MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

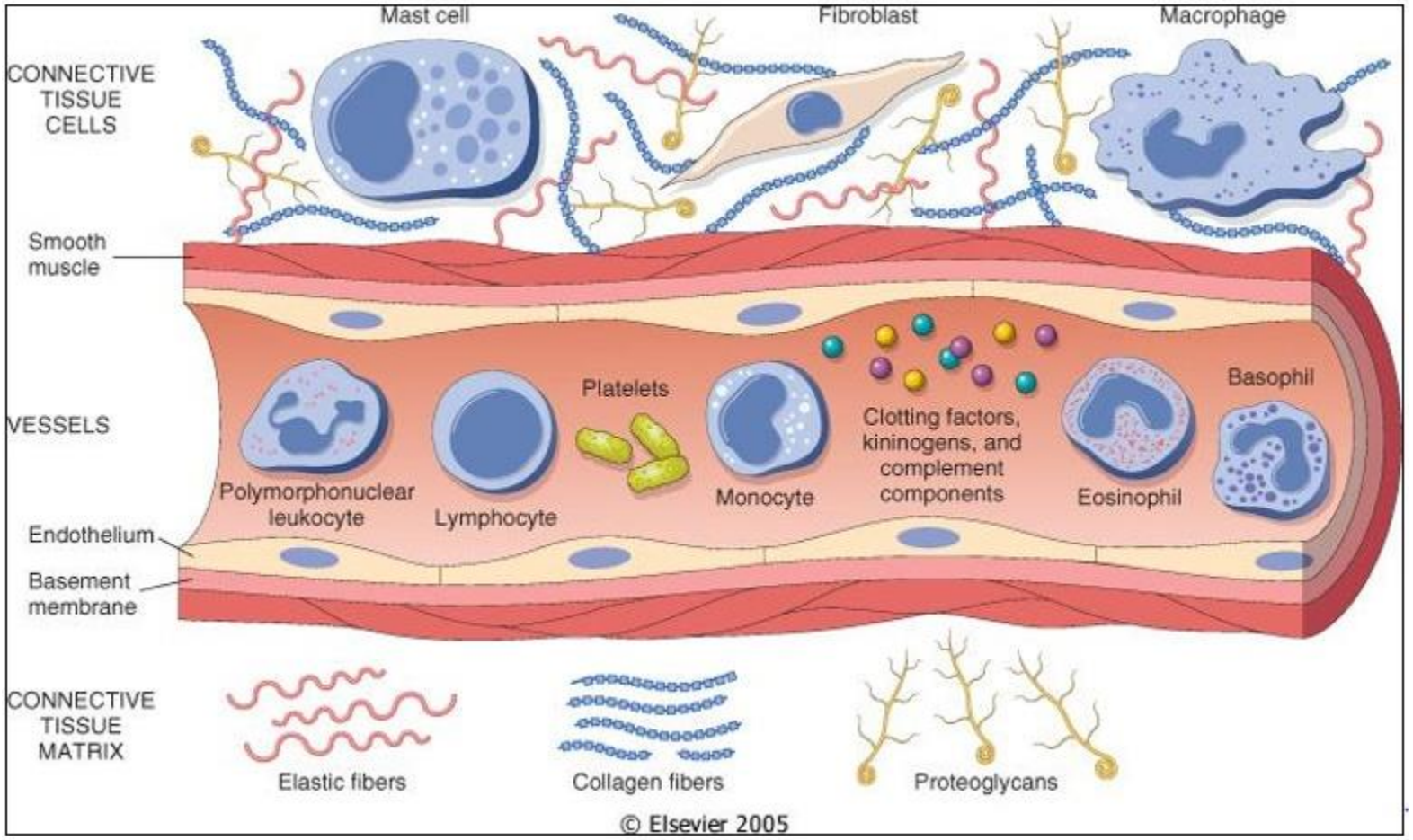
DEPARTMENT OF VETERINARY PATHOLOGY

Inflammation part-1

Acute Inflammation

Primary function:

- Move defense mechanisms out of vasculature to tissues
- Initiate repair



Sequence of Vascular Events

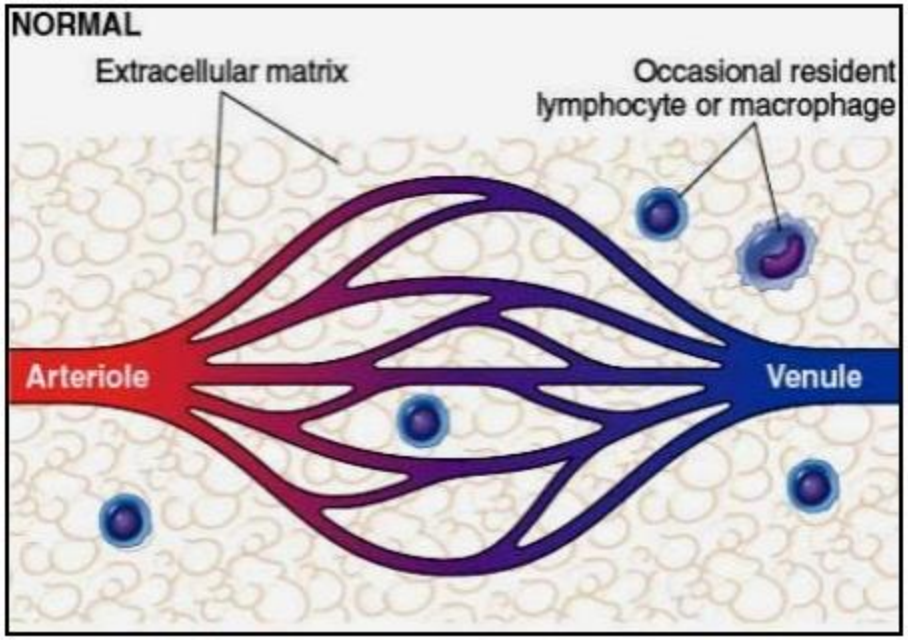
- Julius Cohnheim – first described vascular changes in 1877.

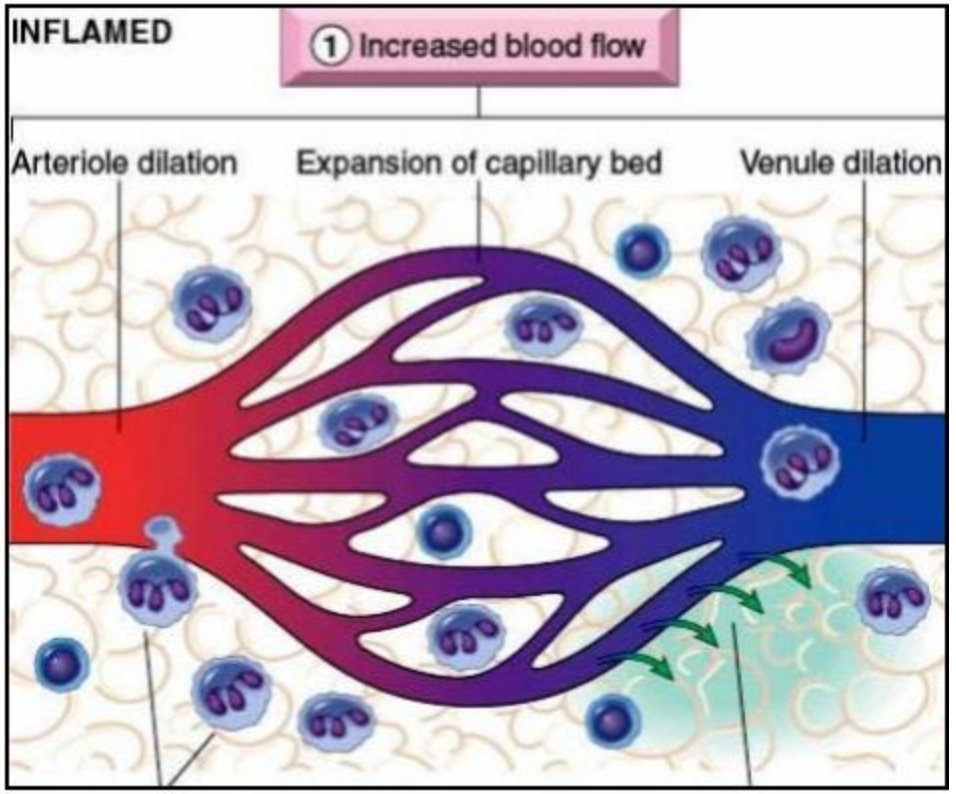
1. Changes in the Blood Vessels
2. Changes in the Rate of Flow
3. Changes in the Bloodstream
4. Exudation of Plasma
5. Emigration of Leukocytes
6. Diapedesis of Erythrocytes

Sequence of Vascular Events

1. Changes in the Blood Vessels

- (a) Momentary constriction:
- (b) Vasodilation (esp Histamine / NO / PG): (first arterioles, then capillaries and post capillary venules)





Sequence of Vascular Events

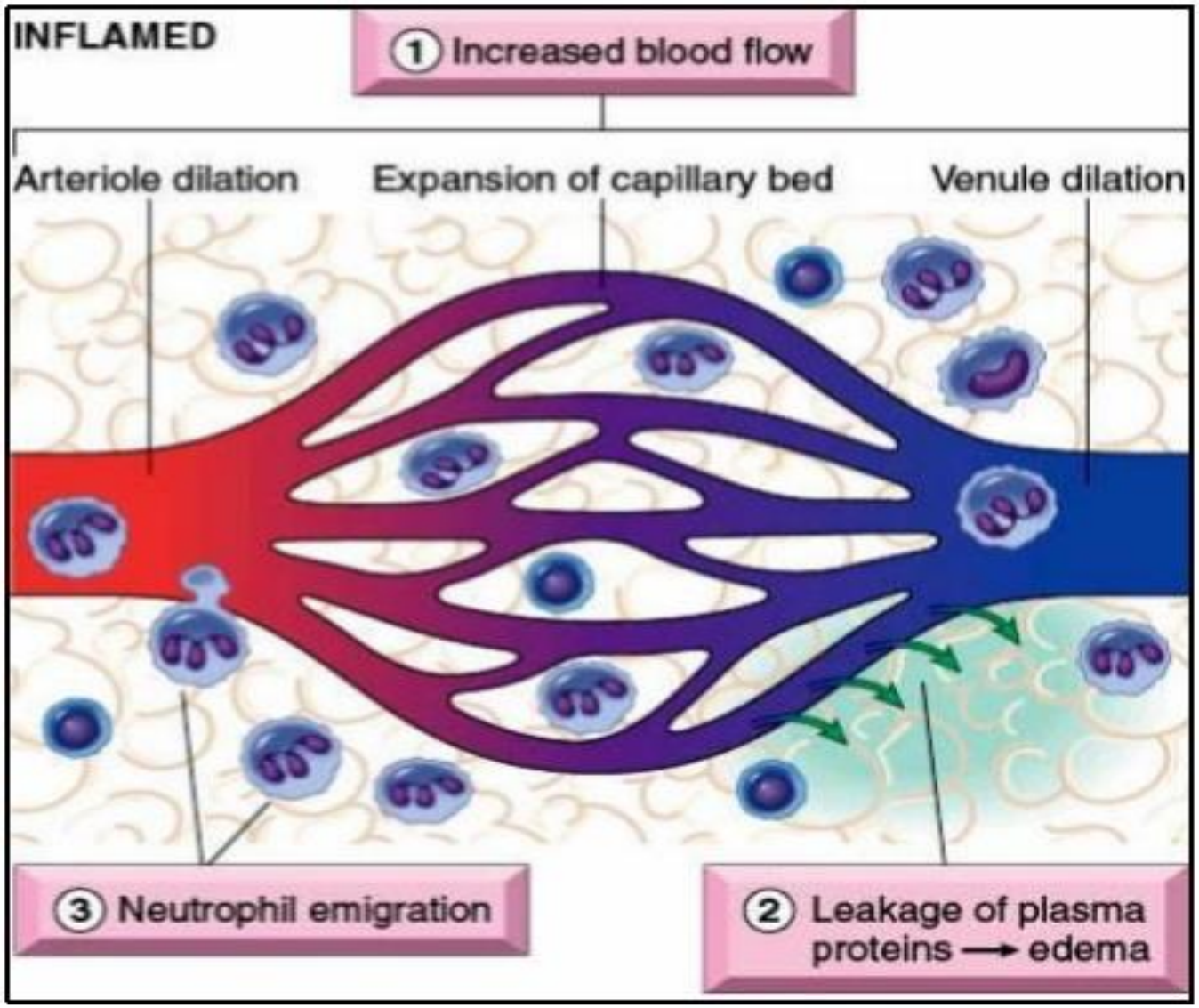
2. Changes in the Rate of Flow
   - Early vasodilation results in increased blood flow, soon followed by slowing of the circulation.
   - Increased Vascular Permeability- slowing of the circulation
- Oedema formation
- Concentration of red cells in small vessels and increased viscosity of the blood
- Stasis

Sequence of Vascular Events

(i) Increased vascular permeability (vascular leakage):

- hallmark of acute inflammation
- normal fluid exchange depends on intact endothelium
- increase of extravascular fluid is called inflammatory oedema.

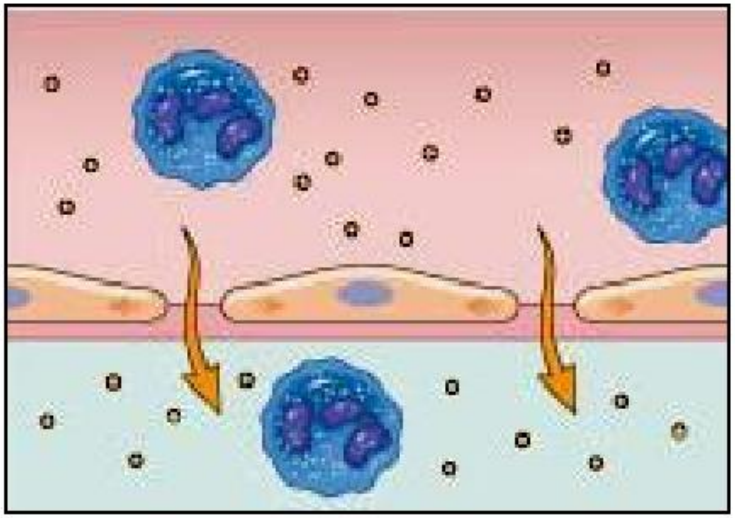
Sequence of Vascular Events



Sequence of Vascular Events

- **Mechanisms of increased Vascular Permeability –**
- **1. Retraction of endothelial cells (gaps) in venules**
- a) Endothelial cell contraction
- immediate and transient (15-30 min)
- mediator binds to receptor → contraction
- affects only venules

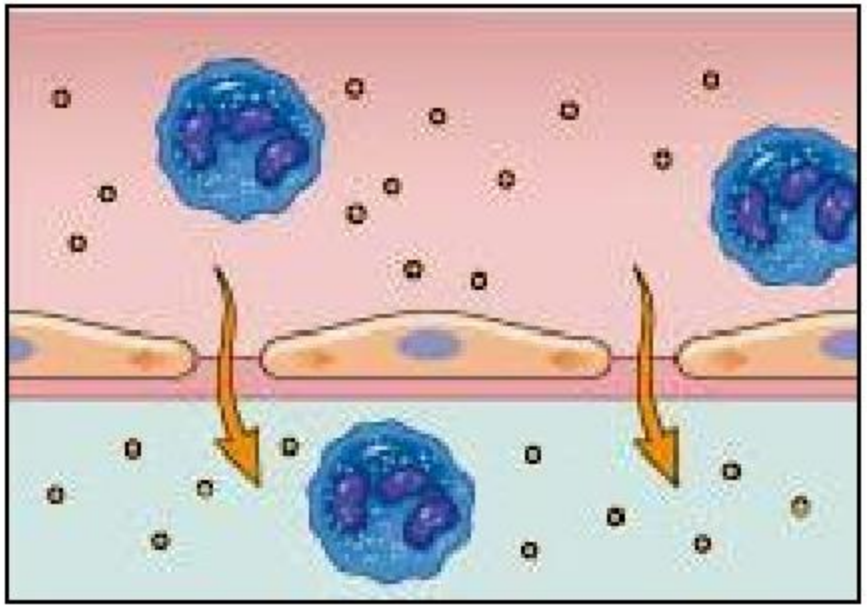
Histamine, Bradykinin, Leukotrienes, etc



Sequence of Vascular Events

- b) Delayed prolonged leakage
- some mild injuries cause vascular leakage
- begins after a delay of 2 to 12 hrs, but lasts for several hours to days
- see endothelial cell contraction &/or mild degeneration

mild burns, UV irradiation

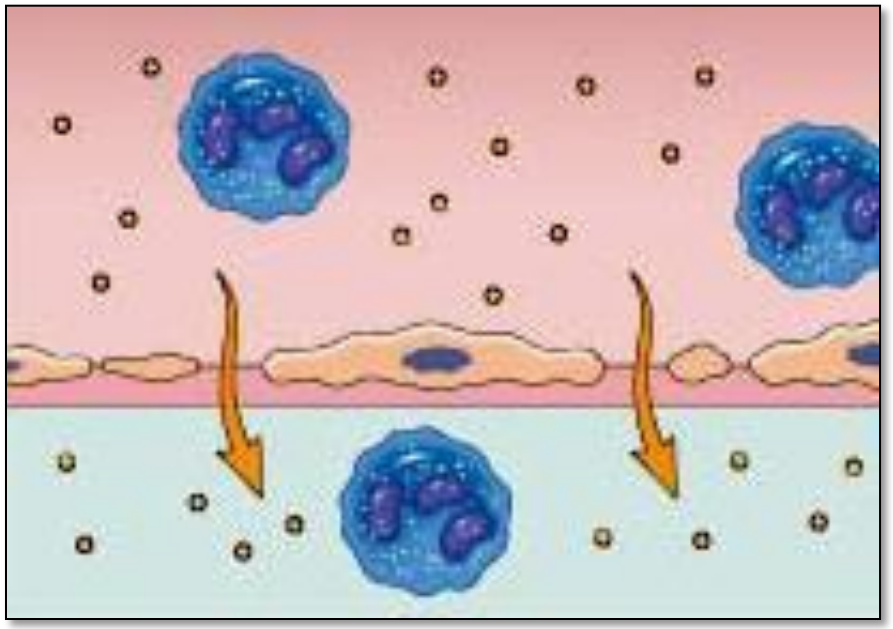


Sequence of Vascular Events

2. Direct endothelial injury

- arterioles, venules and capillaries affected
- direct damage due to severe injurious stimuli
- immediate sustained response, lasts hrs to days until damaged repaired

severe burns, bacterial toxins, viruses, etc

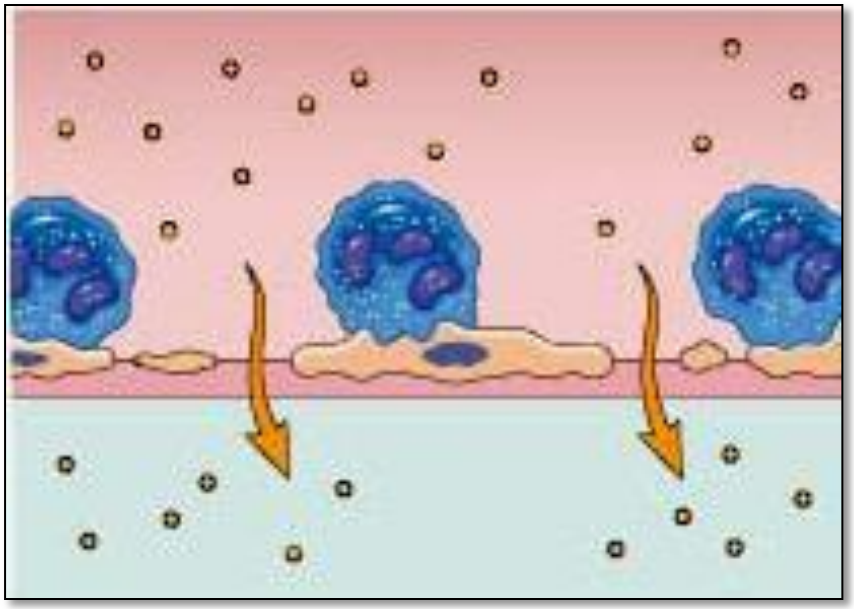


Sequence of Vascular Events

3. Leukocyte Dependent Endothelial Injury

- neutrophils that adhere to the endothelium during inflammation may also injure the endothelial cells and thus amplify the reaction.
- associated with the later stages of inflammation.
- long-lived, lasts hrs to days until damaged repaired

Reactive O2 + proteolytic enzymes released from adhered neutrophils

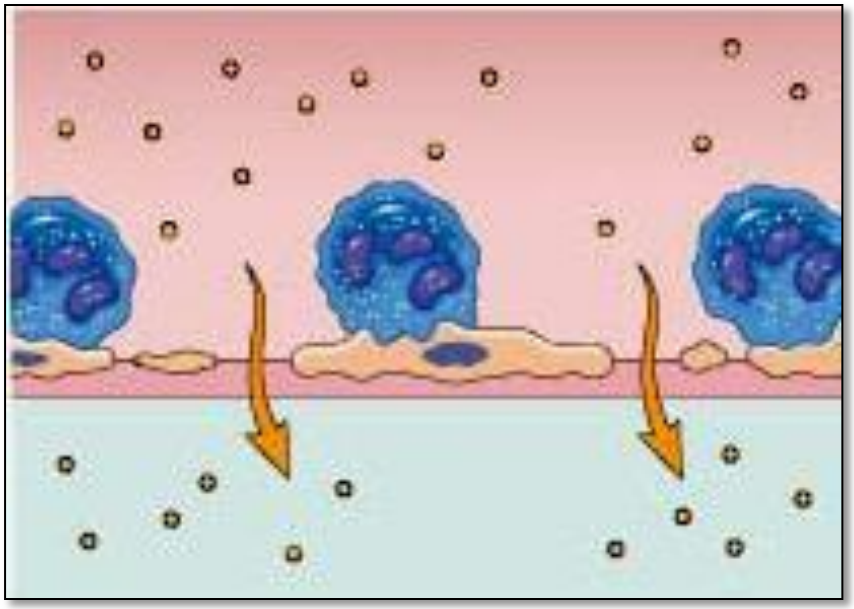


Sequence of Vascular Events

4. Increased Transcytosis (active transport mechanism)

- normally some transport by channels of vesicles & vacuoles (vesiculovacuolar organelles).
- certain factors can increase the number and size of these channels.

VEGF, histamine(?)

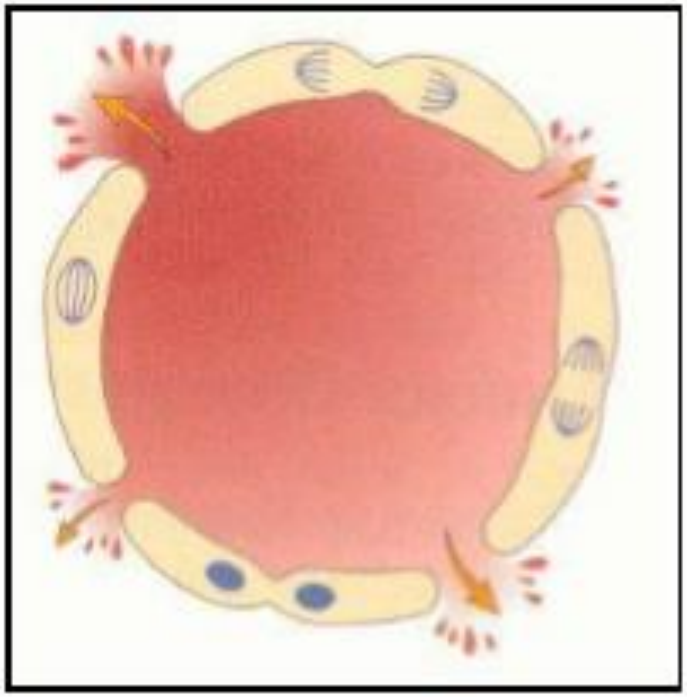


Sequence of Vascular Events

5. Leakage from New Capillaries

- during the repair process, proliferating endothelial cells are leaky
- mediated by VEGF (vascular endothelial growth factor)

VEGF



Sequence of Vascular Events

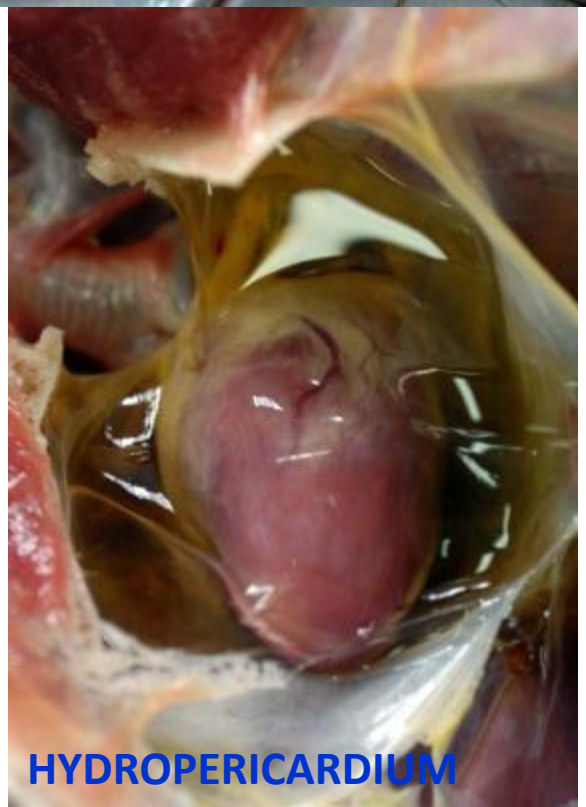
Increased Vascular Permeability - Results

- **Transudate**
- –fluid
- –low protein, few cells
- –non-infectious
- ↑ Hydrostatic pressure in capillaries: more dependant on venous pressure than arterial pressure
- ↓ Oncotic pressure in capillaries: Hypoalbuminemia - Lymphatic obstruction
- **Exudate**
- –fluid + cells
- –high protein, many cells
- –infectious
- Vascular damage & leakage

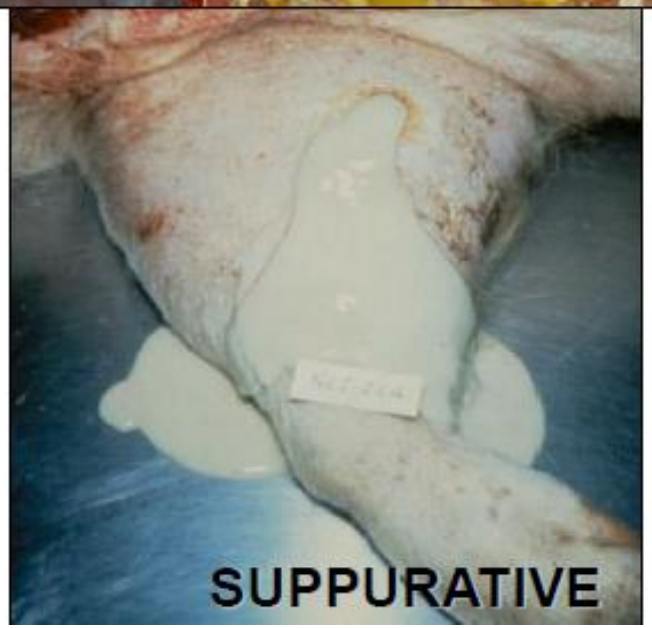
Transudate vs Exudate

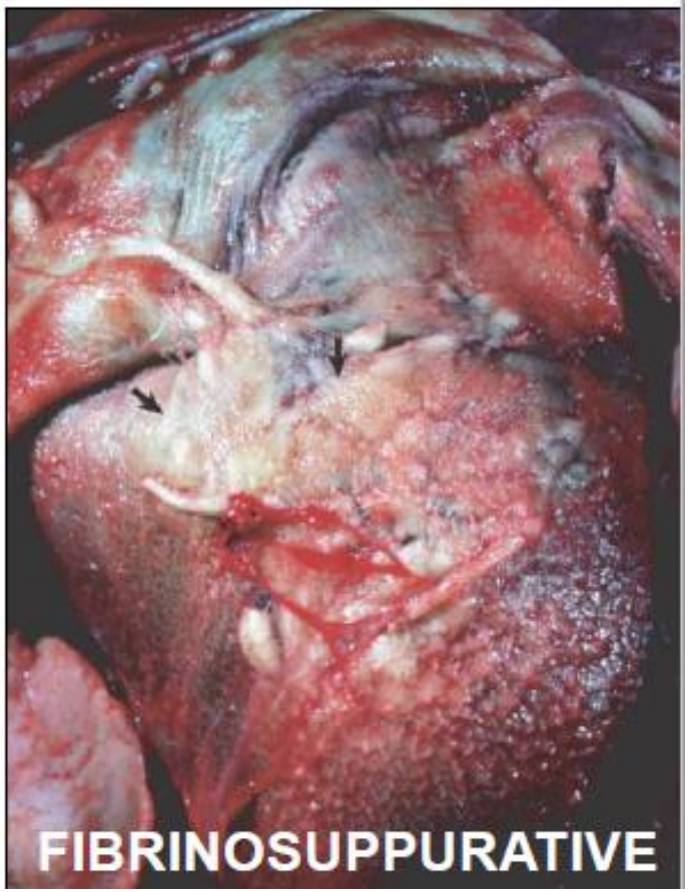
	Exudate	Modified Transudate	Transudate
Definition	Inflammatory		Non-inflammatory
Etiology	Inflammation / Infection	Long-standing transudates or ↑ Vasc. perm.	Non-inflammatory edema
Specific Gravity	>1.025	1.017-1.025	<1.017
Protein Content	> 30 g/L	25-75 g/L	< 25 g/L
Clots?	Often	Varies	Rarely
Inflam. Cells	Many (>5,000-7,000 cell/µL)	Few (1,000-7,000 cell/µL)	Occasional (<1,500/µL)
Bacteria	Often		Rare

TRANSUDATES



EXUDATES





Sequence of Vascular Events

(ii) Slowing of the circulation: essential for emigration of the leukocytes

- (i) by increasing the capillary bed in the area.
- (ii) by swelling of the endothelial cells lining the capillaries.
- (iii) haemoconcentration
- (iv) margination of the leukocytes

Sequence of Vascular Events

(iii) Stasis:

- markedly reduce the blood flow
- situation is ideal for the escape of molecular and cellular elements essential for the formation of inflammatory exudate.

Sequence of Vascular Events

3. Changes in the Bloodstream

- main change consists of a redistribution of the cellular elements of the bloodstream.
- two distinct zones
  1) **axial stream**:- In centre- cellular elements (erythrocytes and leukocytes) - centripetal force
  2) **plasmatic stream**:- mainly of plasma - centrifugal force
- As the blood flow slows, the centripetal force of the bloodstream is overcome by the centrifugal force and the leukocytes fall out of the axial stream.

Sequence of Vascular Events

- Leukocytes better opportunity to interact with the lining endothelial cells.
- This process of leukocyte adhesion at the periphery of vessels is called margination. Afterwards, leukocytes tumble (roll over and over) slowly along the endothelial surface and adhere transiently.
- This process of brief, loose sticking of leukocytes to the endothelium is called **rolling**.
- Finally, leukocytes come to rest at some point where they adhere firmly.
- Firm sticking of leukocytes to the endothelium is called **adhesion**.
- The endothelium is virtually lined by white cells. This appearance is called **pavementing.**

Sequence of Vascular Events

4. Exudation of Plasma

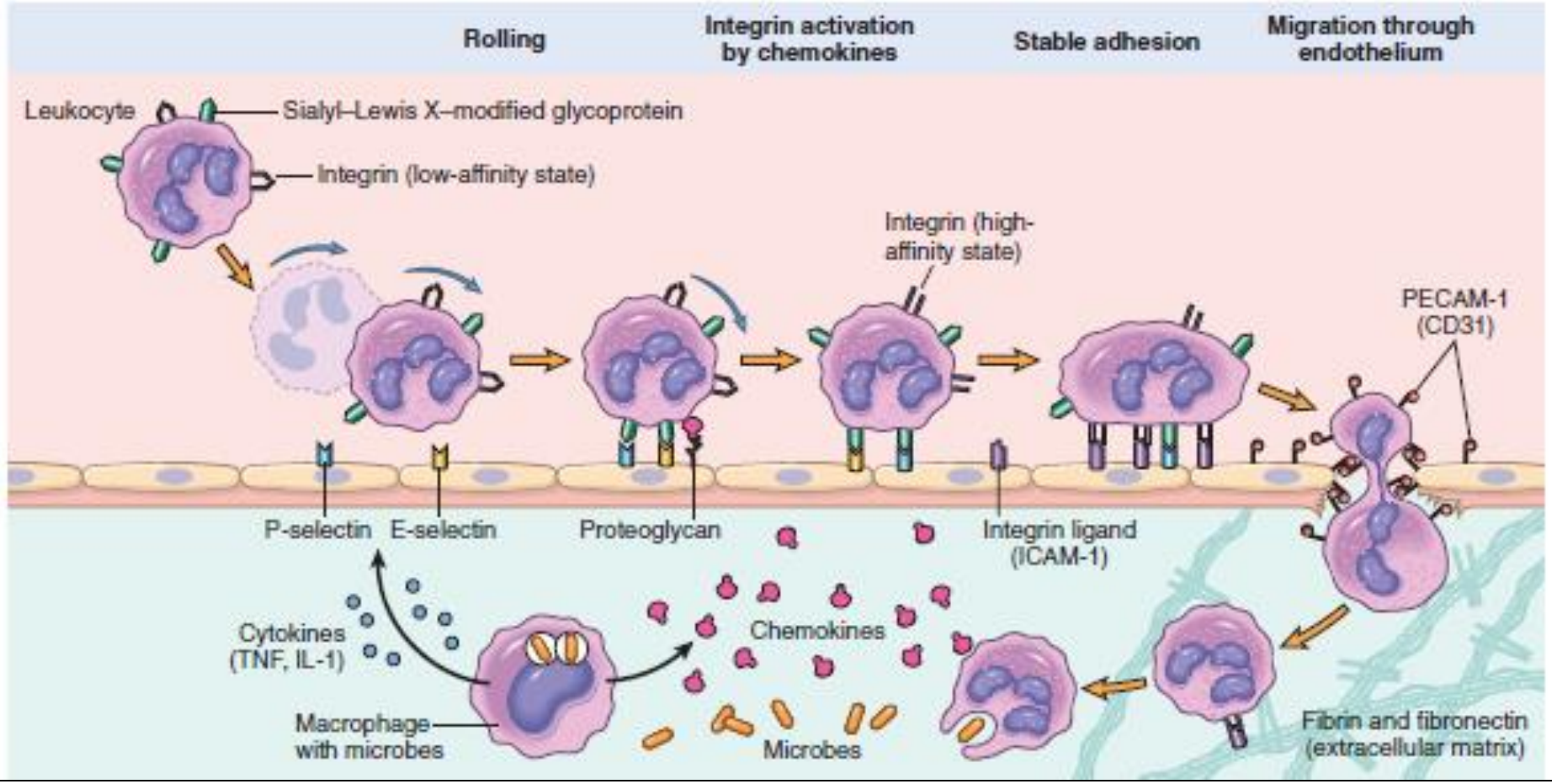
- Following increased vascular permeability, fluid part of the blood escapes into the inflamed area. This is known as exudation.
- The accumulated plasma outside the vessel is known as an inflammatory exudate.

Sequence of Vascular Events

5. Emigration of Leukocytes

- This is the process by which leukocytes come out of the blood vessels into the extravascular space.

Mechanisms of leukocyte migration through blood vessels



Sequence of Vascular Events

- **6. Diapedesis of Erythrocytes**
- Red cells may also leave the intact blood vessels. However, they have no power of movement and are pushed outof the vessel passively by the intravascular pressure following emigration of leukocytes.This is called diapedesis.
- In severe injuries, red cells may also enter the tissue by rhexis (break) of the vessel wall.

Inflammation part-2

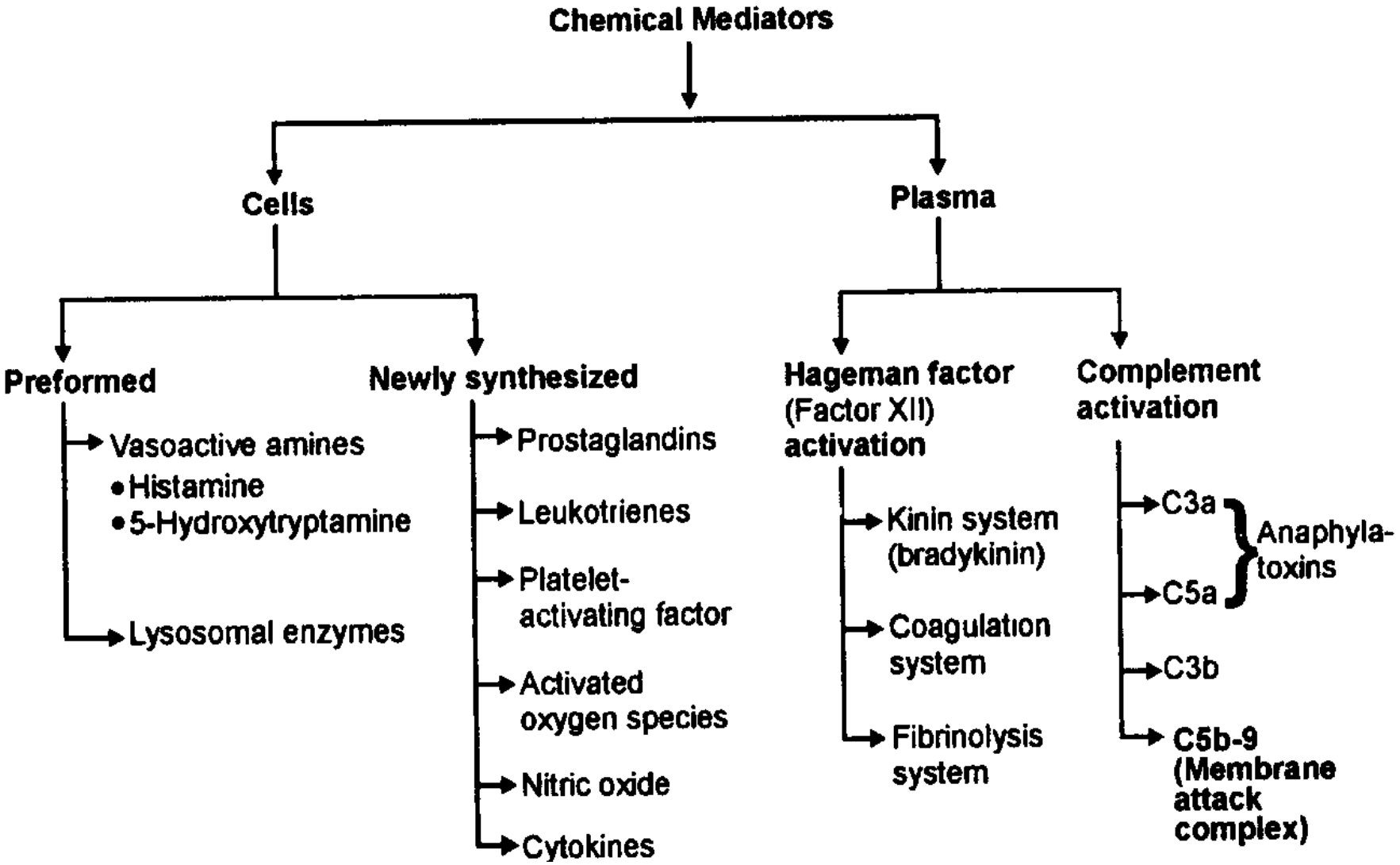
Chemical Mediators of Inflammation

- any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response.
- Sir Thomas Lewis described the 'triple response‘.
- First, within seconds, a dull red line (erythema)
- Second, a bright red halo (the flare) appears around the stroke mark.
- Third, swelling of the stroke mark (the weal) appears.
- Lewis's experiments were the first to suggest the action of chemical mediators in inflammation.

Chemical Mediators of Inflammation

- originate either from cells, or from plasma
- Cell-derived mediators are
  - already formed (preformed) (e.g., histamine in mast cells),
  - synthesized *de novo (i.e., newly* synthesized; e.g., prostaglandins) in response to stimulus.
- Plasma derived mediators are present in
  - inactive or
  - precursor forms that must be activated to acquire their biological properties.

Chemical Mediators of Inflammation



Chemical Mediators Classified by Effect

Effect	Mediator
Vasodilation	Histamine Nitric Oxide Prostaglandins: PGI_2, PGE_2, PGD_2
Increased Vascular Permeability	Histamine Complement: C3a & C5a (anaphylatoxins) Bradykinin Oxygen metabolites (ROS) Leukotrienes: LTC_4, LTD_4, LTE_4 Platelet-activating factor (PAF)
Chemotaxis	Complement: C5a Leukotrienes: LTB_4 & LTC_4 Chemokines such as TNF, IL-1, IL-8 Bacterial products such as LPS
Fever	IL-1, TNF, IL-6 Prostaglandins
Pain	Bradykinin Substance P Prostaglandin (PGF_2)
Tissue Damage	Oxygen metabolites (ROS) Nitric Oxide Lysosomal Enzymes

a) Vasoactive Amines

- **Histamine and serotonin - primary mediators in the immediate active phase of increased permeability.**
- Vasoactive amines cause **vasodilation and increased vascular permeability by**
  - **causing endothelial cells to round up,**
  - **increasing intercellular gaps,**
  - **increasing vesiculovacuolar transfer of fluids.**
- stored within cells for immediate release.

Histamine

- Extensively distributed in tissues, the main source mast cells
- preformed and stored in granules with heparin.
- Present in granules of basophils and in platelets (some species).
- important mainly in early inflammatory responses and in type 1 hypersensitivity reactions (IgE-mediated hypersensitivity).
- increased vascular permeability.

- Imp in allergic reactions as it promotes contraction of extravascular smooth muscles in the bronchi and stimulates stromal cells to synthesize and release chemotaxins for eosinophils.

- stimulate release of histamine from mast cells:
  - Ag (eg pollen) binding to IgE on mast cells
  - Anaphylotoxins (C3a and C5a)
  - Physical injury, mechanical trauma, heat, chemical agents
  - Snake venoms, toxins, bile salts, ATP
  - Histamine-releasing factors from neutrophils, monocytes, and platelets
  - Cytokines (IL-1, IL-8)
  - Neuropeptides, like substance P

Serotonin

- Present in platelets and some mast cells (not in humans).
- Acts primarily on venules during the early phase of acute inflammation,
- released from mast cells, basophils and platelets.
- Release of histamine and serotonin from platelets is stimulated when platelets aggregate after contact with collagen, thrombin, and antigen-antibody complexes

- **Substance P**
- Substance P is produced in some leukocytes and sensory nerve fibres (thus known as a neuropeptide);
- it has similar effects to those of bradykinin

b) Plasma Proteases

- 3 interrelated systems important in the inflammatory response are found within plasma:
  - **Complement**
  - **kinin**
  - **clotting systems.**
- activated by activated Hageman's factor (factor XIIa of the coagulation cascade).

Complement system

- Set of plasma proteins that act together to attack extracellular forms of microbial pathogens.
- Activated directly by certain pathogens or by antibodies binding to a pathogen.
- Activated complement is also involved in:
  - Facilitated removal & killing of targeted microorganisms,
  - Vascular permeability (esp C3a & C5a) – via histamine release from mast cells.
  - Chemotaxis - C5a chemoattractant for neutrophils, monocytes, eosinopils & basophils.

Kinin System

- generates vasoactive peptides from plasma proteins called **kininogens** by the action of specific proteases called **kallikreins** which ultimately leads to activation of **bradykinin.**

- **Bradykinin has the following actions:**
  - Vasodilation and stimulation of histamine release by mast cells increased vascular permeability
  - Contraction of non-vascular smooth muscle
  - Produce pain
  - Activate the arachidonic acid cascade

Clotting system

- The clotting system and inflammation are intimately connected.
- Intrinsic clotting system is a sequence of plasma proteins that can be activated by Hageman factor (factor XII – **produced in liver and circulating in inactive form**).
- The final phase of the cascade is the conversion of fibrinogen to fibrin by the action of **thrombin.**
- Thrombin binds to a receptor on platelets, endothelium, smooth muscle cells and causes them to:
  - Mobilize P-selectin to the cell membrane and express adhesion molecules for integrins.
  - Produce chemokines.
  - Induce cyclooxygenase-2 – production of prostaglandins.
  - Produce platelet activating factor (PAF) & nitric oxide (NO).
  - Change endothelial shape.

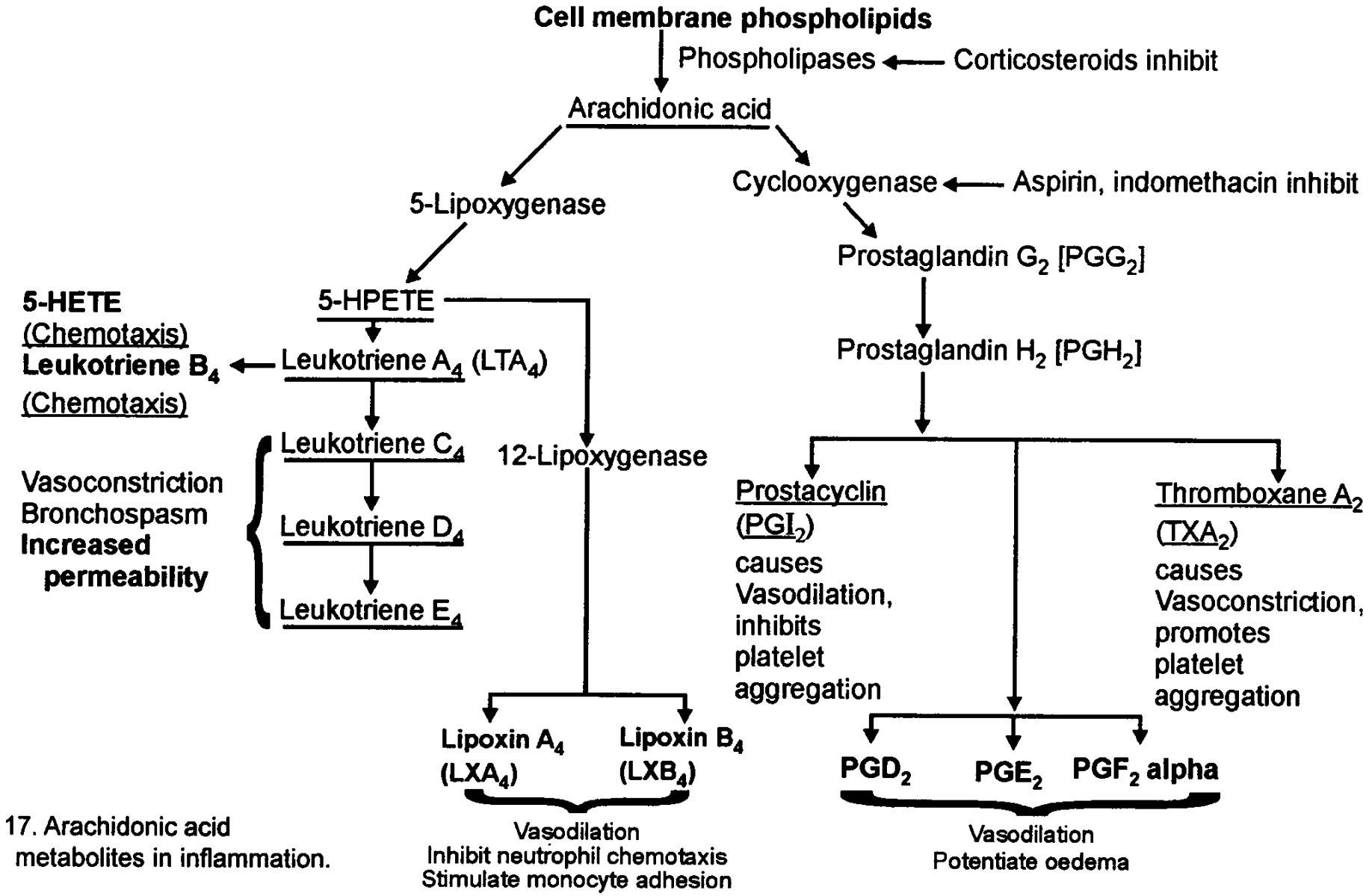
c) Arachidonic Acid Metabolites

- When cells are activated by diverse stimuli, their lipid membranes can be rapidly remodelled to generate biologically active lipid mediators.
- These lipid mediators are like short-range hormones that are formed rapidly and exert their effects locally and then are inactivated.
- Oxygenated arachidonic acid derivatives act in biologic & pathologic processes, one of which is inflammation.
- not free in the cell but esterified in membrane phospholipids;

- released from phospholipids it must be activated by cellular phospholipases, particularly phospholipase A2 (via mechanical, chemical and physical stimuli or by other mediators).
- Following activation, biosynthesis of the metabolites of arachidonic acid occurs by one of two major pathways:
  - cyclooxygenase pathway
  - lipoxygenase pathway.

- Drugs such as **corticosteroids, aspirin and indomethacin** have anti-inflammatory properties because they inhibit specific steps of arachidonic acid metabolism

Arachidonic acid metabolites in inflammation.



17. Arachidonic acid metabolites in inflammation.

Cyclooxygenase Pathway

- Two enzymes : COX-1 and COX-2.
- COX-1 is normally present (constitutively expressed) and necessary for everyday activities; also synthesized at sites of inflammation.
- COX-2 is transcriptionally regulated - present in various circumstances (eg inflammation).
- the main 3 products resulting from this pathway are:
- **Thromboxane A2 is** found in platelets and other cells is a potent platelet aggregator and vasoconstrictor
- **Prostacyclin (PG I2) is** found predominantly in endothelial cells; a potent inhibitor of platelet aggregation and vasodilator.
- **Prostaglandins (PG's E2, D2, F2α) c**ause vasodilation, increased vascular permeability & pain.

Lipoxygenase Pathway

- Results in the production of leukotrienes and lipoxins

Leukotrienes

- Exacerbate acute inflammatory response:
  - 1. Increased vascular permeability (**up to 1000X as potent as histamine**)
  - 2. Chemotaxis for leukocytes
  - 3. Vasoconstriction
  - 4. Also causes bronchoconstriction

Lipoxins

- Secreted mainly by platelets
- Have both pro- and anti-inflammatory effects and can counteract leukotrienes
- Inhibit neutrophil chemotaxis and adhesion to endothelium but promotes macrophage adhesion to endothelium.
- **Lipoxins A4 causes** vasodilation and counteracts leukotriene C4-induced vasoconstriction

d) Lysosomal Constituents

- Found mainly in
  - neutrophils,
  - macrophages and
  - cytotoxic lymphocytes,
  - but also in eosinophils and mast cells

e) Oxygen-Derived Free Radicals

- The main oxygen free radicals are
  - **superoxide anion (•O2-)**
  - **hydroxyl radical (•OH).**
- When released into tissue cause:
  - Endothelial cell damage with resultant increased vascular permeability.
  - Inactivation of antiproteases -unopposed protease activity - increased destruction of ECM
  - Injury to a variety of cell types (tumour cells, red cells, parenchymal cells).

f) Platelet Activating Factor (PAF)

- PAF is of phospholipid origin, derived from the cell membranes of leukocytes, endothelial cells & platelets.
- PAF has several inflammatory effects, including:
  - Platelet aggregation and release.
  - Bronchoconstriction and vasoconstriction (at high concentrations).
  - Vasodilation & increased vascular permeability (low concentrations); much more potent than histamine.
  - Increased leukocyte adhesion to endothelium, chemotaxis, degranulation and oxidative burst.

g) Cytokines and Chemokines

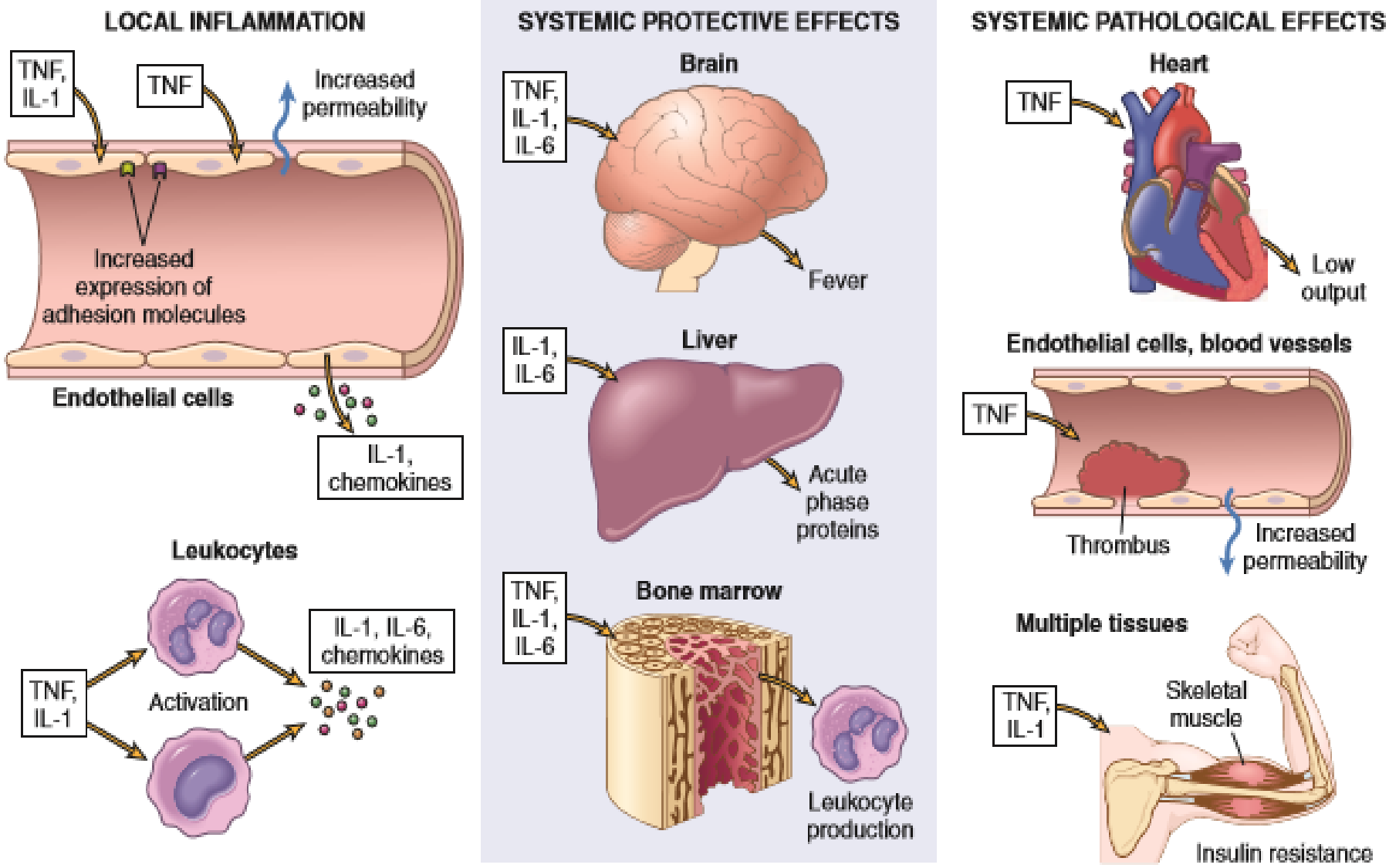
- Cytokines are polypeptides produced by many cells activated macrophages and lymphocytes)
- they function to modulate the function of other cell types.
- Chemokines are cytokines that promotes leukocyte chemotaxis and migration across capillaries and postcapillary venules.

- Cytokines are essential transmitters of cell-to-cell communication in many physiological and patho-physiological processes.

IL-1 and TNF-α

- The “Master Cytokines”, produced by monocyte-macrophages.
- Biochemically and immunologically distinct proteins, but are similar in their biologic activities:
- On endothelial cells, they increase leukocyte adhesion (induction of surface antigens), stimulate the synthesis of PGI2 and PAF, and increase pro-coagulant activity (surface thrombogenicity).
- They induce **systemic acute phase responses**, eg fever, neutrophilia, hemodynamic effects (shock).
- On fibroblasts, they induce proliferation, increased collagen formation, and increased collagenase & protease synthesis.

The roles of cytokines in acute inflammation



IL-5

- Produced by helper T lymphocytes (CD4 Th2) and mast cells.
- Affects proliferation, chemotaxis, **and activation of eosinophils (important in parasitic infections, allergies, etc.).**

IL-6

- Produced by T lymphocytes & macrophages; its major activities include **B and T cell proliferation**.
- Sometimes considered a “Master cytokine”, like IL-1 and TNF.

IL-8

- Produced by leukocytes and endothelial cells.
- It is a powerful chemoattractant and activator of neutrophils and to lesser degree monocytes and eosinophils

IFN-γ

- Produced by T lymphocytes and NK cells.
- Activates macrophages and T lymphocytes, **particularly against viral infections**.

PDGF (Platelet Derived Growth Factor)

- Produced by leukocytes, endothelial cells and fibroblasts.
- It is most important in chronic inflammation but is present from the beginning.
- Acts as a chemoattractant to leukocytes and mesenchymal cells (fibroblasts);
- **stimulating the proliferation of fibroblasts**.

Other growth factors, like Vascular Endothelial Growth Factor (**VEGF**), Transforming Growth Factor beta (**TGF-β**) are particularly important in **tissue regeneration and repair.**

h) Nitric Oxide (NO)

- A tiny molecule produced mainly in endothelial cells, neurons and macrophages.
- NO **relaxes smooth muscles in** vessels (causes **vasodilation**).
- When isoforms of nitric oxide synthase (NOS) is up-regulated in macrophages (& others), as in sepsis, there is **massive vasodilation & shock.**

Cellular Events in Acute Inflammation

- **Rudolph Virchow-** the father of **cellular pathology**
- Most important function of inflammation is delivery of leukocytes, particularly neutrophils and monocytes, to the site of injury.
- Leukocytes engulf and kill bacteria and degrade necrotic tissue and immune complexes.

Cellular Events in Acute Inflammation

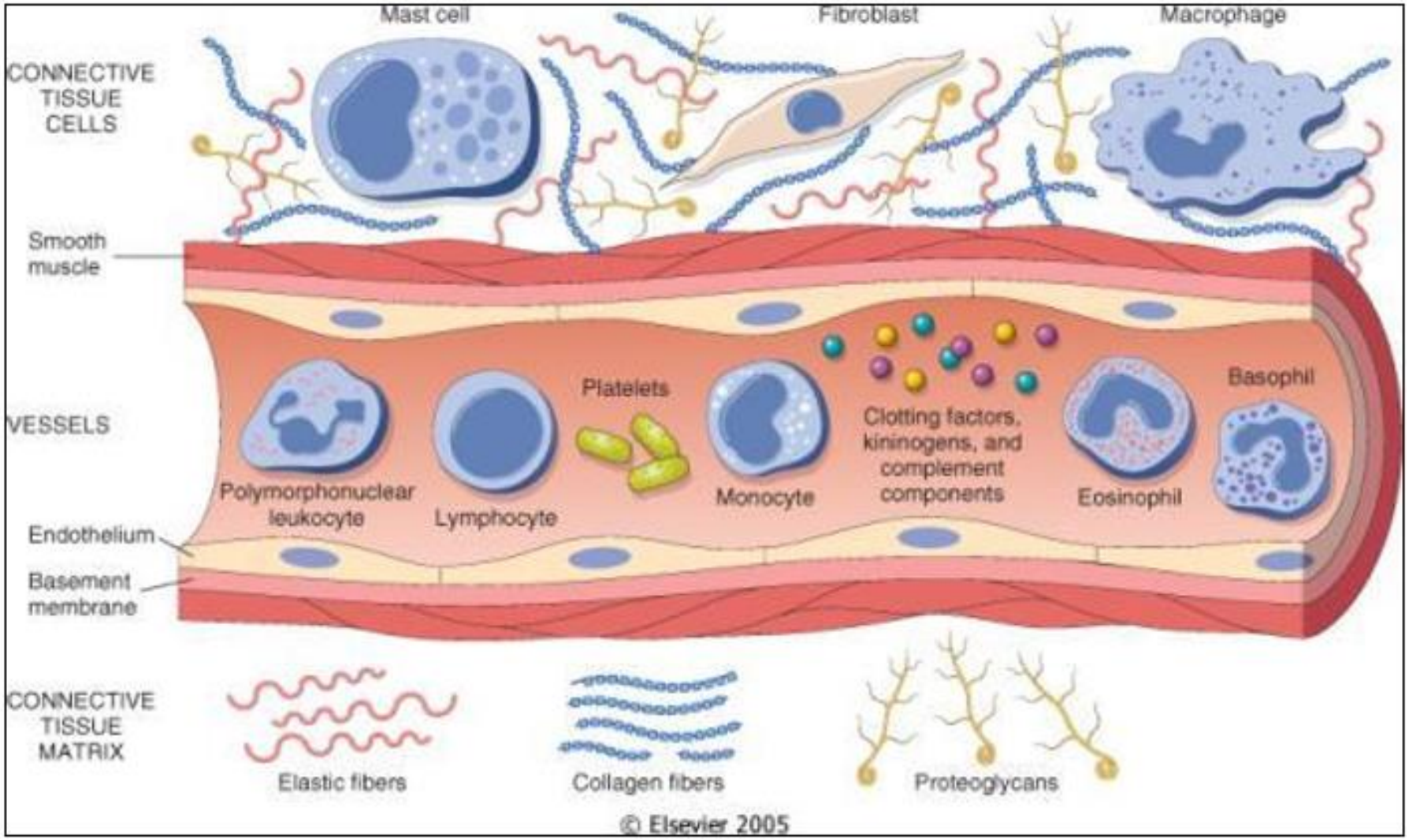
- The sequence of events in the journey of leukocytes from the lumen of blood vessels into the extravascular space is called **extravasation.**
- These events can be divided into:
  - (1) margination,
  - (2) adhesion,
  - (3) emigration (transmigration),
  - (4) phagocytosis, and
  - (5) release of leukocyte products.

Cellular Events in Acute Inflammation

- **Polymorphonuclear Leukocytes**
  - Neutrophils (heterophils)
  - Eosinophils
  - Basophils/Mast Cells

- **Mononuclear Cells**
  - Monocytes/Macrophages
  - Lymphocytes
  - Plasma cells
  - Thrombocytes*

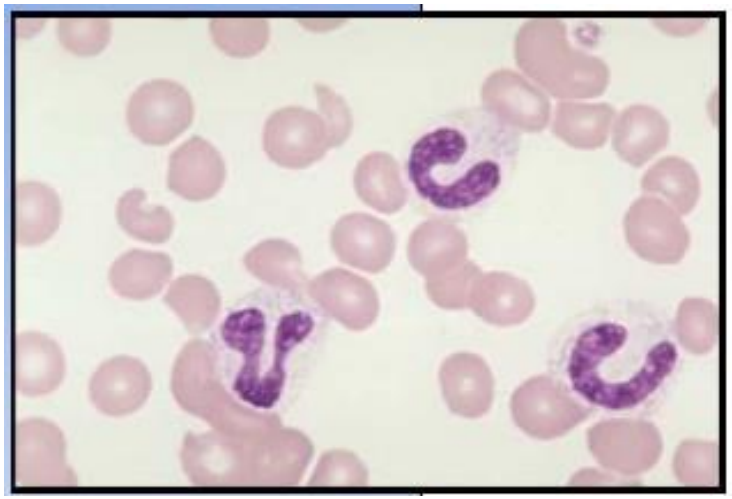
Cellular Events in Acute Inflammation



Neutrophils

Synonyms:

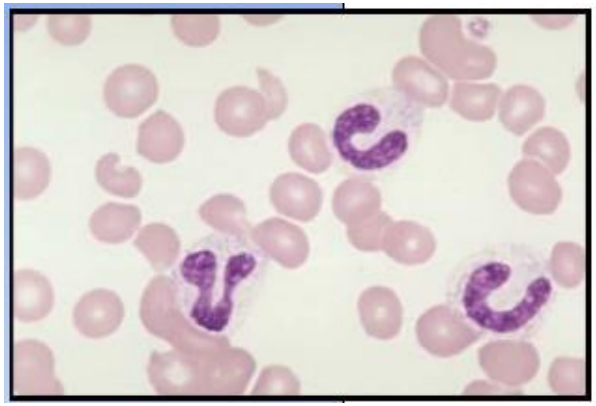
- polymorphs, PMNs, Neuts



Characteristics:

- highly mobile
- respond to lots of chemotaxins
- phagocytic and bactericidal
- 1º cell against bacteria
- **1st line of defense & crucial**
- don't divide in tissue
- their average time in circulation is likely just a few days (until recently thought to be only 6 hrs in circulation)
- Neutrophils are also called microphages of Metchnikoff, being smaller than macrophages.

Neutrophils



Synonyms:

- polymorphs, PMNs, Neuts

Characteristics:

- Neutrophils are also called microphages of Metchnikoff, being smaller than macrophages.
- The neutrophils are highly chemotactic and are usually the first to arrive at the site of inflammation- **first line of cellular defence**
- **Schilling** index helps in determining the immature (juvenile) forms of neutrophils in the blood, and an increase in their number is known as **'shift to the left'**

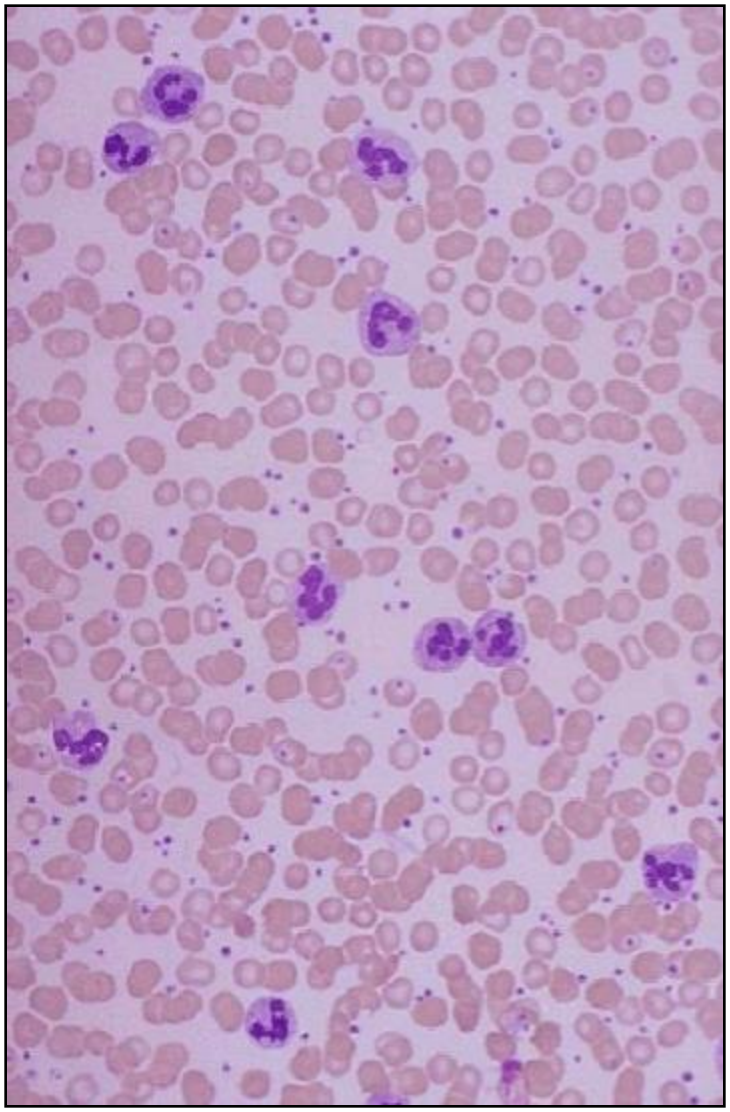
Cellular Events in Acute Inflammation

Originate in Bone Marrow
- 5-10 X 1010 per day

Blood
- Circulating Pool (measured in a CBC)
- Marginating Pool (out of the flow)

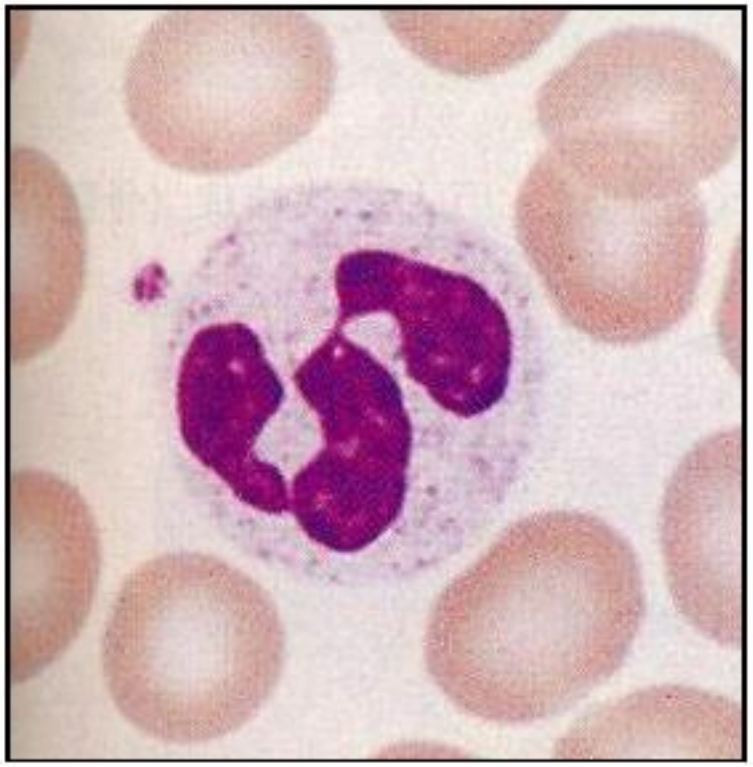
Storage
- pool in bone marrow

Exit into tissue
- die after 1-2 days



Neutrophil - Morphology

- i) Azurophil Granules (primary granules): myeloperoxidase, lysozyme, elastase, etc.
- ii) Specific Granules (secondary granules): leukocyte adhesion molecules, lysozyme, histaminase, etc.
- iii) Tertiary granules (gelatinase granules): gelatinase, lysozyme, leukocyte adhesion molecules, etc.



Functions of neutrophils

i) Phagocytosis:

- Ingest material (opsonized by C3b and Ig), neutralize & destroy it through the following mechanisms:
  - production of oxygen free radicals
  - hydrogen peroxide
  - lysosomal enzymes Non-opsonized material can also be ingested, but in a less efficient manner.

Cont…

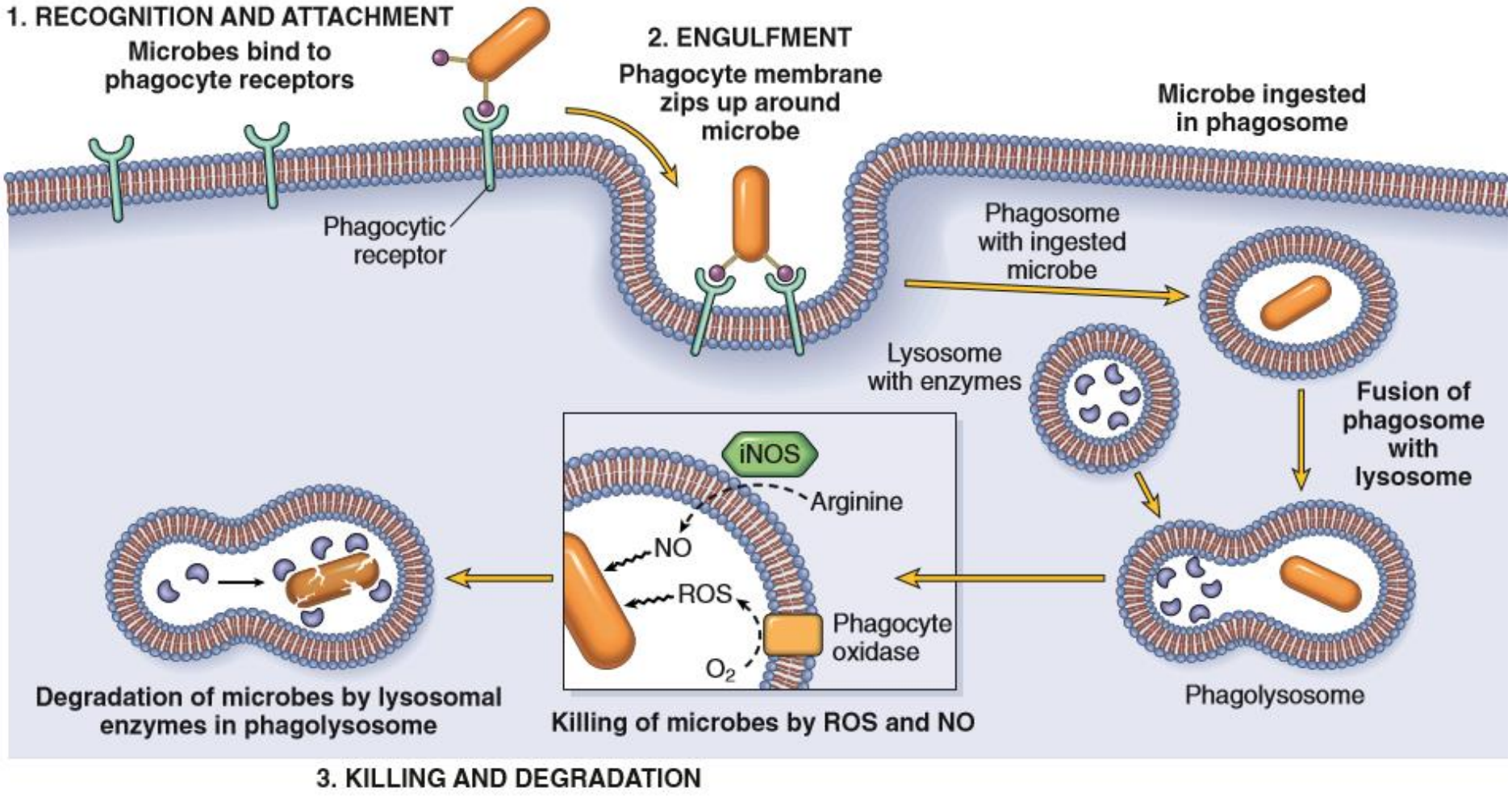
ii) Mediate tissue injury:

- via release of O2 free radicals and lysosomal enzymes into the tissue.

iii) Regulate inflammatory response:

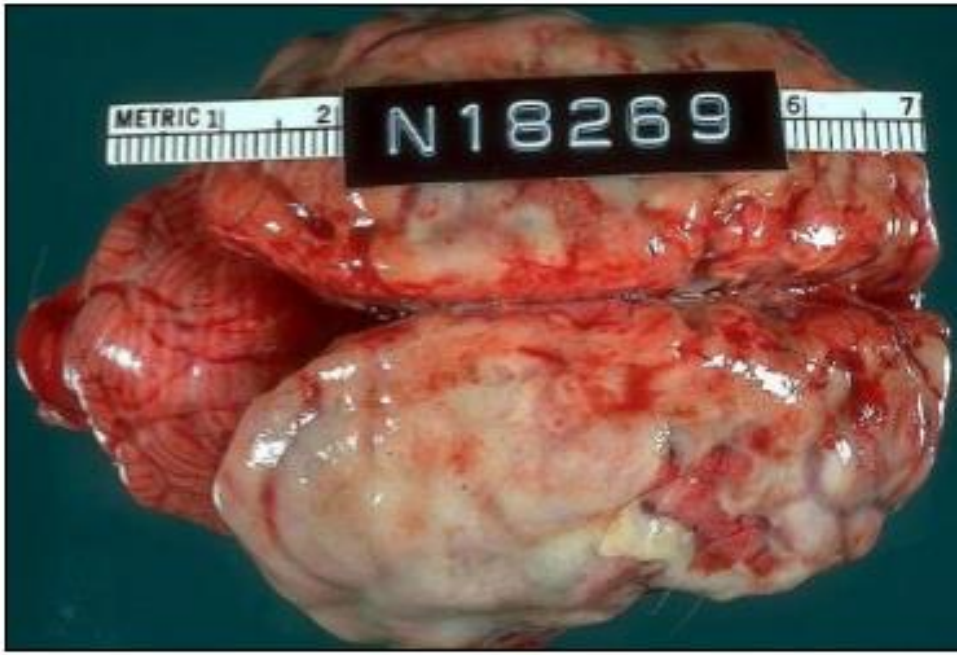
- via releasing mediators (eg leukotrienes, platelet activating factor).

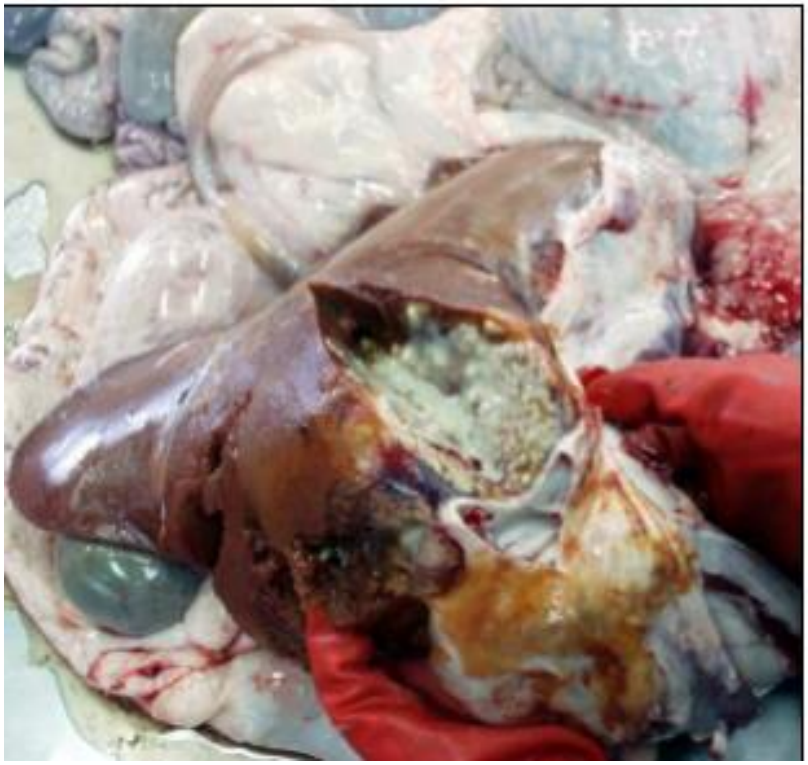
Cont…


1. RECOGNITION AND ATTACHMENT
Microbes bind to phagocyte receptors
Phagocytic receptor
2. ENGULFMENT
Phagocyte membrane zips up around microbe
Microbe ingested in phagosome
Phagosome with ingested microbe
Lysosome with enzymes
Fusion of phagosome with lysosome
iNOS
Arginine
NO
ROS
Phagocyte oxidase
O_2
Phagolysosome
Degradation of microbes by lysosomal enzymes in phagolysosome
Killing of microbes by ROS and NO
3. KILLING AND DEGRADATION

WHEN DO NEUTROPHILS PREDOMINATE?

- acute inflammatory reactions
- suppurative / purulent exudates (esp bacterial infections)





Morph. Dx (pig):

Meningitis, suppurative, focally extensive, acute, severe

Morph. Dx (calf):

Hepatitis, suppurative, focal, acute, severe (abscess?)

Heterophils

- equivalent of neutrophils in some species (eg rabbits, guinea pigs, rats, reptiles, fish and birds),
- but they **do not contain myeloperoxidase** in their granules.
- Differentiating heterophils from eosinophils is difficult because they have prominent eosinophilic granules and thus resemble eosinophils.

2) Eosinophils (aka eo's)

- Present in parasites and allergic / immune-mediate disease, but may be present in any exudate.
- Some live in tissues in contact with environment such as intestine, skin, lung and mucous membranes.
- Corticosteroids cause a reduction in the release of eo's from the bone marrow.
- The most important cytokines for production and recruitment of eo's is IL-5.
- Histamine is very eotactic (attracts eosinophils).

Cont…

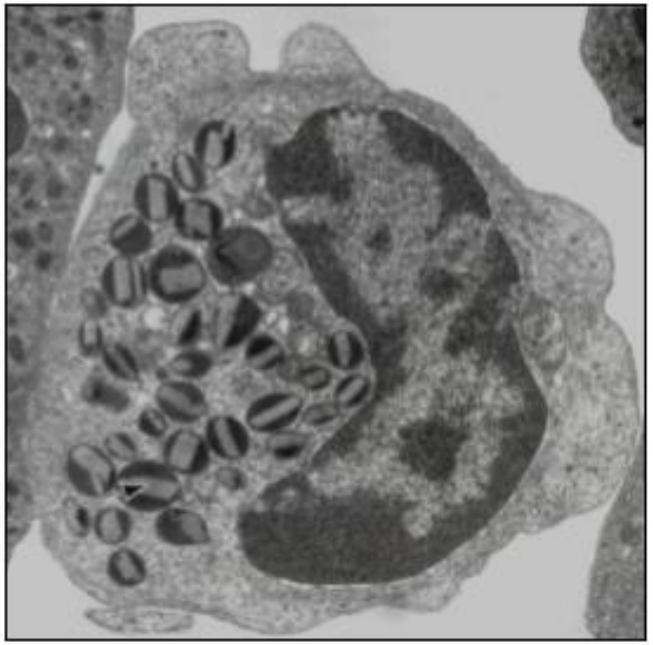
Eosinophil granules

- Vary in size depending on the species but all stain with acid dye eosin - hence their name.
- The main components of the granules are:

1) Major basic protein
   - Parasite (helminth) killing
   - Induce histamine release from mast cells

2) Eosinophil cationic protein
   - Parasite (helminth) killing
   - Shortens coagulation time and alters fibrinolysis

3) Histaminase: inactivates histamine
   Anti-inflammatory



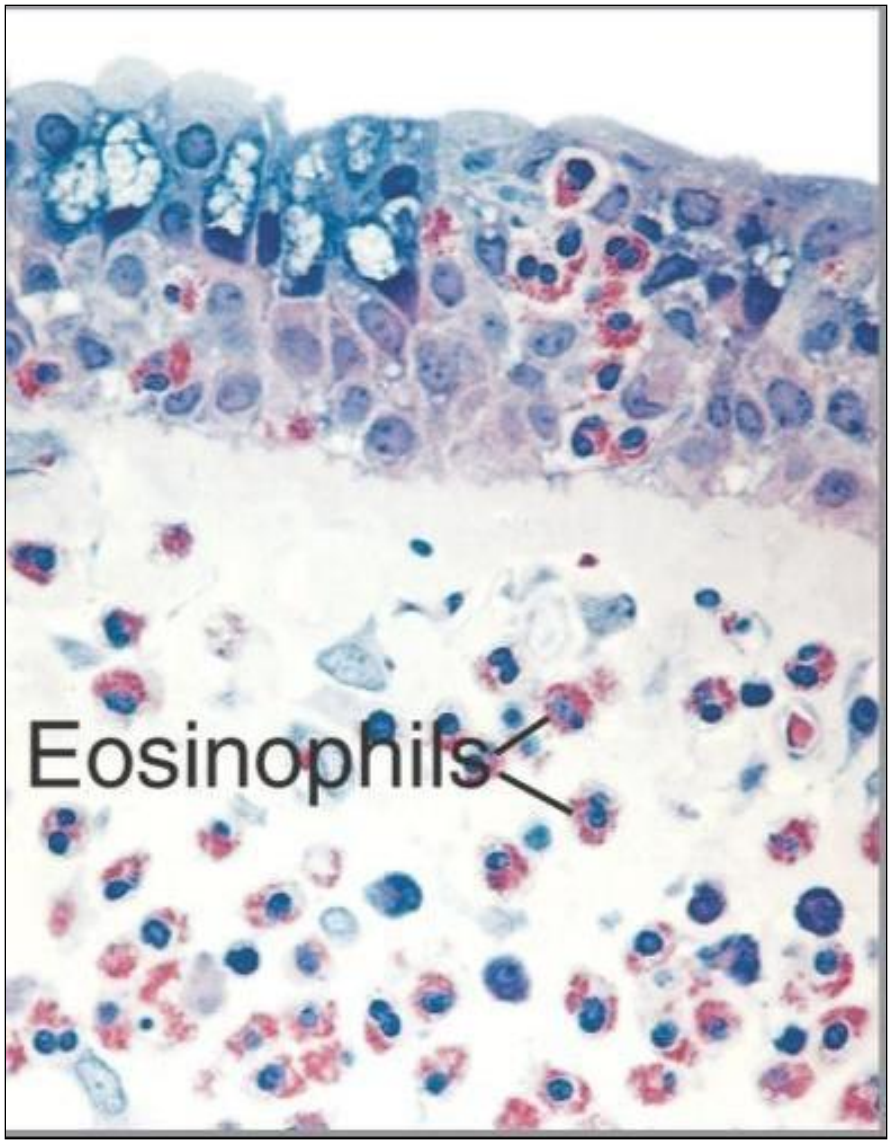
Functions of eosinophils

- i. Kill or damage helminths and other pathogens by antibody-dependent cell-mediated cytotoxicity.

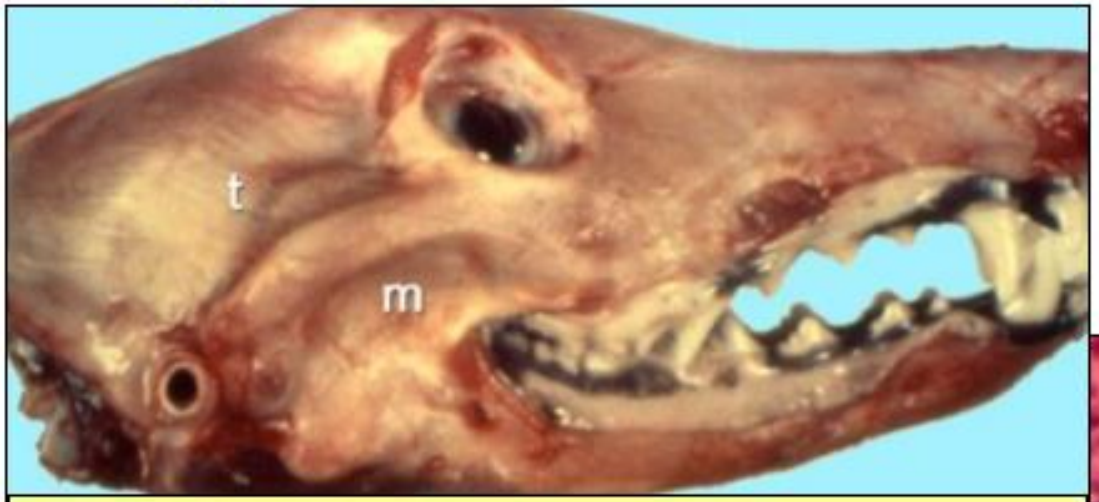
- ii. Cause and assist in hypersensitivity reactions, especially Type I hypersensitivities.

Functions of eosinophils

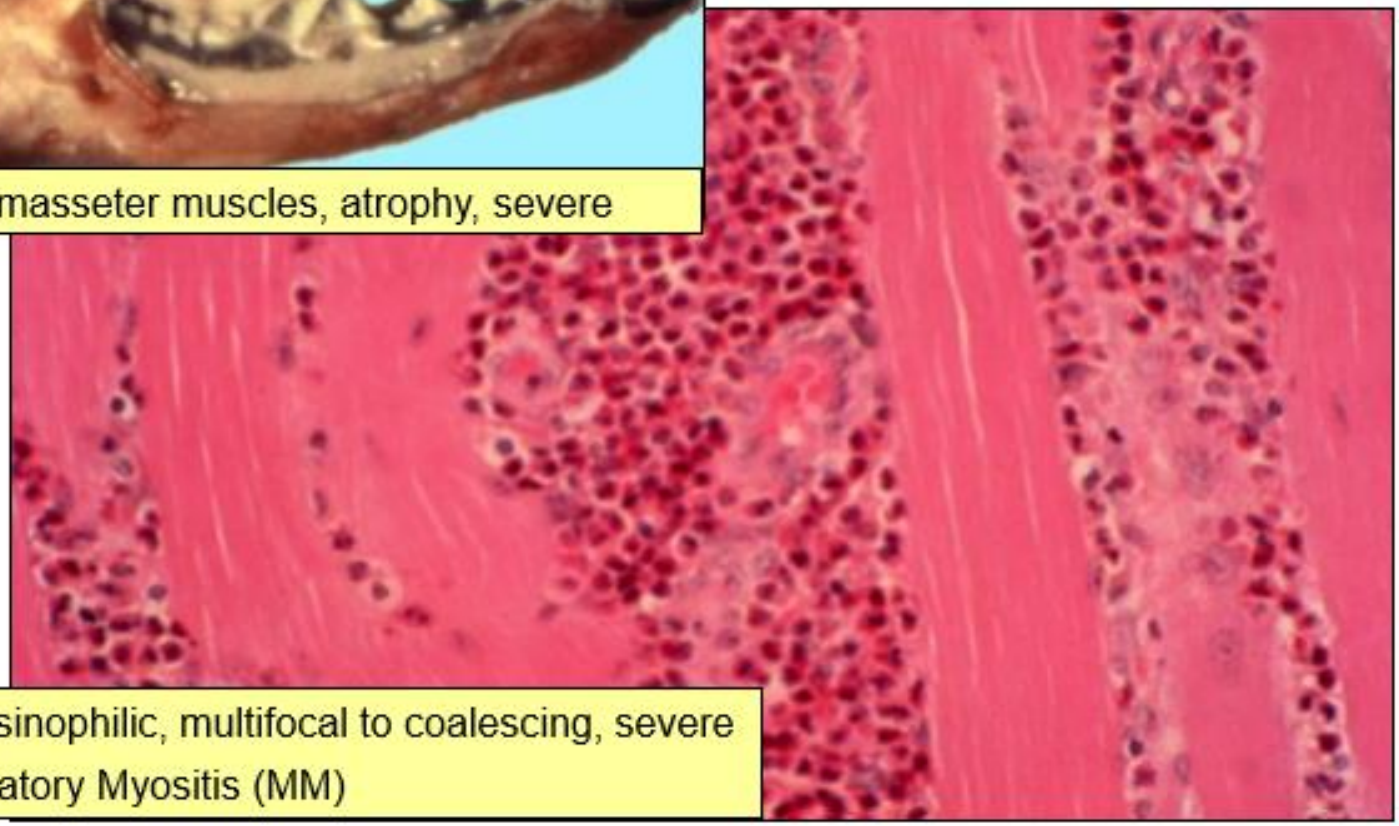
- iii. Regulate inflammation, particularly to mast cell products.
- iv. Phagocytosis, but much less than neutrophils.



Hypersensitivities and autoimmune conditions



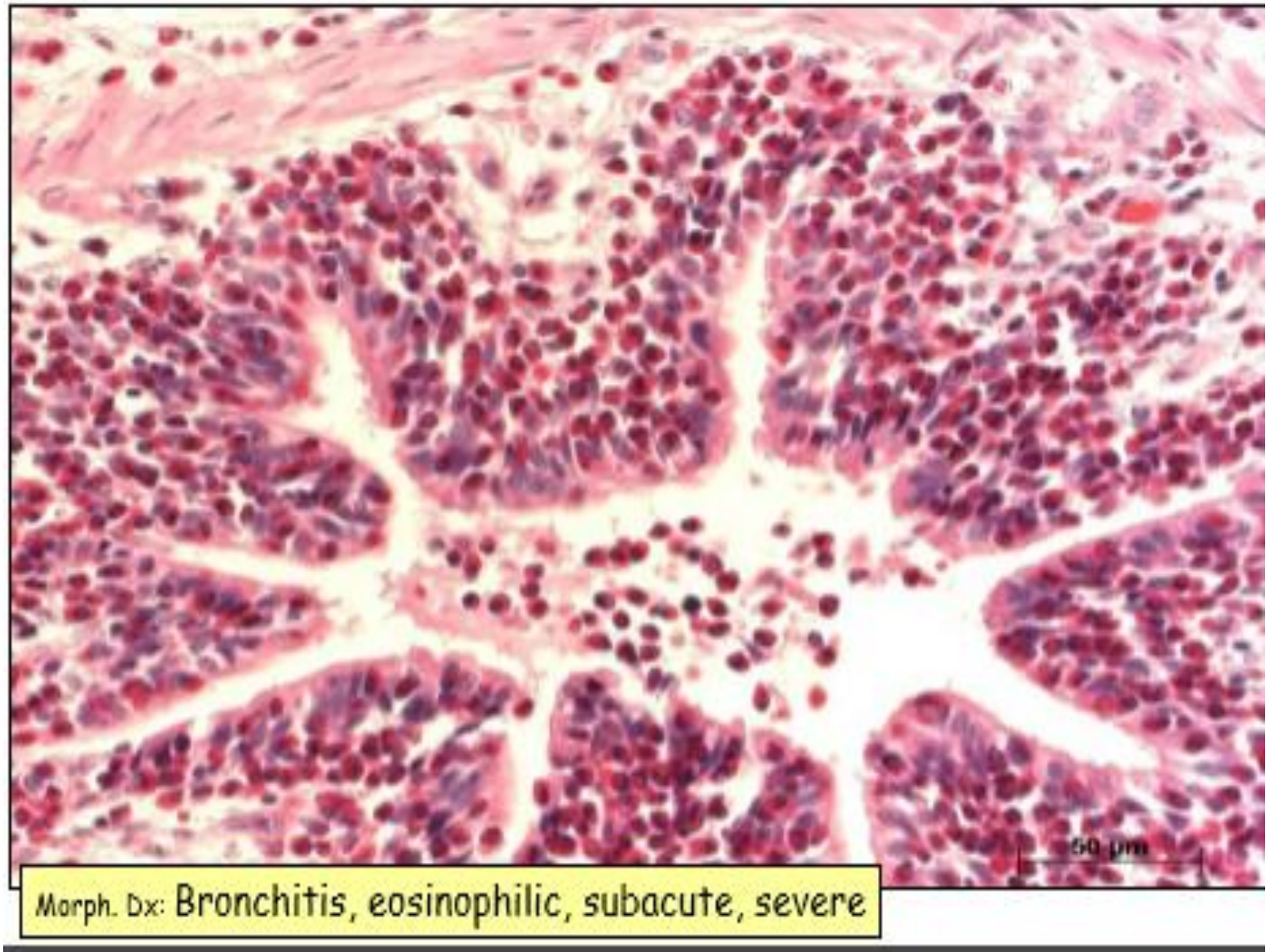
Morph. Dx: Temporal & masseter muscles, atrophy, severe



Morph. Dx: Myositis, eosinophilic, multifocal to coalescing, severe

Name of condition: Masticatory Myositis (MM)

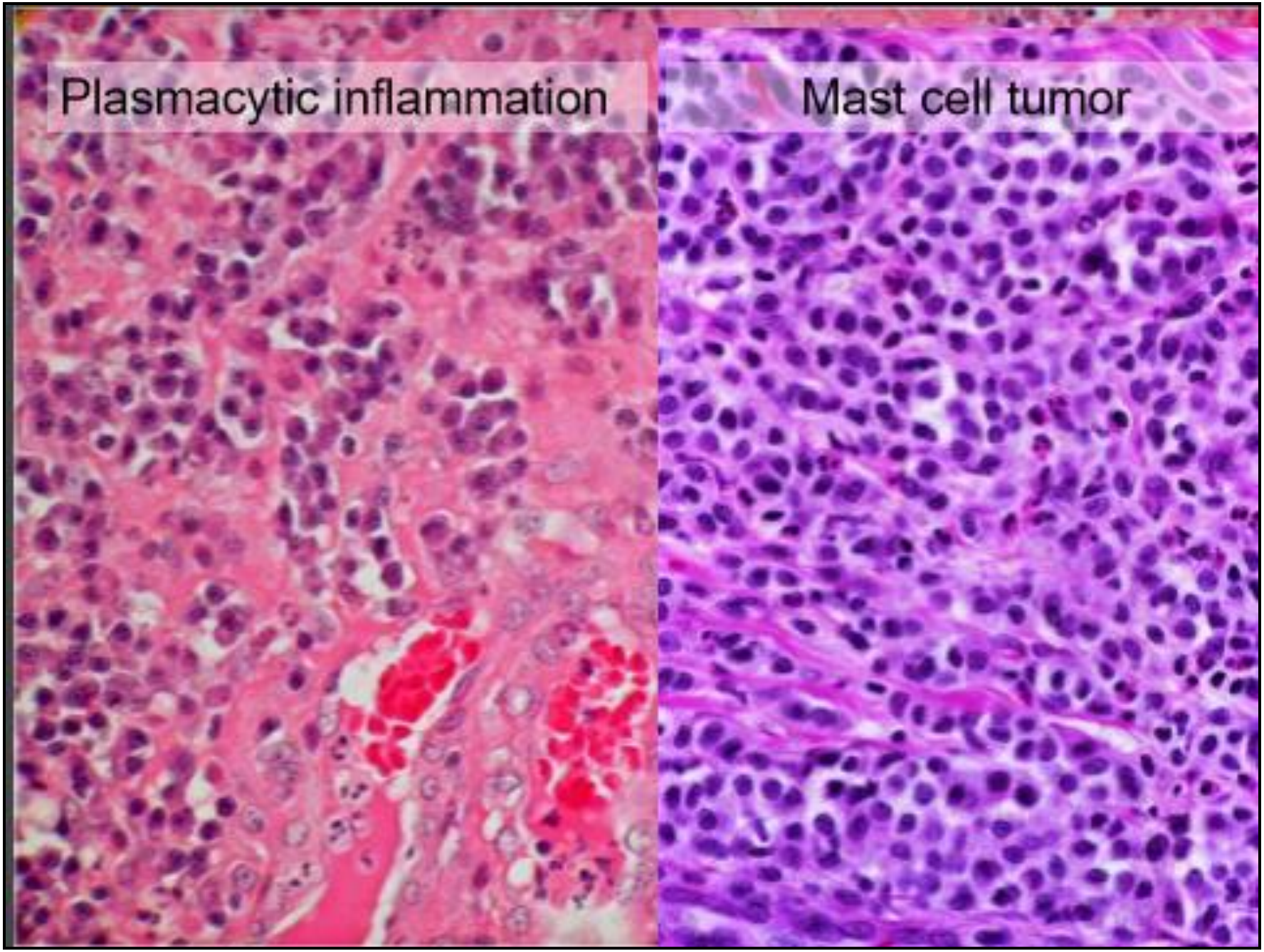
Hypersensitivities and autoimmune conditions



Cont…

- tumor-associated eosinophilia
  - – Mast cell tumors
  - – T cell lymphoma, etc.

- reduced numbers with corticosteroid therapy

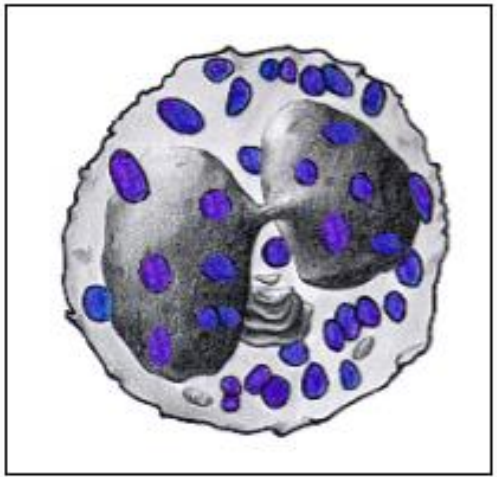

Plasmacytic inflammation
Mast cell tumor

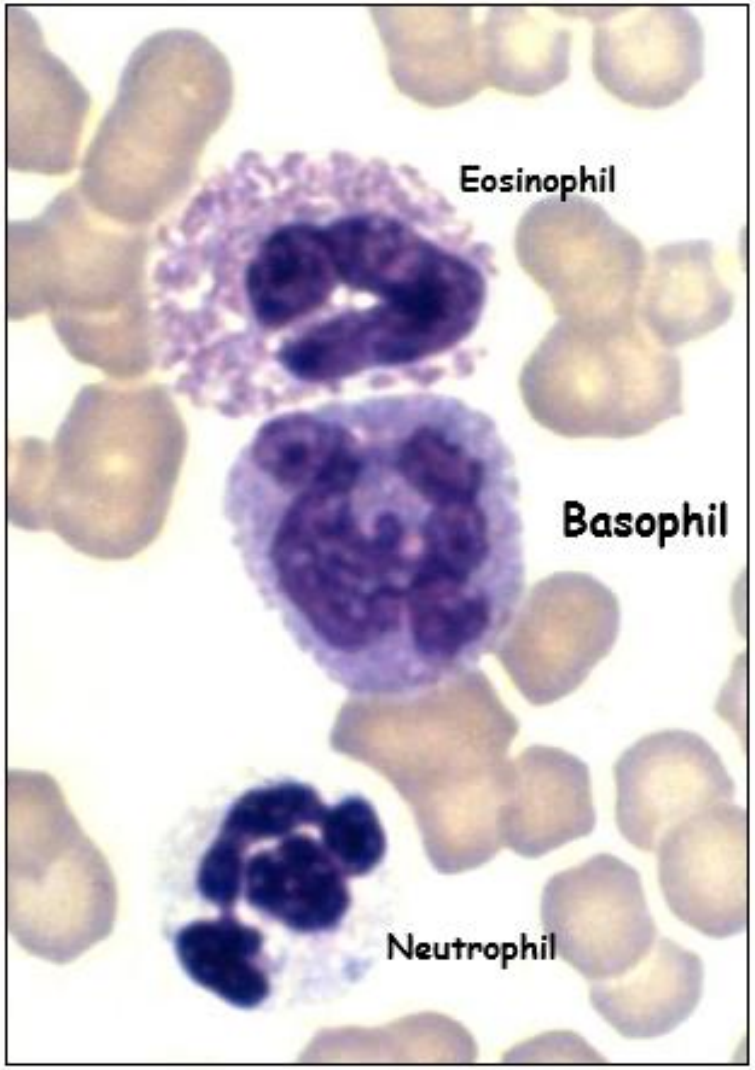
Basophils and Mast Cells

- Basophils are rare circulating granulocytes
- Mast cells are relatively numerous and are found in perivascular sites, particularly in areas of contact with the environment (lung, gut, mucous membranes and skin).
- Both are derived from bone marrow and have similar functions;
- but they come from separate stem cell lineages (ie basophils don’t become mast cells when they move into circulation).

Cont…

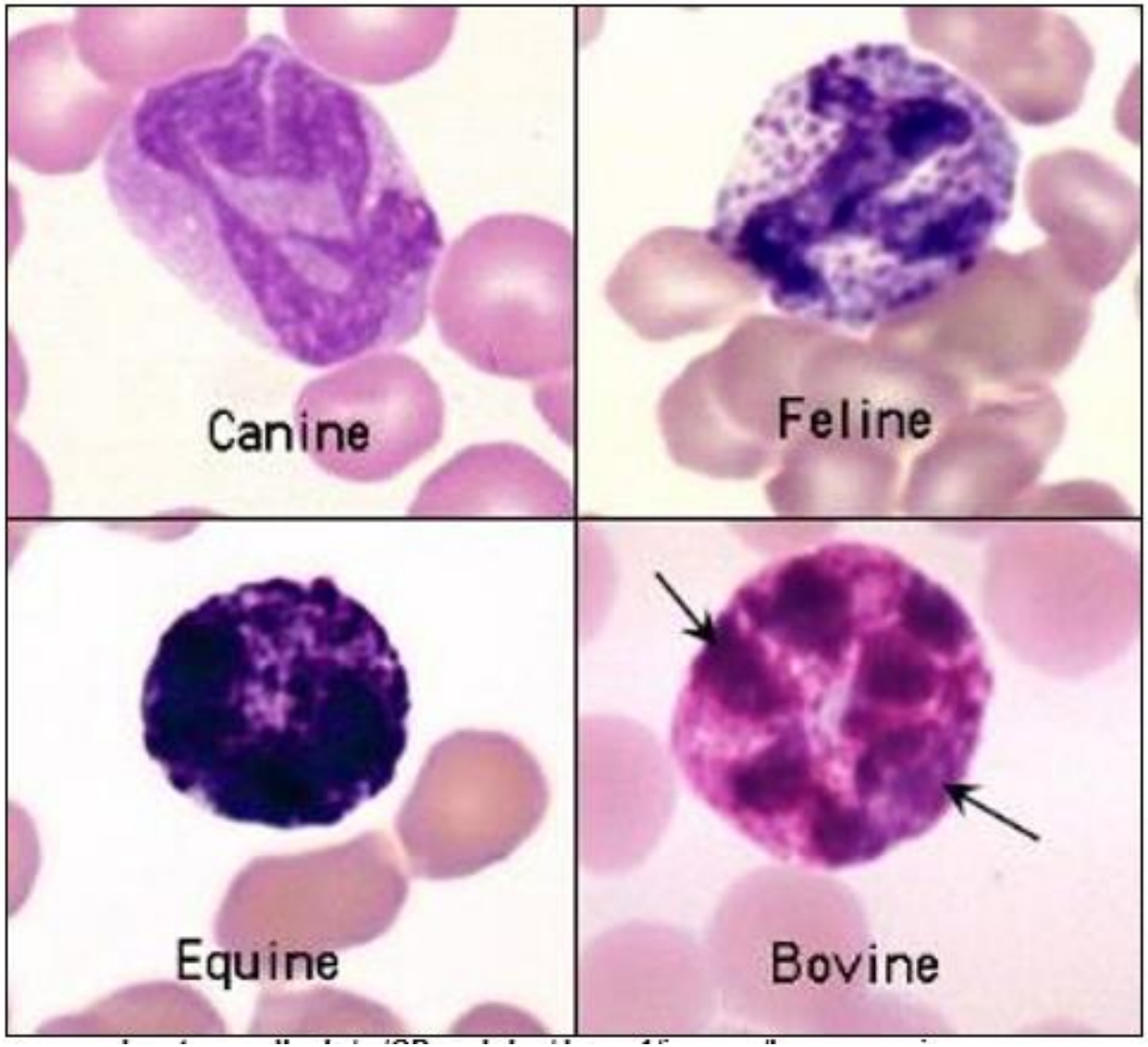
- Basophils and mast cells share many characteristics:
  - They contain abundant cytoplasmic metachromatic granules (**stain magenta with toluidine blue**) that are rich in histamine, proteases, and potent inflammatory mediators (they don't die after releasing their granules, unlike neutrophils).





Basophils

Morphology - Species Differences



Cont…

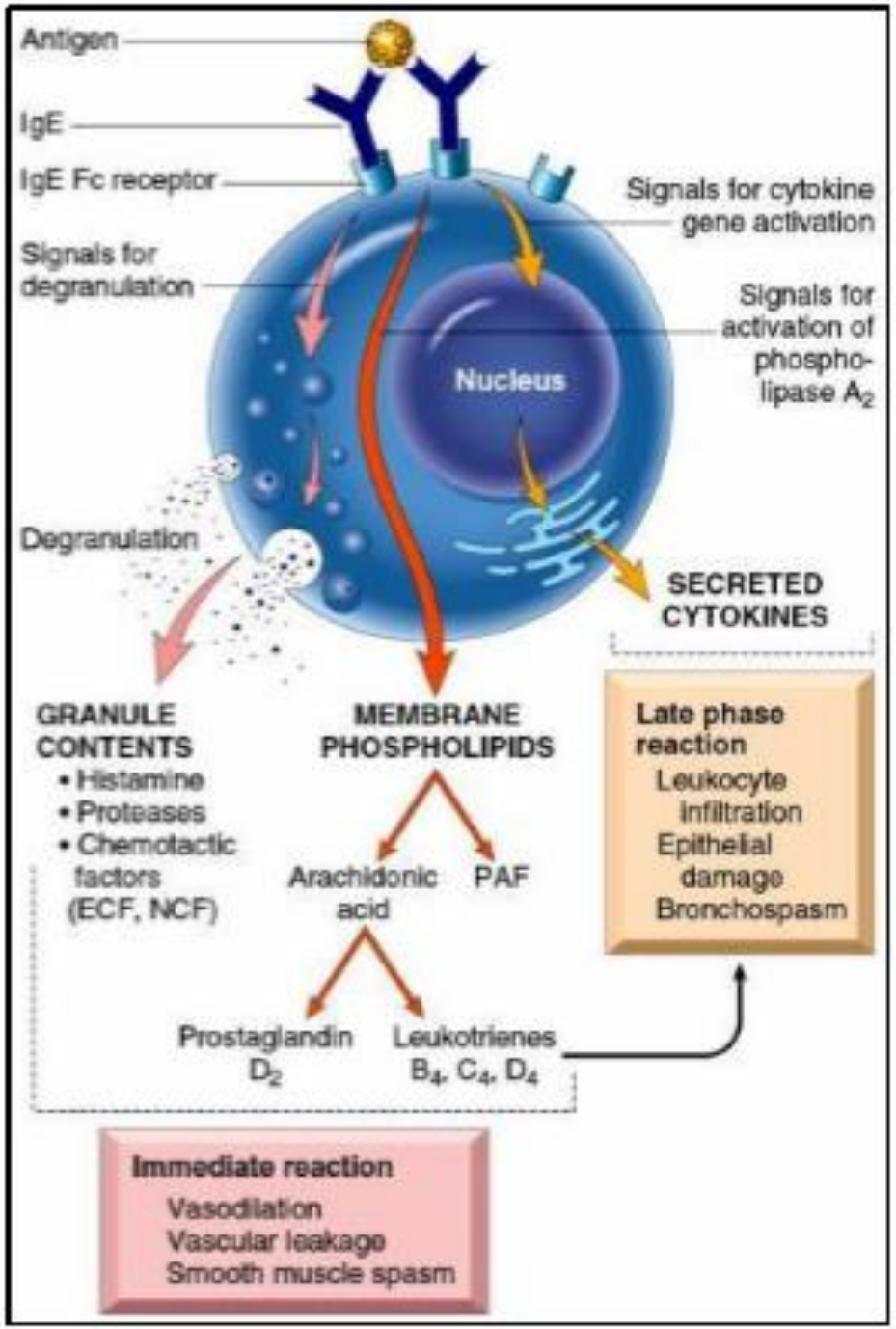
- Basophils and mast cells share many characteristics:
  - Membrane receptors bind the Fc portion of IgE antibody (these cells mediate Type 1 hypersensitivity reactions).
  - Produce cytokines (eg TNF- , IL-3,-4,-5,-10,-13, IFN-γ) and arachidonic acid metabolites (eg leukotrienes).
  - Mast cells are the major source of histamine in acute inflammation.

Acute Inflammation

- activated by IgE (parasities & allergies) and substance P
  - release histamine
  - tryptase (tissue damage)
  - generate cytokines

Recruitment of eosinophils

- IL-5 • LT-C4



Cont…

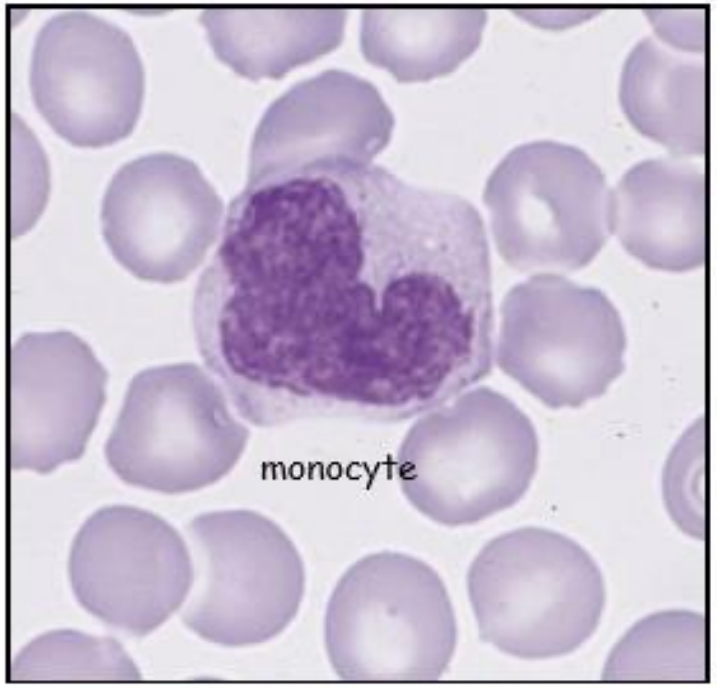
Functions of mast cells

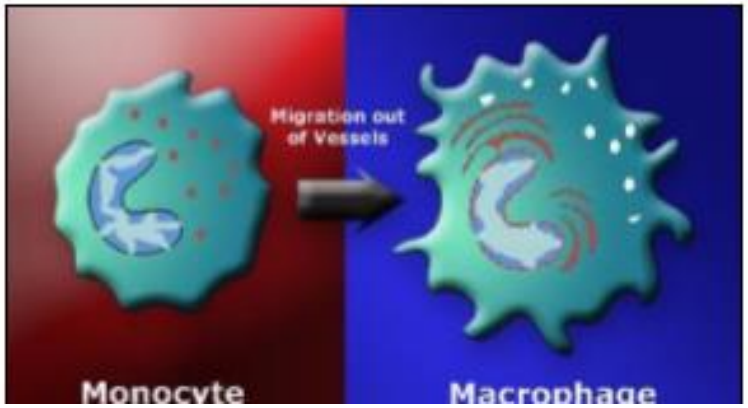
- Intimately involved in acute inflammation, particularly hypersensitivity reactions.
- Activated by IgE-bound antigens (parasites, pollen, and other allergens), as well as Substance P from nerves and macrophages.
- Cross-linking of IgE membrane receptors on mast cells causes the release of histamine (in granules)
- Histamine causes smooth muscle dilation in arterioles (vasodilation) and increased permeability in venules.
- Recruitment of eosinophils via IL-5, leukotriene C4.

Monocytes

Monocyte

- small reserve pool in bone marrow
- in circulation ($t_{1/2}$ = 24-72 hrs)
- functional cells but require activation
- monocytes migrate into tissues → macrophages

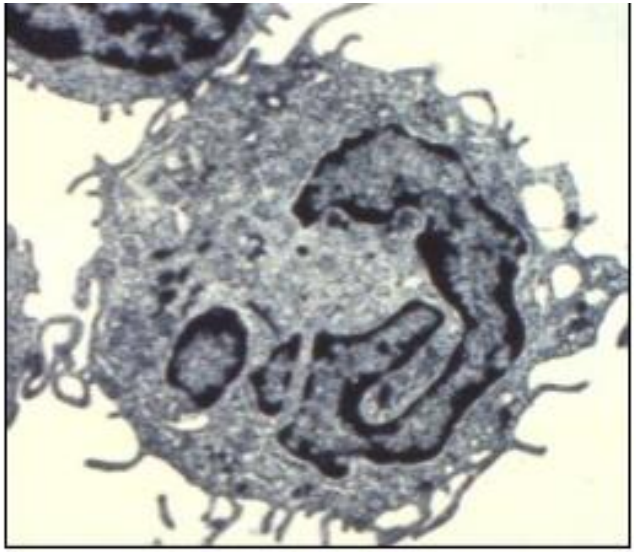


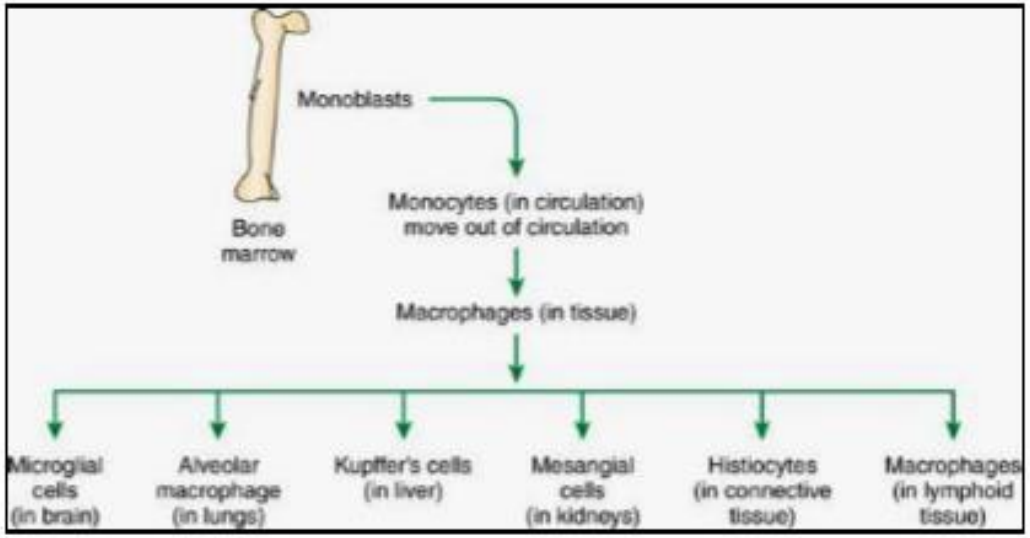


Macrophages

Macrophages (MØ's)

- derived from circulating monocytes (or resident MØ's)
- $t_{1/2}$ 30-60 days in tissue
- motile - but sluggish





Macrophages/Monocytes - Morphology

- larger than neutrophils
- large nuclei (folded or bean-shaped)
- contain lysosomes

Macrophages - Functions

- **"Most dynamic and gifted of the leukocytes“**
  - Antimicrobial and phagocytic (O2 radicals)
  - Recruit other leukocytes (chemokines/cytokines)
  - Stimulate or modulate other cell activity
  - Clean up debris
  - Induce systemic effects

- Source of: • Epithelioid macrophages • Multinucleated giant cells

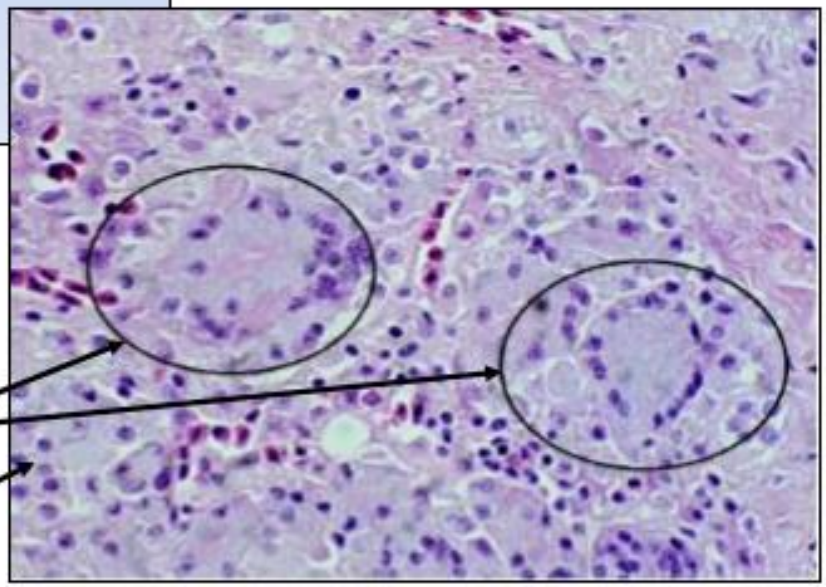
Where do we see MACROPHAGES?

Acute Inflammation (in low numbers)
- with neutrophils

Subacute Inflammation
- with plasma cells/lymphocytes

Chronic Inflammation
- predominate in granulomatous inflammation

Repair



Epithelioid cells

- Specialized macrophages with more abundant eosinophilic cytoplasm and eccentrically located, round to oval nucleus, thus resembling epithelial cells.
- Possess numerous lysosomes and vacuolated cytoplasm.
- Have fewer receptors and less phagocytic activity; they specialize in secretion of cytokines.
- Can fuse together to form multinucleate giant cells.

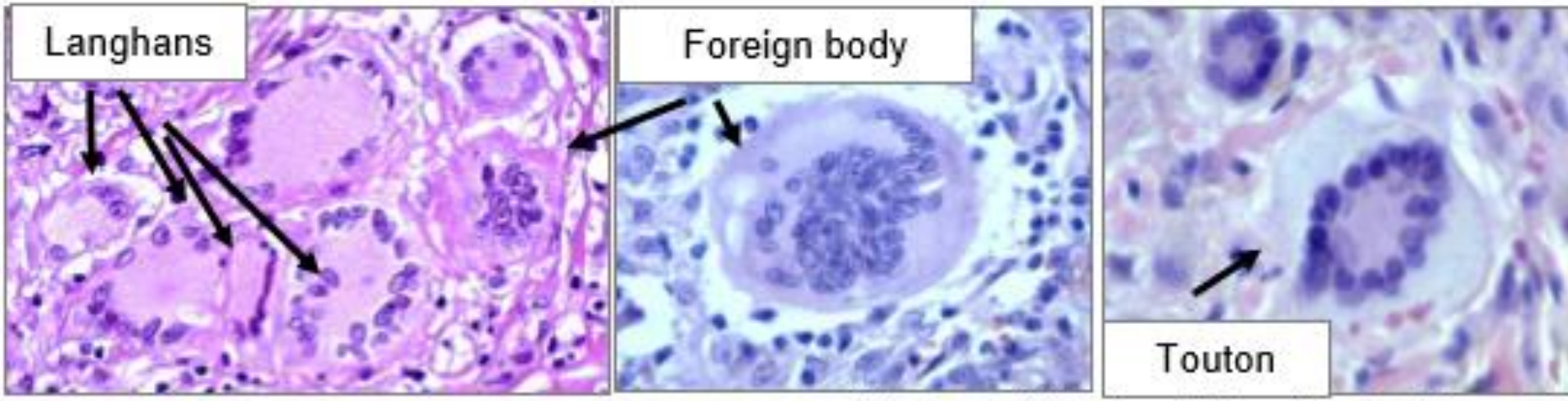
Giant Cells

- Multinucleated Giant Cells
- Result from fusion of macrophages under the influence of IL-4 and IFN-γ.

Types:

- **Langhans:** nuclei located at periphery; found in most types of chronic inflammation.
- **Foreign body:** nuclei scattered throughout the giant cell cytoplasm.
- **Touton:**
  - rosette of nuclei at the centre,
  - can be in tumours of histiocytic (tissue macrophage) origin or xanthomas (masses composed of lipids, foamy macrophages and giant cells;
  - associated with defects in lipid or triglyceride metabolism).

Giant Cells



Yale Rosen, MD "Atlas of Granulomatous Diseases"

Participation of macrophages in chronic inflammation

- Continued recruitment of monocytes from circulation (if persistant agent) thanks to steady expression of chemotactic factors, eg C5a, IL-8, PDGF.
- Macrophage numbers can also increase due to local proliferation (replication).
- Macrophages can be activated by microbial products (eg LPS), cytokines (eg IFN-γ, IL-4) & other mediators.
- Activated macrophages become immobilized and long lived at the sites of chronic inflammation.
- Macrophages cause tissue destruction, even when properly activated.
- **Tissue destruction is one of the hallmarks of chronic inflammation.**

Actions of activated macrophages

- Inflammation & Tissue Injury, due to the release of:
  - reactive oxygen and nitrogen species
  - proteases
  - cytokines / chemokines
  - coagulation factors
  - arachidonic acid metabolites

- Repair / Fibrosis, due to the production of:
  - growth factors (PDGF, FGF, TGFß)
  - fibrogenic cytokines
  - angiogenesis factors
  - "remodelling" collagenases

Lymphocytes

Characteristics

- less motile than PMN's & macrophages
- not phagocytic and possess only limited power of amoeboid movement.
- recirculate (lymph nodes, lymphatics)
- 'perivascular cuffing'.
- They usually appear late in inflammation and are the important cells in chronic inflammation.

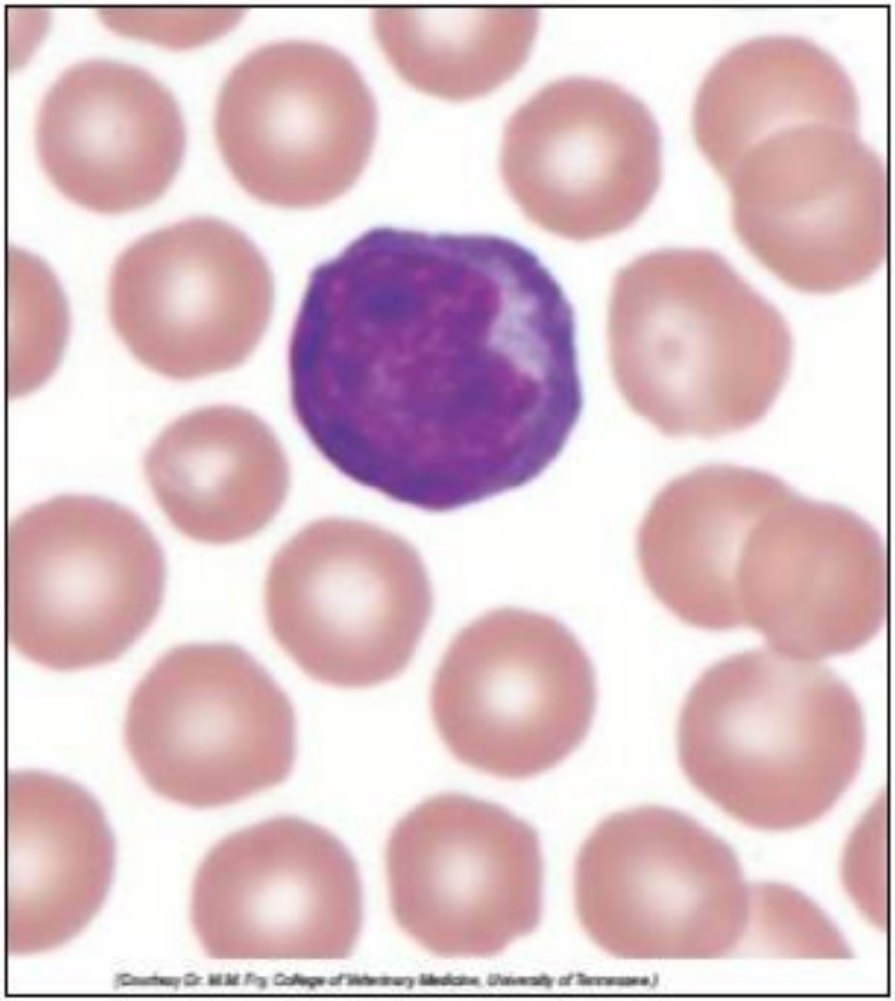
Lymphocytes -

Morphology

Heterogeneous

- T lymphocytes -Thymus
  - Cell mediated immunity
  - Produce lymphokines

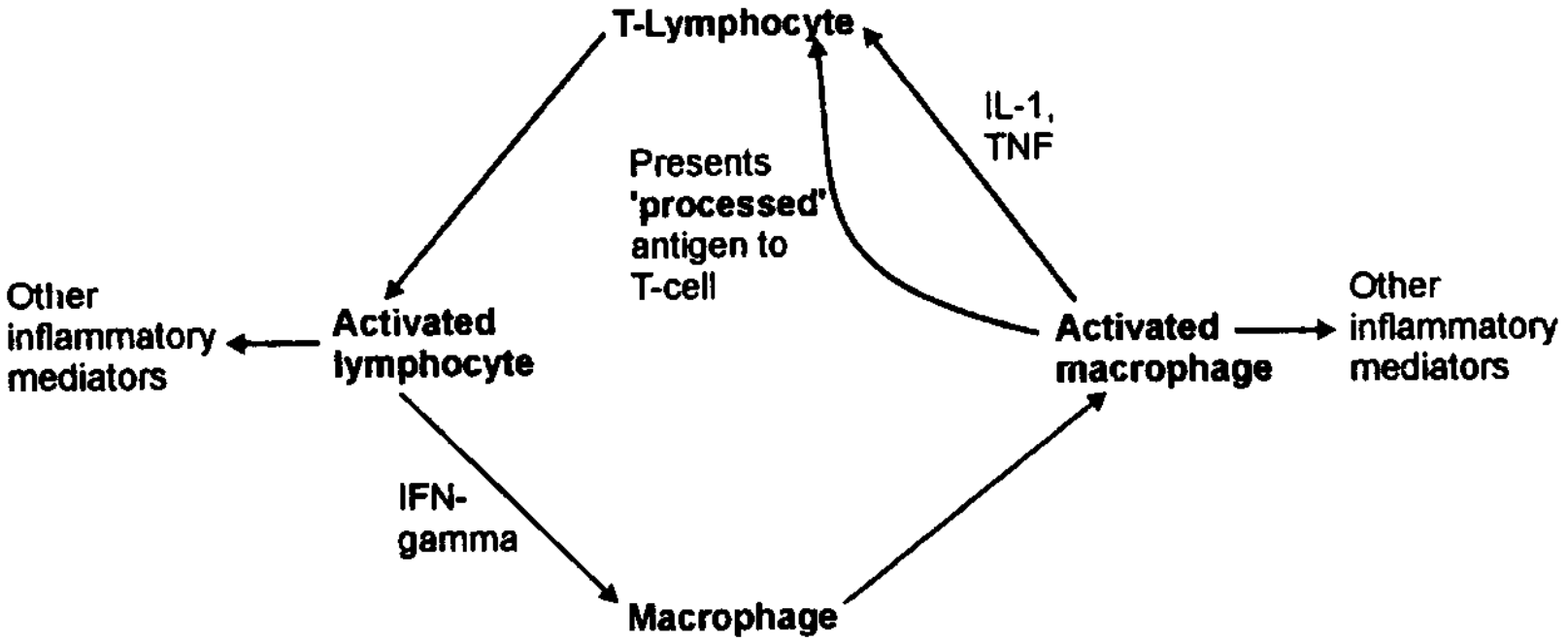
- B lymphocytes- Bone marrow
  - Produce plasma cells
  - Important in antibody production



Cont…

- Lymphocytes comprise 40 to 60 percent of the total blood leukocytes
- Most of the lymphocytes present in blood are
  - T-lymphocytes (60% -70%),
  - B-lymphocytes being 10%-20%,
  - the rest (10%-15%) are natural killer (NK) cells.
- Lymphocytes are mobilized in both
  - antibody-mediated and
  - cell-mediated immune reactions

Macrophage-lymphocyte Interactions In chronic Inflammation



Where do we see LYMPHOCYTES?

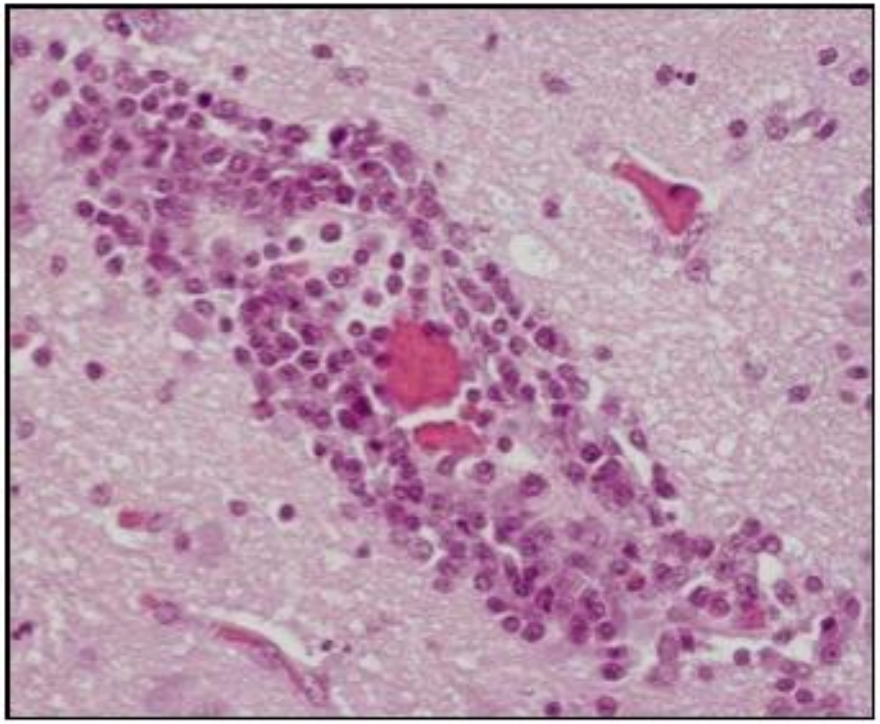
1. Subacute Inflammation
2. Viral Infections
3. Immune-Mediated Diseases
4. Chronic Inflammation

Morph. Dx:

Encephalitis, lymphoplasmacytic, multifocal, subacute, moderate

Name of Disease:

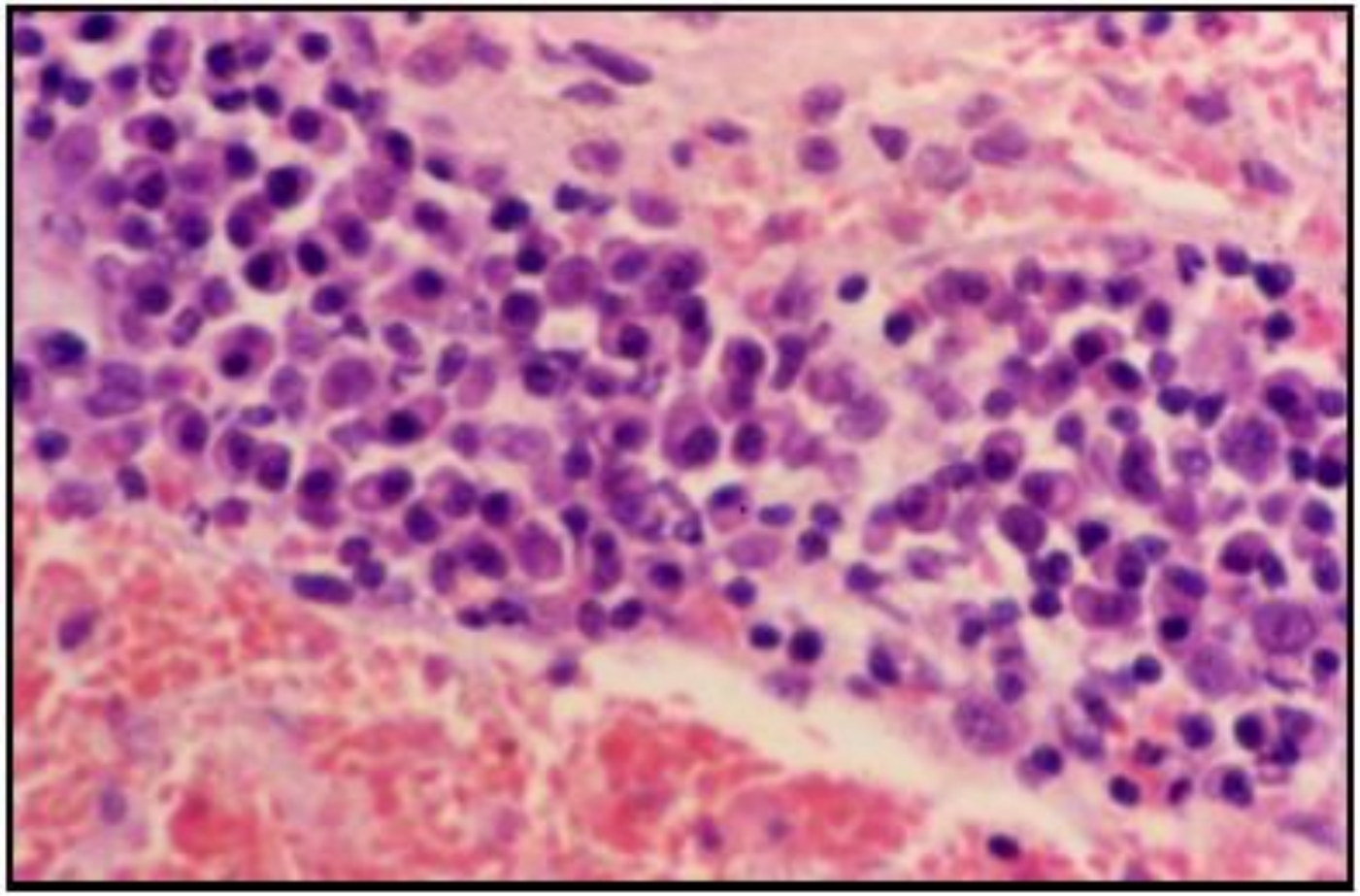
Distemper (Canine Morbillivirus)



Plasma cells

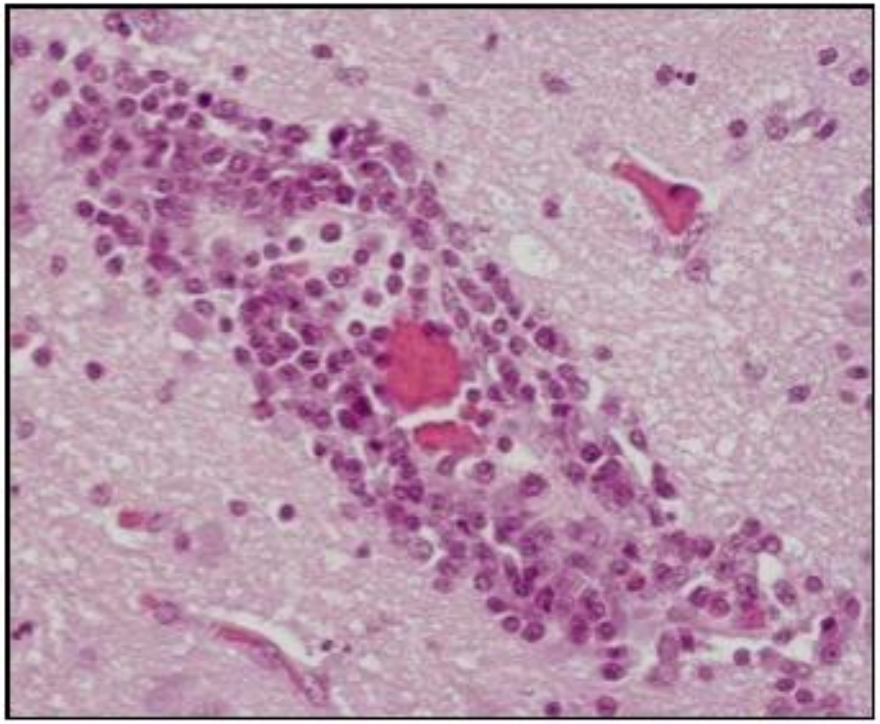
- not found in blood, but are present in tissues.
- important component of many chronic inflammatory reactions.
- possess more cytoplasm than lymphocytes and are therefore larger.
- nucleus is eccentrically placed in the cell (i.e., away from the centre) and is spherical.
- The arrangement of chromatin granules along the nuclear membrane imparts it a clock face or cart-wheel appearance.
- The cytoplasm is highly basophilic;
- basophilia is due to a complex endoplasmic reticulum, which is rich in RNA.

- do not undergo mitosis.
- originate from B-lymphocytes in response to antigenic stimulation
- terminally differentiated end product of B-cell activation.
- The function of plasma cell is production, storage and secretion of antibodies (immunoglobulins).
- Chronic inflammations like
  - Johne's disease, actinomycosis, and actinobacillosis



Where do we see PLASMA CELLS?

1. Subacute Inflammation
2. Viral Infections
3. Immune-Mediated Diseases
4. Chronic Inflammation



Morph. Dx:

Encephalitis, lymphoplasmacytic, multifocal, subacute, moderate

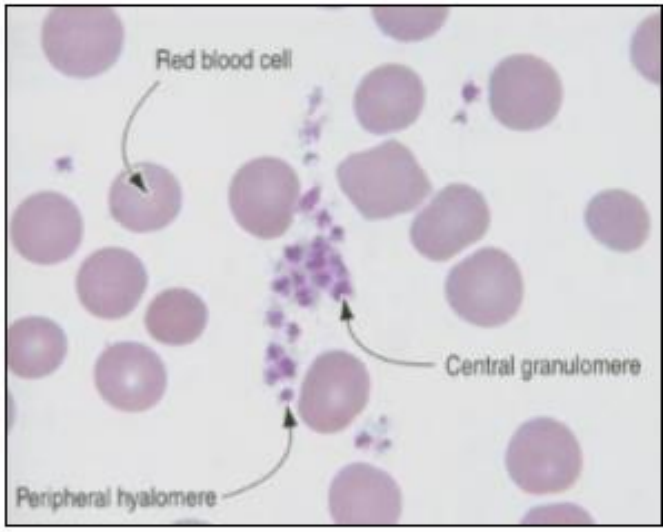
Name of Disease:

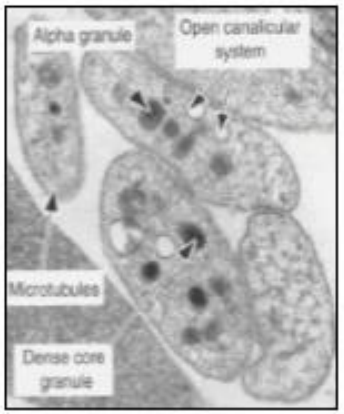
Distemper (Canine Morbillivirus)

Platelets - as inflammatory cells!

Contributions to inflammation

- Increase vascular permeability
  - -Histamine
- Produce adhesion molecules
  - - P selectin
- Release of inflammatory mediators
  - - activates complement (C5)
  - - attracts neutrophils (chemotaxis)





Classification of Inflammation

Organ	[Anatomic subtype]	Exudate (type of inflammation)	Distribution	Duration	Extent
Nephritis	Interstitial	Suppurative	Focal	Peracute	Minimal
	Glomerulo-	Fibrinous	Multifocal	Acute	Mild
Pneumonia	Broncho-	Necrotizing	Locally extensive (segmental)	Subacute	Moderate
Enteritis		Granulomatous	Diffuse	Chronic	Marked
Hepatitis		Catarrhal		Chronic-active	(severe)
Etc.	Etc.	Etc.	Etc.		

WORD ROOT	ORGAN/TISSUE
Arter-	artery
Oste-	bone
Osteomyel-	bone marrow, or bone and bone marrow
Encephal-	brain
Encephalomyel-	brain and spinal cord
Bronch-	bronchi
Burs-	bursa(e)
Typhl-	cecum
Typhlocol-	cecum and colon
Col-	colon
Conjunctiv-	conjunctiva(e)
Cellul-	connective tissue (usually under the skin)
Duoden-	duodenum
Ot-	ear
Endocard-	endocardium
Esophag-	esophagus
Ophthalm-	eye (does not specify area[s] of the eye)
Panophthalm-	eye, the entire eye
Kerat-	eye, just the cornea
Keratoconjunctiv-	eye, cornea, and conjunctivae

WORD ROOT	ORGAN/TISSUE
Lymphaden-	lymph node
Mast-	mammary gland(s)
Mening-	meninges
Meningoencephal-	meninges and brain
Stomat-	mouth
Myocard-	myocardium
Myos- (my-)	muscle
Neur-	nerve
Rhin-	nose
Salping-	oviduct
Pericard-	pericardium
Periost-	periosteum
Periton-	peritoneum/abdominal cavity
Pharyng-	pharynx
Pleur-	pleura
Posth-	prepuce
Prostat-	prostate
Proct-	rectum or anus, or both
Dermat-	skin
Pododermat-	skin (and often deeper structures) of the foot
Funicul-	spermatic cord
Myel-	spinal cord

Uve-	eye, just the uveal tract (iris, ciliary body, choroid)
Blephar-	eyelid
Cholecyst-	gallbladder
Aden-	gland (generic)
Balan-	glans penis
Gingiv-	gum
Valvul-	heart valve
Lamin-	hoof
Enter-	intestine
Arthr-	joint
Nephr-	kidney
Laryng-	larynx
Laryngotrache-	larnyx and trachea
Cheil-	lip
Hepat-	liver
Pleuropneumon-	lung and pleura
Pneumon-	lung Note: inflammation of the lung is usually, by common convention, referred to as "pneumonia," not "pneumonitis." Likewise, inflammation of the pleura and lungs is called "pleuropneumonia," not "pleuropneumonitis."
Splen-	spleen
Gastr-	stomach
Synov-	synovium
Tendin-	tendon
Orch(id)-	testicle
Gloss-	tongue
Tonsil-	tonsil
Odont-	tooth
Trache-	trachea
Omphal-	umbilicus
Ureter-	ureter
Cyst-	urinary bladder
Metr-	uterus
Vagin-	vagina
Phleb-	vein

Catarrhal or Mucous Inflammation

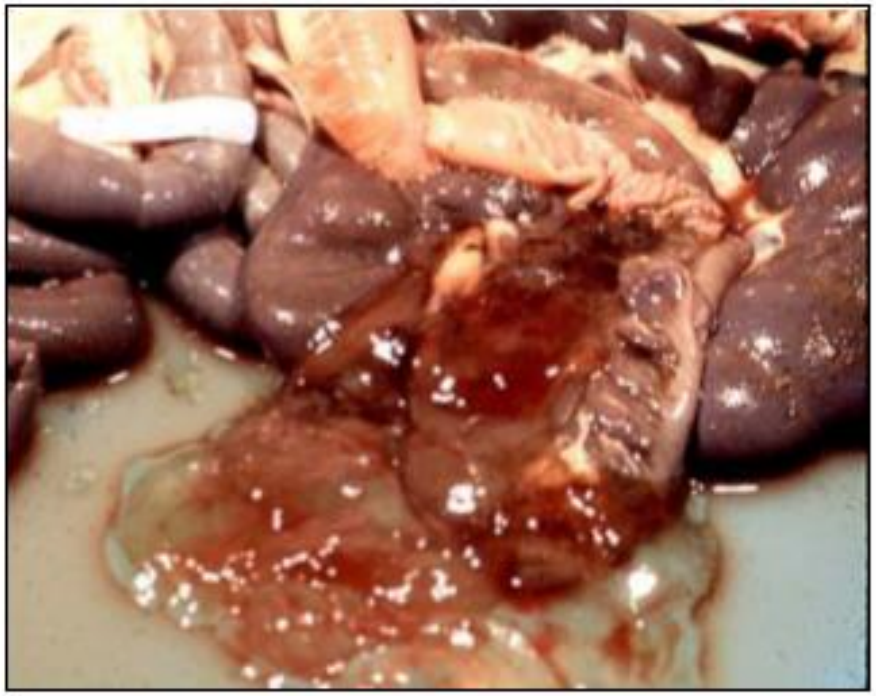
- main component of exudate is mucus
- with few inflammatory cells

Example (rabbit):

Morph. Dx: **Enteritis, mucoid**

Disease name: **Mucoid Enteritis**

Etiology: **Unknown**



Inflammation of a mucous membrane with marked increase in flow of exudate (typically mucoid or mucopurulent exudate)

SEROUS INFLAMMATION

- Main component - Serous exudate
- fluid rich in protein, few cells (inflammatory edema)
- on body surface or mucosa

Time

– usually acute

Causes

– often dominant pattern of exudation for a wide variety of mild injuries.

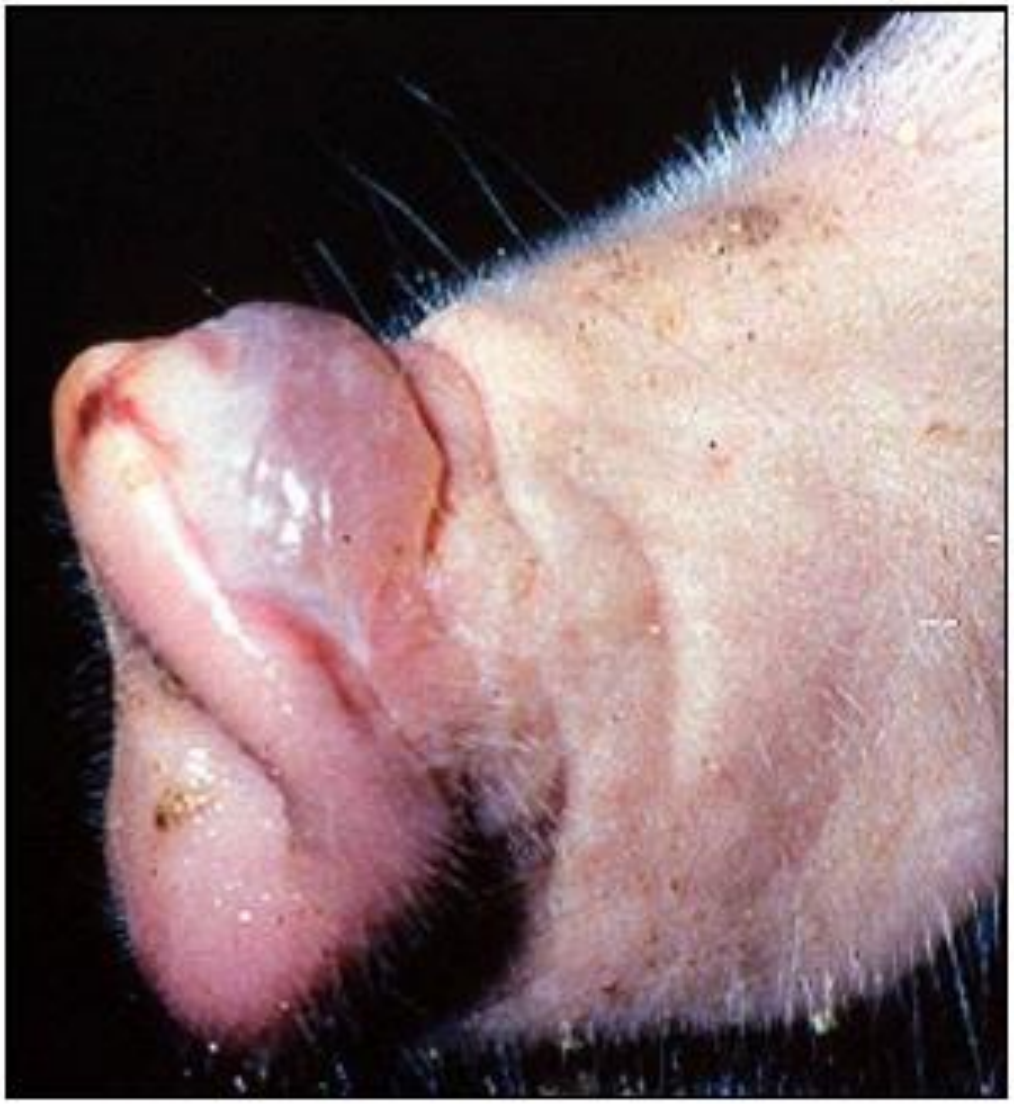
– eg, trauma, cold, blisters, sunburn

Gross Appearance

– straw-yellow or clear fluid

SEROUS INFLAMMATION

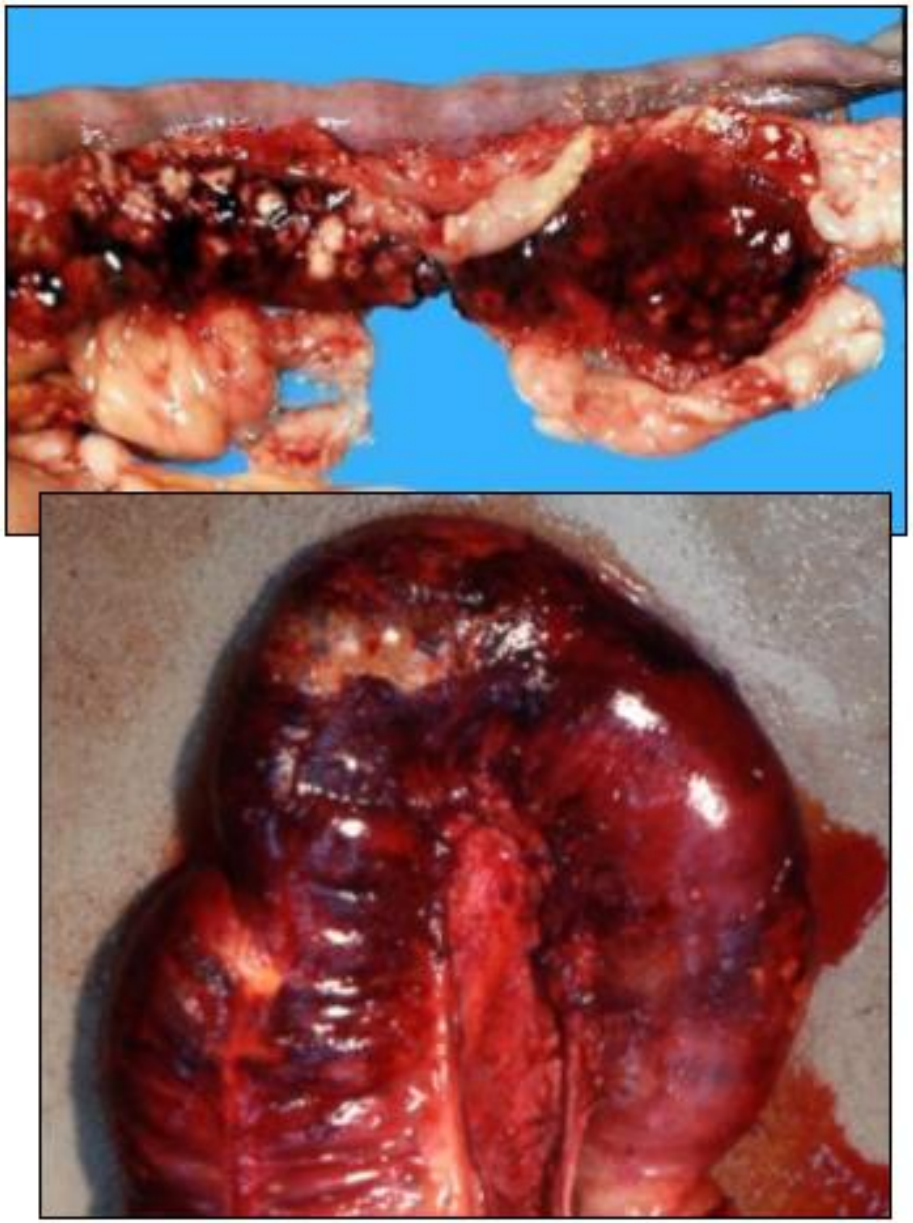
Morph. dx: Dermatitis, vesicular
Etio. dx: Viral dermatitis
Etiology: Vesicular exanthema virus



HEMORRHAGIC INFLAMMATION

- hemorrhage predominates
- severe injury to blood vessels:
  - thromobosis / vascular obstruction
  - bacterial toxins
  - proteolytic enzymes
- most often acute or peracute
- often accompanied by necrosis (necrohemorrhagic)

HEMORRHAGIC INFLAMMATION

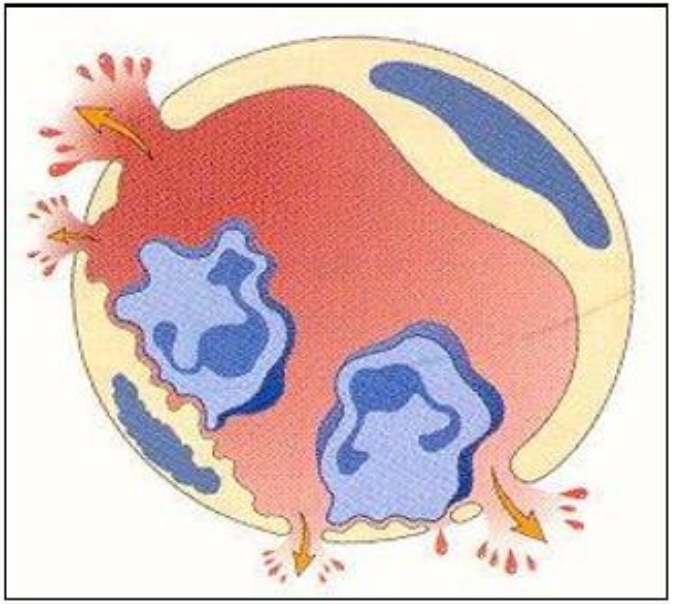


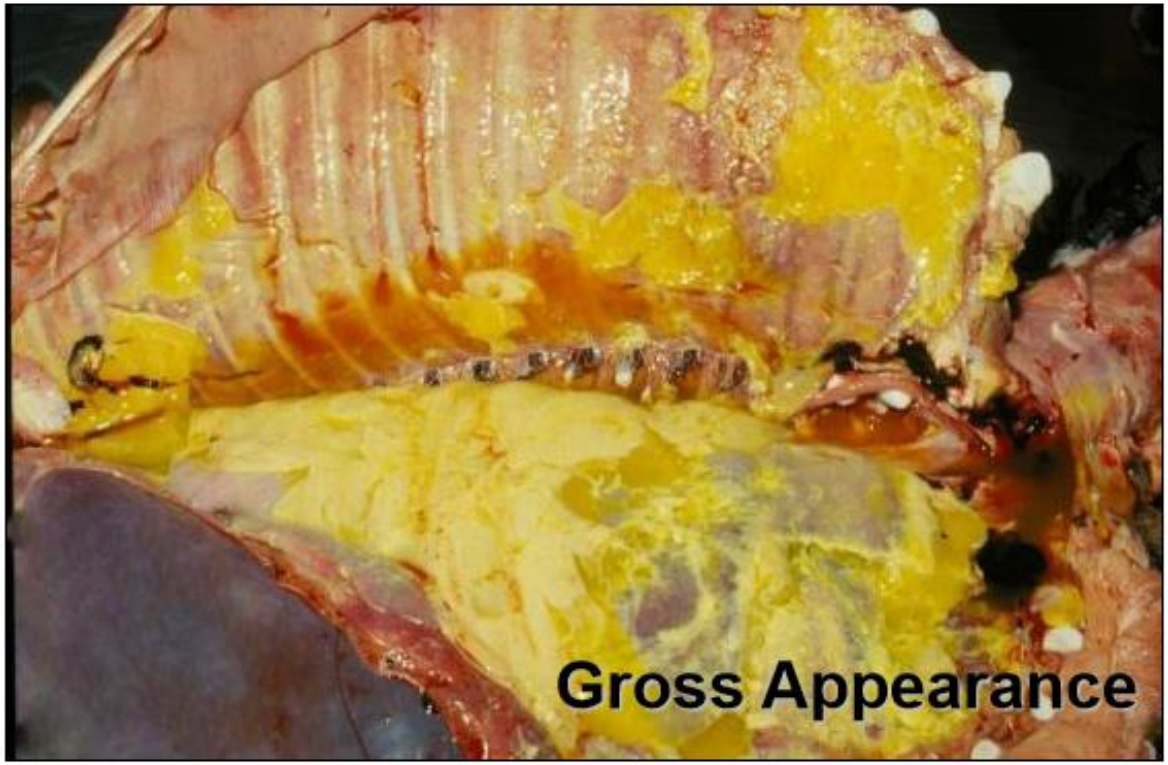
FIBRINOUS INFLAMMATION

Can be in tissue or body cavities

- 1. Increased vascular permeability (inflammatory edema)
- 2. Leakage of fibrinogen
- 3. Fibrinogen turns into fibrin
- 4. Fibrin clots

FIBRINOUS EXUDATE



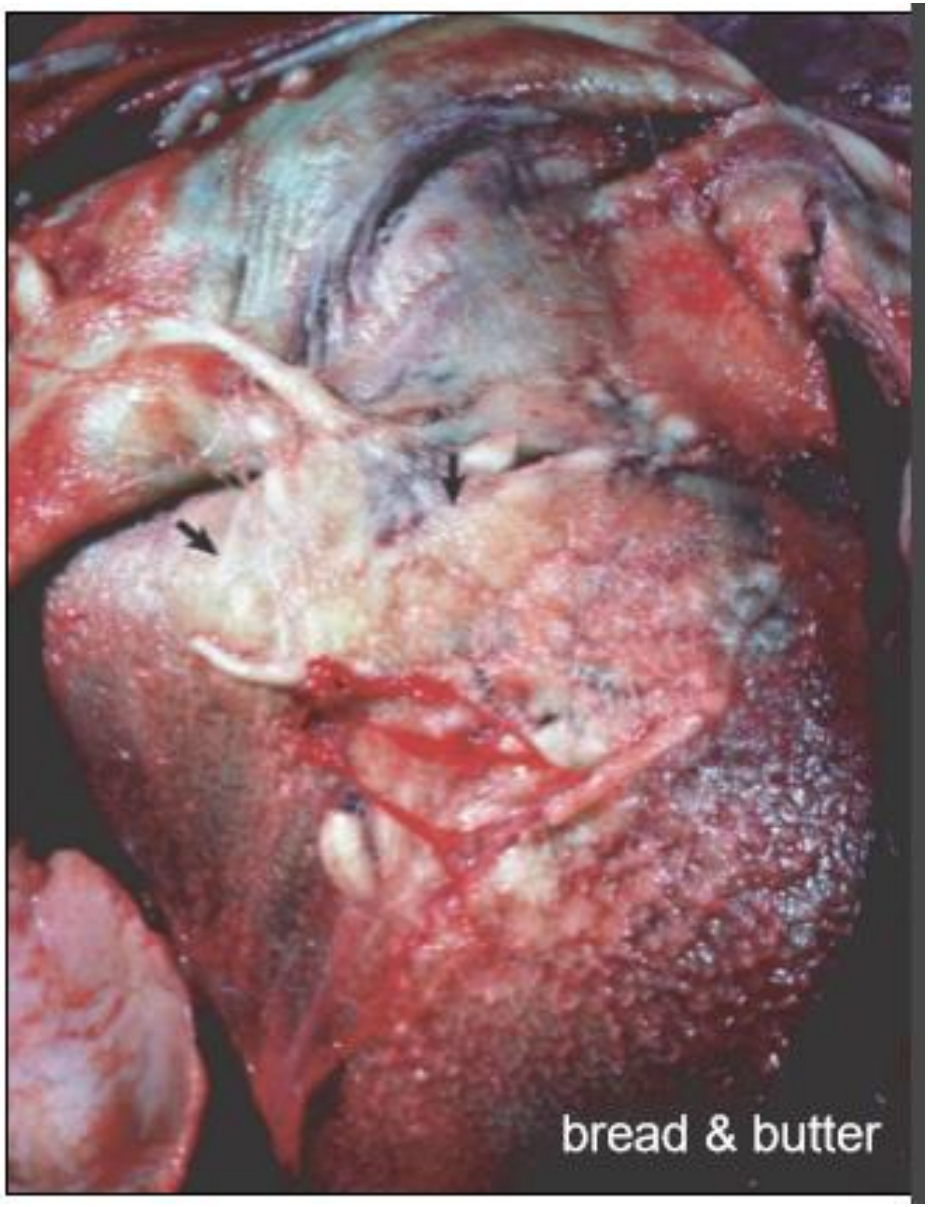


FIBRINOUS INFLAMMATION

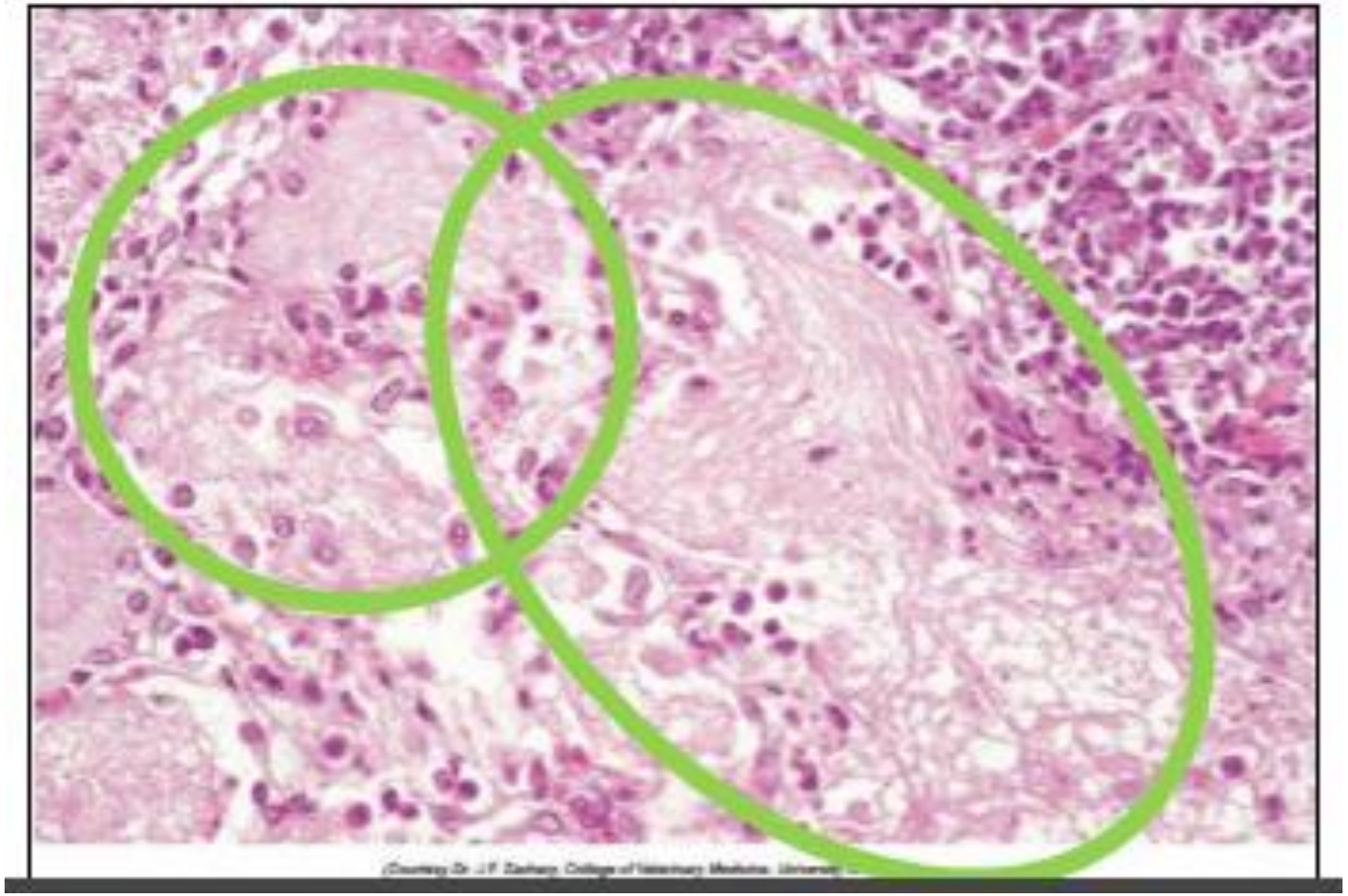
Acute process - can form in minutes

- histo: thread-like eosinophilic meshwork or solid amorphous eosionophilic material (few neutrophils)
- outcome: small amounts removed. - larger amounts provide the support for the eventual growth of fibroblasts and new capillaries (granulation)

FIBRINOUS INFLAMMATION



FIBRINOUS INFLAMMATION



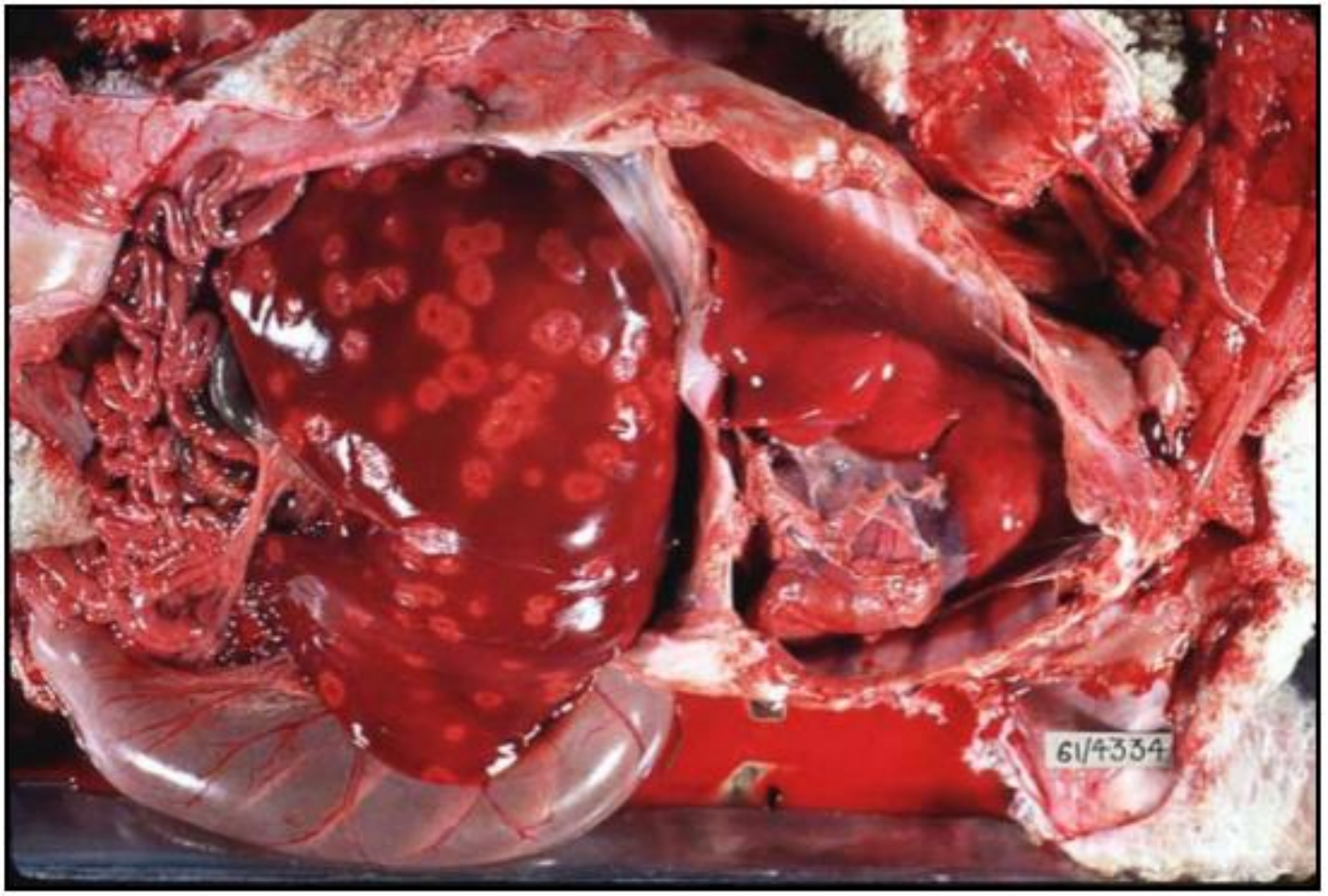
FIBRINOUS VS. FIBROUS

Fibrous adhesions are common sequelae of fibrinous exudate

NECROTIC INFLAMMATION

- characterized primarily by necrosis, with usually minimal exudate

Multifocal necrotizing hepatitis, lamb. The cause of the abortion in this case was *Campylobacter fetus.*

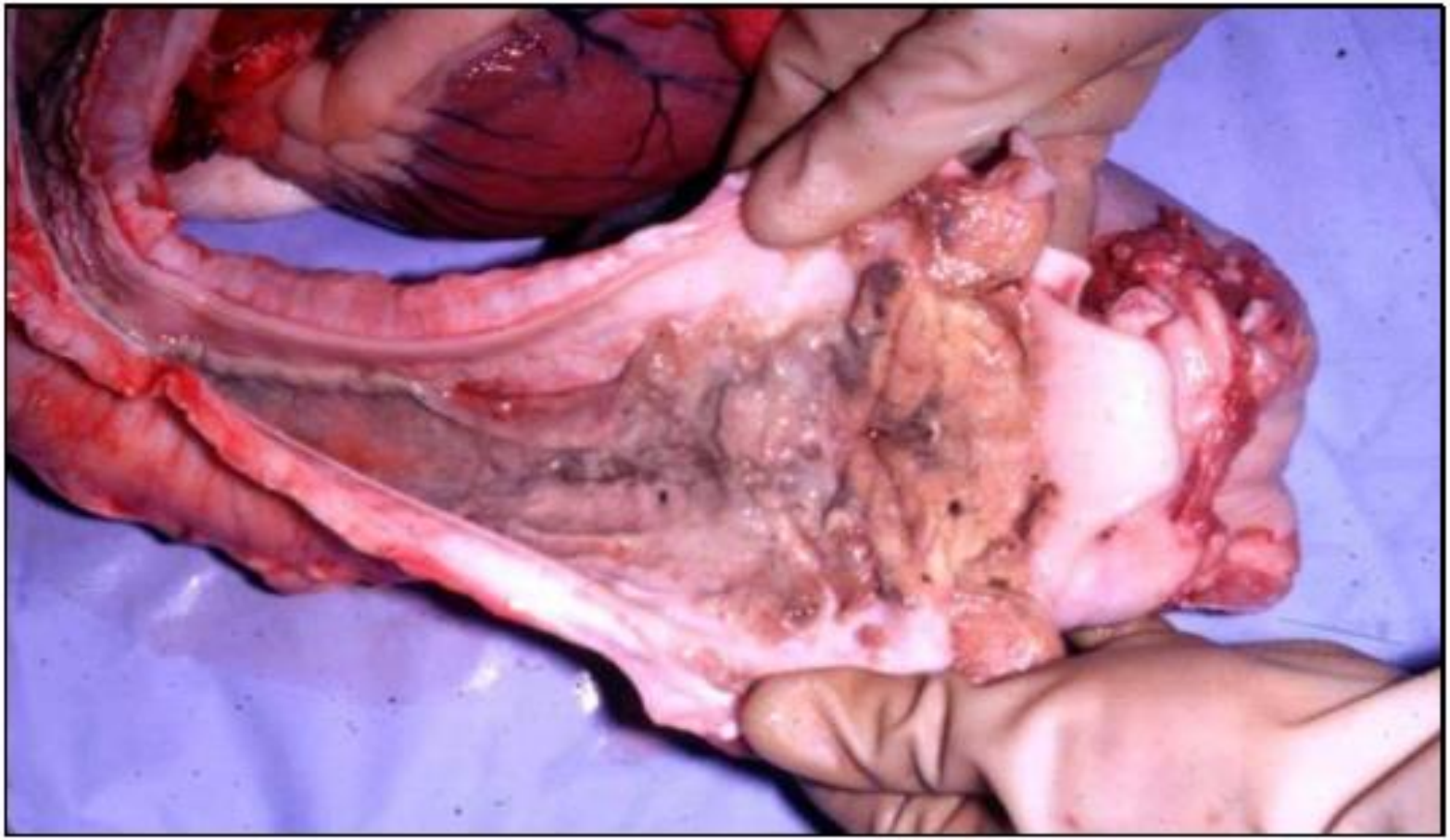


FIBRINO-NECROTIC INFLAMMATION

- necrosis of well-vascularized epithelial surface = necrosis + fibrin exudation

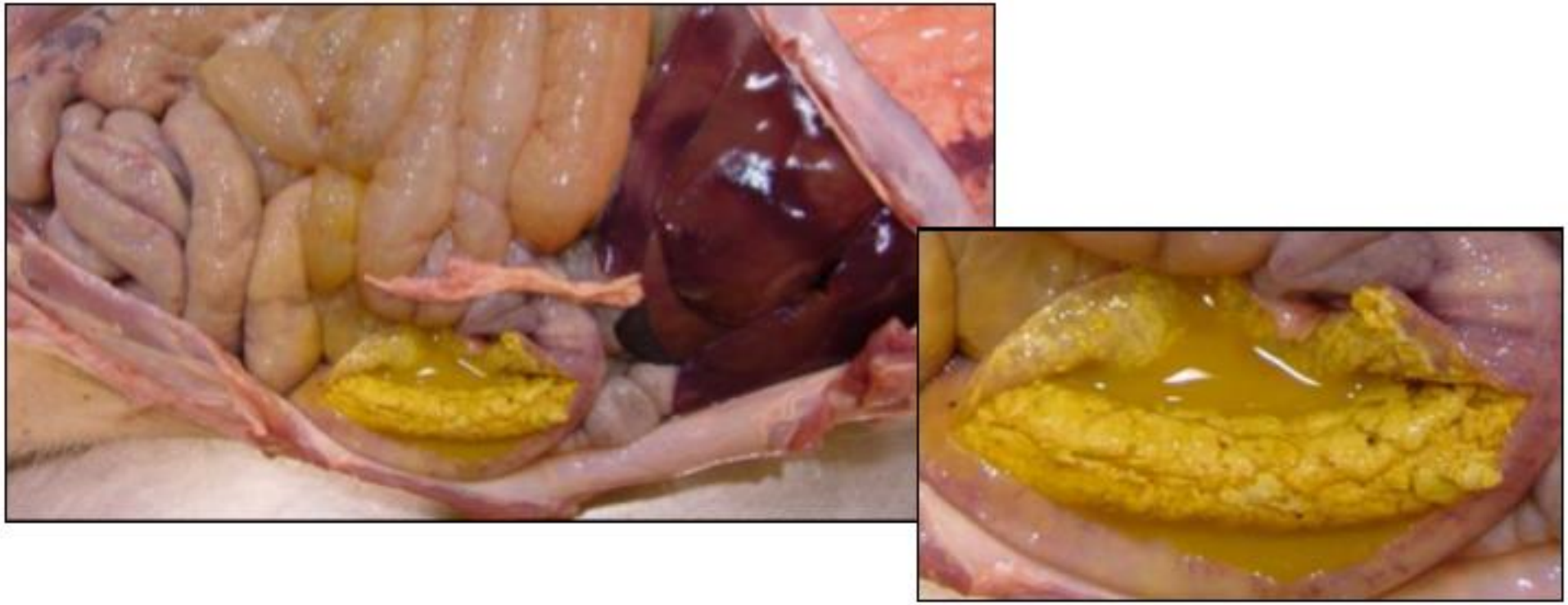
- eg, pseudomembranes (diphtheric membranes) where the fibrinonecrotic exudate forms a membrane like structure on the luminal surface (fibrin + necrotic mucosa)

FIBRINONECROTIC INFLAMMATION



Fibrinonecrotic tracheitis, Bison. Note the pseudomembrane on the luminal surface of the trachea.

in the gut "casts" of friable material (fibrin & necrotic mucosa) can fill the lumen.



Morph Dx: Enteritis, fibrinonecrotizing

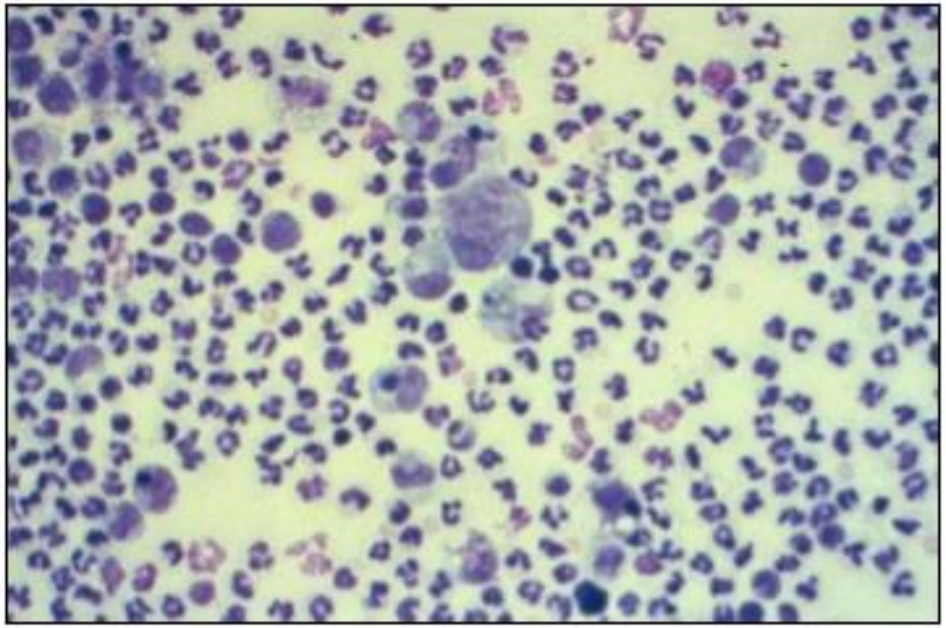
Etiology: *Lawsonia intracellularis*

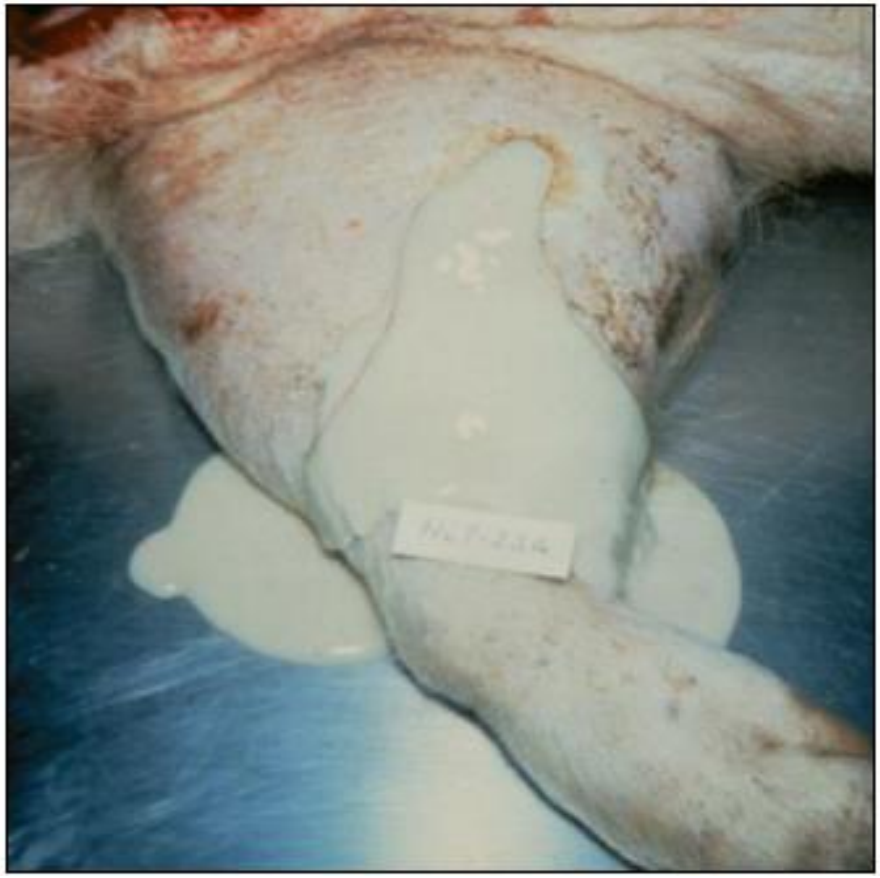
Differ Dx: Salmonellosis

SUPPURATIVE INFLAMMATION

- Main component- **Pus / purulent exudate**
- Pus- dead neutrophils
- composed of many neutrophils, necrotic cells and debris
- formation of pus due to proteolytic enzymes (esp myeloperoxidase)

SUPPURATIVE INFLAMMATION

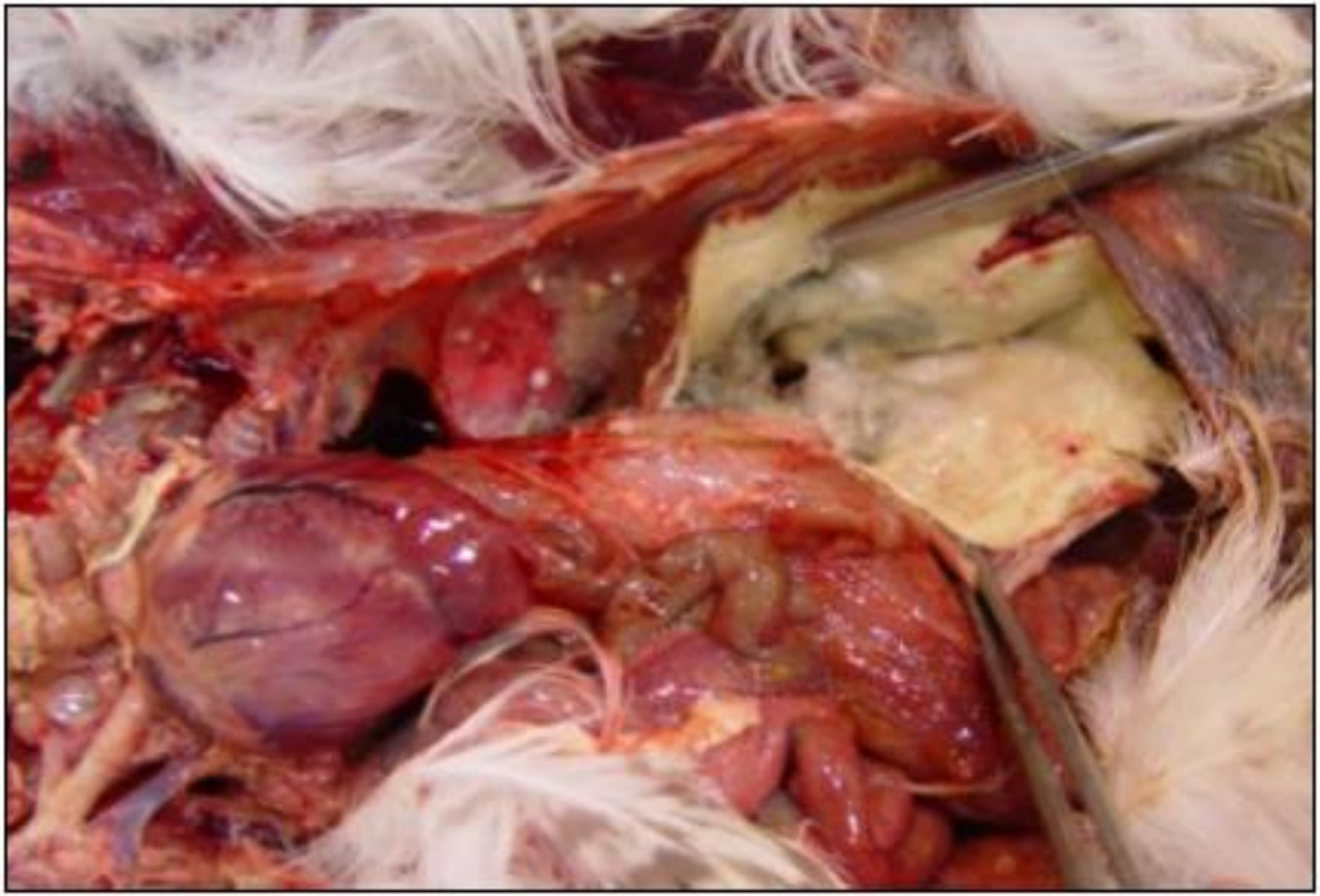




SUPPURATIVE INFLAMMATION

- avian species, amphibians, fish, reptiles & some mammals have heterophils, instead of neutrophils
- lack myeloperoxidase
- caseous exudate (no liquefaction)

SUPPURATIVE INFLAMMATION

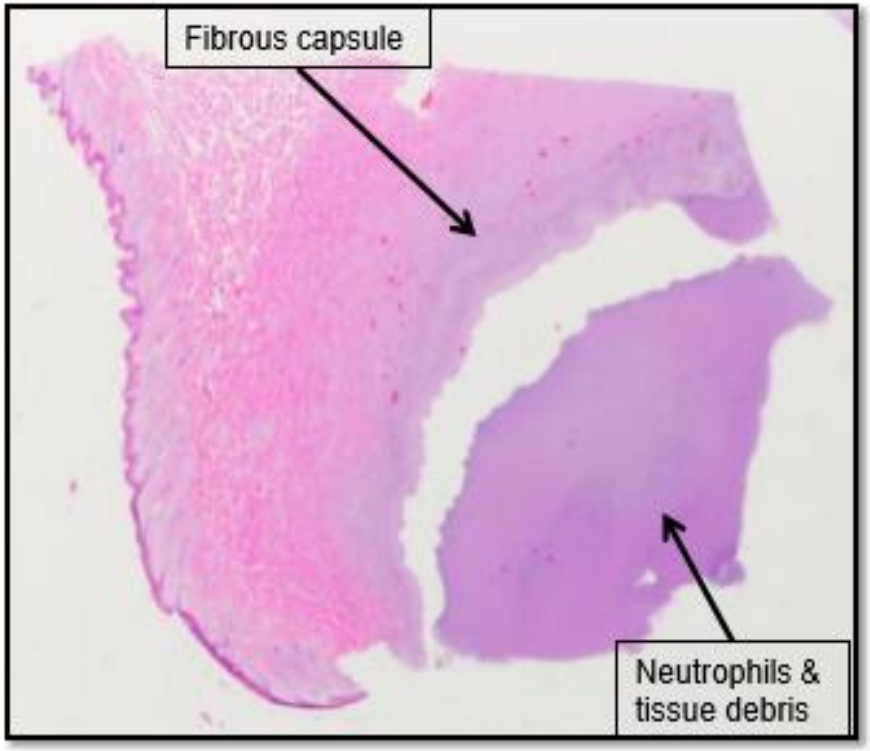


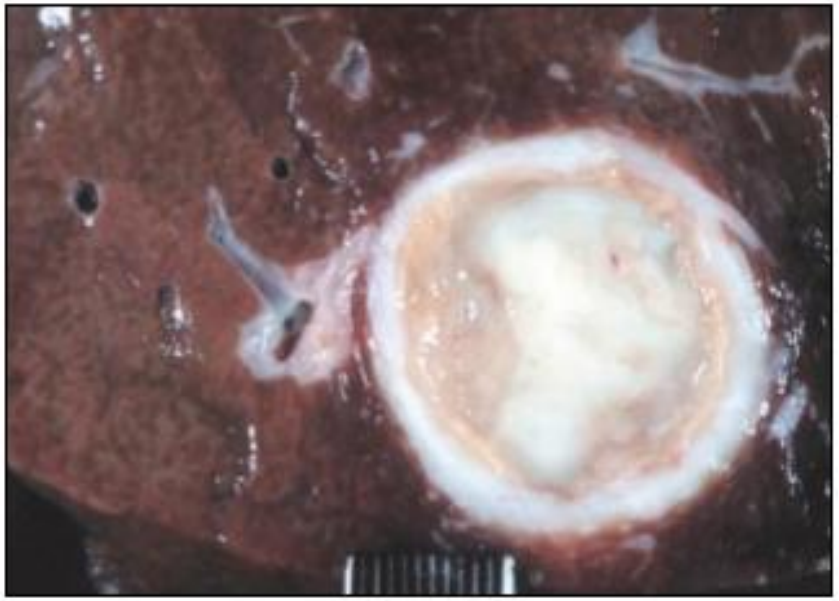
caseous exudate (no liquefaction)

SUPPURATIVE INFLAMMATION

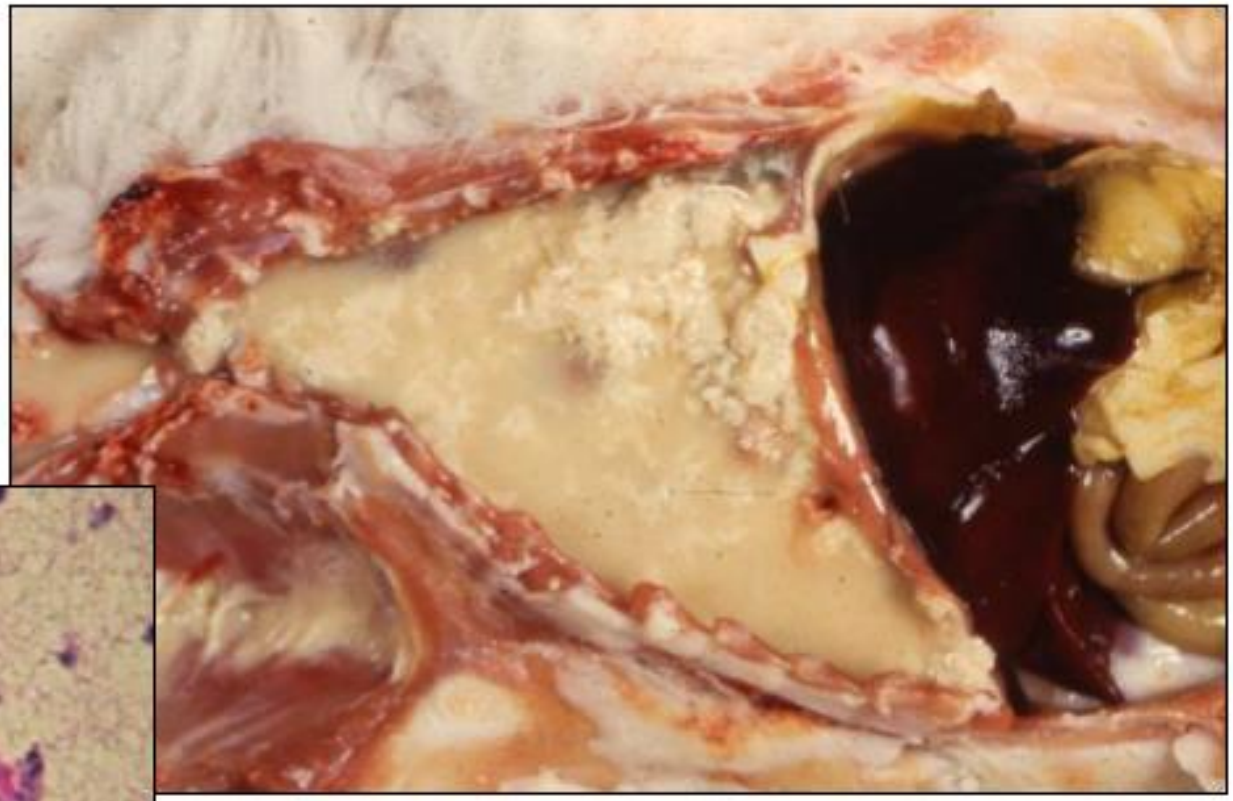
ABSCESS:

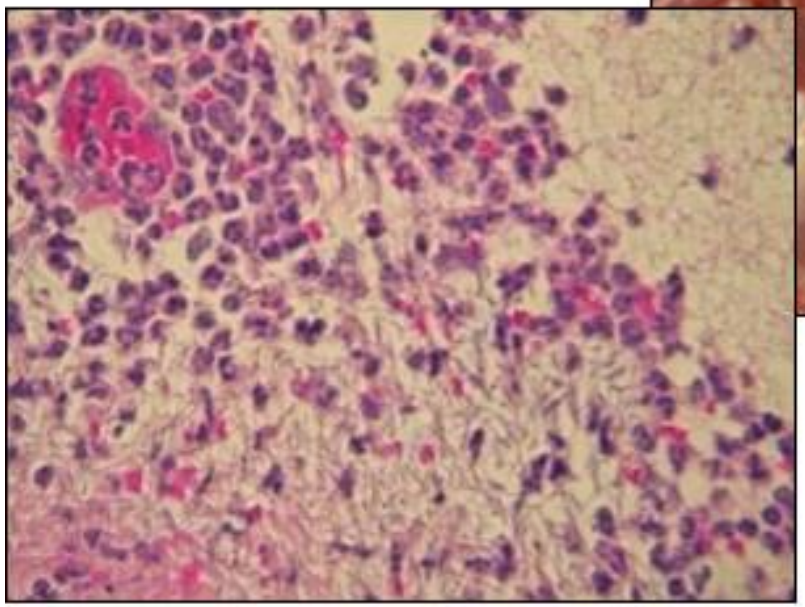
- localized form of suppurative inflammation that is walled off by a connective tissue capsule (ie chronic)
- suppurative lesions are often of bacterial origin!





SUPPURATIVE INFLAMMATION

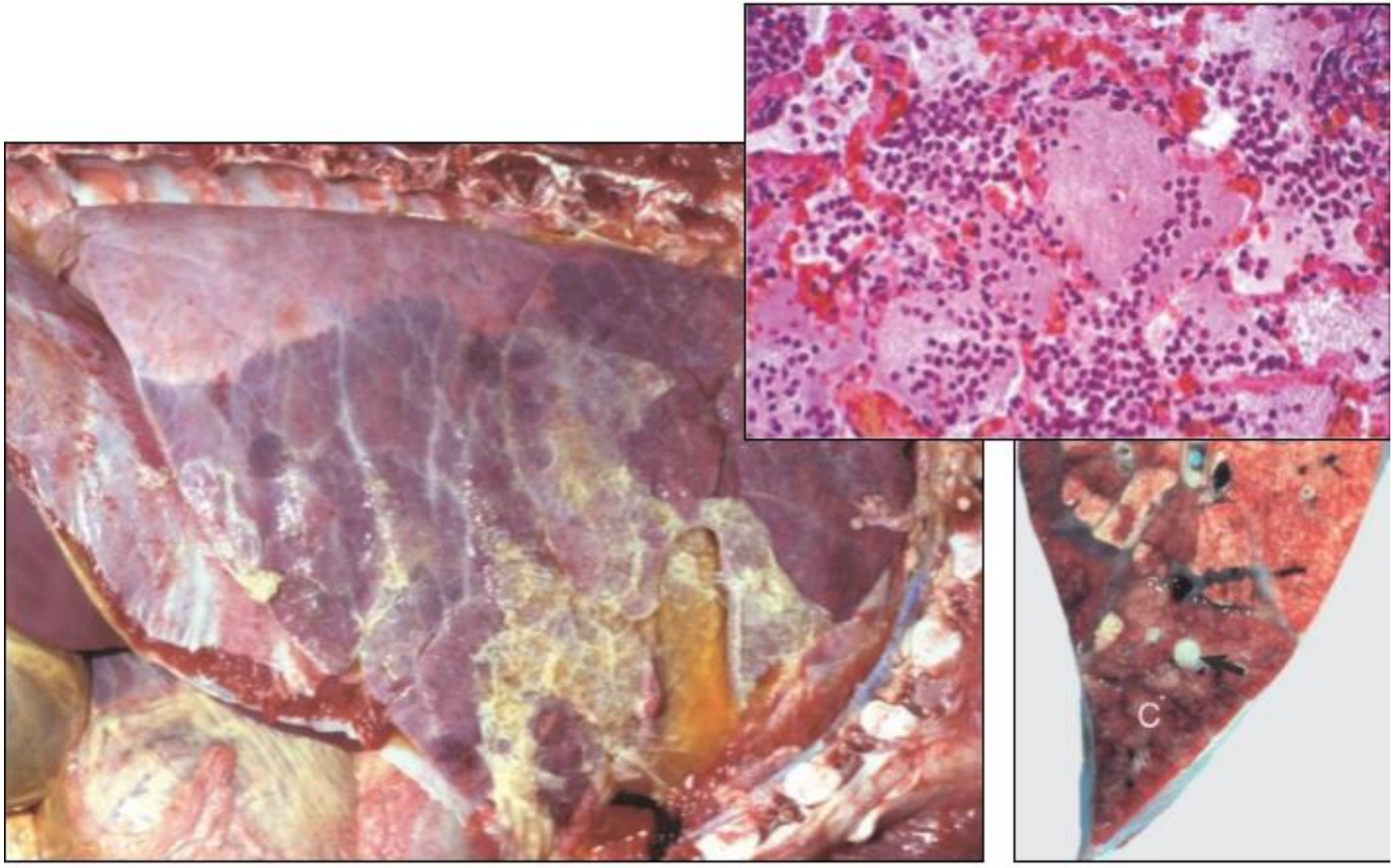




Note large numbers of neutrophils
& a few fibrin strands

Pyothorax (pleural empyema): pus in the thoracic cavity

FIBRINOSUPPURATIVE INFLAMMATION



Inflammatory process rich in both neutrophils and fibrin

GRANULOMATOUS INFLAMMATION

- Histologically dominated by **macrophages; typically epithelioid macrophages &/or multinucleated giant cells.**
- Granulomatous inflammation is a chronic process and is a distinctive pattern of chronic inflammation.
- It is a mechanism for dealing with indigestible substances and certain microorganisms that are difficult to kill.
- The dominant cells are **macrophages and lymphocytes.**
- **Cell-mediated hypersensitivity** can accelerate development and intensity of granulomatous inflammation.

GRANULOMATOUS INFLAMMATION

Gross appearance:

- Usually firm (due to fibrosis) but with variable distribution and demarcation.
- Diffuse (or locally extensive) thickening of tissue (eg. Johne's disease due to *M. paratuberculosis*).
- Nodular lesions (granulomas), often with central caseous necrosis or suppuration (pyogranuloma).

GRANULOMATOUS INFLAMMATION

Granuloma :

- Focal type of granulomatous inflammation, consisting of a central aggregate of macrophages (many being epithelioid macrophages &/or multinucleated giant cells);
- which is surrounded by variable numbers of primarily lymphocytes and plasma cells and often circumferential fibrous connective tissue.

GRANULOMATOUS INFLAMMATION

Types of Granuloma :

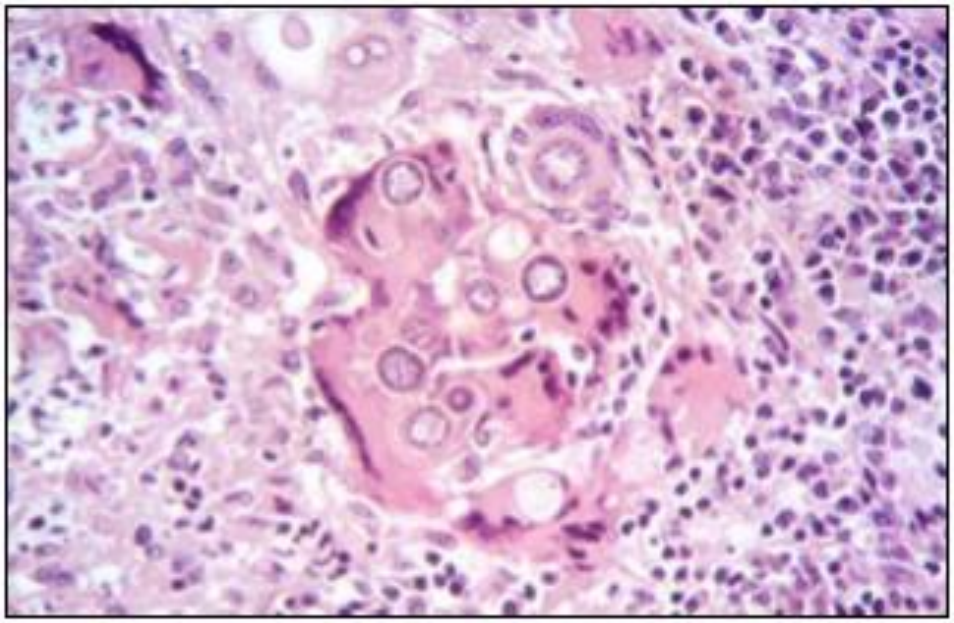
- **Simple granuloma:** organized accumulation of macrophages and epithelioid cells, often rimmed by lymphocytes.
- **Complex granuloma:** granuloma with a central area of necrosis (which may show dystrophic calcification / mineralization). Necrosis may be due to release of oxygen free radicals &/or lysosomal enzymes or ischemia.
- **Pyogranuloma:** core is rich in neutrophils; often these neutrophils have undergone degeneration.
- **Foreign body granulomas:** often characterized by the abundance of foreign body giant cells.
  - Eg. of foreign material: inert particles (eg silica, asbestos, etc), lipids resistant to metabolism (eg mineral oil), plant material (eg wood splinters, grass awns) suture material, hair, keratin, sperm, etc.

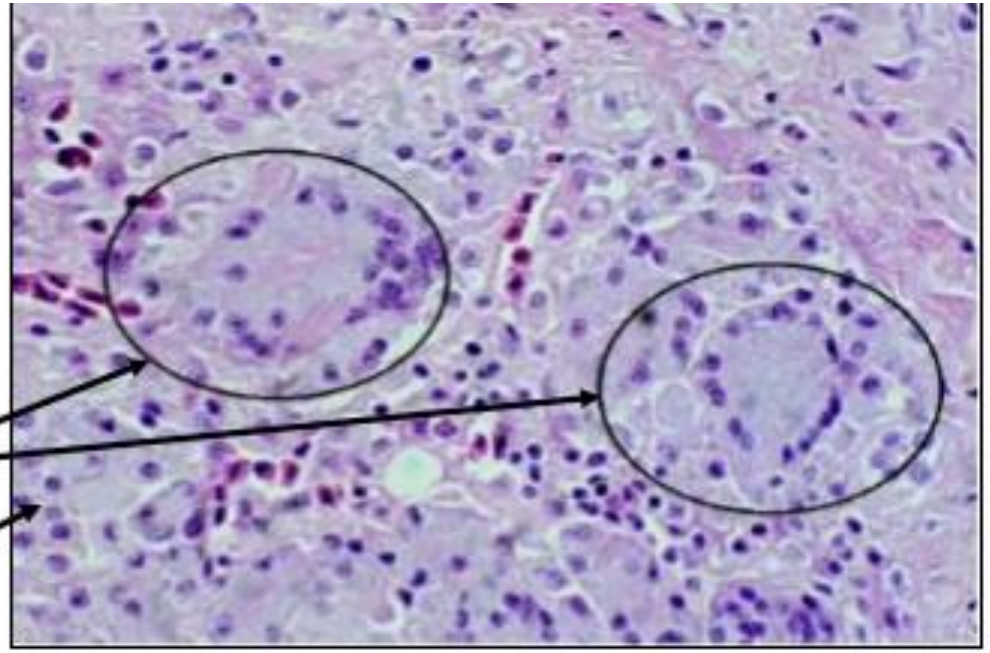
GRANULOMATOUS INFLAMMATION

Pathogenesis

- Certain pathogens (antigens) stimulate macrophages or dendritic cells to activate T lymphocytes (esp by IL-12).
- T cells secrete IFN-γ which promotes transformation of macrophages into epithelioid macrophages, +/- multinucleated giant cells.
- If pathogen persists - inflammation continues (esp maintained by TNF) and will organize into a granuloma.
- Can also have recruitment of neutrophils (esp IL-8 & IL-17) or eosinophils (IL-5).

GRANULOMATOUS INFLAMMATION





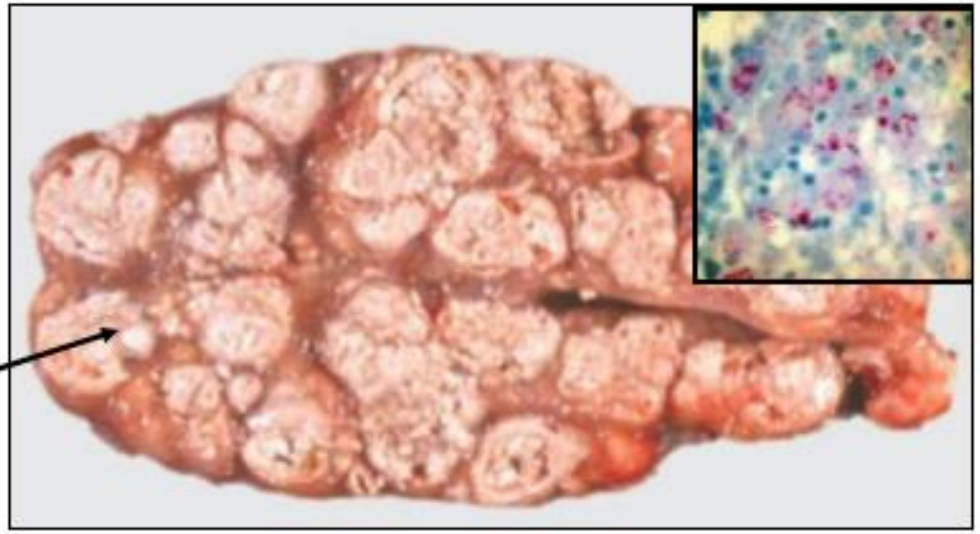
GRANULOMATOUS INFLAMMATION

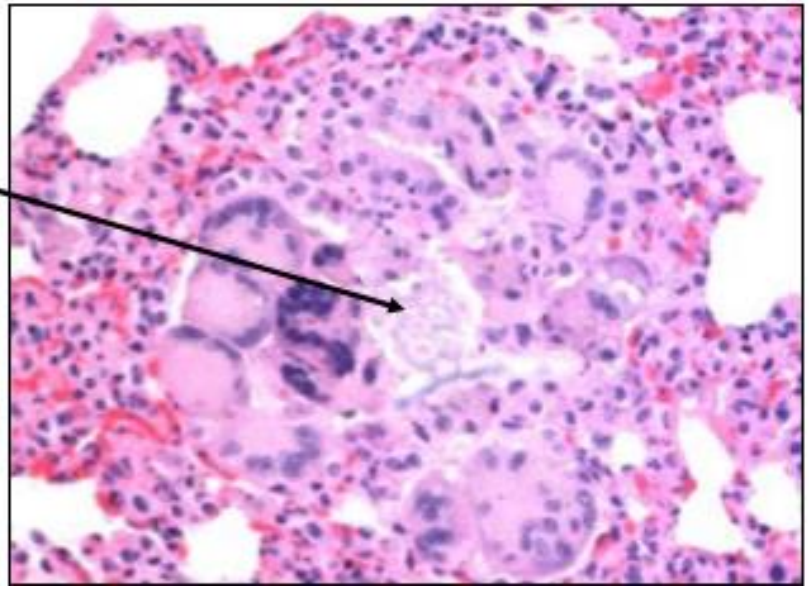
Time: always chronic

Etiology:

- typically non-digestible organism or particles
- eg:
  - Fungus
  - some bacteria (eg Mycobacteria)
  - Plant material
  - Mineral crystals
  - Suture material
  - Sperm
  - Keratin, etc.

- delayed-type HS often required





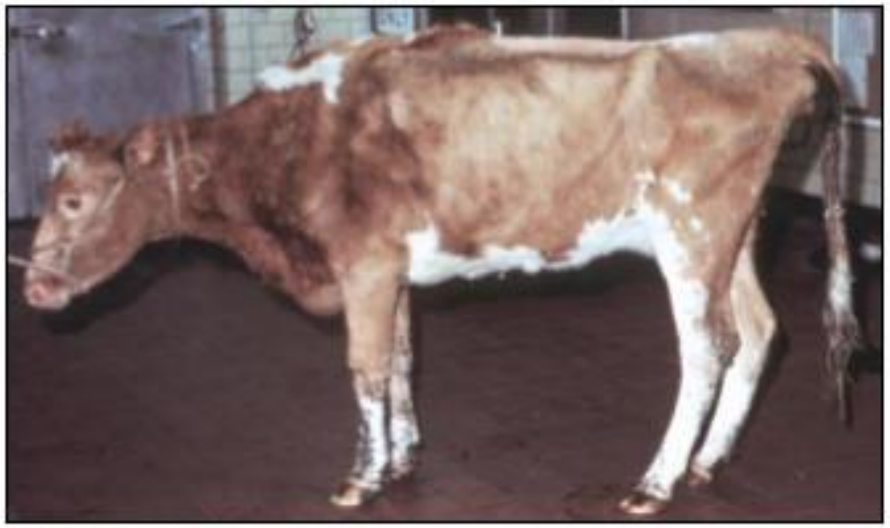
GRANULOMATOUS INFLAMMATION

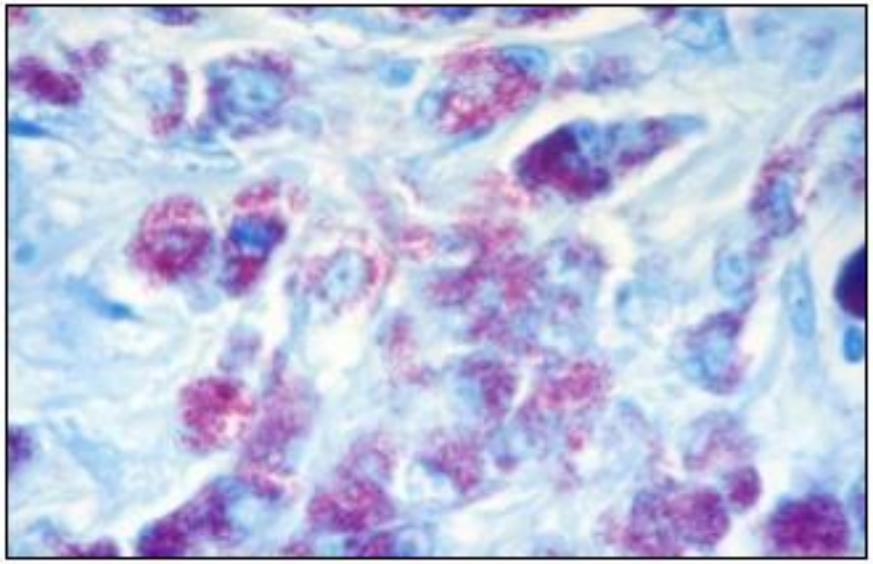
Example: Johne's disease (paratuberculosis)

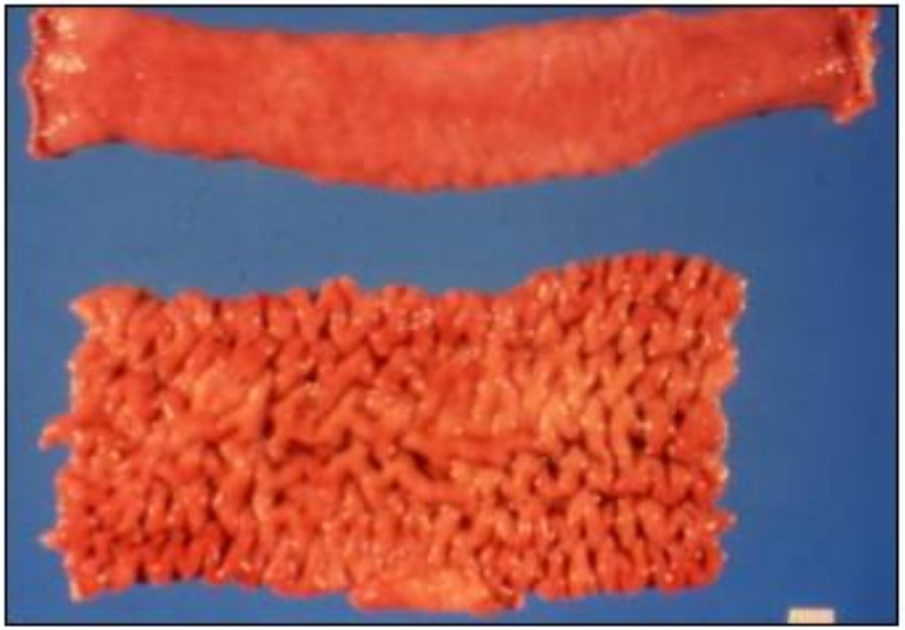
Morph. dx: **Enteritis, granulomatous**

Etio. dx: **Mycobacterial enteritis**

Etiology: ***Mycobacterium avium spp. paratuberculosis***







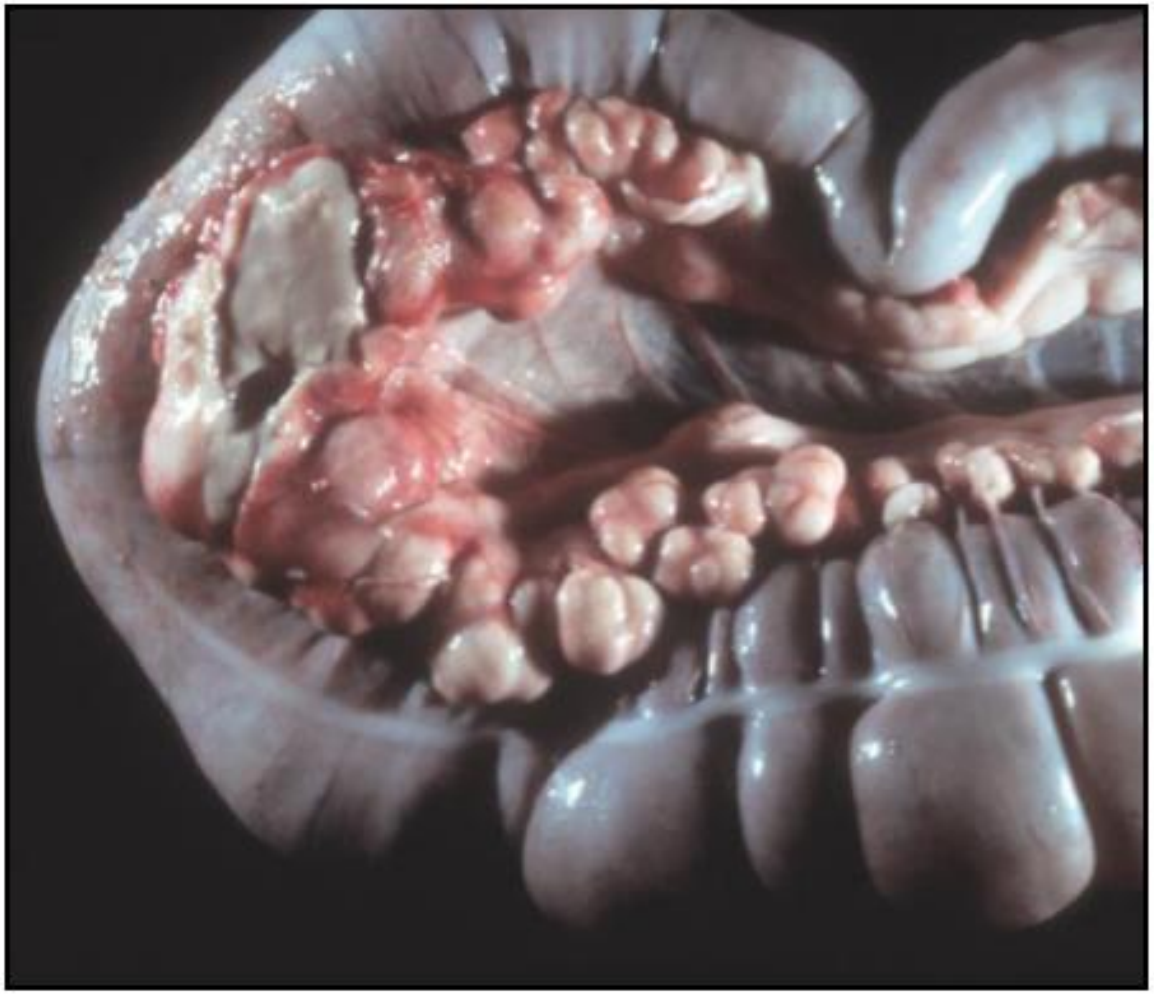
PYOGRANULOMATOUS INFLAMMATION

- neutrophilic + granulomatous inflammation

Morph. dx:
Lymphadenitis, pyogranulomatous

Name dz: Rhodococcosis

Etiologic agent:
Rhodococcus equi



PYOGRANULOMATOUS INFLAMMATION

Example:

Morph. dx: Dermatitis, pyogranulomatous

Etiologic agent: *Actinobacillus spp.*