurs late in inflammation 48 – 72hours
  - Viral infections particulary in CNS
  - Brain & Spinal cord → Perivascular cuffing
  - Endocrine secretions from pituitary and adrenal cortex control the number of lymphocytes e.g. glucocorticoids
  - Have and inflammatory response
- Functions
  - Humoral and cell mediated immunity

Plasma cells

- 12 – 15 µ
- Nucleus similar to lymphocytes, eccentric
- Cart wheel like arrangement of nuclear clnomatin
- Cytoplasm abundant
- Slightly amoeboid and phagocytic

Origin

- From lymphocytes

Function

- Antibody production.

Macrophages

- Synonyms
  - Macrophages of Metchnikoff
  - Second line of cellular defense
- Morphology/ Character
  - 12 – 20 μ in diameter
  - Nucleus round to oral
  - 1 – 2 nucleoli
  - Macrophages may bunch together to form epithelioid cells
  - Amoeboid & phagocytic
- Origin
  - Macrophages originate from monocytes
  - Monocytes emigrate from the blood into the inflammatory lesions and transform into macrophages
  - Capable of reproduction at the site of inflammation
- Monocytic phagocytic system

Histiocytes -	Connective tissue
Kupffer cells -	Liver
Microglial cells -	Nercous system
Alveolar macrophages -	Lung
Fixed / free macrophages -	Spleen / lymphnode
Skin	Langhan's cell
Bone	Osteoclasts

Macrophages + Monocytes - Mononuclear phagocyto system

- Condition encountered
  - Arise 48 – 72 hours in inflammation
  - Late
  - Response to immune mediated reaction, endotoxin, fibronectin, chemical mediators. Activated T cells secretes gamma interferons which activates macrophages
- Function
  - Phagocytosis
  - Second line of cellular defense
  - Chemotactic
  - Produce potent enzymes that degrade connective tissue
  - Release substances responsible for fever & leucocytosis (prostaglandins, endogenous pyrogens)
  - Release factors in wound healing
  - Secrete lysosymes, interferon defense mechanism
  - Serves to process antigens in CMI

Giant cells

- Multinucleated cells formed by fusion of macrophages

Foreign body giant cell

- Fusion of macrophages evoked in response to foreign body

50 – 100 nuclei

- Nuclei arranged in periphery of cells (horse-shoe pattern)
- e.g. Langhan's giant cell → TB, JD, MYCOSES

Tumor - giant cell

- Nuclear division without cytoplasmic division

Reed - Sternberg cells

- Hodgkin's disease (mirror image nuclei – two)

Touton giant cell

Xanthomas

10 – 15 μ diameter

SYSTEMIC EFFECTS OF INFLAMMATION

- Fever
- Suppression of fever
- Leukocytic response

Fever

- Fever is the main response in acute inflammation.

Definition

- Fever is a complex systemic response that includes increased body temperature, respiration and heart rate.
- It is a syndrome of elevated body temperature increased due to that effect of potent cytokines released by inflammatory cells.

Mechanism

- The endogenous pyrogens of leucocytic origin elevate hypothalamic thermostat.
- Bacterial products, immune complexes, toxins, physical injury, other cytokines
- Macrophage (and other cell) activation

- IL-1 / TNF

1. Acute phase reactions

- Fever – increased sleep, decreased appetite
- Increased acute phase proteins
- Haemodynamic effects (shock)
- Neutrophilia

2. Endothelial effects

- Increased leucocyte adhesion
- Increased PGI synthesis
- Increased procoagulant activity
- Decreased anticoagulant activity
- Increased IL-1,IL-6, IL-8, PDGF

3. Fibroblast effects

- Increased proliferation
- Increased collagen synthesis, increased collagenase
- Increased protease, increased PGE synthesis

4. Leukocyte effects

- Increased cytokine secretion (IL-1, IL-6)

The progression of fever depends upon

- release of pyrogen from leucocytes
- suppression of body heat loss by cutaneous vasoconstriction
- increased heat production and shivering

The following changes occur in fever

- Metabolic changes: Secretion of acute phase proteins,
- Endocrine changes: Increased level of glucocorticoids, growth hormone and aldosterone and decreased vasopressin and
- Autonomic changes: Increased blood pressure, pulse rate and decreased sweating.

The most important thermoregulatory mechanism is a redirection of blood flow to skin to deep capillary bed i.e. intended to decrease heat loss from body surface.

Clinical effects in mammals

- Anorexia
- somnolescence
- malaise
- shivering and search for warmth (chills)

Causes

Causes of fever includes

- Bacteria (endotoxin - lipopolysaccharide of gram negative bacteria (multiple causes))
- Viruses
- Protozoa
- Fungi
- Rickettsia
- Hypersensitivity reaction (antigen - antibody complexes) - stimulate pyrogen release
- Mechanical injury - severe crushing, major surgery
- Vascular disorders - infarction

Neoplasm

The benefits of fever includes

- Increased neutrophils production,
- Accelerated distribution of leucocytes
- Increased phagocytosis
- Efficient killing of organisms
- Quick formation of antibodies
- Higher temperature: Bacteriostatic

The acute phase reactions are mediated by interleukin-(IL-1) and tumour necrosis factor (TNF) which induce secretion of acute phase proteins by hepatocytes. Major acute phase proteins are C-reactive protein (CRP) and serum amyloid protein (SAP)

MODULE-14: CELL CYCLE, CYCLINS, GROWTH FACTORS AND HEALING

Learning objective

- In this module, the learner will be taught about cell cycle, growth factors and healing processes in injury. The first two are required for proliferation of cells in healing.

CELL CYCLE AND CYCLINS

Proliferationof cells are important in degeneration and repair and also a feature in neoplasia. The dividing cells undergo cyclical change i.e cell cycle and it has four phases.

- Presynthetic growth phase or G1 phase – this is thetime gap between end of mitosis and start of DNA synthesis.
- Synthetic phase or S phase – this is period of DNA synthesis and beginning of mitosis.
- A premitotic growth phase or G2 phase
- Mitotic phase or M phase
    - A cell takes16 hours to give rise to another cell.

The time taken by different phases is as follows

Phases	Time taken (hours)
G1	5
S	7
G2	3
M	1

- The cell is usually in interphase and G1 is most variable period. G0 state wherein cell proliferation is arrested.
- The cyclins synthesized during specific phase of cell cycle are involved in activating cyclin dependant kinases (CDK).
- Once the job is completed, the cyclins leave their activity. This is the way the cell cycle or proliferation is regulated.
- G1 to S phase is regulated by cyclin D/CDK4, cyclin D/CDK6, cyclin D/CDK2.
- S phase is regulated by cyclin D/CDK2, cyclin A/CDK1.
- G2 to M phase transistion is regulated by cyclin B/CDK1.
- Cyclin D gene sare overexprssed in many cancers so that neoplastic transformation occurs. e.g.hepatic tumour, breast cancer.
- Cell cycle is also regulated by CDK inhibitors. e.g. CDKNIA (p21), p27 and p57. The members acting on cyclin D/CDK4 and cyclin D/CDK6 are p15, CDKN2A (p16), p18 and p19.

Cell cycle checkpoints

- Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. Checkpoints prevent cell at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met. The checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints are: the G1/S checkpoint and the G2/M checkpoint. G1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point. An alternative model of the cell cycle response to DNA damage has also been proposed, known as the post replication checkpoint. p53 plays an important role in triggering the control mechanisms at both G1/S and G2/M checkpoints.

GROWTH FACTORS

Growth factors (GF) act by autocrine, paracrine, endocrine or signalling pathways. GFs play a role in the movement of inflammatory cells, in contractility of cells, differentiation and in wound healing. These are polypeptides found in the serum and/or elaborated by cells.

The main growth factors are:

- Epidermal growth factor (EGF): It is a polypeptide of 6-kDa, a progression factor which acts by combining with EGF receptors in the cell membrane. Transforming growth factor-alpha (TGF-α) is homologous to this factor. Both are mitogenic for epithelial cells and fibroblasts.

- Platelet derived growth factor (PDGF): It is stored in platelets and of 30-kDa size. PDGF may be released upon activation of platelets, macrophages, endothelium and tumour cells. It is a complement factor and requires a progression factor for its activation. PDGF is responsible for migration and proliferation of fibroblasts, macrophages and smooth muscle cells. Therefore, PGDF is important in angiogenesis.
- Fibroblast growth factor (FGF): It includes acidic and basic FGFs. These are involved in angiogenesis, cell migration and proliferation of endothelial cells. Besides, they are involved in wound repair, development and haematopoesis. Basic FGFs are found in many organs and released by activated macrophages. Acidic FGF is usually found in neural tissue.
- Transforming growth factor-beta (TGF-β): It is derived from platelets, endothelium, T cells and macrophages.It induces fibrosis by stimulating fibroblast chemotaxis, collagen and fibronectin synthesis and inhibition of collagen degradation. It is also inhibitory to most epithelial cells' growth.
- Vascular endothelial growth factor (VEGF): It promotes formation of blood vessels (Angiogenesis), also plays a role in angiogenesis of chronic inflammation and healing of wounds. Specifically, lymphatic endothelial cell proliferation induced by VEGF.
- Tumour necrosis factor-alpha (TNF-α) and Interleukin-1 (IL-1) : These cytokines play a role in fibroplasia by attracting fibroblasts and increasing collagen synthesis. TNF-α is also angiogenic in nature.

HEALING (TISSUE REPAIR)

Tissue - Proliferating potential of cell types

Labile cells - Continuously dividing cells. **e.g**. epidermis, epithelial cells, bone marrow cells

Stable cells

- Quiescent cells
- Undergoes division occasionally
- Liver, kidney pancreas, fibroblasts, endothelial cells

Permanent cells

- Non–dividing cells
- Neurons, muscle cells (cardiac, skeletal)

Hence, healing occurs by

1. Healing by regeneration
2. Healing by substitution

Depending on the proliferation potential of the cellsas described above.

Wound healing

Wound healing is not a separate process and occurs along with the inflammatory reaction. It is a complex but orderly phenomenon involving a number of processes.

Namely,

- Acute inflammatory reaction following initial injury
- Parenchymatous cellular regeneration
- Migration and production of parenchymatous and connective tissue cell
- Extracellular matrix, protein synthesis
- Remodelling of connective tissue

Healing by primary union or first intention

- This type of healing occurs in clean surgical approximated incision ie. limited bleeding and tissue destruction.
- The sequence of events occurring in primary union is given below

Clot filling the incised area
Neutrophilic infiltration
Basal cell proliferation and epithelial closure takes place by 24-48 hours
Macrophages replace neutrophils. Granulation tissue begins to appear. Collagen is arranged vertically
ncised space is filled with granulation tissue. Neovascularisation is maximal. Collagen fibre begin to appear and epith roliferation is maximal
Proliferation of fibroblast with continuous collagen accumulation producing a scar. Type III collagen is deposited earl issue and is replaced by adult type I collagen which accounts for wound strength. Newly formed blood vessels disapp
car tissue consists of granulation tissue which is devoid of inflammation covering intact epidermis.

Healing by second intention

- The wound involved shows extensive loss of cells and tissue. e.g. infarction, ulceration, abscesses, surface wound with large defects. The wound is filled with tissue debris, a few erythrocytes and bacteria. Abundant granulation tissue (soft, pink, granular appearance of wound surfaces) grows in from the margin to fill the defect but at the same time the wound contracts i.e., the defect is marked by depression and decrease from its original size. Microscopically granulation tissue consists of new capillaries, fibroblasts, collagen and proteoglycan rich ground substance. Initially granulation tissue is soft and spongy due to leaky blood vessels.

Injury – open wound – excess loss of tissue – infected – necrosis – inflammation

↓

Blood clot

↓

24 hours – neutrophils infiltrate to destroy irritant

↓

48-72 hours – macrophages and lymphocytes infiltrate

↓

Removal of necrotic and cellular debris by liquefaction by macrophages

↓

Red granules from underneath (granulation tissue) represent proliferating capillaries. Fibroblast also proliferate to fill the gap

↓

There is a definite order.

Base - capillaries grow vertically and project towards the surface. Fibroblast grows perpendicular to capillary and parallel to surface – pulling pressure of the wound

↓

Surface – fibroblasts are arranged parallel to capillaries exerting tension towards wound surface for easy closure. This arrangement differentiates granulation tissue from fibrosarcoma which lacks orderly arrangement

↓

The surface is closed by the epithelium proliferating from the margin

↓

The tissue is devoid of sweat gland, sebaceous gland, hair and hair follicles and pigment. So, the scar appears dry and unpigmented white and puckered as it becomes avascular and shrinkage of collagen

Septic wound - Healing by substitution

Exuberant granulation or proud flesh

- Sometimes the granulation continues to grow in abnormally large amount due to irritant, movement or trauma which prevents healing. This condition is called proud flesh or excess granulation tissue.

Keloid

- Keloid is another condition. Reason for its development is not known. The connective tissue below the epithelial covering continues to proliferate. This condition may recur after the removal. This is found in horses and black people having some genetic or familial predisposition.

Systemic and local factors influencing wound healing

Systemic factors

- Nutritional
  - Vitamins – vitamin C is required for collagen synthesis
  - Proteins deficiency – starvation
  - Sulphur containing amino acids (methionine and cystine) are important and required for intermediate forms of collagen
  - Zinc – as metalloenzyme, it is essential for remodelling of extracellular matrix
- Metabolic factors
  - Diabetes mellitus – delays healing
  - Hyperadrenocortism
- Circulatory stasis or adequacy of blood supply
  - Inadequate blood supply – delays healing
- Hormones – concurrent glucocorticoid therapy hinders inflammatory and reparatory process

Local factors

- Infection can delay healing
- Mechanical – movements directly affect wound healing
- Foreign bodies impede healing
- Size, location and type of wound
- Cold inhibits wound healing

Others

- Old age-Healing is slower than young ones.
- Chemotherapeutic agents
- Radiation
- Immunodeficiency

MODULE-15: PHOTOSENSITISATION AND GROWTH DISTURBANCES

Learning objective

- This module deals with autoimmune (Antibodies to its own tissues) diseases and amyloidosis.

AUTOIMMUNE DISEASES

Definition

The animal reacts to its own tissue (endogenous antigen) to incite production of antibodies or sensitized lymphocytes. There is breakage of tolerance to the self-proteins.

- Autoimmune diseases are prevented by elimination of sensitized T and B lymphocytes by the process of apoptosis in the thymus and bone marrow during development (**Central tolerance**; clonal deletion), in the peripheral tissues (**Peripheral tolerance**) and clonal anergy (**Clonal avoidance**) by defective presentation of cells.
- The tolerance of CD4+ T_H cells is critical in preventing autoimmunity. Two major autoimmune diseases are thyroiditis and haemolytic anemia. Other conditions are rare in animals.

Autoimmune thyroiditis

Causes

- Genetic predisposition (Doberman dogs)
- Autoantibodies
- Lymphocyte mediated mechanisms

Pathogenesis

- Exact mechanism is not known. There is involvement of T lymphocytes.
- Microscopically, thyroid shows interstitial lymphoplasmacytic infiltration with germinal centres. The thyroid follicular epithelial cells are destroyed by T cells in dogs, causing hypothyroidism.
- Signs: Obesity, lethargy, alopecia, hyperlipidosis and pyoderma in dogs.

Autoimmune haemolytic anaemia

- The disease is characterized by severe haemolytic anaemia and thrombocytopenia, regenerative anaemia with high reticulocyte counts. Erythrocytolysis occurs following antigen-antibody attachment to the surface membrane of erythtocytes or by removal of such cells by the splenic macrophages. There will be low haemoglobin with spherocytosis and direct Coombs test (antiglobulin) is positive.
- **Cryopathic autoimmune haemolytic anaemia** (Cold haemagglutinin disease in dogs and horses): The dog is anaemic. Anaemia is observed only when the animal is having IgM auto-antibodies or exposed to cold.
- Grossly, lesion is seen in the nose, ears and extremities in dogs.
- Microscopically, capillary stasis, agglutination and lysis of erythrocytes are seen.

Myasthenia gravis

- The autoantibodies bind to acetylcholine receptors at motor endplates resulting in progressive muscular weakness and low exercise tolerance.
- Lymphocytic infiltration in synaptic clefts occurs at a later stage interfering with release of acetylcholine and diminishing the total area of postsynaptic contents. Congenital disease occurs in Jack Russell and smooth fox terrier dogs.

Pemphigus

- It is characterized by bullae formation in the skin and mucous membrane of dogs and humans.
- Oral mucosa is affected in dogs with loss of epithelial cell coherence and acantholysis. Autoantibodies are produced against epithelial cell glycopoteins. The variant of pemphigus is known as *pemphigus foliaceous* in which painful skin disease develops in the face and ears.
- The bullae form under the stratum corneum progressing to scabs and alopecia. Footpad lesions are common e.g. Bearded collies.
- In autoimmune **pemphigoid**, antiglycocalyx antibodies are produced against keratinocytes which affect basement membrane of epithelium.

Idiopathic polyradiculoneuritis

- It is a group of diseases of inflammation of peripheral nerves, nerve roots and ganglia, characterized by mononuclear cell infiltration, axonal degeneration and axonal reactions in lower motor nerves

Idiopathic polyneuritis in dogs (Coonhound paralysis)

- There is ascending symmetrical paralysis beginning 7-14 days after scratches or bites of raccoons, progressed to tetraparesis. The animals are alert and show initial signs of

weakness to flaccid symmetric quadriplegia and may be segmental demyelination with perivenular lymphoid infiltration in the ventral nerve roots of spinal cord and some peripheral nerves.

Neuritis of the cauda equine

- Neuritis of the cauda equine (Guillain-Barre syndrome-idiopathic polyneuritis, a postinfectious paralytic disease that typically follows Influenza infection) in which segmental demyelimation is seen in spinal nerve roots of horses. Disintegration of myelin and infiltration of mononuclear phagocytes and macrophages into the sacral intradural rootlets, resulting in paralysis of tail and urinary and anal sphincters.

Systemic autoimmune diseases

- **Canine lupus erythematosus:** A rare disease in which progressive haemolytic anaemia, thrombocytopenic purpura, proteinuria and polyarthritis are seen. Renal failure causes death due to glomerulonephritis and plasma cell infiltrations.
- Thymus shows medullary lymphoid follicular development. Lymphocytic infiltration is seen around the dermal blood vessels of dogs. The anaemia is acute with severe haemolysis and positive antiglobulin (Coombs) test.
- Platelet destruction (autoantibodies to platelets) leading to thrombocytopenic purpura is manifested as haematuria, epistaxis, petechiae and ecchymoses in the skin and mucous membrane.

AMYLOIDOSIS

Amyloid (G. Amylon; Amyl(o) - STARCH) means starch-like. Amyloid is a pathologic glycoprotein deposited in the extracellular spaces and forms fibrils on polymerization.

Histological characteristics

- Amyloid is specially stained with Congo Red. Under polarized light, green birefringence is noticed because of alignment of fibrils. Amyloid fibrils are 7.5 to 10 nm in diameter, rigid, non-branching hollow-cored tubules of unknown length. β-pleated sheet configuration is seen in X-ray diffraction.
- The P-component which is a glycosa-amino-glycan (GAG) facilitates polymerization of amyloid. The GAG makes the amyloid to stain with iodine. The amyloid is resistant to enzymatic digestion and progressively accumulate in tissues until the underlying disease process persists.

Types/Sources of amyloid

- Amyloid associated (AA): It occurs in chronic diseases and septic conditions. Precursor is serum amyloid associated protein (SAA).
- Amyloid light-chain (AL): It is produced in plasmacytoma and the precursor is immunoglobulin light-chain.
- The AI occurs in pulmonary arteries and derived from apolipoprotein AI.
- IAPP is associated with pancreatic islets and derived from islet amyloid polypeptide.

In the brain of aged animals, beta amyloid protein is produced from beta

Amyloidosis

- It is an immunological disorder in which homogeneous, translucent amyloid substance is deposited between capillary endothelium and adjacent cells.

Pathogenesis

- The main event occurring in amyloidosis is the deposition of amyloid fibrils due to abnormality of protein processing.
- The sources of amyloid may be acute phase proteins, immunoglobulins and endocrine secretes.
- The abnormal variant proteins are continuously incorporated to form fibrils. The preamyloid substances are soluble and synthesised in the cytoplasm and deposited in the extracellular spaces.
- The amyloid forms a β-pleated sheet despite their chemical heterogeneity. This makes the fibril resistant to digestion by macrophages and phagocytic cells and hence accumulates in tissues.
- The fibrils may disappear following the removal of cause. Splenic active macrophages remove the amyloid fibrils, but not in the kidneys.
- The amyloid, deposited around the blood vessels is more dangerous. Pressure atrophy of the adjacent cells and ischaemic anoxia results in degeneration and necrosis. Due to interference with gaseous exchange, supply of nutritents and removal of waste products and stenotic vessels, degeneration and necrosis of cells will occur amyloid precursor protein.

Types of amyloidosis

- Primary amyloidosis
- Secondary amyloidosis

Primary amyloidosis

- It results from antigen-antibody reaction and deposition of its precipitates. The condition is not associated with any diseases e.g. repeated exposure to antigens as in antisera and antitoxin production in horses and B cell dyscrasia (plasmacytoma) in humans in which immunoglobin light chain deposition occurs.
- The soluble immunoglobulin becomes insoluble with defective degradation.

Secondary amyloidosis

- The condition may be associated with chronic diseases like tuberculosis, septic conditions and neoplasia. The serum amyloid associated proteins increase (SAA) and converted to insoluble amyloid associated substances. This occurs in two phases. In the initial preamyloid phase, there is accumulation of reticular cells and macrophages in the spleen and other lymphoid tissue with consequent rise in plasma SAAs and globulins. Probably, the cytokines, interleukin-1and interleukin - 6 from macrophages stimulate the liver to synthesize SAAs.
- During the second phase, known as amyloid phase, PAS staining cells, amyloid deposition and fall in the SAAs level are found. Animals affected are dogs, cattle, horses and chickens. Spleen, liver, kidney, lymph node and adrenals are commonly affected.

- **Grossly**, the amyloid deposition may be diffuse or focal. The amyloid is deposited around the central artery of splenic follicles and it forms sheet like deposits which is referred as **bacon spleen** and it may protrude resembling like a grain of sago known as **sago spleen**.
- The organ is waxy in consistency and the cut surface is grayish. Splenic corpuscles become large, gray and translucent. Liver is enlarged with rounded edges, doughy in consistency, pits on pressure and ruptures easily because of its friable nature.In renal amyloidosis, the organ is swollen, mottled, pale and yellow to orange in colour

Effects of amyloidosis

- Hypovolumic or haemorrhagic shock may occur following hepatic rupture. The deposition of amyloid is found between the endothelium of sinusoids and cords of hepatic cells. Hepatocellular atrophy occurs from pressure and nutritional deficiency. In renal amyloidosis, amyloid deposition occurs between capillary endothelium and epithelium of glomeruli interfering with glomerular filtration. The enlargement and ischaemic anoxia leads to tubular epithelial degeneration and necrosis, marked proteinuria, nephrotic syndrome, uremia and death
- In pancreatic amyloidosis, the deposition of amyloid is found between capillary and islet cells leading to islet cell destruction and development of Diabetes mellitus. Blindness may be encountered in horses in with conjuctival amyloid deposition.

MODULE-16: NEOPLASIA-I

Learning objective

- Neoplasia is dealt in two lessons. In this module, the learner will learn about what is neoplasia?; its characteristics, classifications, differences between benign and malignant neoplasms and causes of neoplasia.

NEOPLASM

Neoplasm (G. neo-new; plasia- development or formation)

Definitions

The simple meaning of neoplasia is new growth. Out of many definitions offered, the following definition given by Mallory (1914) is satisfactory.

“A neoplasm is a new growth of cells which

- Proliferate continuously without control
- Bearing a considerable resemblance to the healthy cells from which they arise
- Have no orderly structural arrangement
- Serve no useful function
- Have no clearly understood cause (Now a few causes of neoplasms have been identified)”.

Sastry (1986) added that neoplasm continues to grow even after the cessation of the stimuli which evoked the growth response. Tumour the term meaning swelling is currently restricted to neoplasms. The term cancer is used to indicate malignant tumours.

CLASSIFICATION OF NEOPLASM

- Tumours are classified based on histogenesis (Cell of origin) and behavioral pattern (Dangerous to life or not). Based on histogenesis, the neoplasms are classified as simple tumours (Involvement of one cell type), mixed tumours (Involves more than one cell type arising from a single germinal layer) and compound tumours (Cells arising from all germinal layers). Tumours are further classified based on behavioral pattern as benign (not ordinarily fatal) and malignant (usually fatal).
- **Histological classification of neoplasms**

	Benign	Malignant
Epithelial		
i. Epidermis	Papilloma	Squamous cell carcinoma
ii. Basal cell (Skin adnexae)	-	Basal cell carcinoma
Adnexae		
i. Hair follicle	Trichoepithelioma	Adenocarcinoma
ii. Sebaceous/Sweat/Perianal gland	Adenoma of respective gland	Adenocarcinoma
Non glandular epithelium	Papilloma	Carcinoma
Glandular surface	Polyp	Adenocarcinoma
Glandular epithelium	Adenoma	Adenocarcinoma
Mesenchymal		
i. Fibrocyte	Fibroma	Fibrosarcoma
ii. Mucoid connective tissue	Myxoma	Myxosarcoma
iii. Adipose connective tissue	Lipoma	Liposarcoma
iv. Cartilage	Chondroma	Chondrosarcoma
v. Bone	Osteoma	Osteosarcoma
Blood vessel	Angioma or haemangioma	Haemangiosarcoma
Lymph vessel	Lymphangioma	Lymphangiosarcoma

Smooth muscle	Leiomyoma	Leiomyosarcoma
Striated muscle	Rhabdomyoma	Rhabdomyosarcoma
Histiocyte	Histiocytoma	Malignant histiocytoma or histiocytic sarcoma
Mast cell	Mastocytoma	Malignant mast cell tumour or mast cell sarcoma
Haemopoietic tissue		
i. Lymphocyte	Lymphocytoma	Lymphosarcoma
ii. Plasma cell	-	Myeloma
iii. Monocyte	-	Monocytic leukemia
iv. Granulocyte	-	Myelogenous leukemia or granulocytic leukemia
v. Reticulum cells	-	Reticulum cell sarcoma
vi. Erythroblasts	-	Erythroid leukemia
vii. Myoloblast	-	Myeloid leukemia
Mesothelium		
i. Synovial membrane	Synovioma	Synovial carcinoma
ii. Meninges	Meningioma	Meningioma or invasive meningioma
iii. Bronchial epithelium	-	Bronchogenic carcinoma
Nervous tissue		
i. Astrocyte	Astrocytoma	Astrocytoma
ii. Oligodendroglia	Oligodendroglioma	Oligodendroglioma
iii. Ependyma	Ependymoma	Ependymoma
iv. Schwann cells	Schwannoma (neurilemmoma)	Neurilemmoma
v. Nerve cell	Neuroblastoma or Ganglioneuroma	Malignant neuroblastoma or Malignant ganglioneuroma
vi. Chromaffin paraganglia (adrenal medulla)	Pheochromocytoma	Malignant pheochromocytoma

vii. Non chromaffin paraganglia (Carotid body, aortic body)	Chemodectoma or Non chromaffin paraganglioma	Malignant chemodectoma or Non chromaffin paraganglioma or meduloblastoma
Others		
i. Neuroectoderm-Melanocyte	Melanoma	Malignant melanoma
ii. Renal epithelium	Renal tubular adenoma	Renal cell carcinoma
iii. Urinary tract epithelium (Transitional)	Transitional cell papilloma	Transitional cell carcinoma
iv. Placental epithelium (Trophoblast)	Hydatidiform mole	Choriocarcinoma
v. Spermatogonic epithelium (Testicular epithelium; germ cells)	Seminoma	Seminoma or Embryonal carcinoma
vi. Kidney	Nephroblastoma	Malignant nephroblastoma
vii. Islet cell	Insulinoma (β cell adenoma)	Malignant insulinoma
viii. Liver ix. Sertoli cell	Hepatoma Sertoli cell tumour	Hepatocellular carcinoma Sertoli cell tumour

Nomenclature

- The nomenclature of neoplasm has two components: an initial part (Prefix) that indicates the type of cell (Histogenesis) and the following part (Suffix) indicates the benign or malignant nature of neoplasm. All benign tumours have the suffix –oma, while malignant tumours originating from epithelial cells carry the suffix carcinoma and mesenchymal cells carry the suffix sarcoma.

S. No.	Histogenesis	Behaviour	
		Benign	Malignant
I.	Simple tumours:	-oma	-carcinoma

	Epithelial cells Mesenchymal cells Others	-oma -oma	-sarcoma -oma
I I.	Mixed tumours	Benign mixed tumour	Malignant mixed tumour
I I I.	Compound tumours	Mature teratoma	Immature teratoma

DIFFERENCE BETWEEN BENIGN AND MALIGNANT TUMOURS

S. No.	Features	Benign	Malignant
1	Occurrence of nodule or mass	Single	Single or multiple
2	Shape of nodule	Round, elliptical or wart-like and pedunculated	Irregular
3	Encapsulation	Present	Absent
4	Rate of growth	Slow	Rapid
5	Growth	Limited	Unceasing
6	Spontaneous regression	Occurs	Do not occur
7	Invasion	Absent	Present
8	Metastasis	Absent	Present
9	Basement membrane	Intact	Broken
10	Blood vessel formation	Moderate	Numerous
11	Degenerative and necrotic changes	Absent as the blood supply is adequate	Present because of inadequate blood supply
12	Recurrence	Do not recur	Recurs after apparent removal
13	Destruction of adjacent tissues	Little	Extensive
14	Cell structure	Typical to adult tissue	Not typical to that of adult tissue
15	Anaplasia	Absent, resembles cells from which they originate	Present

16	Polarity	Maintained	Lost
17	Cellular pleomorphism	Absent	Present
18	Anisokaryosis	Absent	Present
19	Number of nucleus	Not altered	Multiple (Tumour giant cell)
19	Nucleolus	No change	Enlarged, prominent and multiple
20	Nucleolar to nucleus ratio	Not altered	Increased
21	Cytoplasm to nuclear ratio	Not altered	Decreased
22	Mitosis	A few in number; Typical	Abundant, some are atypical
23	Death	Do not occur except if the tumour involves vital organs like heart, brain	Usually occurs depending on the invasion, metastasis and tissue destruction

CAUSES / ETIOLOGY OF NEOPLASMS

Predisposing causes	Definite causes
• Hereditary • Breed • Age • colour • Hormones	• Physical • Chemical • Biological

Predispoing causes

Hereditary

- Hereditary predisposition is observed for some tumours.
- Certain strains of mice are highly susceptible to mammary and liver tumours. e.g. C3H. This is due to simple recessive Mendelian factor.
    - Human e.g. - Neuroblastoma, retinoblastoma and colon, ovarian, prostate, mammary and uterine cancer.
    - Lymphoid leucosis in poultry

Age

The period of life at which cancer appears is called cancer age. The malignant tumours usually occur in old age.

Species	Cancer age
Dog	5 years
Cattle	8-10 years
Human	50 years

- Older age
    - This may be attributed to exposure to carcinogen and accumulation of somatic mutations. Epithelial neoplasms are common in old age. However some tumour occurs at young age. e.g. sarcomas
    - Congenital e.g. nephroblastoma

Colour (Pigmentation)

- Melanin pigment produced by melanocyte protects skin against UV rays of sun. Hence, lack of pigmentation may lead to occurrence of tumours.
- eg. Grey and white horses - malignant melanoma (especially old age); Hereford cattle - ocular squamous cell carcinoma

Hormones

- Hormones like estrogen and progesterone may play a role to predispose animals to cancer. e.g. Estrogen - Mammary tumour, ovarian carcinoma. Progesterone - Mammary tumour in dogs ans cats.

Mammary tumour - Dog - Primary tumour

Definite causes

Physical

- Solar radiation- cutaneous tumours

- It is associated with areas where sunlight is intense, light skinned animals and exposure of the area.
- UV radiation (UVB 280-320 nm) causes pyrimidine dimers injuring DNA causing mutation and tumours.
- **Xeroderma pigmentosum** - a genetic disease of human in which enzymes required for DNA repair are lacking, hence exposure to UV ray of sunlight results in dry pigmented skin. Whole body radiation can cause leukaemia
- Radiation includes elctromagnetic radiation (UV rays, X rays and gamma radiation and particulate radiation (α, β, proton and neutrons) which are carcinogens.
- X ray - skin tumour
- I^{131} - thyroid adenoma
- Radium - osteosarcoma and leukaemia (painters of watches and clocks)

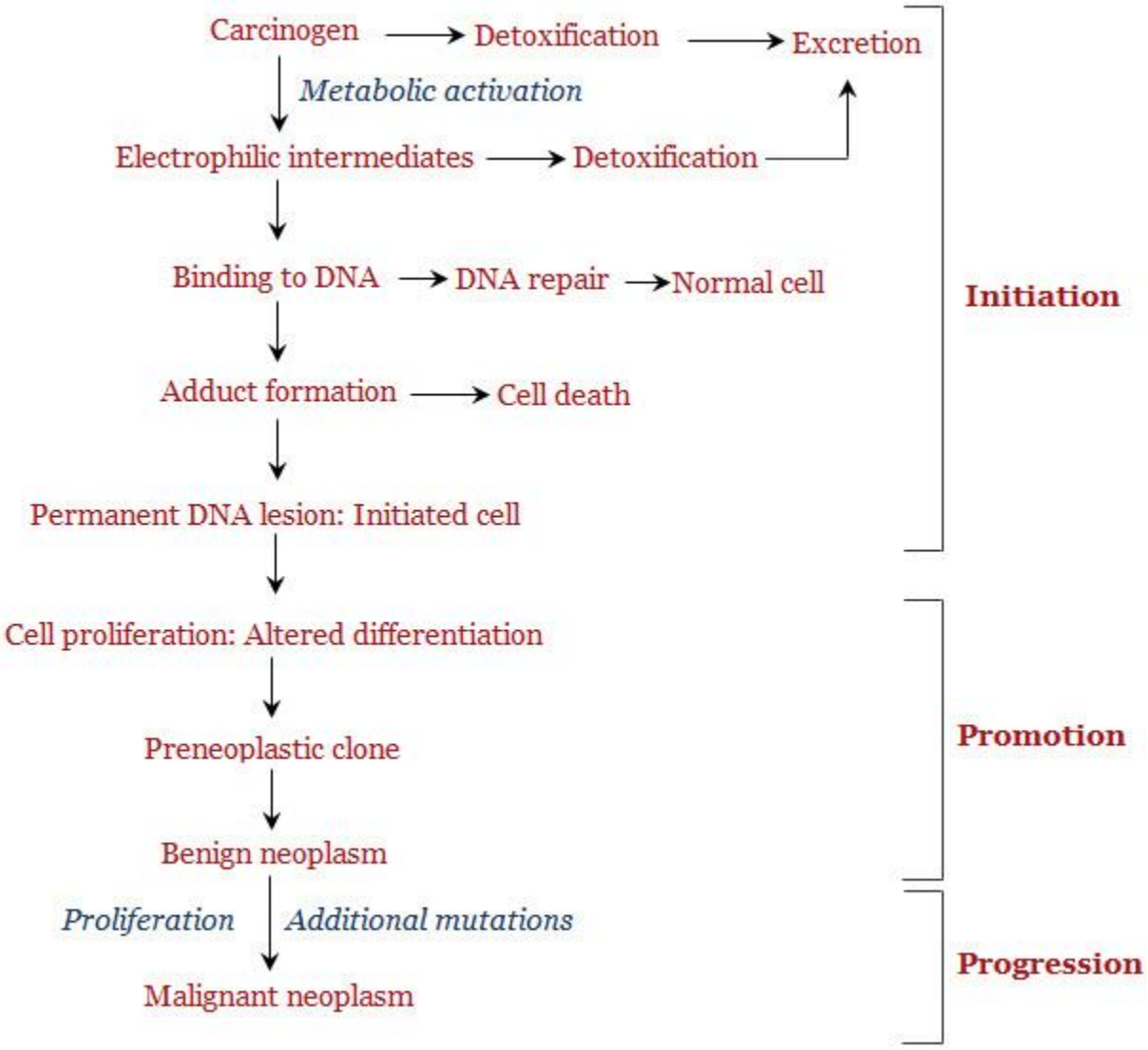
Chemicals

Sir Percival Pott (1775) was the first scientist to identify chemical agent to cause of cancer. In 1915, Yamagiwa and Itchikawa produced cancer in rabbit ears with repeated application of coal tar i.e. experimental carcinogenesis. Kenneway and Cook purified the carcinogen 3, 4 - benzapyrene from crude tar. Other potent chemical carcinogens are benzanthracene, methylcholanthracene (Chlorinated hydrocarbons).

Examples of major chemical carcinogens

- **Direct acting**
  - Alkylating agents - β propiolactone, Dimethylsulfoxide
  - Aceylating agents - 1 acetyl imidasone
- **Indirect acting or procarcinogen** - It requires metabolic conversion to become ultimate carcinogen to induce cancer.
  - Polycyclic and heterocyclic hydrocarbons - benzanthracene
  - Nitrosoamines and nitrosoamides - vinyl chloride, aldrin, dieldrin

Mechanism of chemical carcinogenesis



Initiation promotion model

S.No.	Initiator	Promoter	Tumour produced
1	Aflatoxin B1	Methyl sterculate	Hepatocellular carcinoma in trout
2	Benzapyrene	Croton oil	Squamous cell carcinoma inn mouse skin

Biological causes

- **Bacteria**: *Helicobacter pylori* – gastric cancer and lymphoma in man; *Helicobacter hepaticus* – hepatocellular carcinoma in mice
- **Parasites**
  - *Spirocera lupi* - Oesophageal fibrosarcoma and osteosarcoma in dogs

    - *Cysticercus fasciolaris* – fibrosarcoma in rat liver
    - *Eimeria stiedae* – bile duct tumour in rabbits
    - *Schistosoma haematobium* – bladder cancer in man
- **Viruses**
    - **DNA viruses** – Papova, Shope papilloma, canine oral papilloma, bovine papilloma, human papilloma
        - Pox viruses – fibroma, myxoma in rabbit
        - Herpes virus – Marek's disease chicken
    - **Oncogenic RNA viruses**
        - Retroviruses – Lymphoid leucosis
        - Rous sarcoma virus – Tumours in poultry

Ellerman and Bang (1908) were the first to demonstrate viral carcinogenesis and later by Rous. Peyton Rous (1910) produced similar results with fowl sarcomas. Gross (1953) induced leukaemia with cell free filtrate in mice.

MODULE-17: NEOPLASIA-II

Learning objective

- In this module, the learner will learn about spread of neoplasms, tumour and immunity, clinical effects of neoplasia, diagnosis and stages and grades of neoplasia.

SPREAD OF NEOPLASM

The neoplasm spreads by

- Invasion
- Metastasis

These are hall marks of malignant tumour.

Metastasis can occur by

- Implantation
- Haematogenous spread
- Lymphatic spread

The invasion and metastasis are characteristic features of malignant neoplasm. Invasion is defined as movement of neoplasm directly through tissue planes. Implantation is establishment of neoplasm on new surfaces especially body cavities. Metastasis is defined as spread of neoplasm from primary to a distant site. Invasion of neoplasm into an adjacent tissue is facilitated by breaking of basement membrane by proteolysis (collagenases) and migration through interstitial tissue through the help of proteolytic enzymes or proteases. Increased negative charges on plasma membranes, decreased calcium ion content and lack of cohesiveness facilitate the process of invasion.

Infiltration of neighbouring tissues: The malignant tumour infiltrates and invades the adjacent tissues because of rapid multiplication of cells, neoplastic cell motility (amoeboid movement of

fibroblast due to lack of contact inhibition and cohesiveness) and accumulation of metabolites (e.g. Lactic acid) and enzymes like hyaluronidase which hydrolyse cementing substance.

Infiltration

- Infiltration into tissue spaces
    - Invasion depends upon the type of tissue. Soft and loose tissue can be infiltrated easily while it is difficult to infiltrate hard tissues.
- Intracellular infiltration
    - Tumour cells can also traverse cell. e.g. Penetrate muscle fibres

Lymphatic spread

- This occurs by emboli formed by clumping of neoplastic cells. Permeation can also occur wherein the tumour cells extend along lymphatics by growing along endothelium. Neoplastic cells reach regional lymph nodes and are trapped in the cortical sinuses and following proliferation of cells lead to secondary tumours. Carcinomas spread by lymphatics.

Blood spread

- Neoplastic cells frequently invade veins and capillaries. Tumour emboli involving portal vein induces tumour in the liver and those spread through systemic vein produce metastases in lungs.

Transcoelomic spread (Spread in body cavities)

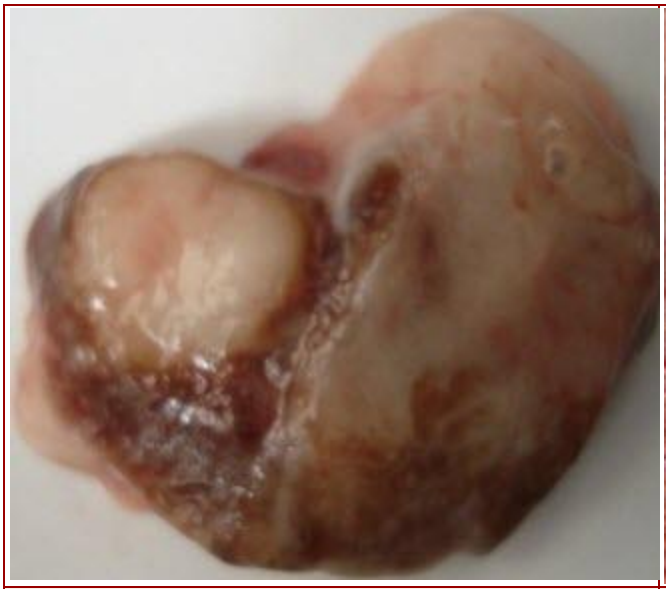
- In implantation, the lack of cohesiveness of neoplastic cells favours implantation into the surrounding body cavities i.e. transcoelomic spread or soil theory or seeding into pericardial, pleural, peritoneal and subarachnoid membranes. e.g. Cancer of ovary and stomach

Implantation

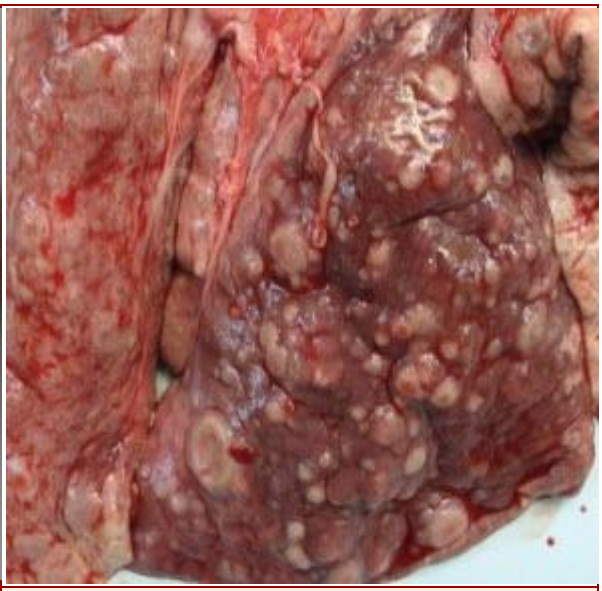
- By natural passages - In hollow organs, the tumour cell casts get and implanted. e.g. Tumour of renal pelvis get washed down in bladder and implanted to form tumours.
- Inoculation - rare hazard in surgery where tumour cell can be implanted in edges of the wound and new tumour develops.
- Coitus - venereal tumour of dogs gets transmitted by this way.

Spread by nerves

- This occurs by permeation through perineural lymphatics with degeneration of nerves.



Mammary tumor - Dog - secondary tumour - Lymph node metastasis



Mammary Tumour - Dog - Metastasis - Lung

Mechanism of invasion and spread

The spread of tumour is divided into two phases.

1. Invasion of extracellular matrix
2. Vascular dissemination and homing of cells

Invasion of extracellular matrix

Extracellular matrix is divided into two types

- Basement membrane
- Interstitial connective tissue

Extracellular matrix is composed of collagen, glycoproteins and proteoglycans. Invasion of extracellular matrix by tumour cell is an active process involving

- Detachment of tumour cells from each other
- Attachment of tumour cells to matrix
- Degeneration of extracellular matrix
- Migration of tumour cells

Detachment of tumour cells from each other which occur due to loosening of tumour cells which lack adhesion molecules. e.g. E-cadherin. Attachment of tumour cells to matrix by proteins like laminin and fibronectin through the receptors. Normal epithelial cells have receptors for basement membrane laminin on basal surface while carcinoma cells have many more receptors. Degeneration of extracellular matrix occurs due to proteolytic enzymes elaborated by tumour cells. Migration of tumour cells: The locomotion of tumour cell is by amoeboid movement by throwing pseudopodia through the degraded basement membrane.

Vascular dissemination and homing of cells

- Once in the circulation those tumour cells which survive host immunity by binding with circulating lymphocytes and platelets adhere to vascular endothelium and exits through basement membrane. Site of metastasis depends on location of primary tumour and its vascular and lymphatic drainage and organ tropism depends on cellular attraction, etc. e.g. Lung cancer spreads to adrenals and do not affect skeletal muscle. This phenomenon is called homing of tumours.

Spread of tumours

Clonal expansion, growth, diversification, angiogenesis

↓

Metastatic subclone

↓

Adhesion to and invasion of basement membrane

↓

Passage through extracellular matrix

↓

Intravasation

↓

Interaction with host lymphoid cells

↓

Tumour cell embolus

↓

Adhesion to the endothelium

↓

Breaking the basement membrane

↓

Extravasation

↓

Metastatic deposit

↓

Angiogenesis

↓

Growth

TUMOUR IMMUNITY

- The genetic alteration that occurs during malignant transformation may result in expression of proteins that are regarded as non-self of foreign by the immune system. The immune surveillance mechanism recognises and destroys non-self tumour cells.

Tumour antigens

- The tumour cells may differ antigenically from normal cells and can either gain or lose cell membrane molecules.
- They are of two types
    1. Tumour specific antigen (TSA), present only on tumour cells and not on any other cells
    2. Tumour associated antigen (TAA), present on tumour and also some normal cells

Tumour specific antigens are found in chemically induced tumour of rodents which express unique antigen not shared by other histologically identical tumour induced by the same chemical even in the same animal. Tumour specific antigen is an altered form of normal protein occurring due to mutation of gene. Each mutated protein combines with MHC class I protein to become an antigen. These are recognised by CD8+ cytotoxic T lymphocytes.

Tumour associated antigen are not specific to individual tumour and shared by similar tumour in other animal.

Two types of tumour associated antigen (TAA)

1. **Oncofetal antigen -** Embryonic antigens which are normally expressed in developing embryos. e.g. Alpha fetoprotein, Carcinoembryonic antigen (CEA)
2. **Differentiation antigens** are peculiar to different stage in which cancer cells are arrested and useful differentiator marker in diagnosis of cancer. e.g. Prostatic and lymphoid tumour in man

Since tumour associated antigens are normal self protein they do not evoke immune response but of value in diagnosis of certain and immune therapy.

Anti-tumour effector mechanism

Both cell mediated immunity and hormonal immunity (activation of complement, ADCC) have anti-tumour activities.

- Cytotoxic T lymphocytes (CD8+ T cells)
    - Cytotoxic T lymphocytes are important in chemically induced tumours. It plays a protective role in virus associated neoplasms. The cells destroy the tumour cells by recognising MHC class I antigen expressed on tumour cells.
- Natural killers cells (NK cells)
    - These cells can destroy tumour cells without prior sensitization thereby provides first line of defence against tumour cells. After activation with interleukin 2, natural killer cells can destroy a wide range of animal and human tumours.
- Macrophages
    - Activated macrophages show selective cytotoxicity against tumour cells. T cells, NK cells and macrophages may work together in anti-tumour activity. γ interferon, a cytokine secreted by T cells and NK cells, is a potent activator of macrophage. These cells kill the tumour cells through reactive oxygen species or secretion of tumour necrosis factor (TNF).
- Antibody dependant cellular cytotoxicity (ADCC)
    - It is involving killing those cells that bear receptor for Fc portion of IgG. Target cell coated by antibody are destroyed without phagocytosis or complement fixation. ADCC may be mediated by neutrophils, eosinophils, macrophages and NK cells.

Immunosuppression

- Many oncogenic substances suppress host immune response (chemicals, ionising radiation) and tumours or tumour products e.g. TGF β, potent immunosuppressor

Evasion of immune system (Immunosurveillance)

This may occur through different mechanisms.

- Non expression of new antigens that are immunogenic
- Failure to express host immune stimulatory molecules required for activation of T-cells
- Lack or poor expression of MHC antigen by tumour cells
- Overwhelming the immune system and rapid proliferation of malignant cells or too small tumour cells in initial stage to evoke immune response
- Secretion of immunosuppression molecules
- Expression of death inducing ligands (Fas L, CD95 L)
- Inactivation or mutation of tumour suppressor and apototic genes. e.g. p53, BCL - 2

EFFECTS OF NEOPLASIA

The effects of neoplasia primarily may be due to the size, location and tissue of origin and secondarily due to spread to other organs.

- **Pressure atrophy** - The expanding tumour may cause pressure atrophy of some organs especially through pressure on blood and lymphatic vessels thereby interfering with nutrition and fluid exchanges to tissues.
- **Location** - The tumour may cause obstruction of luminal organs by narrowing luminal space interfering with functional activity.
    - Obstruction Effect
        - Ureter Hydronephrosis
        - Bronchus Collapse of lung
        - Intestine Intussusception
- **Tissue of origin** - If the tumour involves vital organs like heart and brain, it causes death.
- **Cancer cachexia** - It is due to loss of body fat and wasting besides profound weakness. The TNFα plays a role on suppressing the appetite and inhibition of action of lipoprotein. This will lead to excessive protein degradation and negative nitrogen balance.
- **Infection** - Surface tumours may be ulcerated and subsequently infected.
- **Exudate in serous cavities** – Tumour cells deposited on serous membranes incites an inflammatory response with exudation. Eg. Malignant ascites.
- **Hormonal effects**
    - Parathyroid tumour – Osteoporosis and big head in horses
    - Tumour of sertoli cells – Feminization
    - Hypoglycaemia - Insulinoma
    - Arrhenoblastoma in female - Masculinisation
- **Anaemia** may be due to decreased bone marrow response, haemorrhages and haemolysis
- **Thrombocytopenia** may also occur.
- **Monoclonal gammopathies** occur in plasma cell tumours.
- **Paraneoplastic syndrome** the symptoms that are not directly related to spread of tumour or elaboration of hormones indigenous to the tissue from which tumours arise.
    - Ectopic hormone production or syndrome
        - The production of hormones by the neoplastic cells which are not of endocrine origin.
        - e.g. Lung cancer - ACTH production
        - Fibrosarcoma - insulin production
- **Hypercalcaemia** occurs when neoplastic cell synthesises and secretion of peptides that mimick parathyroid hormones or tumour affecting producing humoral factors and stimulating osteoclasts. e.g. Lymphoma in dogs and cats.

DIAGNOSIS OF CANCER

Early diagnosis of cancer will help in treatment by therapy or surgical intervention.

- Clinical diagnosis
    - Based on the gross features (Papilloma, cystic, fibrotic, nodular tumour)
    - Any nonhealing growth or lesion and growth of profusely bleeding nature are to be suspected for possible cancer.
- Biopsy (histopathology)
    - Reliable method by which diagnosis can be made based on cellular characteristics (microscopically - anaplasia, invasion, mitosis, metastasis, loss of polarity) which indicate malignancy (Also see staging and grading)
- Radiology

    - Radiological examination of viscera and bone may show primary or secondary lesions. However, this is much applicable in small animals and of limited use in veterinary practice.
- Cytology
    - It is examination of cells which can be applied in diagnosing cancer. Cells can be collected most commonly through fine needle aspiration biopsy. Other methods are impression, scraping and brushing. This can be stained by Romanowsky's stains and haematoxylin and eosin stain. Cellular characteristics for malignancy have to be seen.
    - Acridine orange staining - The cytoplasm of malignant cells will show brick red fluorescence and nucleus will show apple green fluorescence.
- Exfoliative cytology
    - Neoplastic cells show loss of cohesiveness and those arising on the surface are easily detached and exfoliated. These cells can be collected and suitably stained for making a diagnosis. This technique is used in human medicine for early diagnosis of cervical, uterine and bronchogenic tumours. Pappanicolaou is considered as father of cytology. This test is known as papa test.
- Chemical and serological tests
    - No such reliable tests are available in veterinary practice. However, in human medicine chemical/enzyme tests are available to diagnose prostatic cancer and bone cancer.
- Molecular methods
    - Polymerase chain reaction (PCR) will be helpful in differentiating monoclonal tumours.
- Flow cytometry
    - This can identify cell population. e.g. immunophenotyping of lymphocytes
- Immunohistochemistry method
    - Immunohistochemistry can be used to identify the type of cell (epithelial cell-cytokeratin marker) and malignancy of tumours.
    - DNA probe analysis, northern, southern and western blot analysis can be used.

Tumour markers

- This is biochemical indicator to identify the presence of tumours. This may be cell surface proteins, cytoplasmic proteins, enzymes and hormones.

Uses

- To confirm diagnosis
- To determine response to therapy
- To indicate relapse after treatment

Markers	Associated tumours
Oncofetal proteins	
Alpha fetoprotein	Hepatocellular carcinoma Germ cell tumour of testes

Carcinoembryonic antigen	Carcinoma of colon, pancreas and stomach
Hormones	
Calcitonin	Thyroid medullary carcinoma
Catecholamines	Pheochromocytoma
Isoenzymes	
Prostatic acid isophosphatase	Prostatic tumour (human)
Specific protein	
Immunoglobulins	Multiple myeloma
Mucin	
CA 125	Ovarian tumour

GRADING AND STAGING OF CANCERS

Based on the extent of malignant features like cellular characters (differentiation and anaplasia), invasion, metastasis and number of mitoses, tumours can be classified as grade I, II, III, IV (grade I for the least and grade IV for the most anaplastic)

Clinical staging of cancer (TNM classification)

This is based on

- Size of the primary tumour -T
- Extent of spread to regional lymph node -N
- Presence or absence of metastasis -M

Clinical staging should be combined with histological analysis such as grading of tumours which is helpful in prediction of survival of cancer patients.

Primary tumour

- T0 – no evidence of tumour
- T1 – tumour confined to primary site
- T2 – tumour invades adjacent tissues

Lymph nodes

- N0 – no evidence of tumour
- N1 – regional lymph node involvement
- N2 – distant lymph node involvement

Metastases

- M0 – no evidence of tumour

- M1 – tumour in same organ or cavity as primary
- M2 – distant metastases