INFLAMMATION

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Inflammation

- Definition
  - A dynamic process of chemical and cytological reactions that occur in response of vascularized tissue to stimuli that cause cell injury.

Inflammation results in:

- accumulation of leukocytes and fluid in extravascular tissue.

- systemic effects.

Effects of Inflammation

- Elimination of the cause of cell injury.
- Elimination of the necrotic cells.
- Paves the way for repair.
- May lead to harmful results.

Inflammation

Nomenclature

- -itis (- after name of tissue) e.g.
  - Appendix — Appendicitis
  - Dermis — Dermatitis
  - Gallbladder — Cholecystitis
  - Duodenum — Duodenitis
  - Meninges — Meningitis, etc.

Inflammation

Causes:

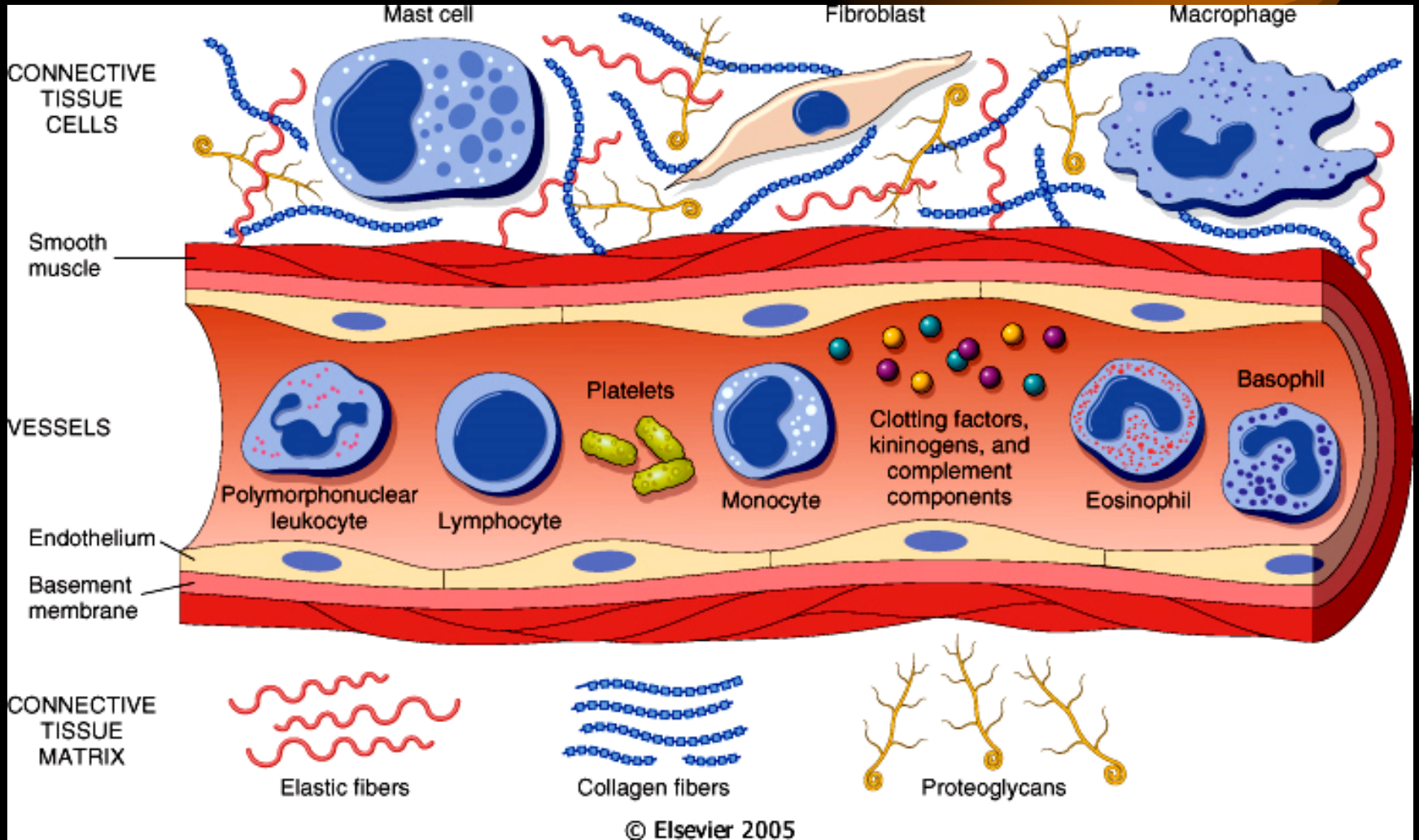
- Microbial infections: bacteria, viruses, fungi, parasites.
- Immunologic: hypersensitivity (contact with some substances), autoimmune reactions.
- Physical agents: trauma, heat, cold, ionizing radiation, etc.
- Chemical agents: acids, alkali, bacterial toxins, metals, etc.
- Foreign materials: sutures, dirt, etc.
- Tissue necrosis: ischemic necrosis.

Inflammation

The participants

1. White blood cells and platelets
   - Neutrophils, monocytes, lymphocytes, eosinophils, basophils.
2. Plasma proteins
   - Coagulation / fibrinolytic system, kinin system, complement system.
3. Endothelial cells and smooth muscles of vessels.
4. Extracellular matrix and stromal cells
   - Mast cells, fibroblasts, macrophages & lymphocytes.
   - Structural fibrous proteins, adhesive glycoproteins, proteoglycans, basement membrane.

Components of Inflammation



Inflammation

Acute inflammation

- Duration: minutes to days.
- Predominance of neutrophils.
- Fluid & plasma protein exudation.

Chronic inflammation

- Duration: days to years.
- Predominance of lymphocytes and macrphages.
- Vascular proliferation and fibrosis.

Acute Inflammation

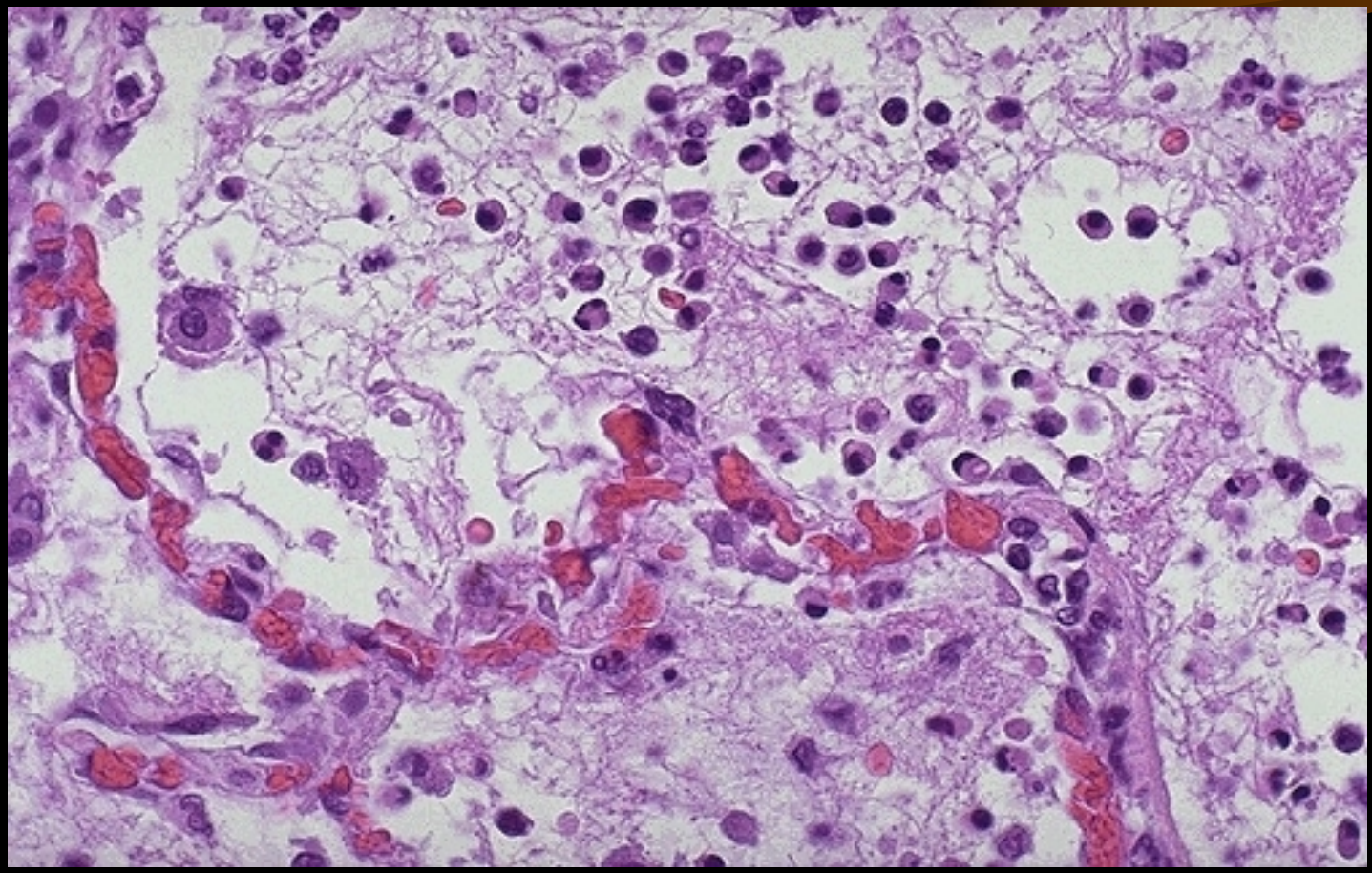
- Early response of vascularized tissue to injury.
- Aim of acute inflammation:
  - Recruitment of neutrophils (1st 3 days), and monocytes (after 3 days) to clear the cause of injury and remove necrotic cells.
  - Deliver plasma proteins: antibodies, complement, others.

The two components of acute inflammation

- Vascular changes
  - Vasodilatation.
  - Increased vascular permeability.
  - Stasis.
- Cellular events
  - Emigration of cells from microvessels.
  - Accumulation at sites of injury.

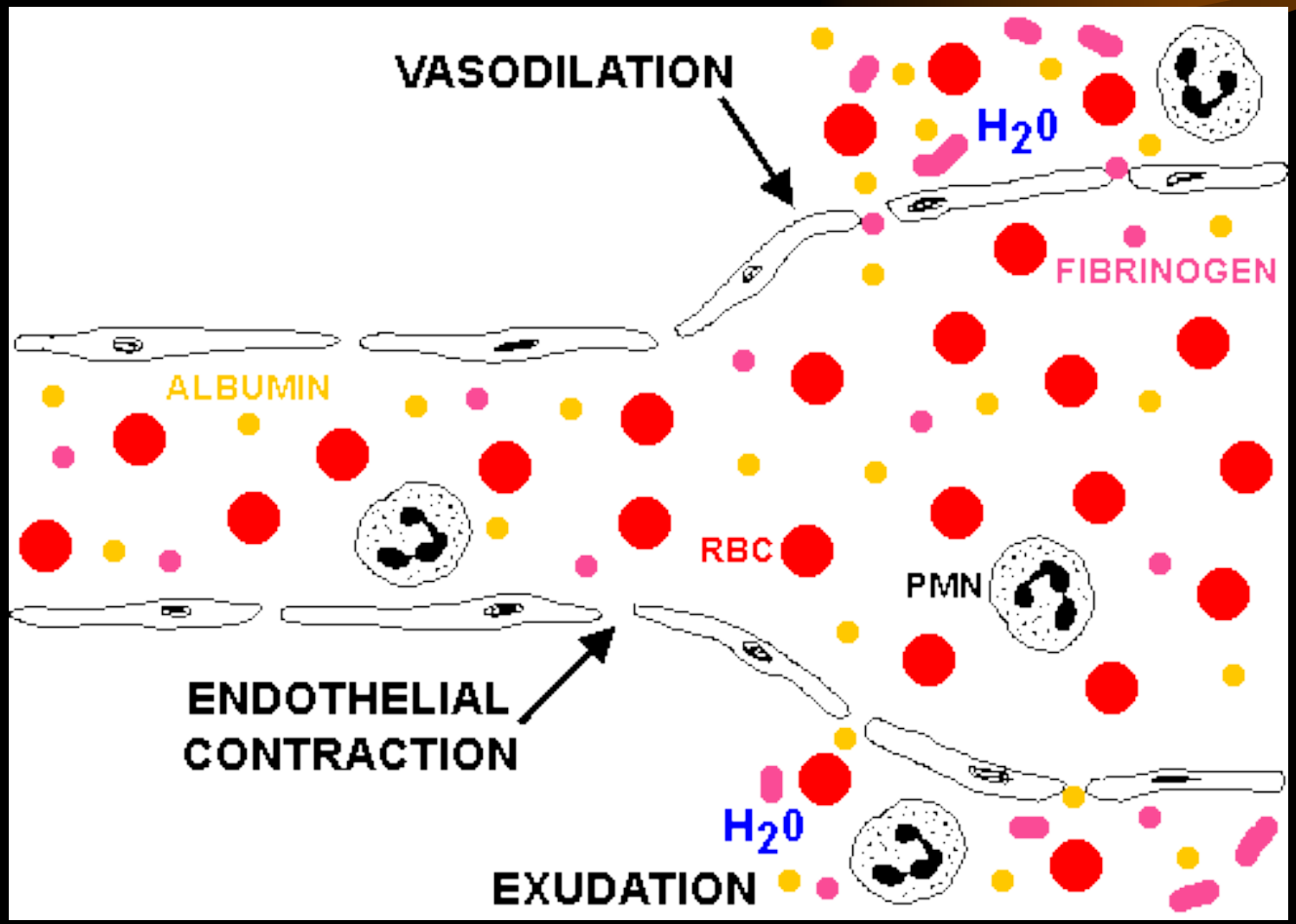
The process is orchestrated by release of chemical mediators

Acute Inflammation (pneumonia)



Local Manifestations of Acute Inflammation

Vascular Changes



The five classic signs of acute inflammation

- Heat.
- Redness.
- Swelling.
- Pain.
- Loss of function.

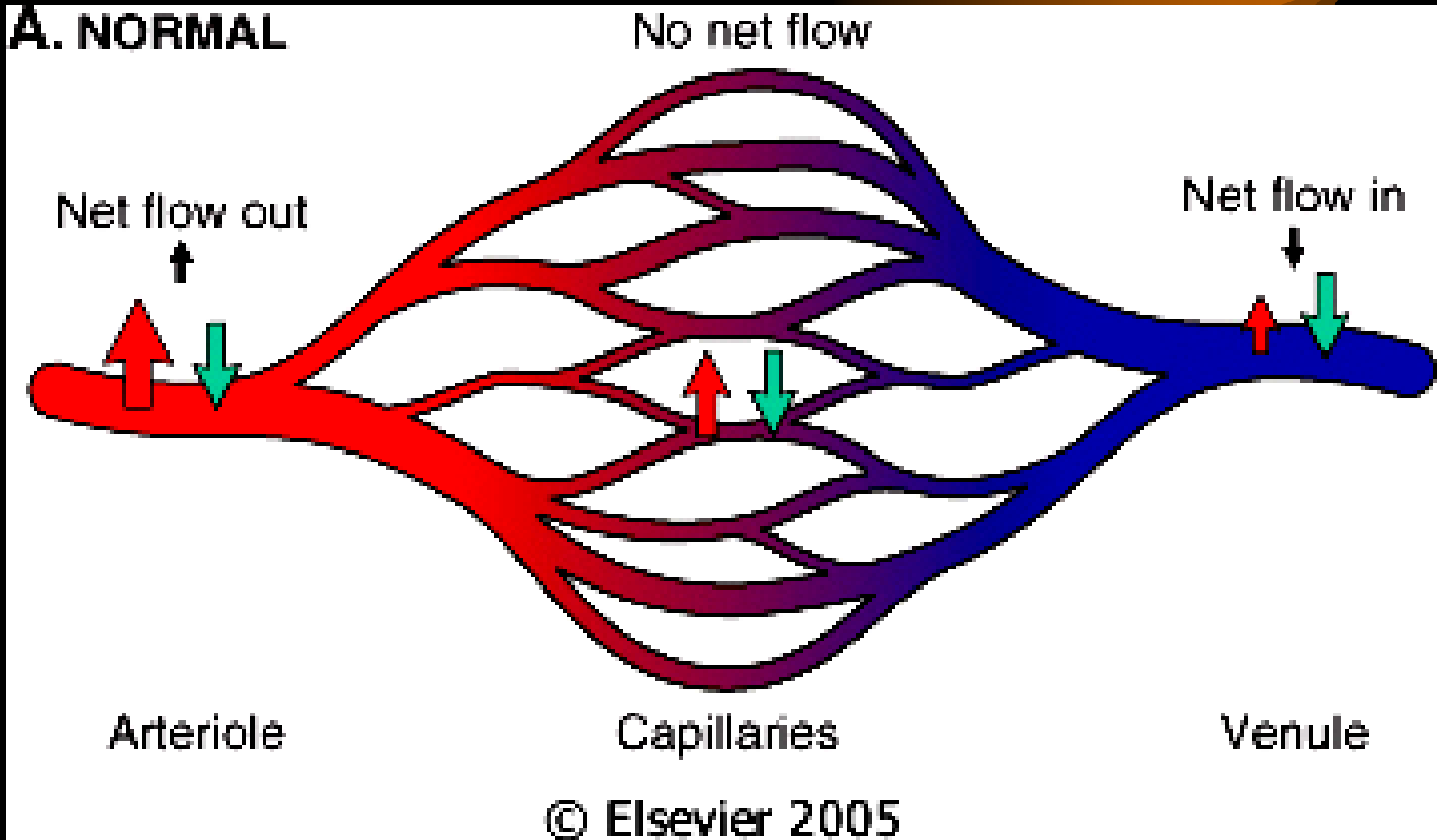
The five classic signs of acute inflammation

Heat **Redness** **Swelling** **Pain** **Loss Of Function.**

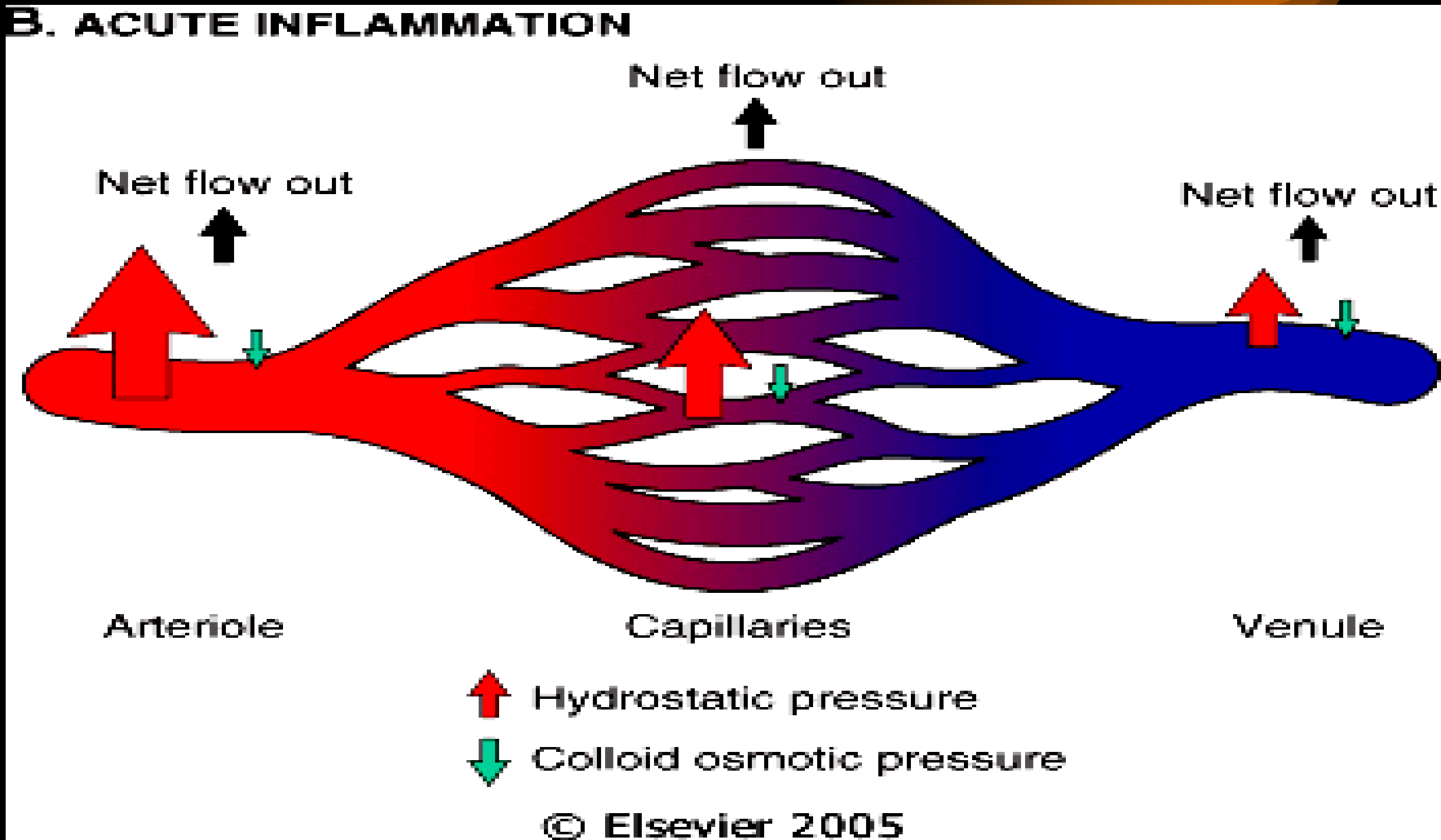
Vascular Changes

- Arteriolar dilatation follows transient vasoconstriction.
- Increased vascular permeability and stasis:
  - Arteriolar vasodilatation → ↑hydrostatic pressure → transudate.
  - Late phase: leaky vessels → loss of proteins → exudate.
- Margination of leukocytes.

Fluid Movement in Microcirculation in Normal Tissue



Fluid Movement in Microcirculation in Inflamed Tissue



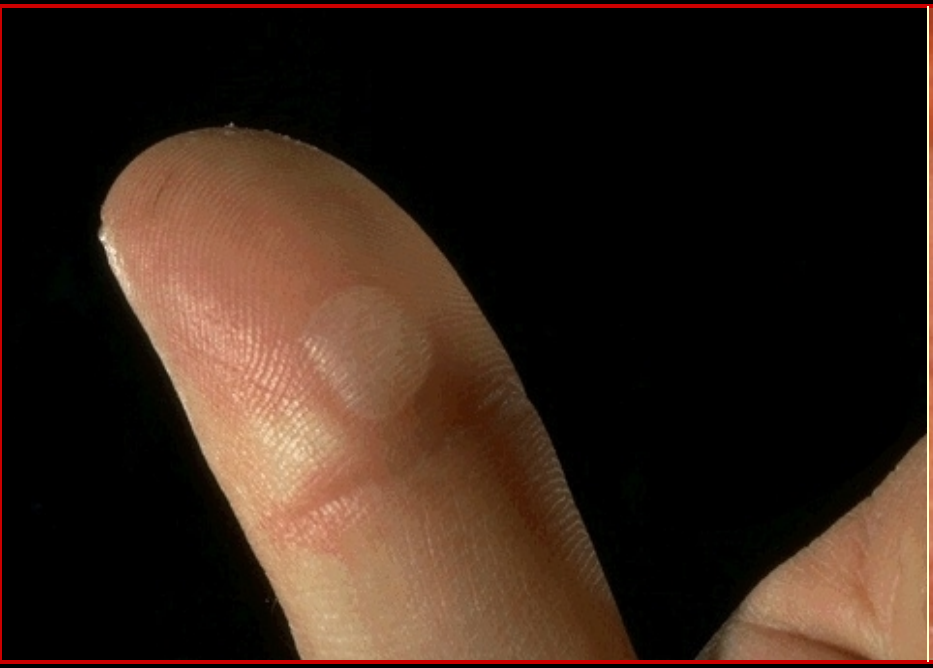
How does inflammation lead to leakiness of endothelial cells? (1)

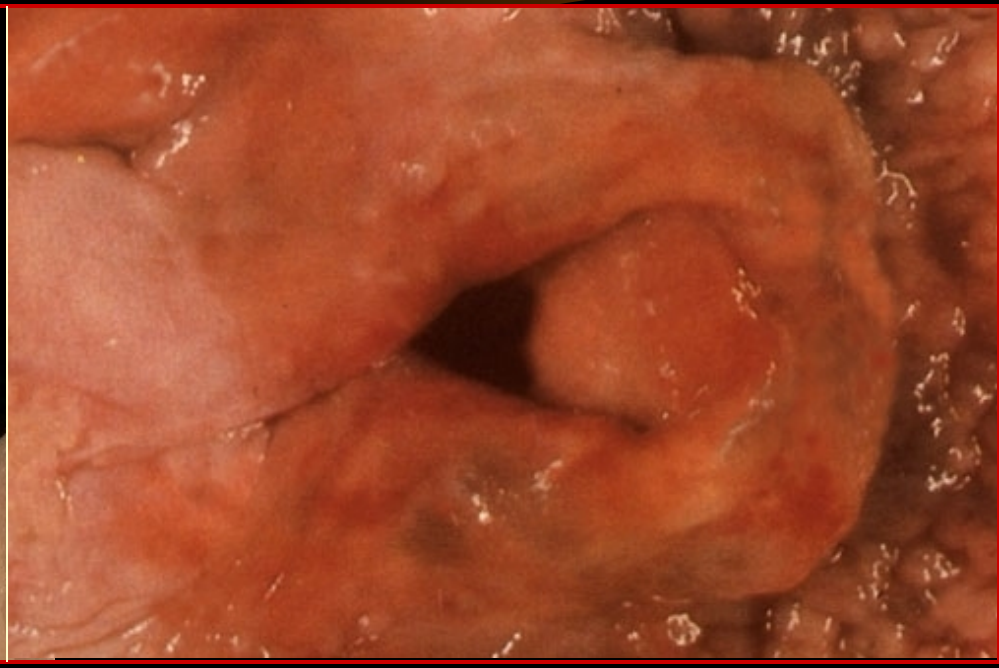
- Endothelial cell contraction
  - Reversible.
  - Immediate transient response with short life (15-30 minutes).
  - Induced by: histamine, bradykinin, leukotrienes, neuropeptide substance P.
  - Mostly in postcapillary venules.
- Endothelial cell retraction
  - Reversible.
  - Starts 4-6 hours after injury and stays for 24 hours.
  - Induced by: IL-1, TNF, and IFN-γ.

How does inflammation lead to leakiness of endothelial cells? (2)

- Direct endothelial injury
  - Severe injury.
  - Immediate sustained response.
  - All microvessels can be affected.
- Delayed prolonged response
  - Begins after delay (2-12 hours), lasts for hours or days.
  - Caused by thermal injury, UV radiation, bacterial toxins.
- Leukocyte-dependent endothelial injury.
- Increased intracytosis (transcytosis) of proteins through vesiculovacuolar pathway
  - Stimulated by VEGF.
- Leakage from newly formed blood vessels.

Edema in Inflammation





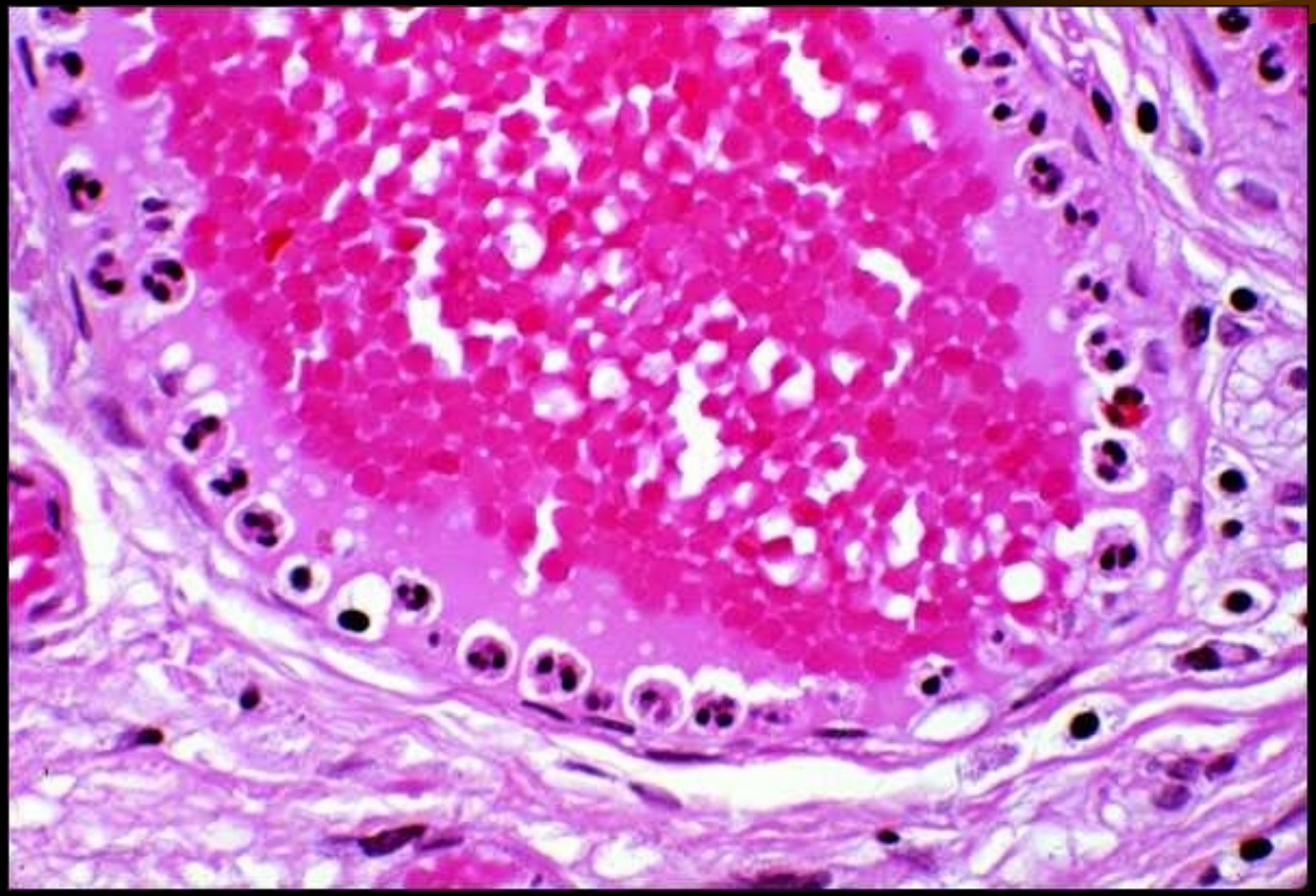
Edema in Inflammation

TRANSUDATE	EXUDATE
• Mechanism: hydrostatic pressure imbalance across vascular endothelium.	• Mechanism: alteration in normal permeability of small blood vessels in area of injury.
• Fluid of low protein content (ultrafiltrate of blood plasma).	• Fluid of high protein content (>.3g/dl) & increased cellular debris.
• Specific gravity <1.012.	• Specific gravity >1.020.

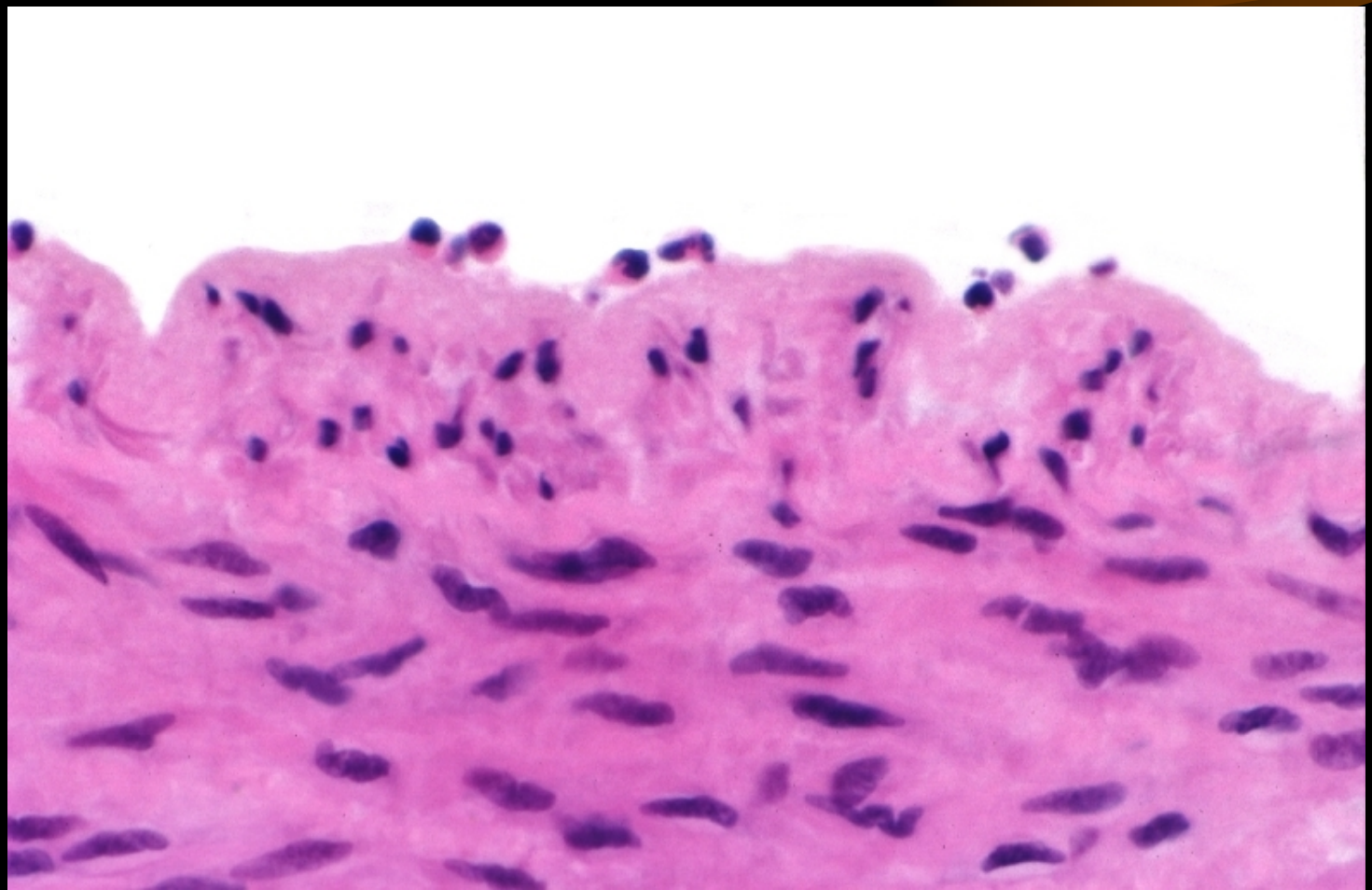
Cellular Events

- Margination, rolling and adhesion.
- Transmigration between endothelial cells.
- Migration in the interstitium toward the site of stimulus.
- Phagocytosis and degranulation.
- Release of leukocyte products.

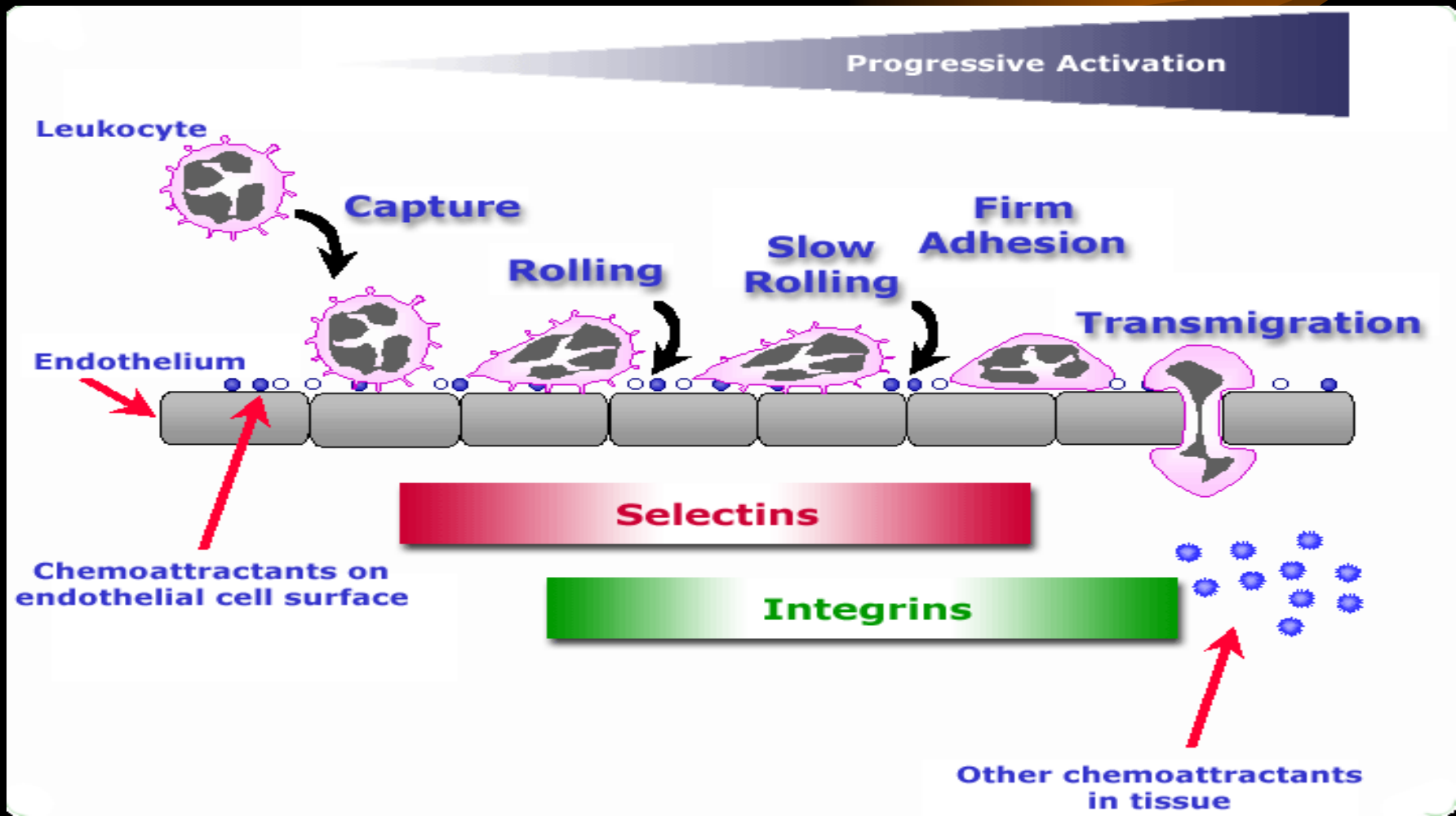
Neutrophil Margination



Neutrophil Margination



The Process of Extravasation of Leukocytes



Selectins

- Receptors expressed on the surfaces of endothelial cells and leukocytes that bind selected sugars (sialylated oligosaccharides).
- Not expressed on resting endothelial cells, but expressed within 30 minutes of stimulation.
- Low affinity binding with a fast-off-rate.
- Single chain transmembrane glycoprotein.
- Binding to ligand needs Ca.
- Distribution:
  - E-selectin (CD62E): endothelial cells.
  - P-selectin (CD62P): platelets & endothelial cells.
  - L-selectin (CD62L): leukocytes.

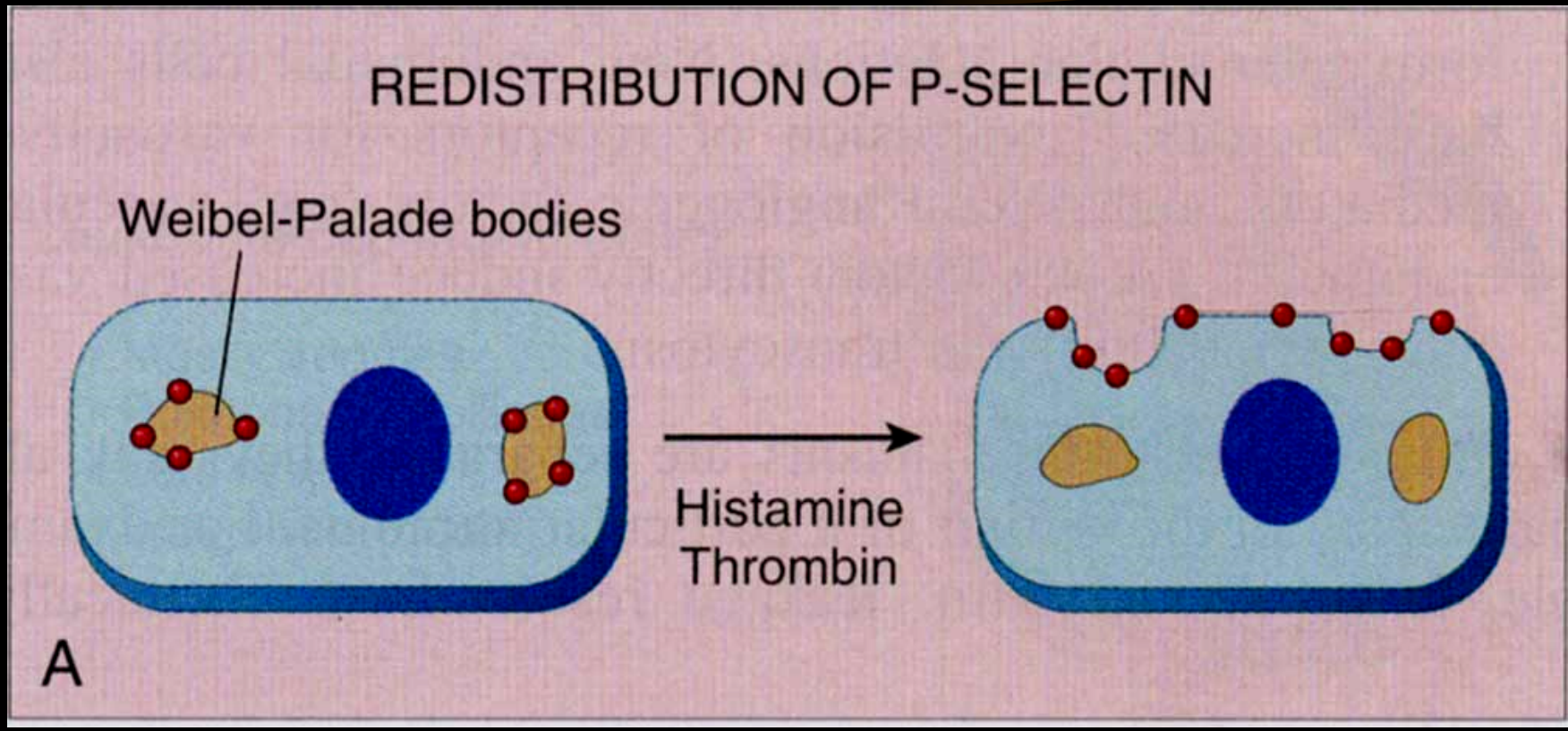
Integrins

- Heterodimeric cell surface proteins (α & β chains).
- Bind to ligands present in:
  - Extracellular matrix.
  - Complement system.
  - Surface of other cells.
- Many integrins recognize the RGD sequence.
- Cytoplasmic domains bind with cytoskeleton.

Adhesion between leukocytes and endothelial cells

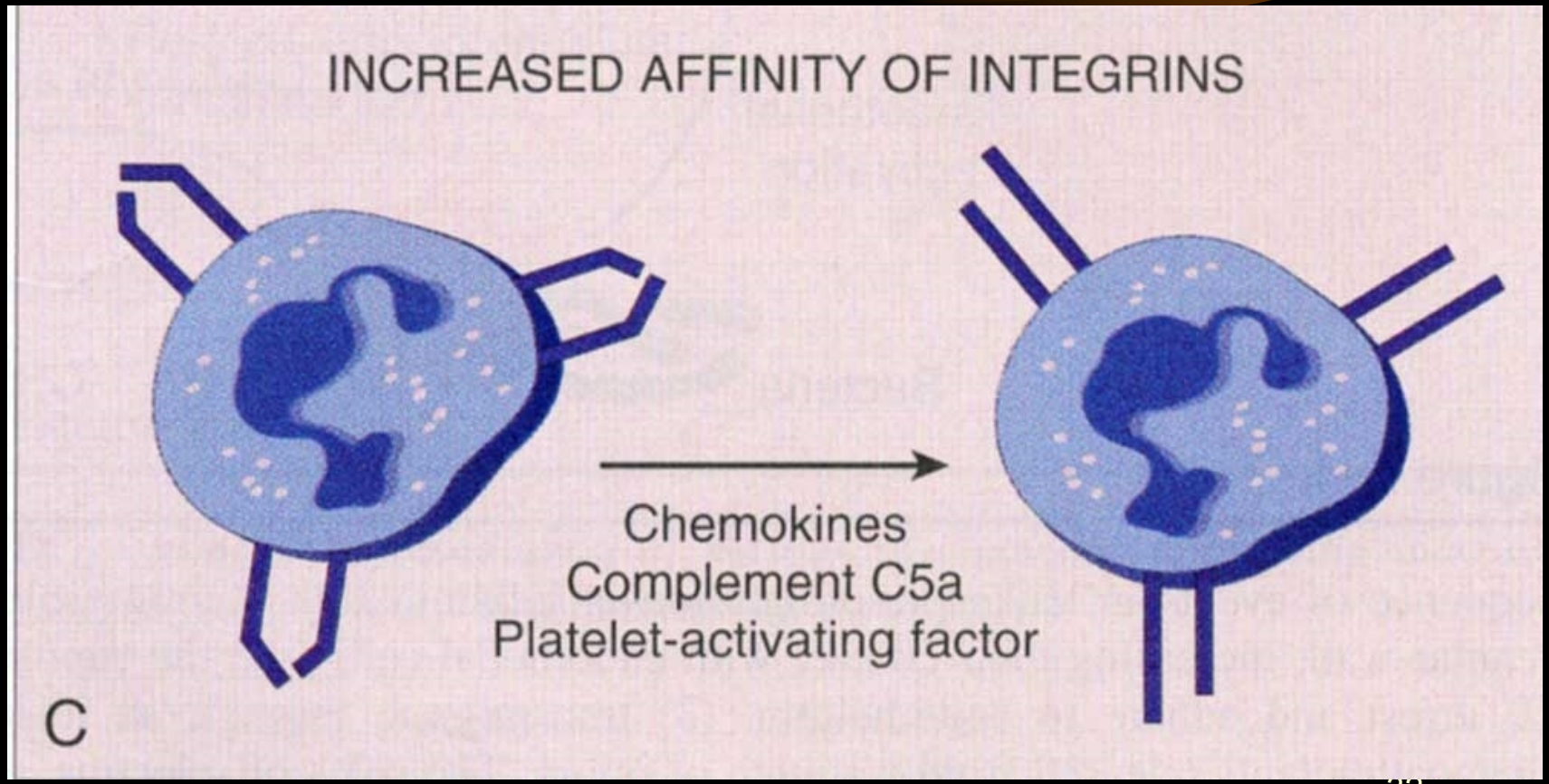
- Weak adhesion and rolling
  - Mediated by selectins.
- Firm adhesion
  - Ig superfamily molecules expressed on endothelial cells such as:
    - ICAM-1
    - VCAM-1
  - Integrins expressed on leukocytes:
    - LFA-1 (CD11a/CD18)
    - Mac-1 (CD11b/CD18)
    - P150,95 (CD11c/CD18)
    - VLA-4

Upregulation of Selectins



Cytokine Induction of Adhesion Molecules

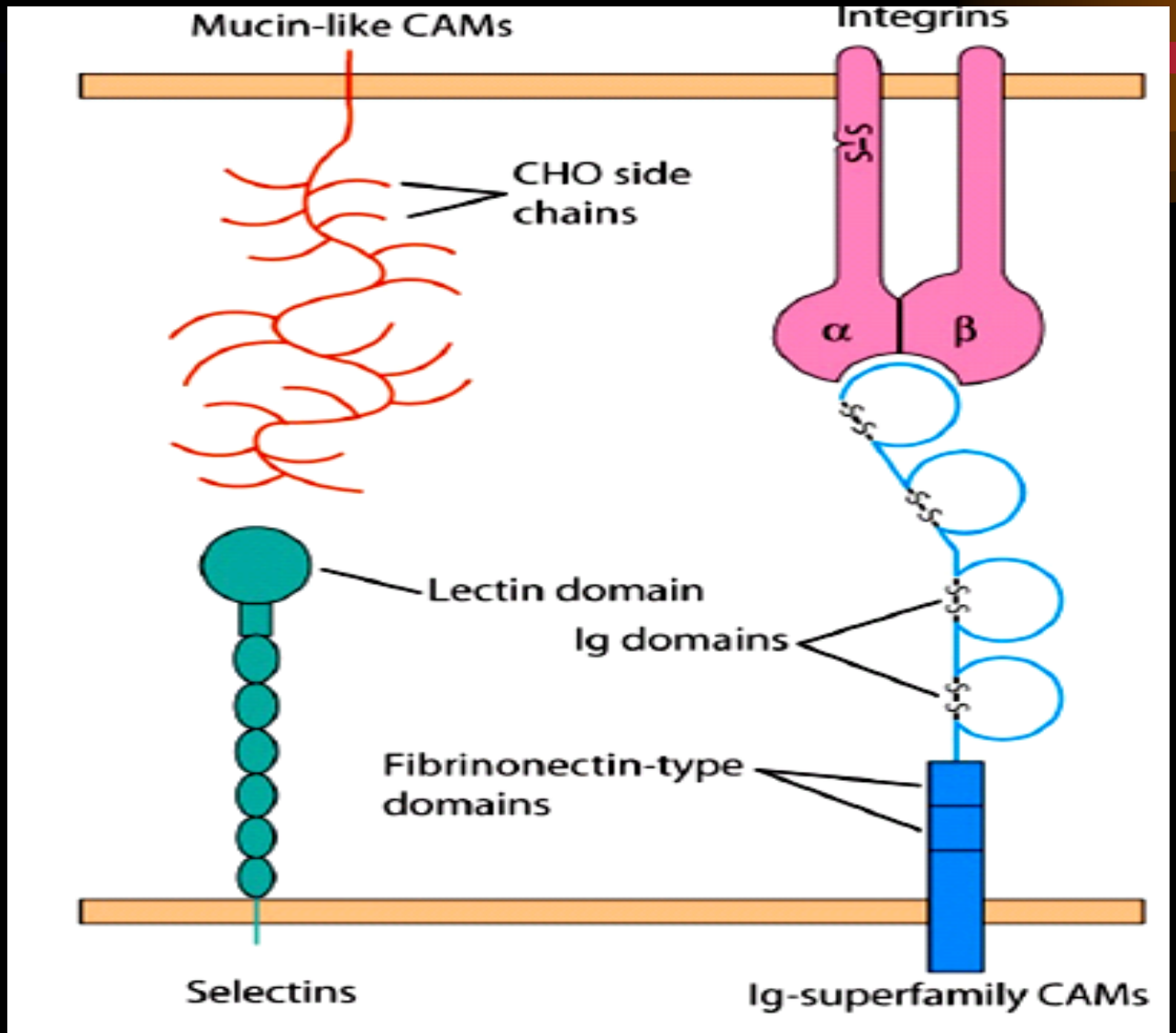
Chemotactics Increase Affinity of Integrins to Adhesion Molecules



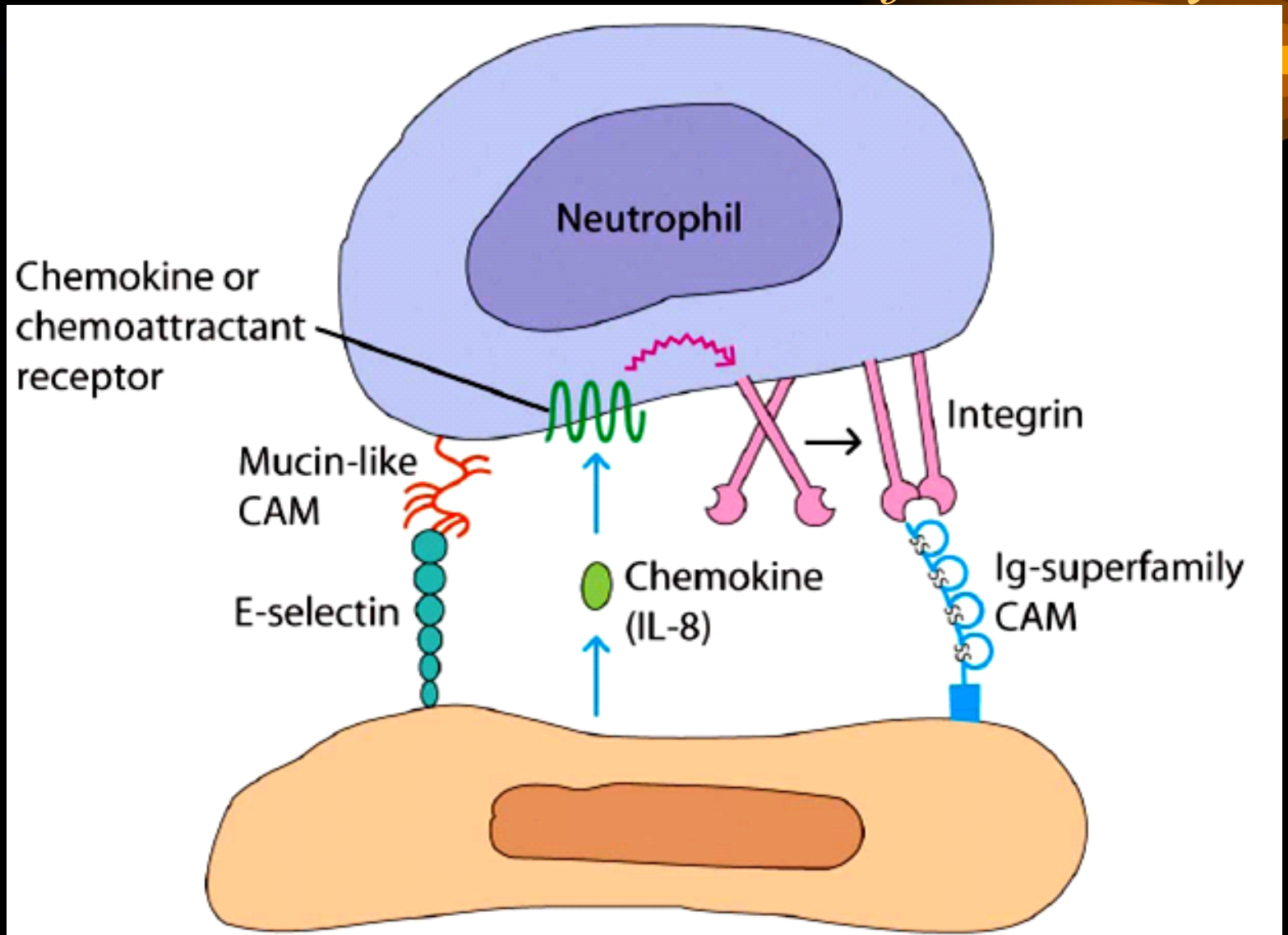
Endothelial and Leukocyte Adhesion Molecule Interactions

ENDOTHELIUM	WBC	FUNCTION
• P & E-selectins	Sialyl-Lewis X	Rolling
• GlyCAM-1, CD34	L-selectin	Rolling
• VCAM-1	VLA-4	Adhesion
• ICAM-1	CD11/CD18 (LFA1, MAC1)	Adhesion,
• CD31 (PECAM-1)	CD31	Transmigration

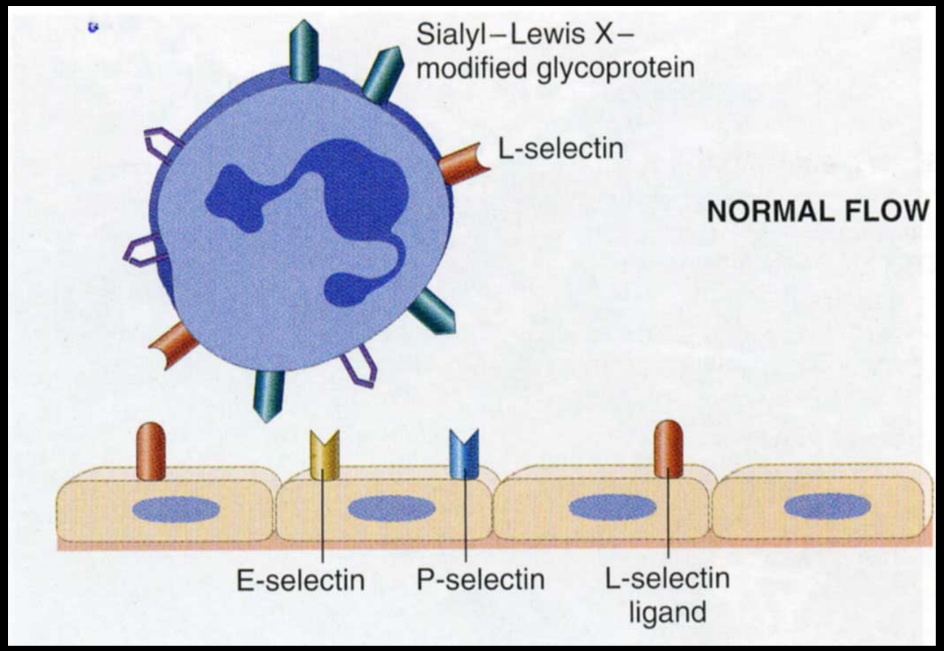
General Structure of CAM Families

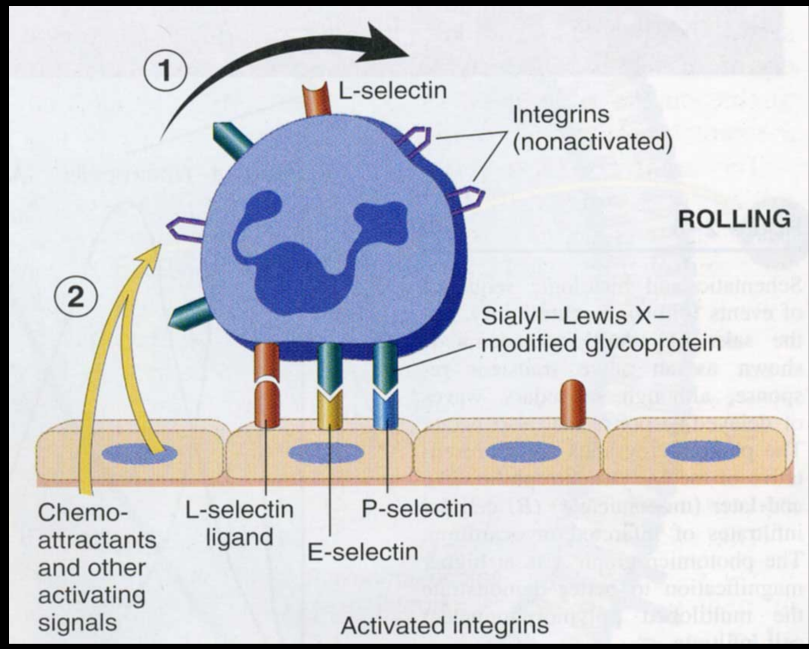


The Process of Rolling, Activation and Firm Adhesion of Leukocytes



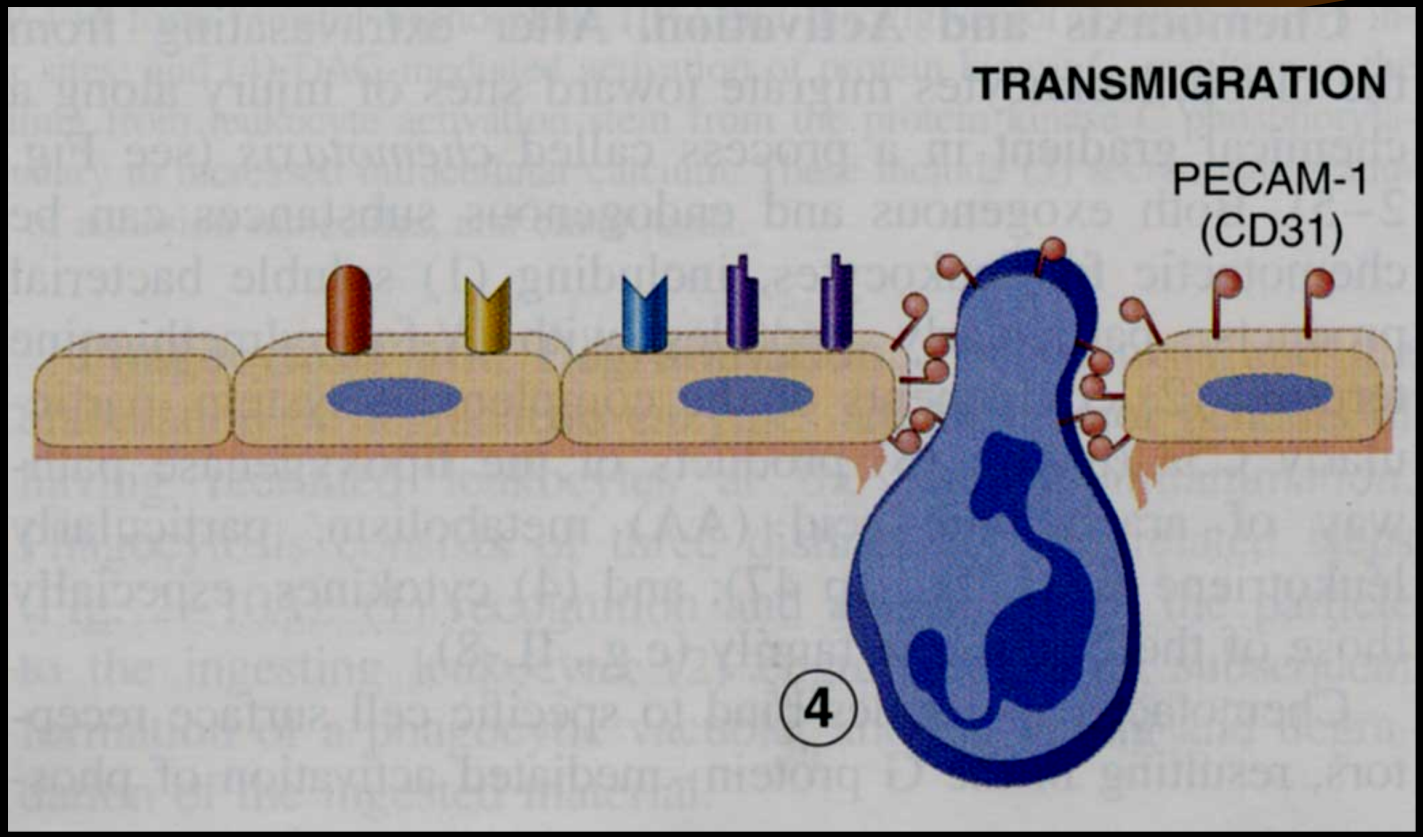
Molecules Mediating Endothelial-Neutrophil Interaction





Firm Adhesion via Integrin ICAM Interactions

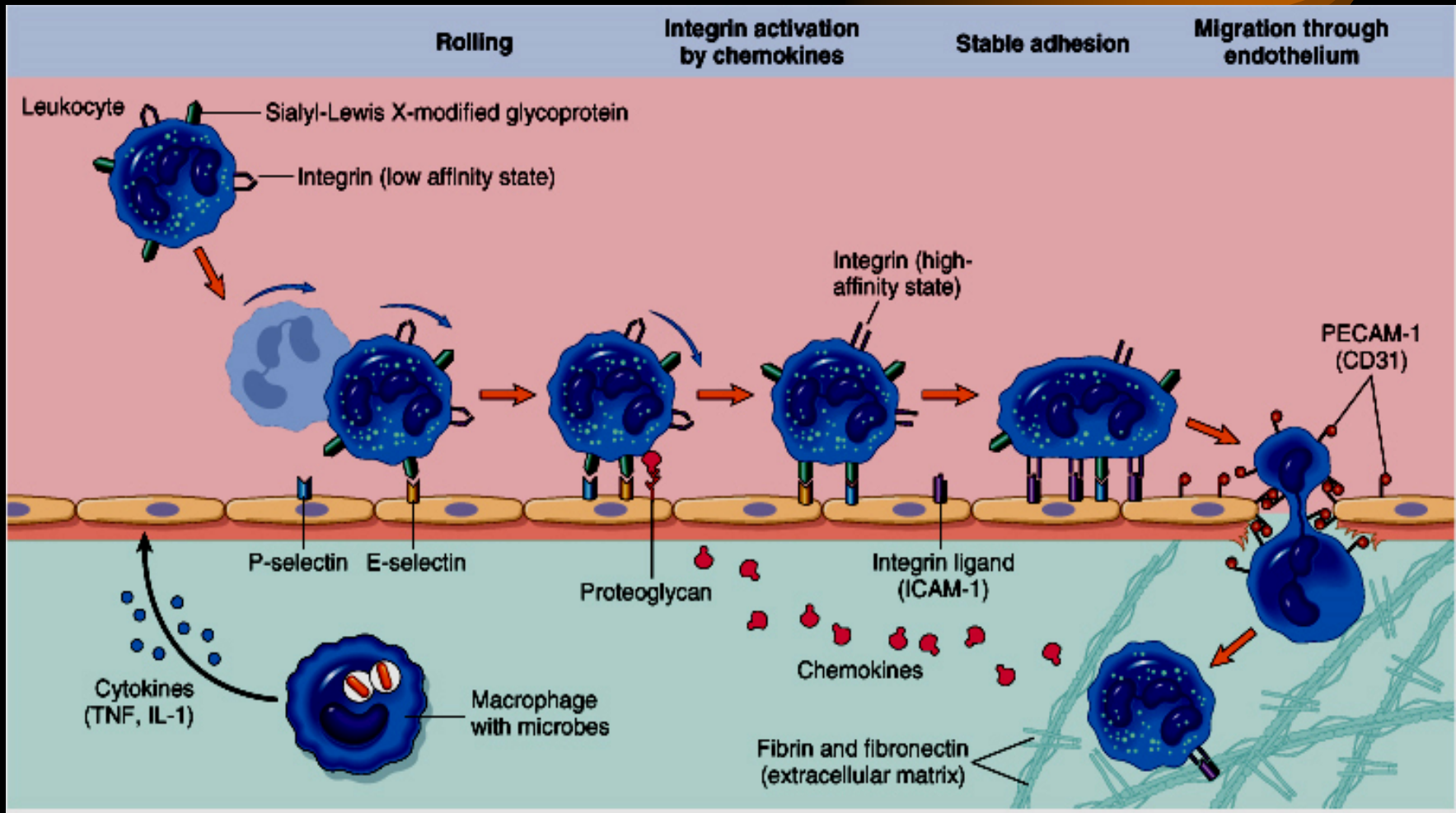
Transmigration of Neutrophils



Diapedesis

- Transmigration of leukocytes between endothelial cells at the intercellular junctions
- Facilitated by PECAM-1 (CD31)/PECAM-1 interaction

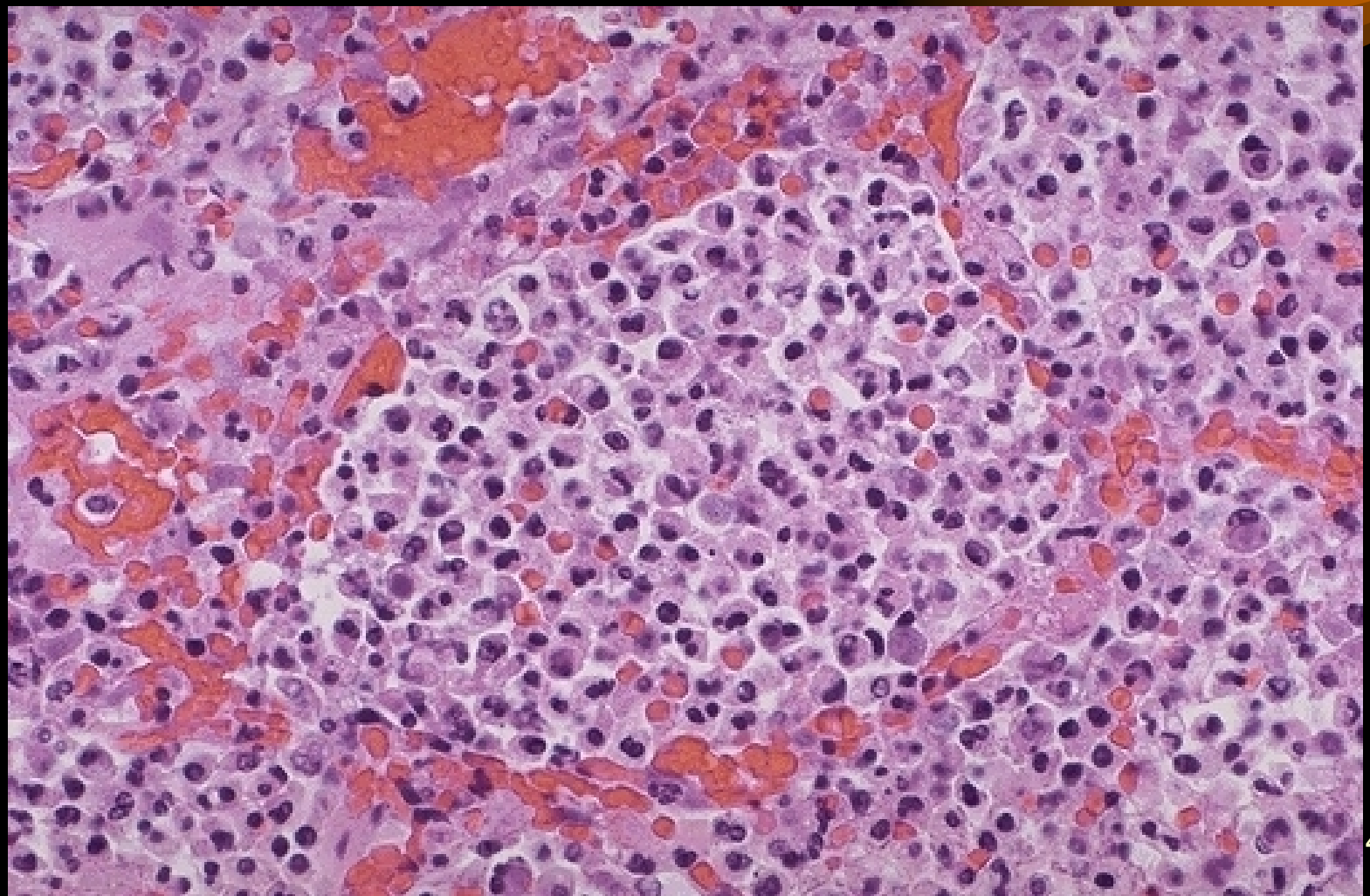
The Process of Extravasation of Leukocytes



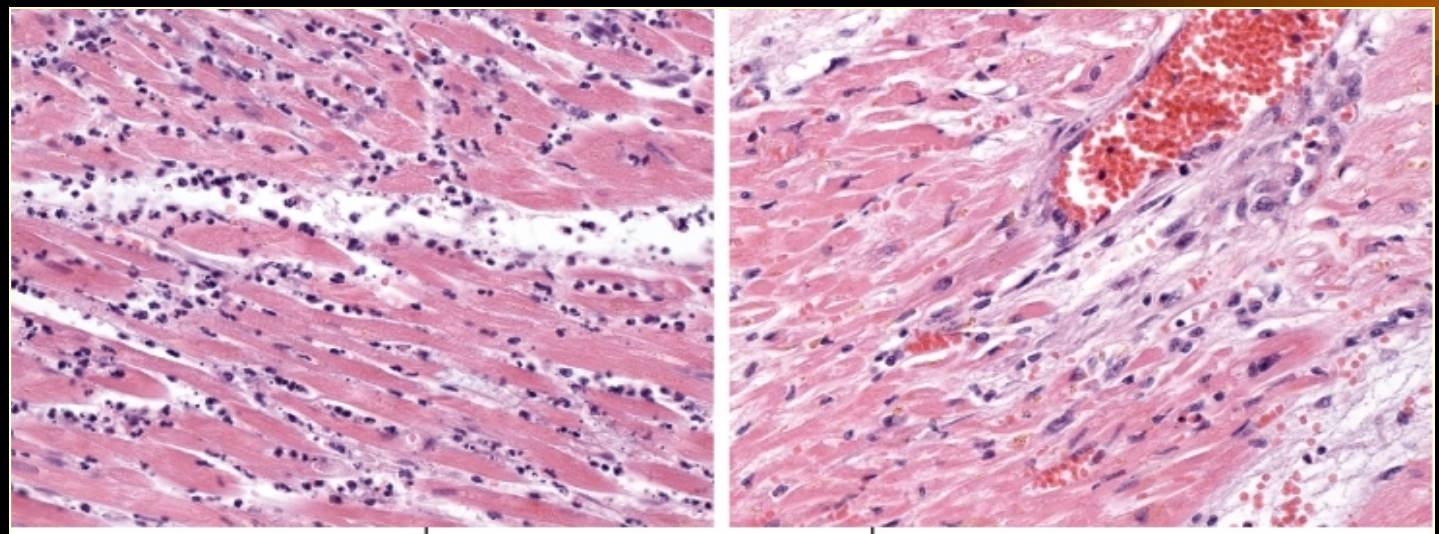
The Process of Extravasation of Leukocytes

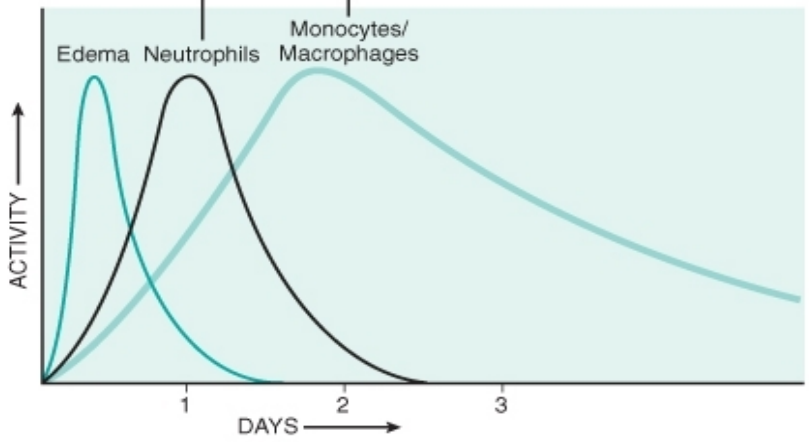
1. Selectins and their carbohydrate counterligands mediate leukocyte tethering and rolling.
2. Leukocyte integrins and their ligands including immunoglobulinlike intercellular adhesion molecules, mediate firm adhesion.
3. Chemokines play a role in firm adhesion by activating integrins on the leukocyte cell surface.
4. The leukocytes are directed by chemoattractant gradients to migrate across the endothelium, and through the extracellular matrix into the tissue.

Lobar Pneumonia



Sequence of Events Following injury

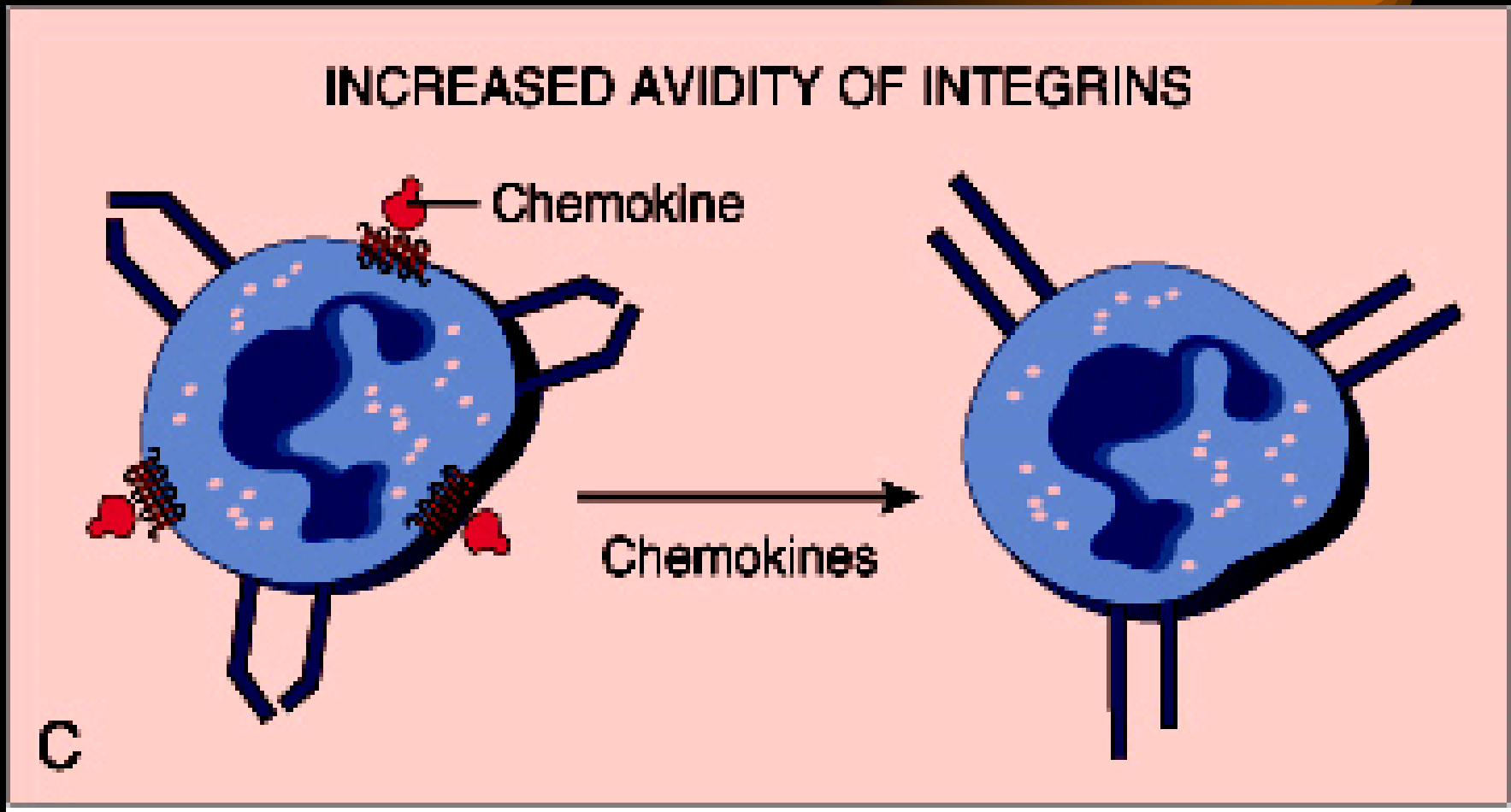




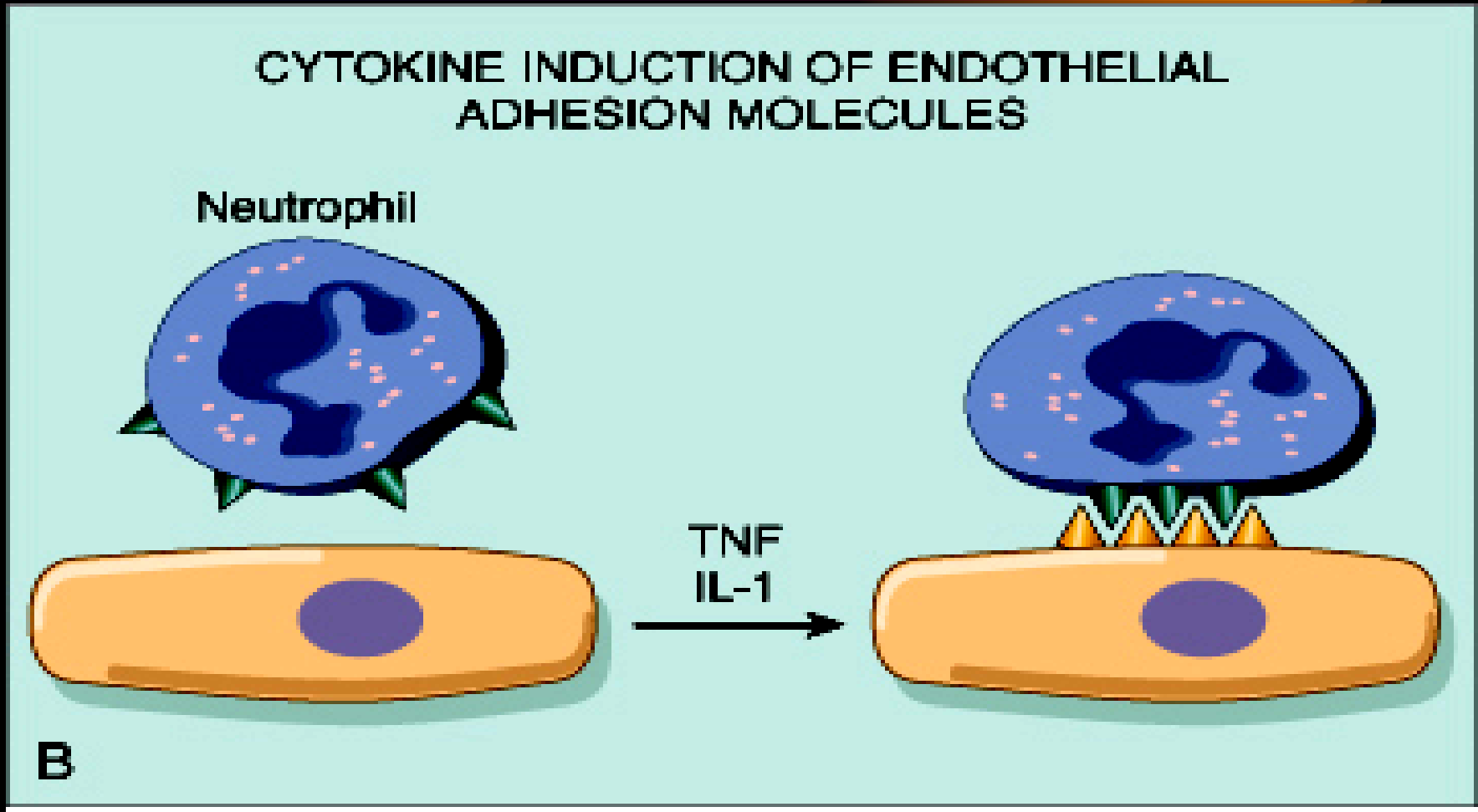
Chemotaxis

- Migration of cells along a chemical gradient
- Chemotactic factors:
  - Soluble bacteial products, e.g. N-formyl-methionine termini
  - Complement system products, e.g. C5a
  - Lipooxygenase pathway of arachidonic acid metabolism, e.g. LTB4
  - Cytokines, e.g. IL-8

Effects of Chemotactic Factors on Leukocytes



Effects of Chemotactic Factors on Endothelial Cells



Effects of Chemotactic Factors on Leukocytes

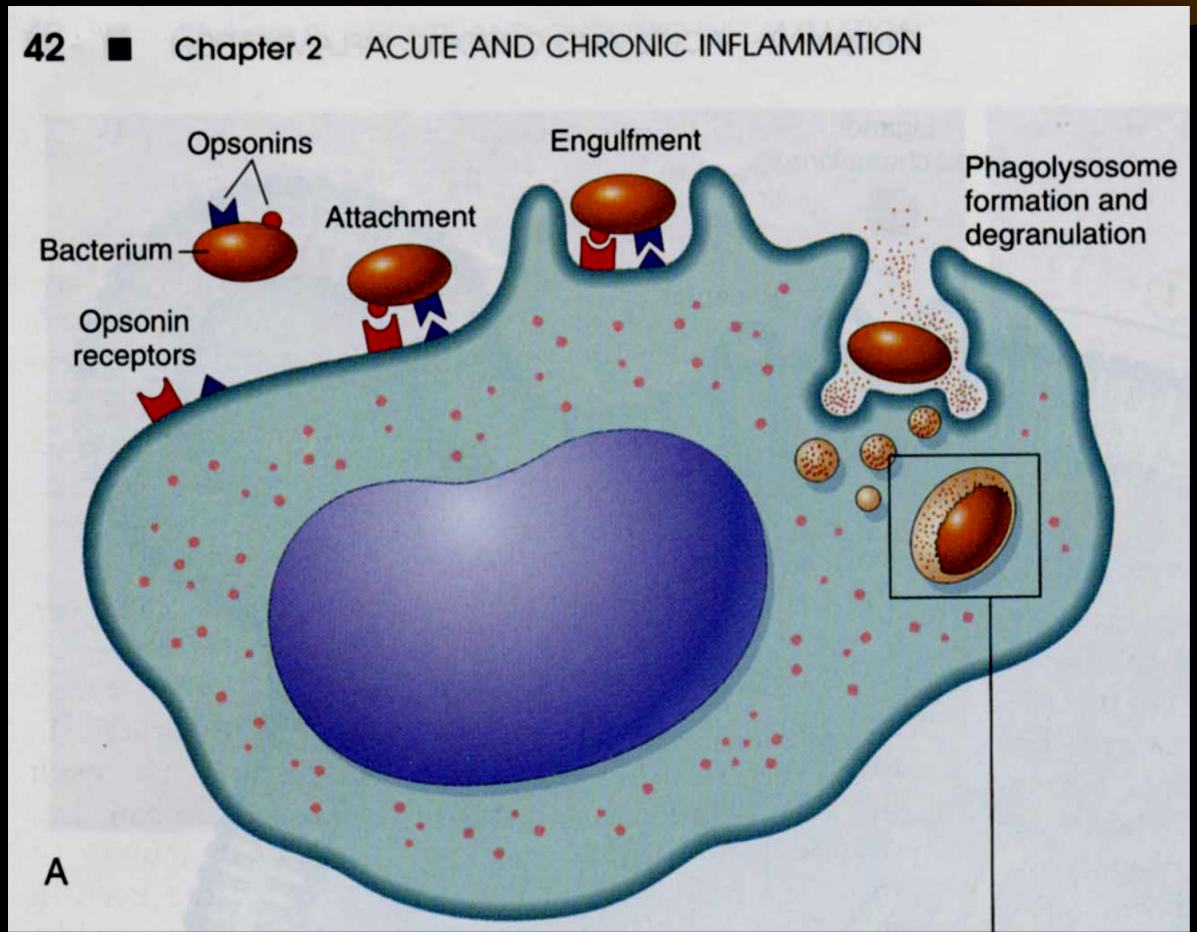
- Stimulate locomotion
- Degranulation of lysosomal enzymes
- Production of AA metabolites
- Modulation of the numbers and affinity of leukocyte adhesion molecules

Biochemical Events in Leukocyte Activation

Phagocytosis

- The process of ingestion and digestion by cells of solid substances, e.g., other cells, bacteria, necrotic tissue or foreign material
- Steps of phagocytosis:
  - Recognition, attachment and binding to cellular receptors
    - IgG, C3b, MBL
  - Engulfment
  - Fusion of phagocytic vacuoles with lysosomes
  - Killing or degradation or ingested material

Phagocytosis

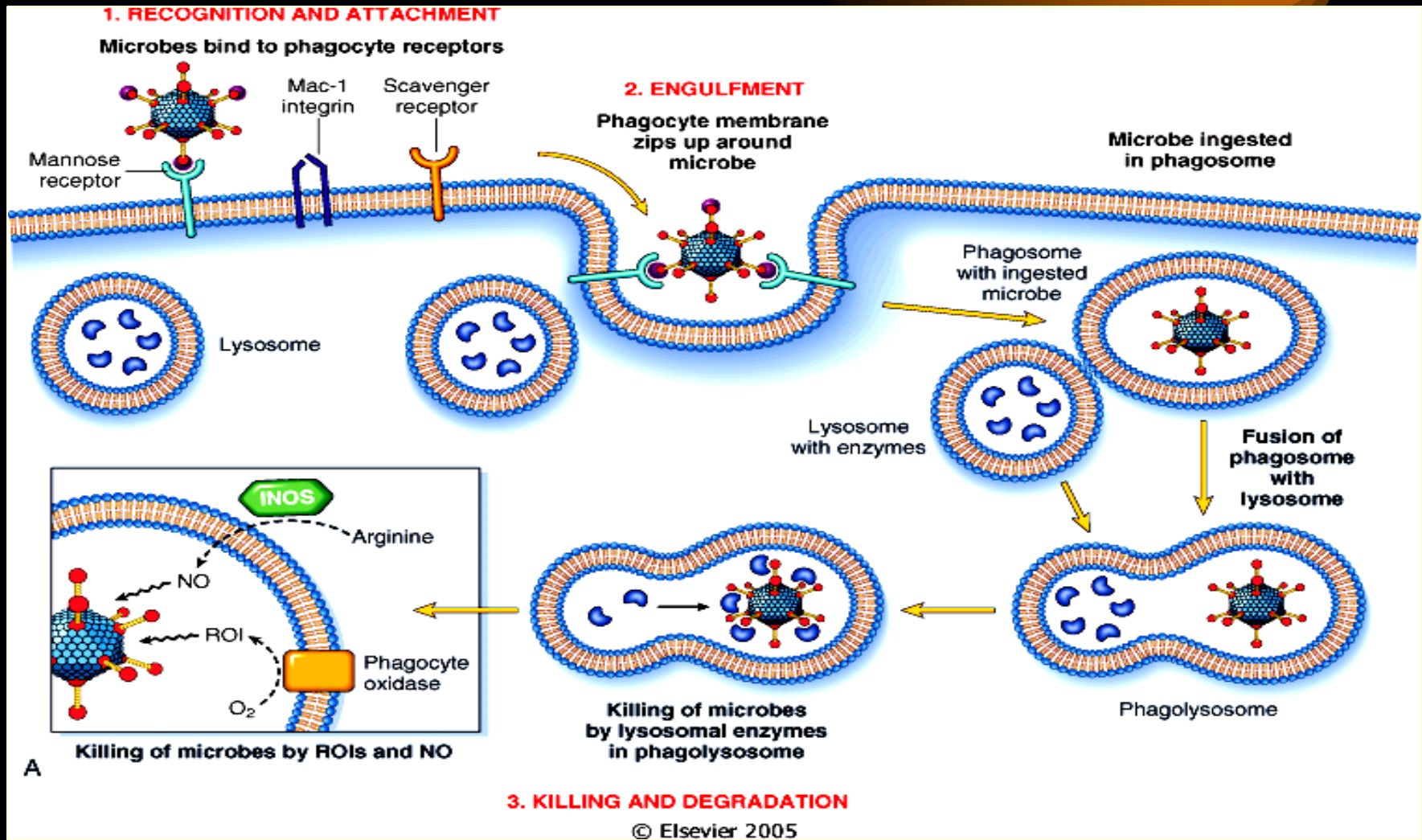


Phagocytosis

- Recognition and attachment by receptors:
  - Mannose receptors: bind to terminal mannose residues on microbes cell walls.

    Mammalian cells are not recognised by mannose receptors because they contain terminal sialic acid and N-acetyl galactosamine.
  - Scavenger receptors: oxidized LDL, and microbes.
  - Opsonin receptors (high affinity): IgG, C3b, MLB.

Phagocytosis

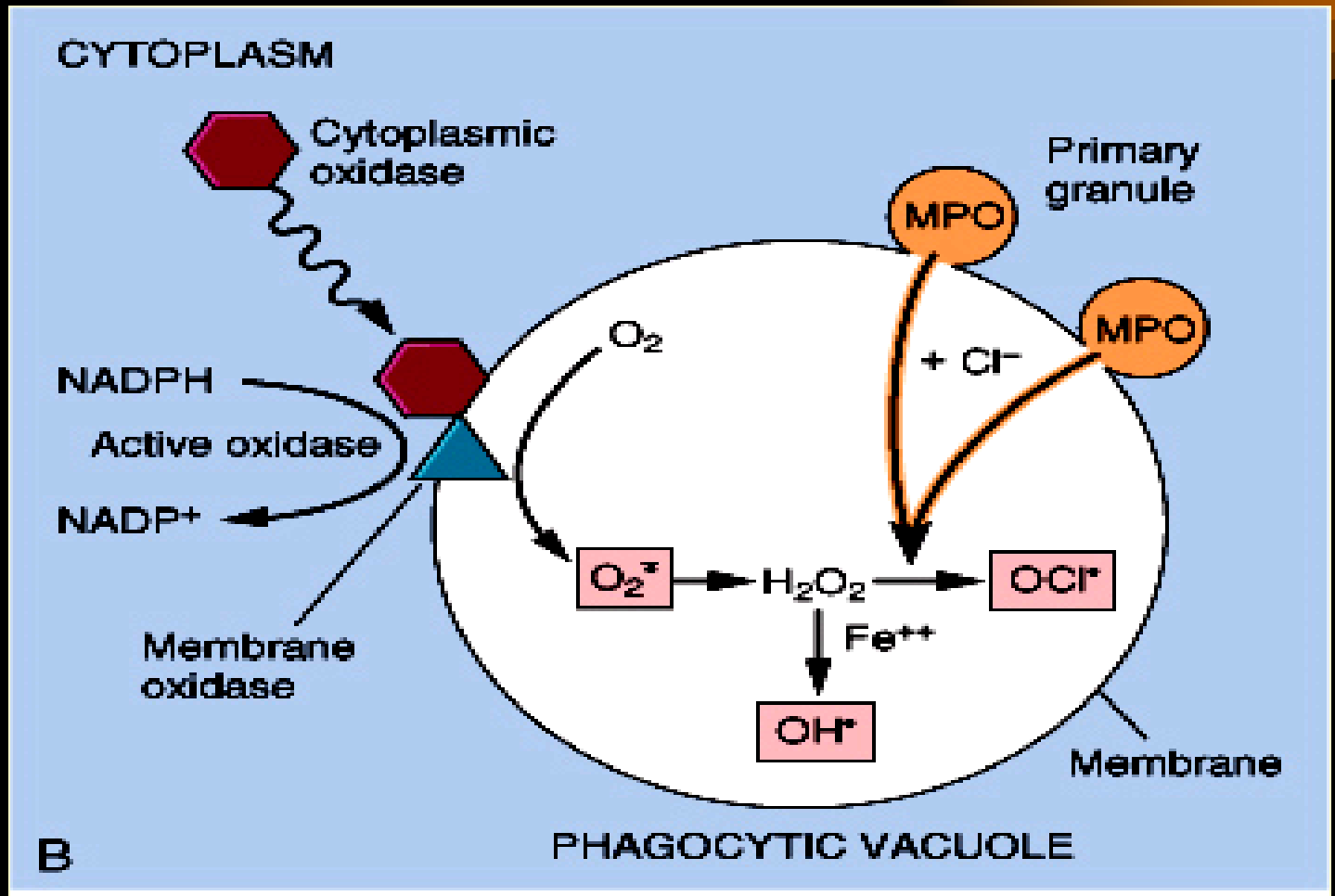


Generation of Oxygen Metabolites

- $2O_2 + NADPH \xrightarrow{\text{NADPH oxidase}} 2O_2^- + NADP^+ + H^+$
- $O_2^- + 2H^+ \xrightarrow{\text{Dismutase}} H_2O_2$
- $H_2O_2 + Cl^- \xrightarrow{\text{Myeloperoxidase}} HOCl^-$

The H_2O_2-MPO-halide is the most efficient bactericidal system in neutrophils

Oxygen Dependent Bactericidal Mechanisms



How Do Leukocytes Kill Infectious Agents?

- Oxygen burst products
- Bactericidal permeability increasing protein
- Lysozyme
- Major basic protein
- Defensins
- Lactoferrin

Genetic defects in leukocyte function

Disease	Defect
Leukocyte adhesion deficiency 1	CD18 unit of integrin
Leukocyte adhesion deficiency 2	Sialyl-Lewis X
Neutrophil-specific granule deficiency	Absent specific granules
CGD, X-linked	Membrane component of NADPH oxidase
CGD, autosomal recessive	Cytoplasmic component of NADPH oxidase
MPO deficiency	Absent MPO-H_2O_2 system
Chediak-Higashi disease	Organelle trafficking

Acquired defects in leukocyte function

- Chemotaxis defects
  - burns, diabetes, sepsis, etc.
- Adhesion
  - hemodialysis, diabetes
- Phagocytosis and microbicidal activity
  - leukemia, sepsis, diabetes, malnutrition, etc

Chemical Mediators of Inflammation

- What are their sources?
  - Circulating plasma proteins
    - Coagulation / fibrinolytic factors
    - Complement
    - Kinins
  - Cell derived
    - Formed elements normally equestered in granules:
      - Vasoactive amines
    - Newly synthesized in response to stimulation
      - PGs, LT, O_2 species, NO, Cytokines, PAF

Cellular Derived Mediators of Inflammation

Systemic Mediators of Inflammation

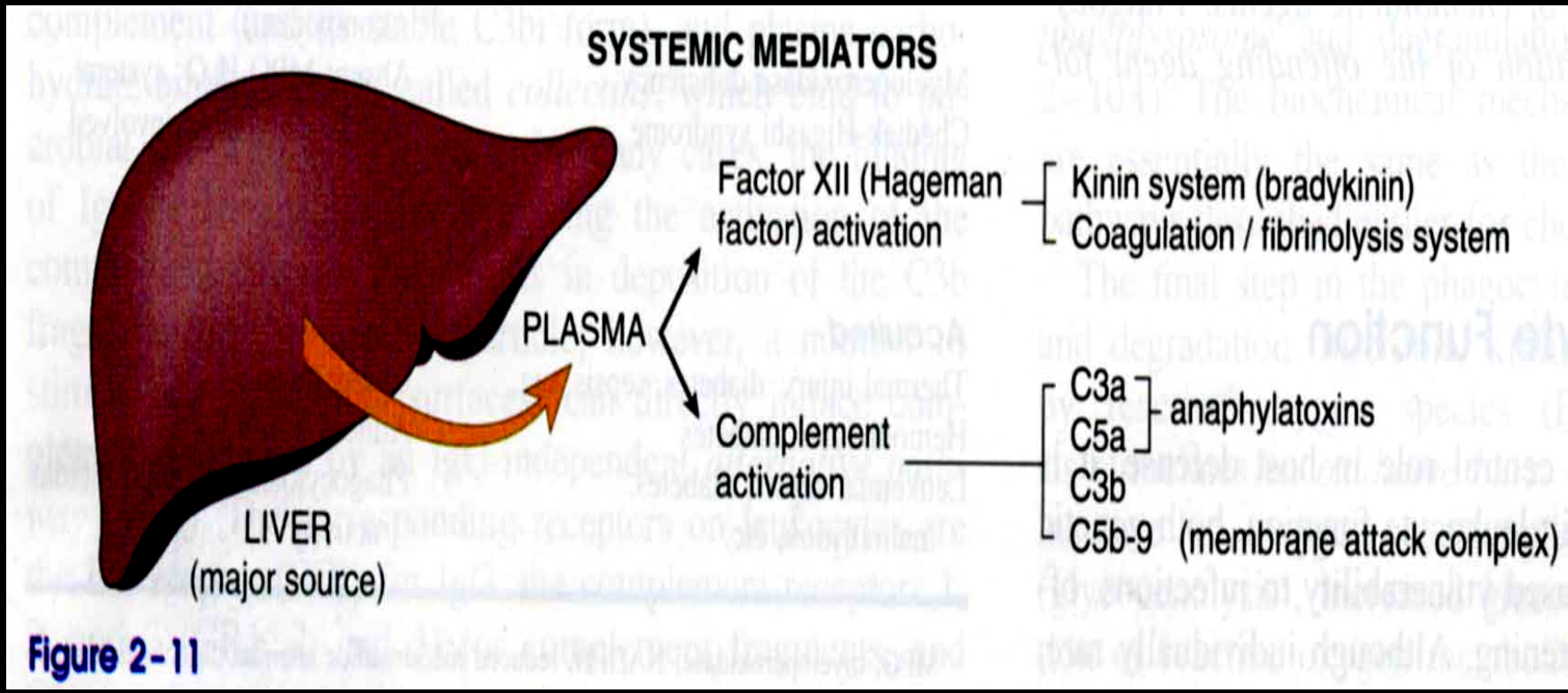


Figure 2-11

Chemical Mediators of Inflammation

- General characteristics
  - Bind to specific cellular receptors, or have enzymatic activity
  - May stimulate target cells to release secondary mediators with similar or opposing functions
  - May have limited targets, or wide spread activities
  - Short lived function
    - Short half-life (AA metabolites)
    - Inactivated by enzymes (kininase on bradykinin)
    - Eliminated (antioxidants on O2 species)
    - Inhibited (complement inhibitory proteins)
  - If unchecked, cause harm

Vasoactive Amines

Release of histamine

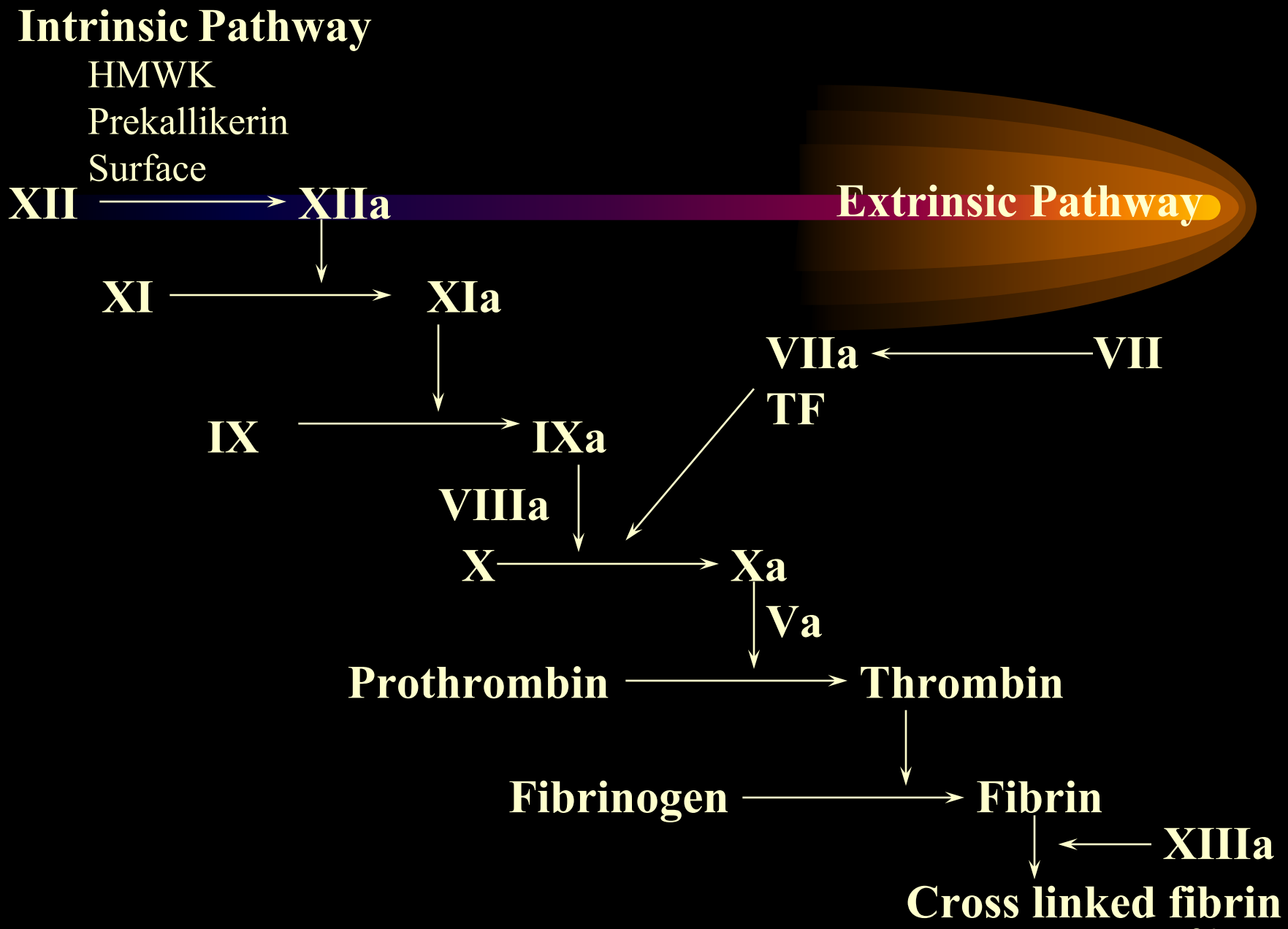
- Physical injury
- Binding of IgE to Fc receptors
- Anaphylatoxins (C3a, C5a) binding
- Histamine releasing ptn derived from PMNs
- Neuropeptides (substance P)
- Cytokines (IL-1, IL-8)

Release of serotonin

- Platelets aggregation
- PAF

Histamine and Serotonin

- Stored in granules in mast cells (histamine), and platelets (serotonin)
- Cause arteriolar dilatation and increases permeability (immediate phase reaction)
- Induce endothelial cell contraction in venules
- Binds to H1 receptors
- Inactivated by histaminase


Intrinsic Pathway
HMWK
Prekallikerin
Surface
XII
XIIa
Extrinsic Pathway
XI
XIa
VIIa
VII
TF
IX
IXa
VIIIa
X
Xa
Va
Prothrombin
Thrombin
Fibrinogen
Fibrin
XIIIa
Cross linked fibrin

Clotting / fibrinolytic system

- Fibrin clot at site of injury helps in containing the cause
- Fibrin clot provides a framework for inflammatory cells
- Xa causes increased vascular permeability and leukocytes emigration
- Thrombin causes leukocytes adhesion, platelets aggregation, generation of fibrinopeptides, and is chemotactic
- Fibrinopeptides are chemotactic & induce vasopermeability

Clotting / fibrinolytic system

(continued)

- XIIa also activates the fibrinolytic pathway to prevent widespread thrombosis.
- Fibrin split products increase vascular permeability
- Plasmin cleaves C3 to form C3a, leading to dilatation and increased permeability
- Plasmin activates XIIa amplifying the entire process

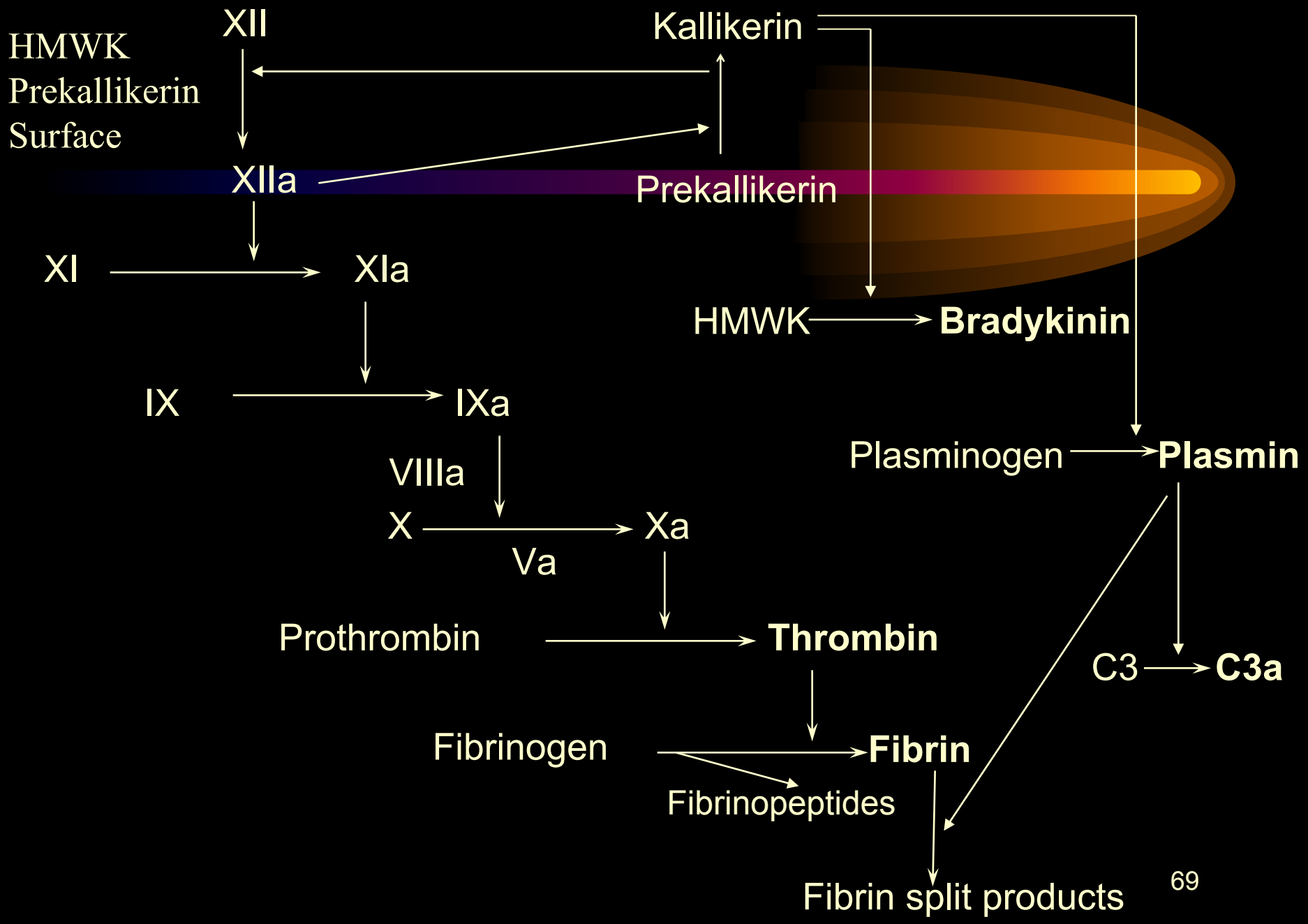
Thrombin as an Inflammatory Mediator

- Binds to protease-activated receptors (PARs) expressed on platelets, endothelial cells, sm. muscles leading to:
  - P-selectin mobilization
  - Expression of integrin ligands
  - Chemokine production
  - Prostaglandin production by activating cyclooxygenase-2
  - Production of PAF
  - Production of NO

Kinin System

- Leads to formation of bradykinin from HMWK
- Effects of bradykinin
  - Increased vascular permeability
  - Arteriolar dilatation
  - Bronchial smooth muscle contraction
  - Pain
- Sort half-life (inactivated by kininases)

Interaction between the four plama mediator systems



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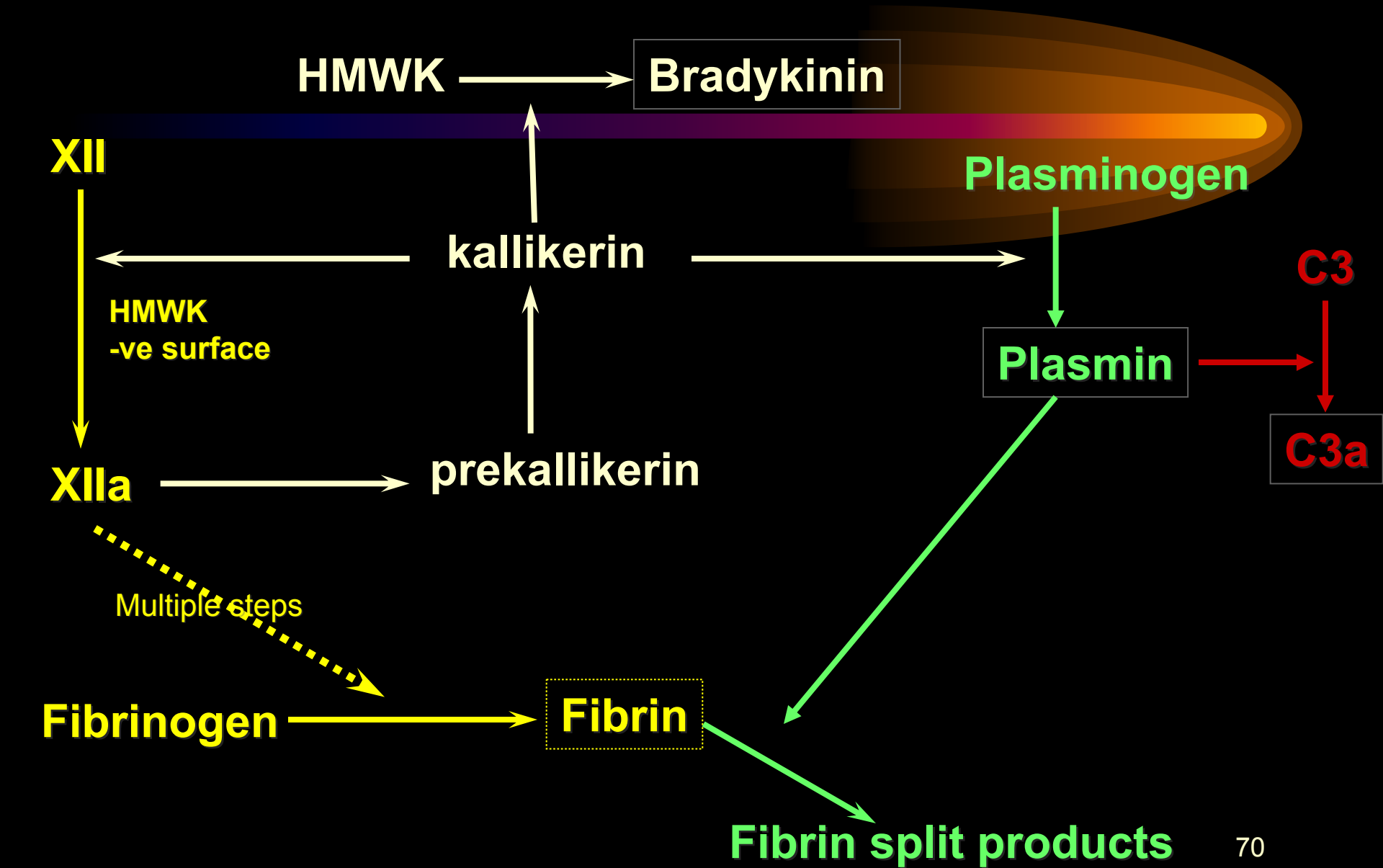
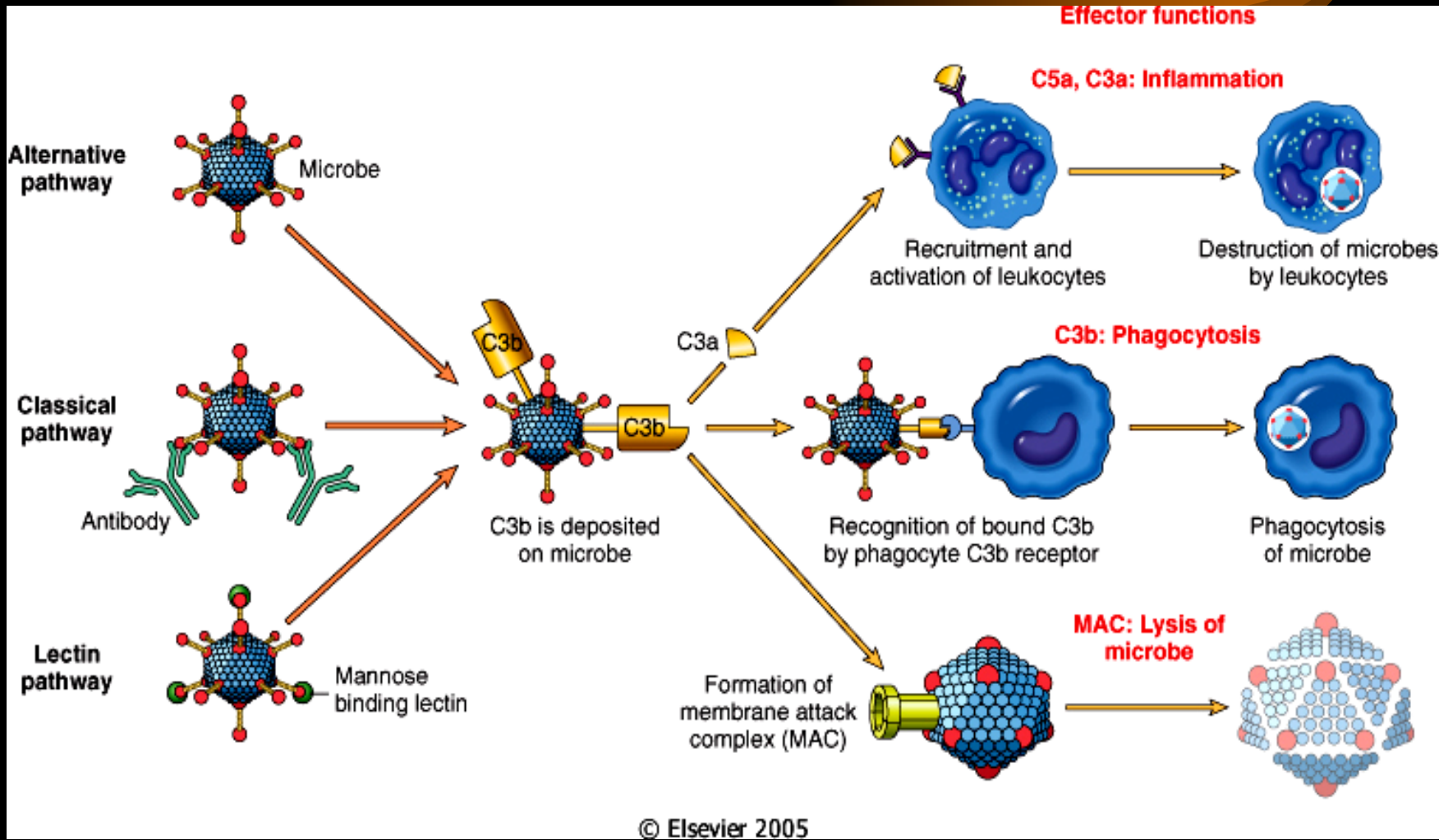


The Complement System in Inflammation

- C3a and C5a (anaphylatoxins) increase vascular permeability, and cause mast cell to secrete histamine.
- C5a activates lipoxygenase pathway of AA
- C5a activates leukocytes, increased integrins affinity
- C5a is chemotactic
- C3b and C3bi are opsonins
- Plasmin and proteolytic enzymes split C3 and C5
- Membrane attack complex (C5-9) lyse bacterial membranes

Complement Activation Pathways

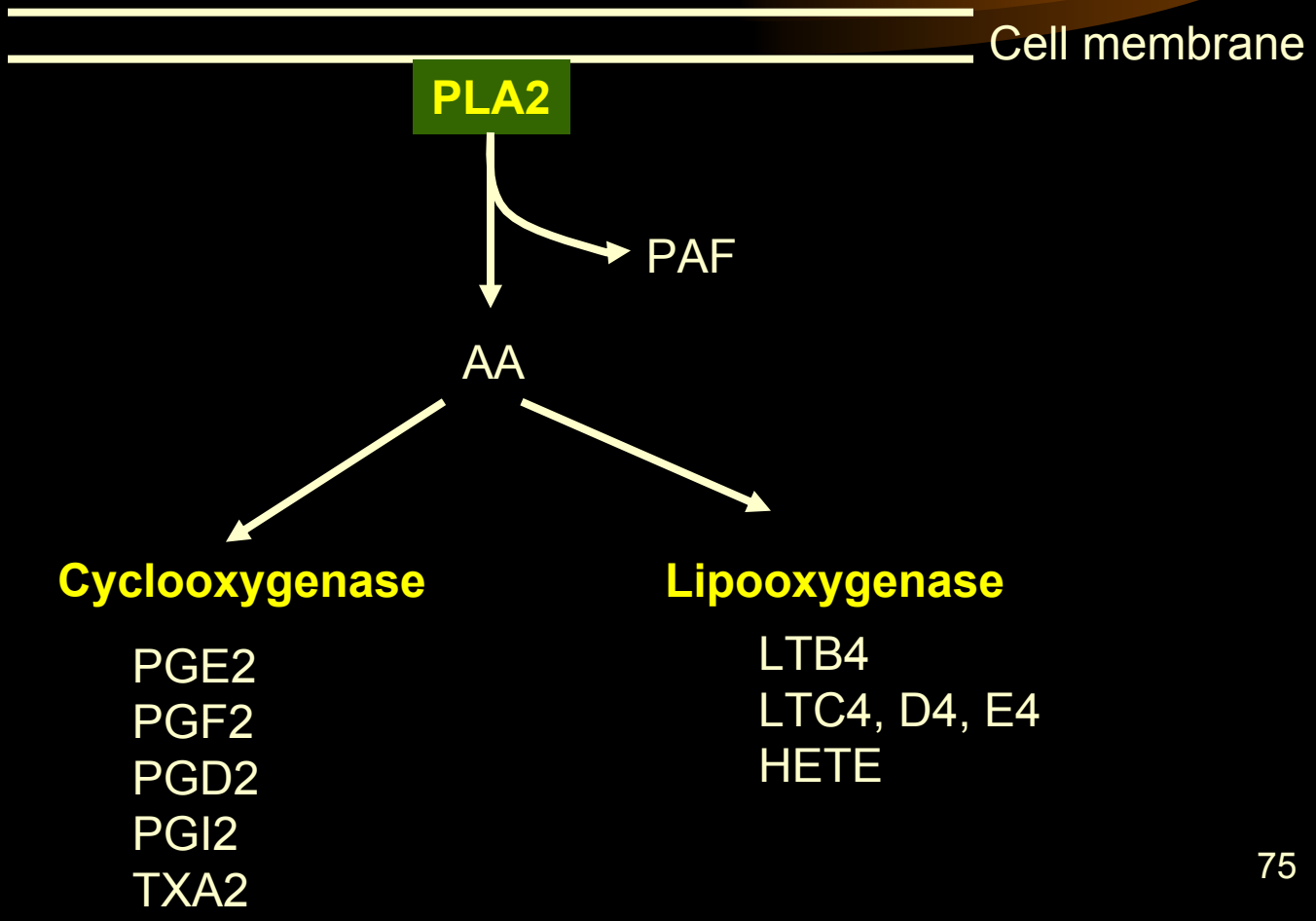
Complement Role in Inflammation



Defects in the Complement System

- Deficiency of C3 → susceptibility to infections.
- Deficiency of C2 and C4 → susceptibility to SLE.
- Deficiency of late components → low MAC → Neisseria infections.
- ↓ inhibitors of C3 and C5 convertase (↓ DAF) → hemolytic anemia
- ↓C1 inhibitor → angioneurotic edema

Arachidonic Acid Metabolism



Products of the cycloxygenase pathway of AA metabolism

- TXA2
  - Vasoconstriction
  - Simulates platelets aggregation
- PGI2
  - Vasodilatation
  - Inhibits platelets aggregation
- PGD2, PGE2, PGF2a
  - Vasodilatation
  - Edema formation
  - Pain (PGE2)

Products of the lipoxygenase pathway of AA metabolism

- 5-HETE and LTB4
  - Chemotactic
- LTC4, LTD4 and LTE4
  - Vasoconstriction
  - Bronchospasm
  - Increased vascular permeability
- Lipoxins (LXA4 & LXB4)
  - Vasodilatation
  - Inhibit neutrophil chemotaxis and adhesion
  - Stimulate monocyte adhesion

Generation of AA Metabolites

Platelet-activating Factor

- Generated from memranes phosphlipids by Phospolipase A2
- Aggregates and degranulates platelets
- Potent vasodilator and bronchoconstrictor
- Increase vascular permeability
- Effects on leukocytes
  - Increase adhesion to endothelial cells
  - Chemotactic
  - Degranulation
  - Oxygen burst

Cytokines

- Hormone-like polypeptides produced by cells, involved in cell to cell communication
- Pleiotropic effects
- Secretion is transient
- Redundant
- Effects: autocrine, paracrine, endocrine

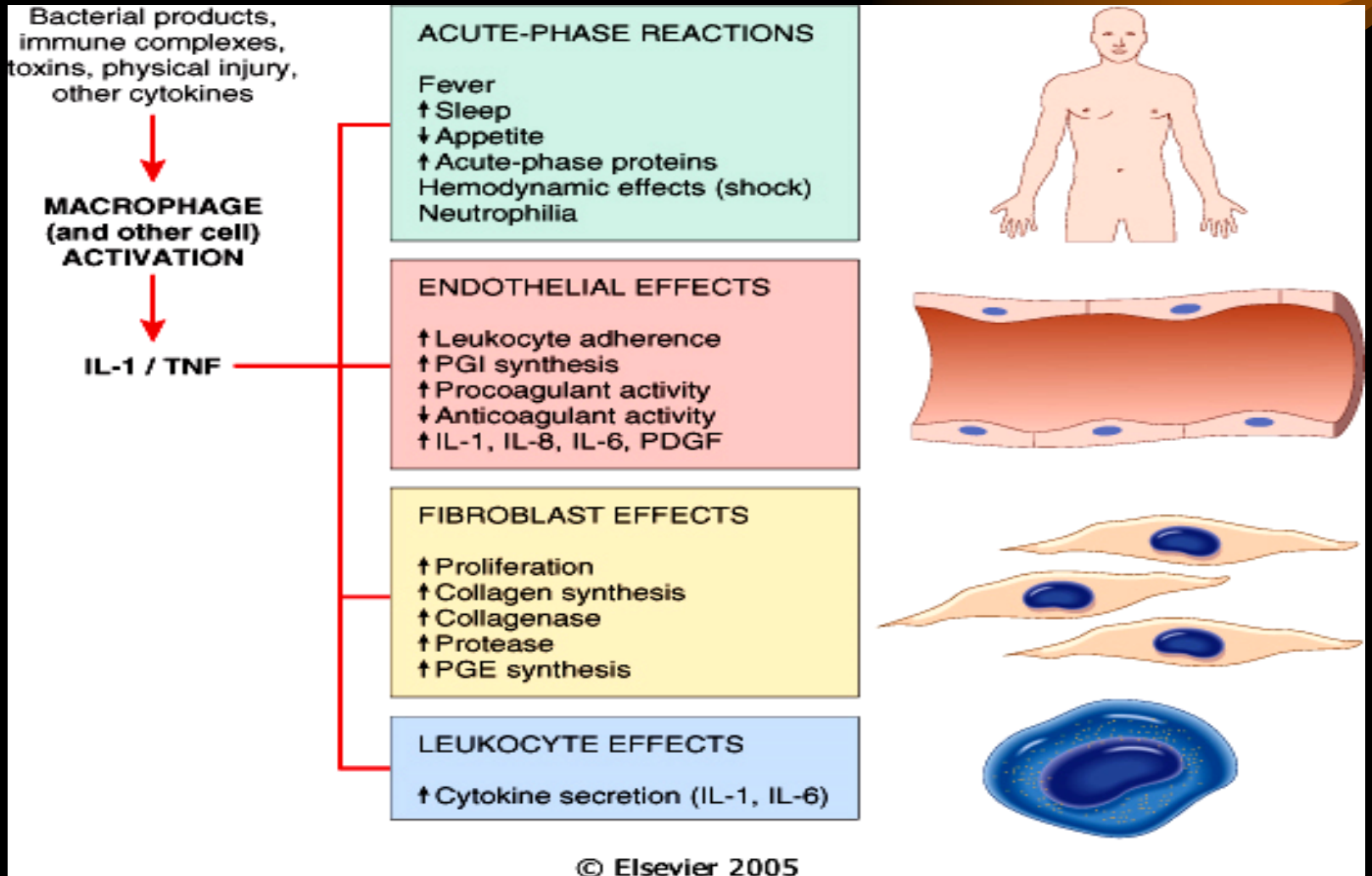
Classes of cytokines

- Regulators of lymphocyte function
  - IL-2 stimulates proliferation
  - TGFβ inhibits lymphocytes growth
- Primary responders to injury (innate immunity)
  - IL-1 & TNF
- Activators of cell mediated immunity
  - INF-γ & IL-12
- Chemotactics
  - IL-8
- Hematopoietic growth factors
  - IL-3 & GM-CSF

TNF & IL-1

- Produced mainly by macrophages
- Secretion stimulated by: bacterial products, immune complexes, endotoxins, physical injury, other cyotkines.
- Effects on endothelial cell, leukocytes, fibroblasts, and acute phase reactions

Major Effects of IL-1 & TNF



Chemokines

- A group of related chemotactic polypeptides, all of which have 4 cysteine residues
- Regulate adhesion, chemotaxis and activation of leukocytes
- Important for proper targeting of leukocytes to infection sites
- The largest family consists of CC chemokines, so named because the first 2 of the 4 cysteine residues are adjacent to each other.
- Examples of CC chemokines:
  - CCL2: Monocyte chemoattractant protein 1 (MCP-1)
  - CCL3 & CCL4: Macrophage inflammatory protein 1 (MIP-1a & 1b)
  - CCL5: RANTES
  - CCL11: Eotaxin
- Examples of CXC chemokines:
  - CXCL8: IL-8, neutrophil chemotactic

Chemokines

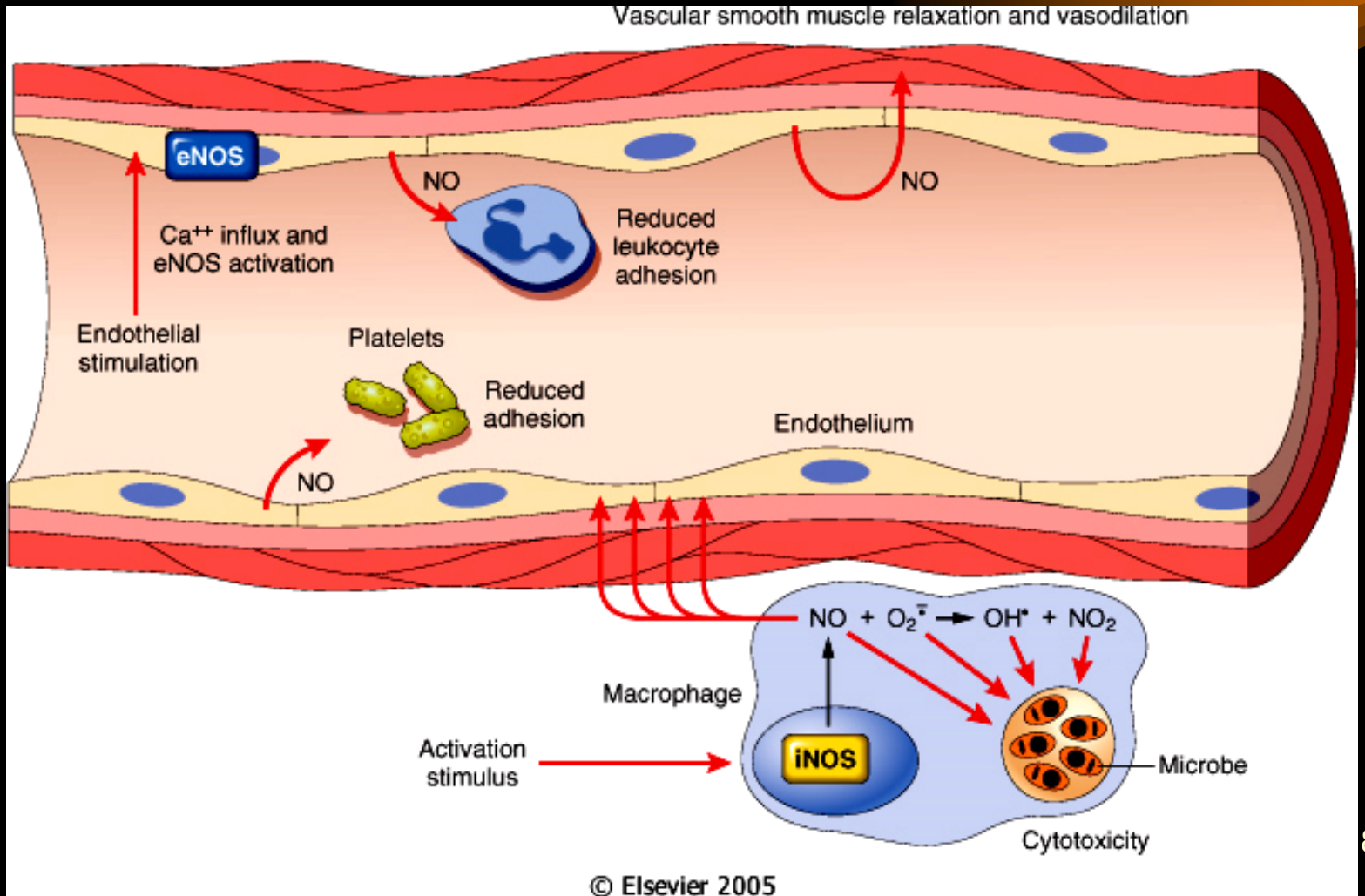
- Chemokines released in extravascular tissue move by trascytosis to the luminal surfaces of endothelial cells
- Buildup of chemokine at the luminal surface of the endothelium occurs by chemokine immobilization mediated by interactions with cell surface proteoglycans such as heparan sulfate.
- The chemokines interact with the G-protein coupled receptors on the leukocyte cell surface, resulting in activation of integrins and firm attachment to the endothelium.

Nitric Oxide

- Produced from arginine by the effect of nitric oxide synthase (NOS)
- Role in inflammation:
  - Vasodilator (smooth muscle relaxant)
  - Antagonist of platelets adhesion, aggregation and stimulation
  - Reduces leukocytes adhesion and recruitment
  - Microbicidal in activated macrophages

↓ **inflammatory response**

Nitric Oxide



Oxygen derived free radicals

At low levels

- Increase:
  - Chemokines
  - Cytokines
  - Adhesion molecules

At high levels

- Endothelial damage & thrombosis
- Protease activation & inhibition of antiproteases
- Direct damage to other cells

Protective mechanisms against free radicals include: transferrin, ceruloplasmin, catalase, superoxide dismutase, and glutathione

Lysosomal constituents

- Released in:
  - After cell death
  - Leakage upon formation of phagocytic vacuoles
  - Frustrated phagocytosis (fixed on flat surfaces)
  - After phagocytosis of membranolytic substance, e.g. urate
- Neutral proteases effects:
  - Elastases, collagenases, and cathepsin
  - Cleave C3 and C5 producing C3a & C5a
  - Generate bradykinin like peptides
- Minimizing the damaging effects of proteases is accomplished by antiproteases:
  - Alpha 2 macroglobulin
  - Alpha 1 antitrypsin

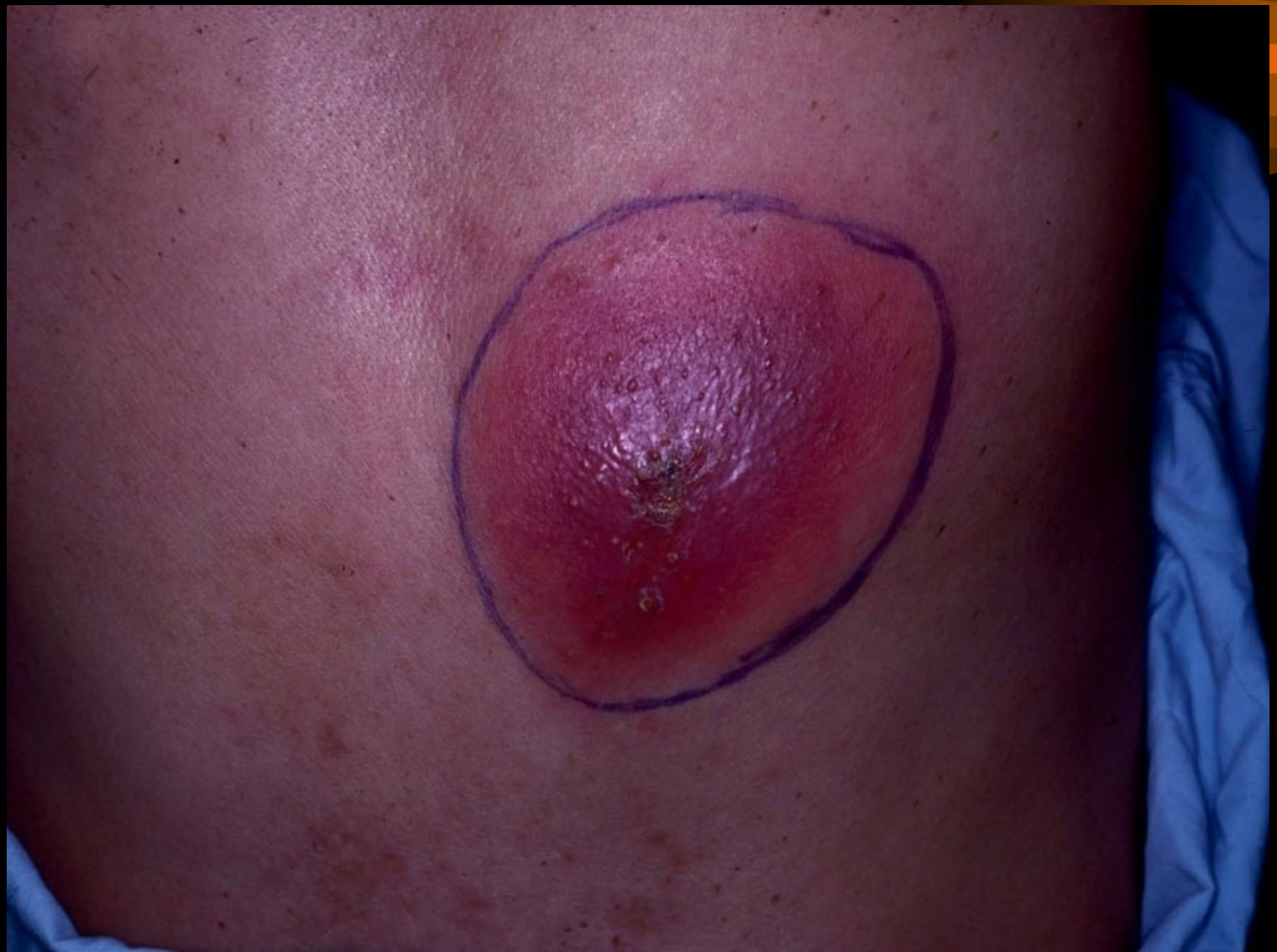
Morphologic Appearance of Acute Inflammation

- Catarrhal
  - Acute inflammation + mucous hypersecretion (e.g. common cold)
- Serous
  - Abundant protein-poor fluid with low cellular content, e.g. skin blisters and body cavities
- Fibrinous:
  - Accumulation of thick exudate rich in fibrin, may resolve by fibrinolysis or organize into thick fibrous tissue (e.g. acute pericarditis)

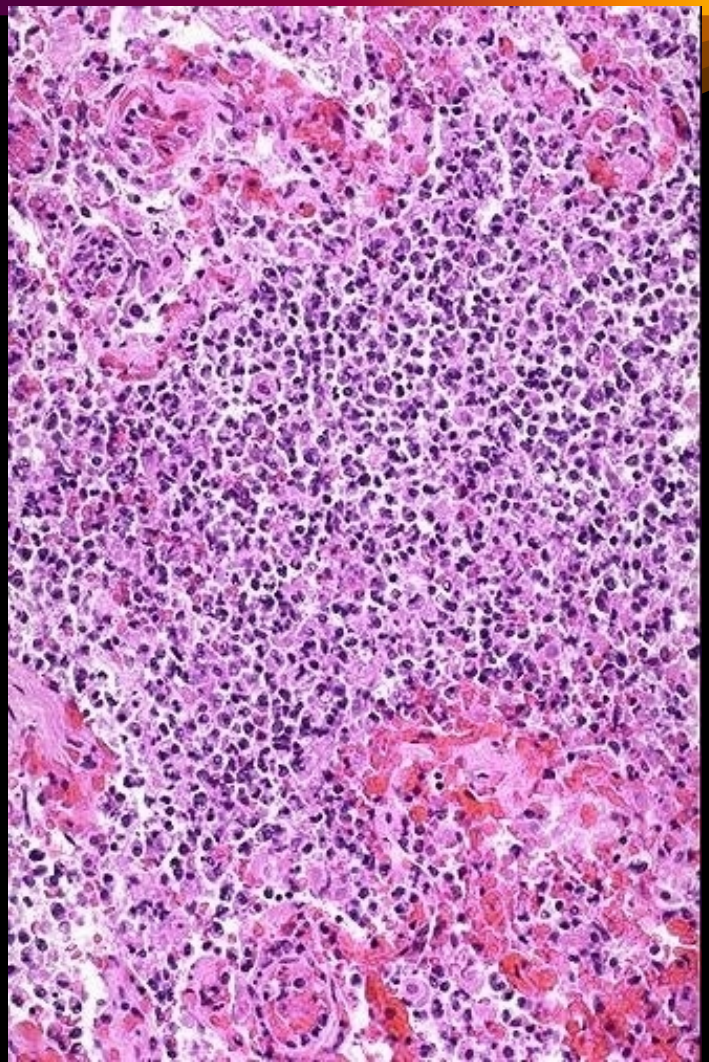
Morphologic Appearance of Acute Inflammation

- Suppurative (purulent):
  - Pus: Creamy yellow or blood stained fluid consisting of neutrophils, microorganisms & tissue debris e.g. acute appendicitis
  - Abscess*:* Focal localized collection of pus
  - Empyema: Collection of pus within a hollow organ
- Ulcers:
  - Defect of the surface lining of an organ or tissue
  - Mostly GI tract or skin

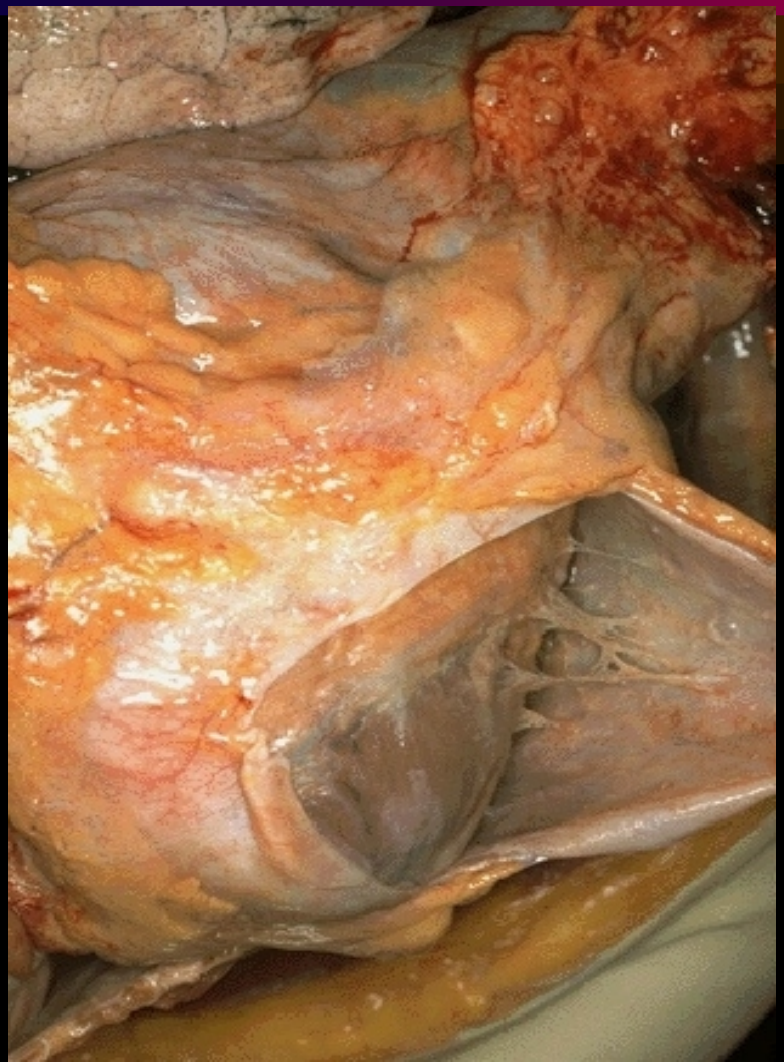
Subcutaneous Abscess



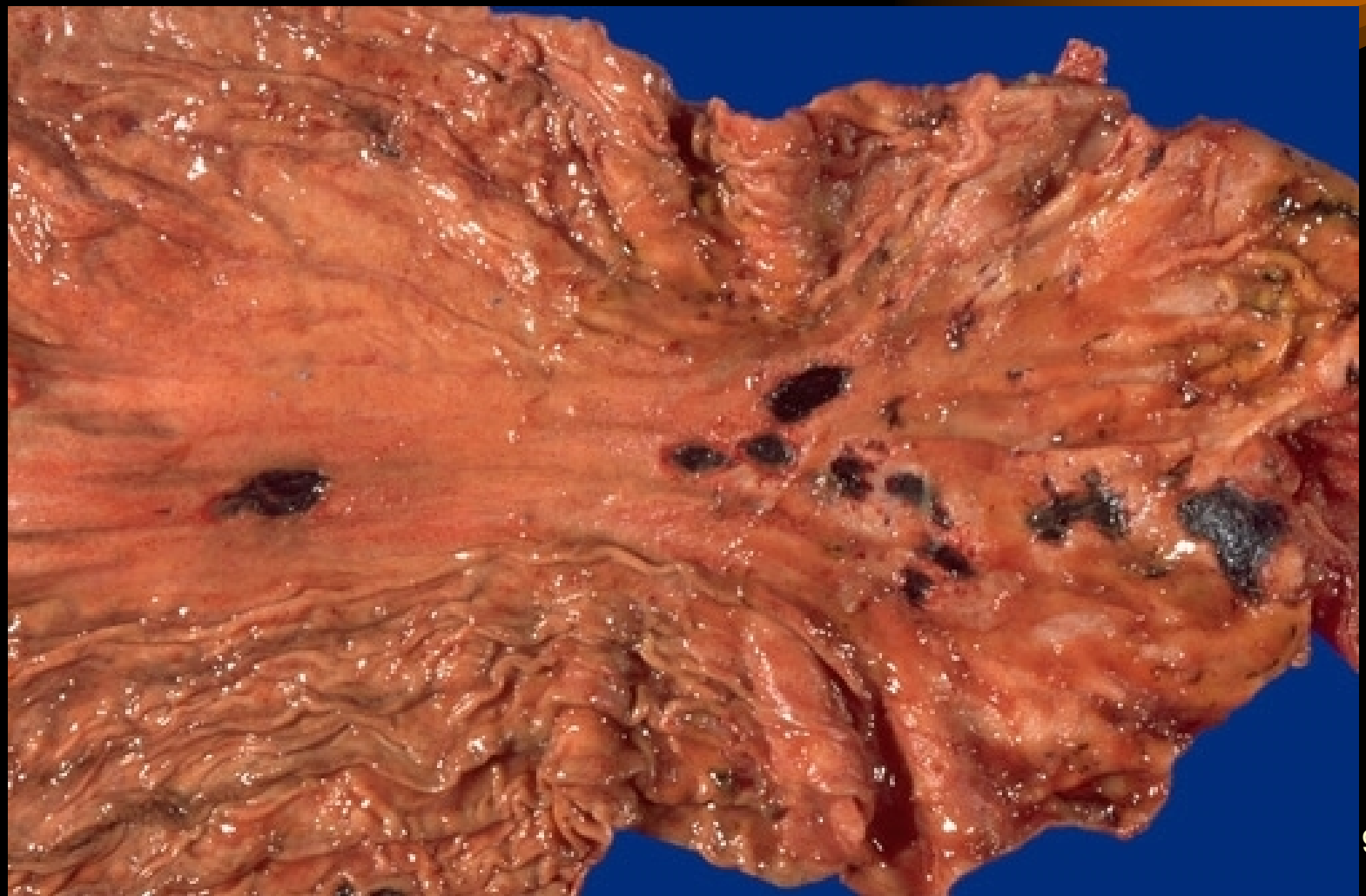
Lung Abscess



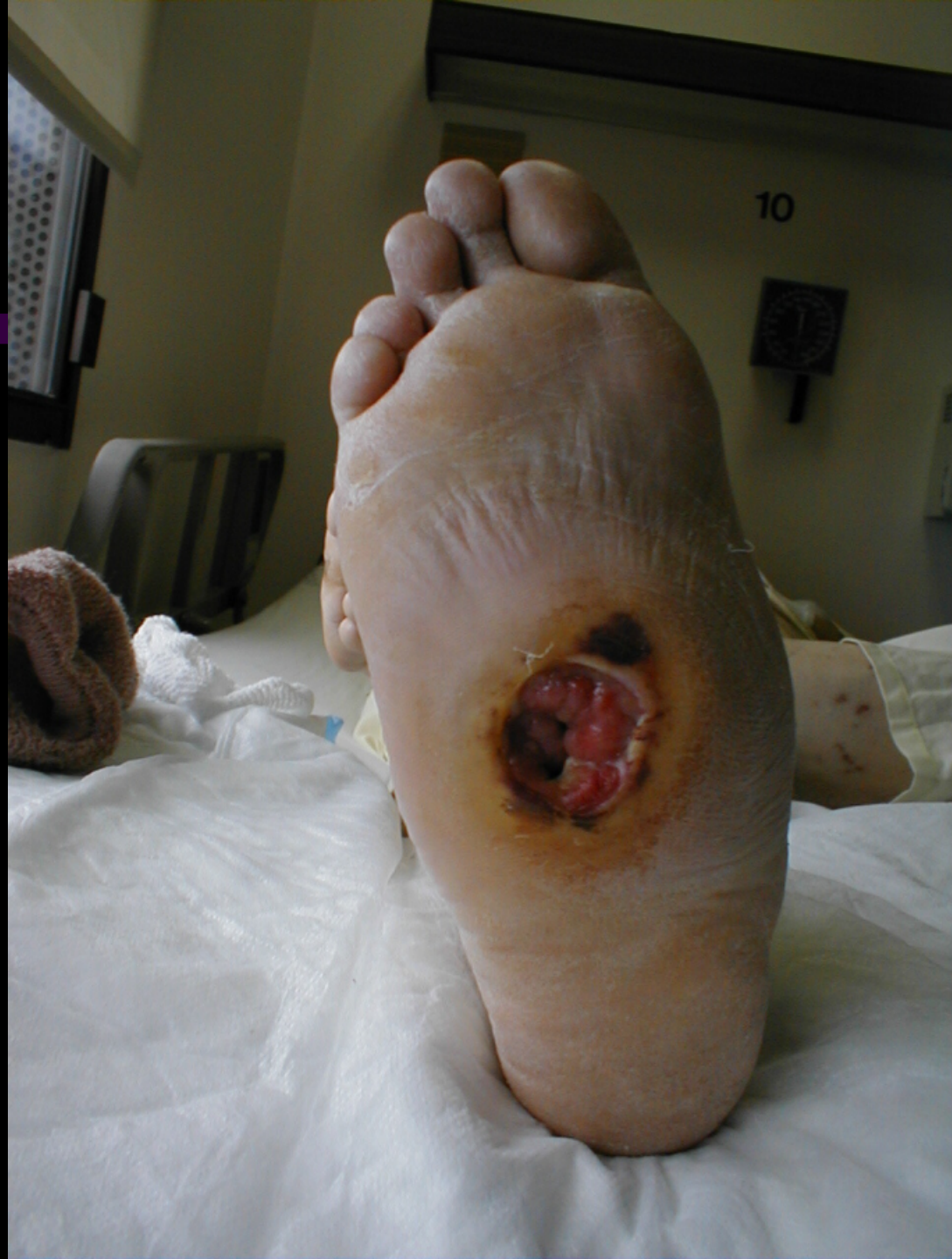
Fibrinous Pericarditis



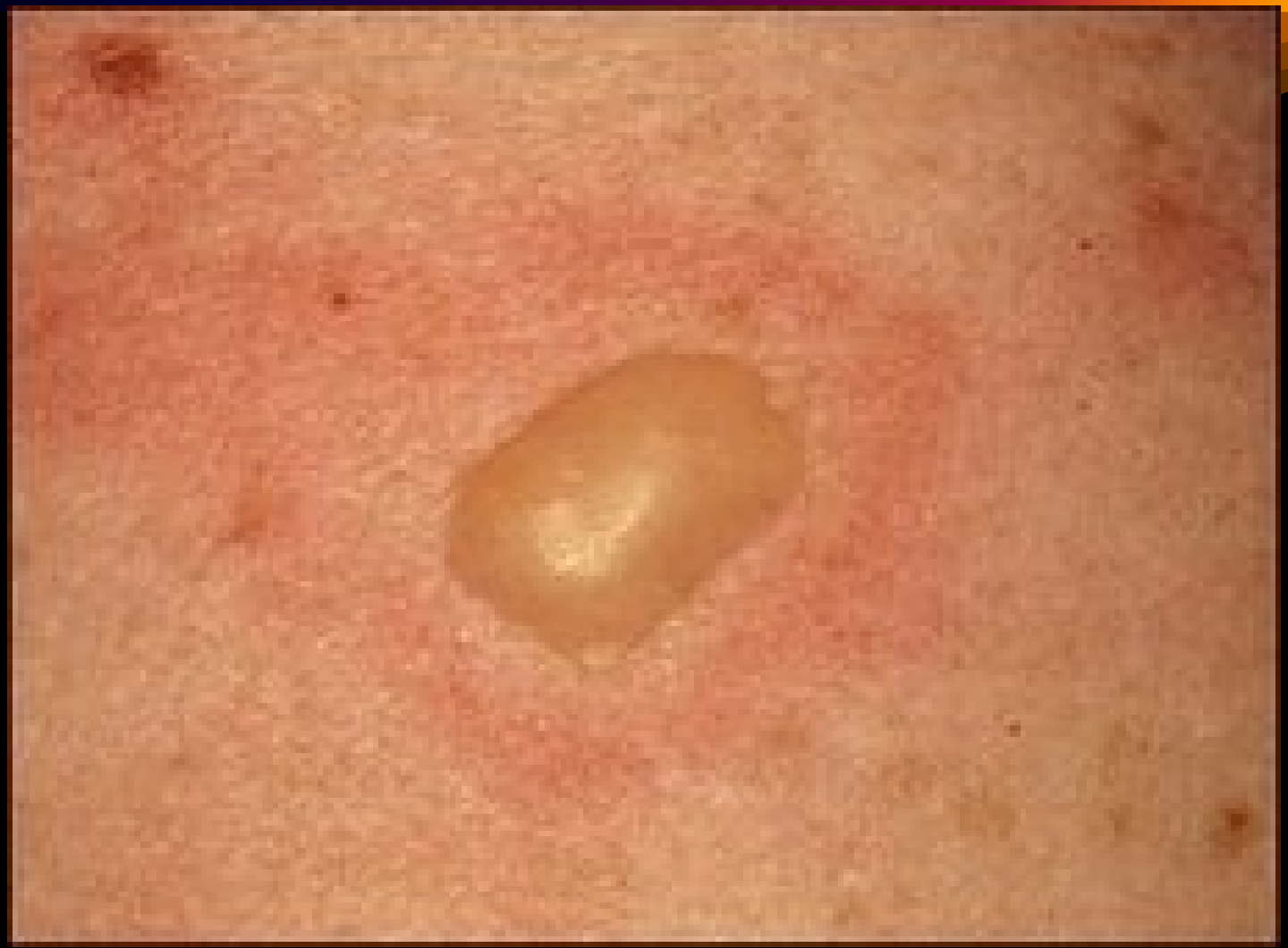
Gastric Ulcers



Foot Ulcer



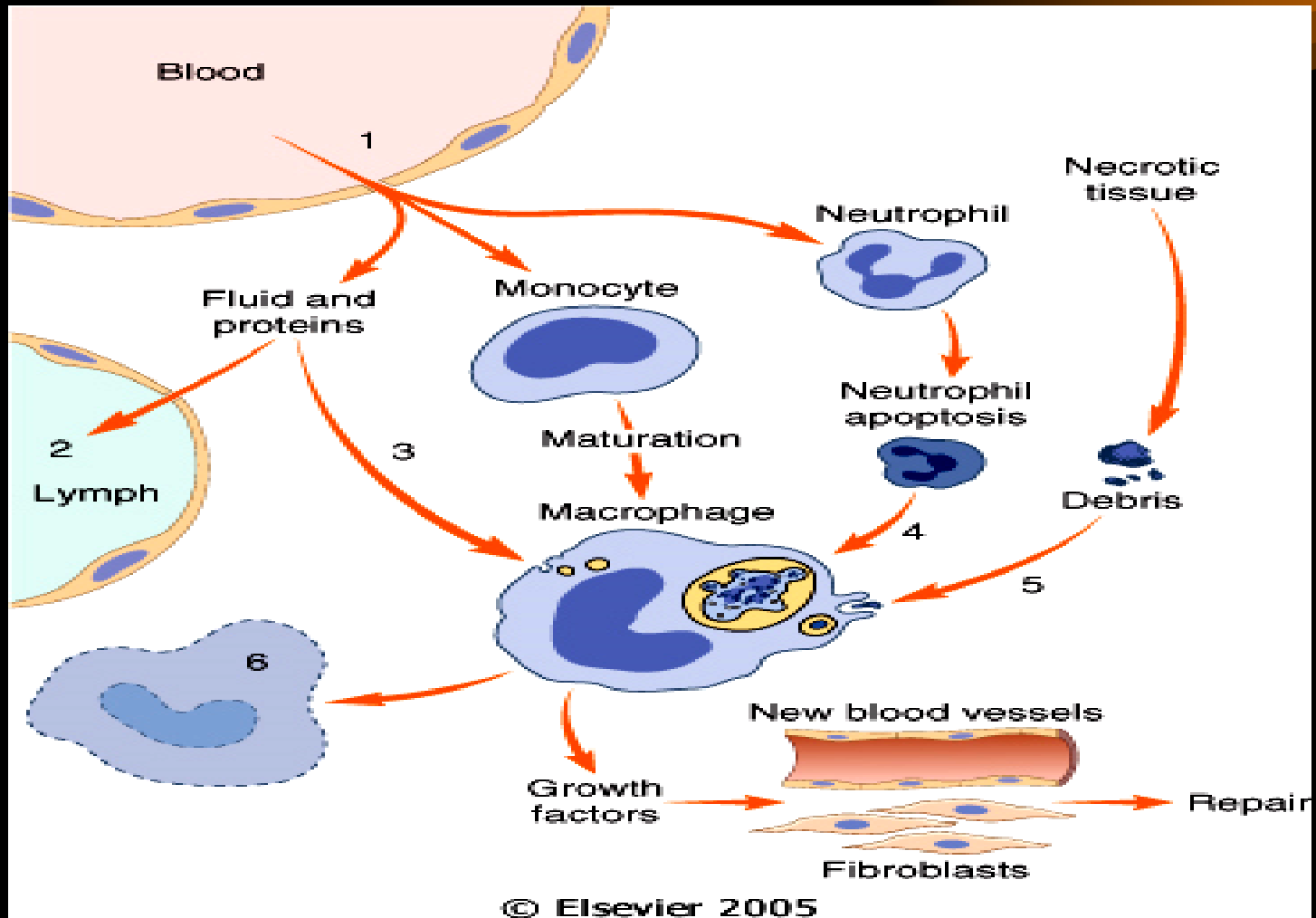
Burn Blister



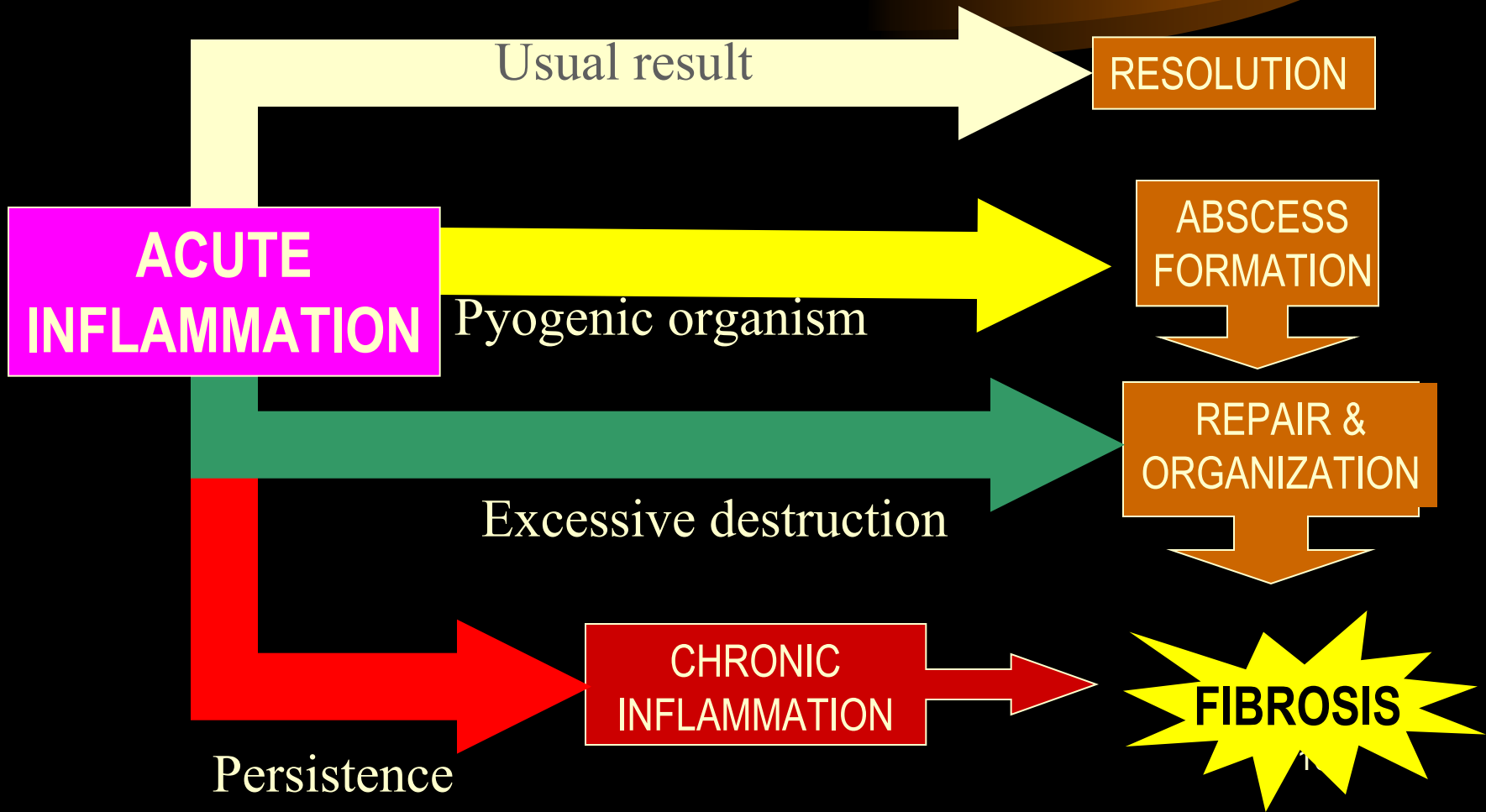
Outcomes of Acute Inflammation

- Complete resolution (back to normal)
  - Clearance of injurious stimuli
  - Removal of the exudate, fibrin & debris
  - Reversal of the changes in the microvasculature
  - Replacement of lost cells (regeneration)
- Healing
  - organization by fibrosis through formation of Granulation tissue. Why?
    - Substantial tissue destruction or
    - Tissue cannot regenerate or
    - Extensive fibrinous exudates
- Abscess formation
- Progression to chronic inflammation

Complete Resolution of Inflammation



Outcomes of Acute Inflammation



Role of Lymphatic System in Inflammation

- The local inflammatory reaction may fail in containing the injurious agent
- Secondary lines of defense:
  - Lymphatic system:
    - Lymphatic vessels drain offending agent, edema fluid & cellular debris, and may become inflamed (LYMPHANGITIS).
    - Lymph nodes may become inflamed (LYMPHADENITIS).
    - Secondary lines of defense may contain infection, or may be overwhelmed resulting in BACTEREMIA.
  - MPS:
    - Phagocytic cells of spleen, liver & BM
- In massive infections, bacterial seeding may occur in distant tissues.

Effects of Acute Inflammation

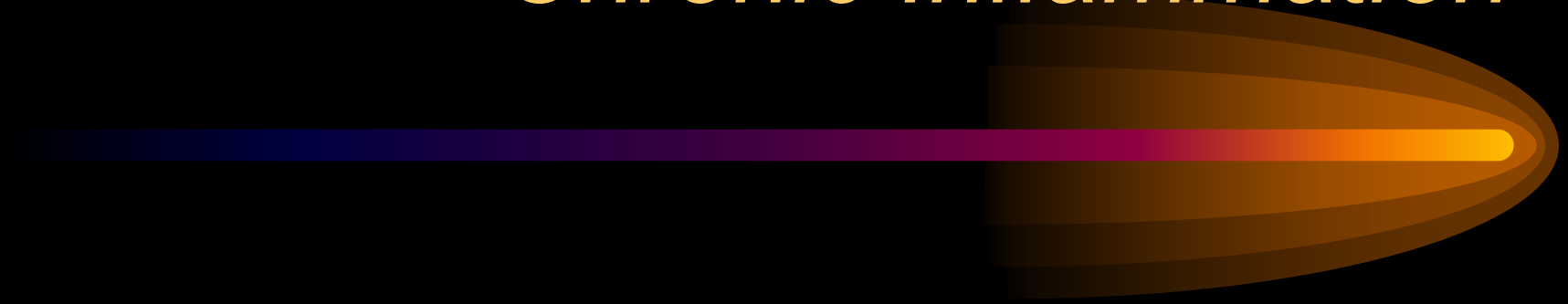
BENIFITIAL:

- Elimination of injurious stimulus
- Dilution of toxins
- Entry of antibodies
- Drug transport
- Fibrin formation
- Delivery of nutrients & oxygen
- Stimulation of the immune response

HARMFUL:

- Digestion of normal tissues
- Swelling
- Inappropriate inflammatory response

Chronic Inflammation



Chronic Inflammation

- Inflammation of prolonged duration (weeks, months, or years) that starts either rapidly or slowly.
- Characterized by an equilibrium of:
  - Persistent injurious agent
  - Inability of the host to overcome the injurious agent

Chronic Inflammation

- Characteristics:
  - Chronic inflammatory cell infiltrate
    - Lymphocytes
    - Plasma cells
    - Macrophages
  - Tissue destruction
  - Repair
    - Neovascularization
    - Fibrosis

Inflammation

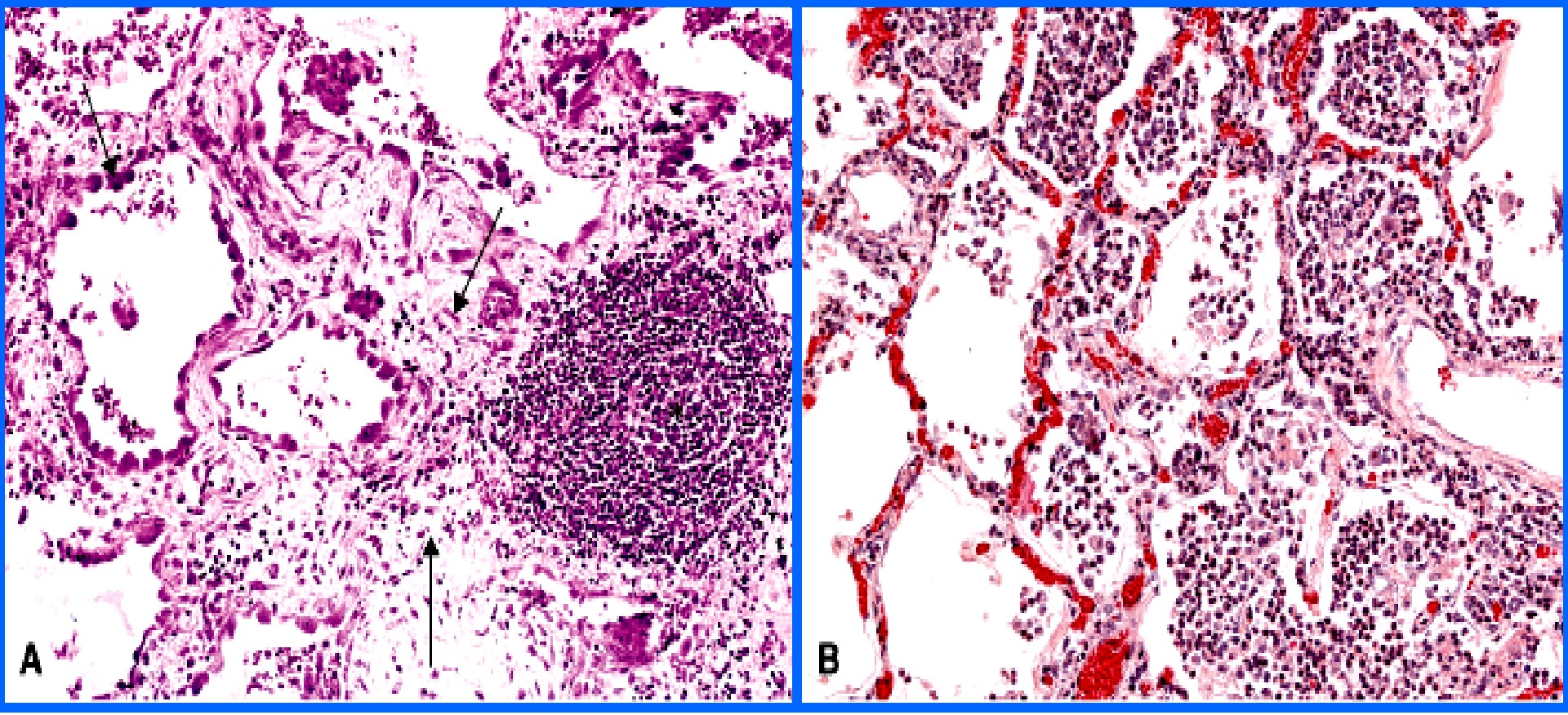
Acute inflammation

- Duration: minutes to days
- Predominance of neutrophils
- Fluid & plasma protein exudation

Chronic inflammation

- Duration: days to years
- Predominance of lymphocytes and macrphages
- Vascular proliferation and fibrosis

Chronic and Acute Pneumonia



Chronic Inflammation

- Under what circumstances, does it develop?
  - Progression from acute inflammation
    - Tonsillitis, osteomyelitis, etc.
  - Repeated exposure to toxic agent
    - Silicosis, asbestosis, hyperlipidemia, etc.
  - Viral infections
  - Persistent microbial infections
    - Mycobacteria, Treponema, Fungi, etc.
  - Autoimmune disorders
    - Rhumatoid arthritis, SLE, systemic lupus, etc.

Macrophages & the Mononuclear Phagocytic System

- Macrophages:
  - Derived from circulating monocytes
  - Scattered in tissues:
    - Kupffer cells (liver),
    - sinus histiocytes (spleen & LN),
    - alveolar macrophages (lung),
    - microglia (CNS)
- Activated mainly by IFN-γ secreted from T lymphocytes
  - Increased cell size
  - Increased lysosomal enzymes
  - more active metabolism, i.e. greater ability to kill ingested organisms
  - Epithelioid appearance

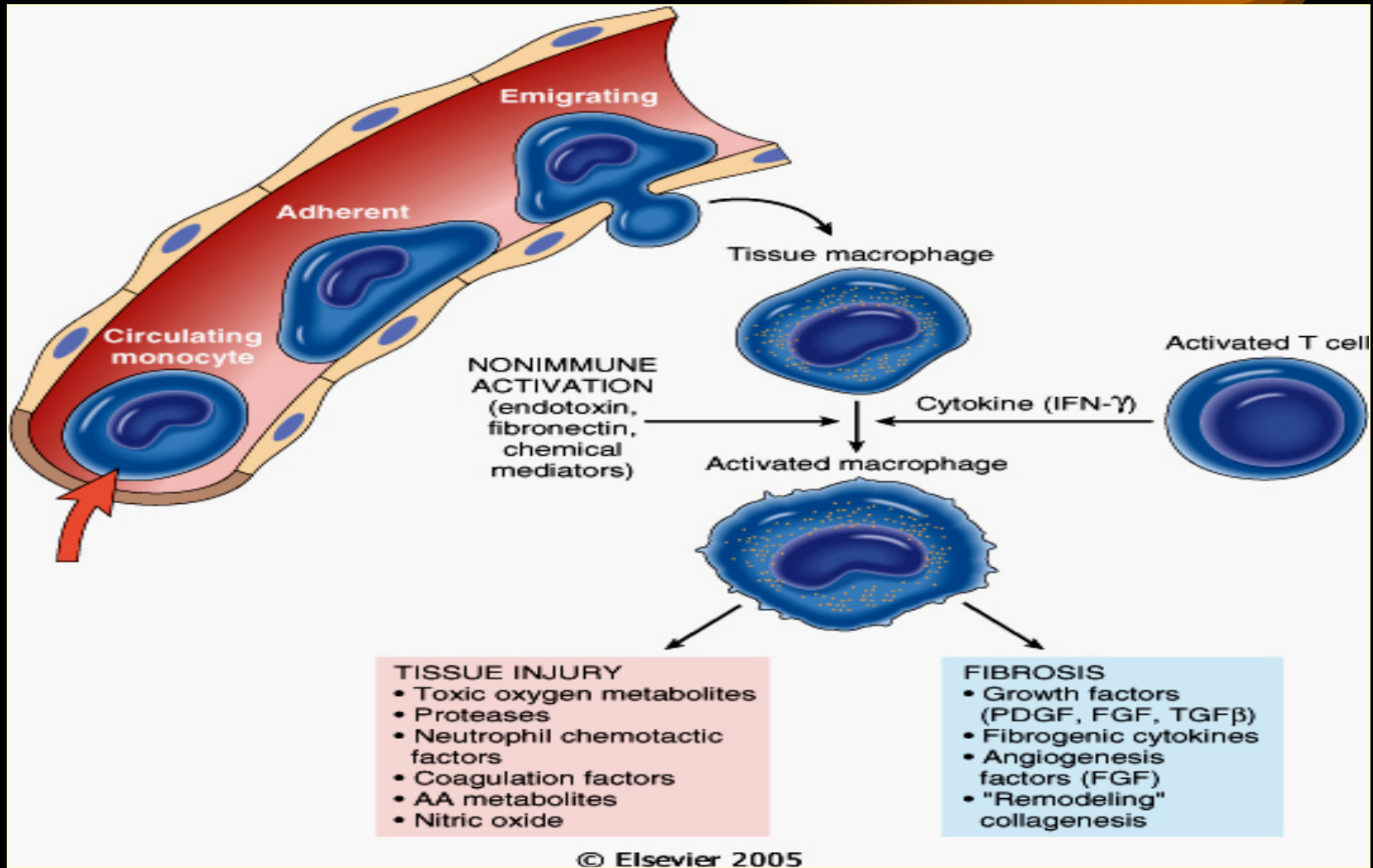
How do Macrophages Accumulate at Sites of Chronic Inflammation?

- Recruitment of monocytes from circulation by chemotactic factors:
  - Chemokines, C5a, PDGF, TGFα, fibrinopeptides, fibronectin, collagen breakdown fragments.
- Proliferation of macrophages at foci of inflammation
- Immobilization of macrophages at sites of inflammation

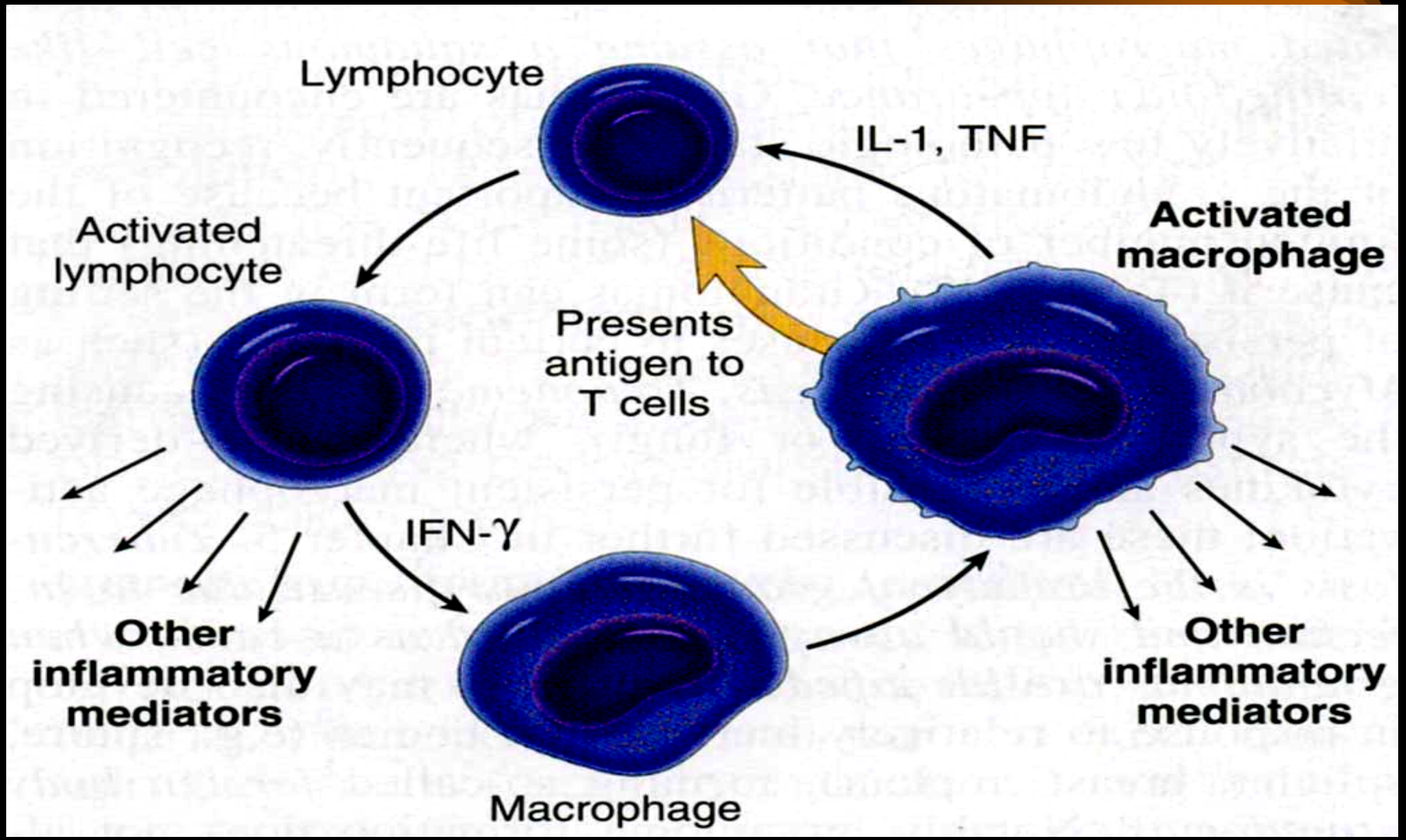
Products Activated macrophages

- Proteases
- Complement and clotting factors
- Oxygen species and NO
- AA metabolites
- IL-1 & TNF
- Growth factors (PDGF, FGF, TGFβ)

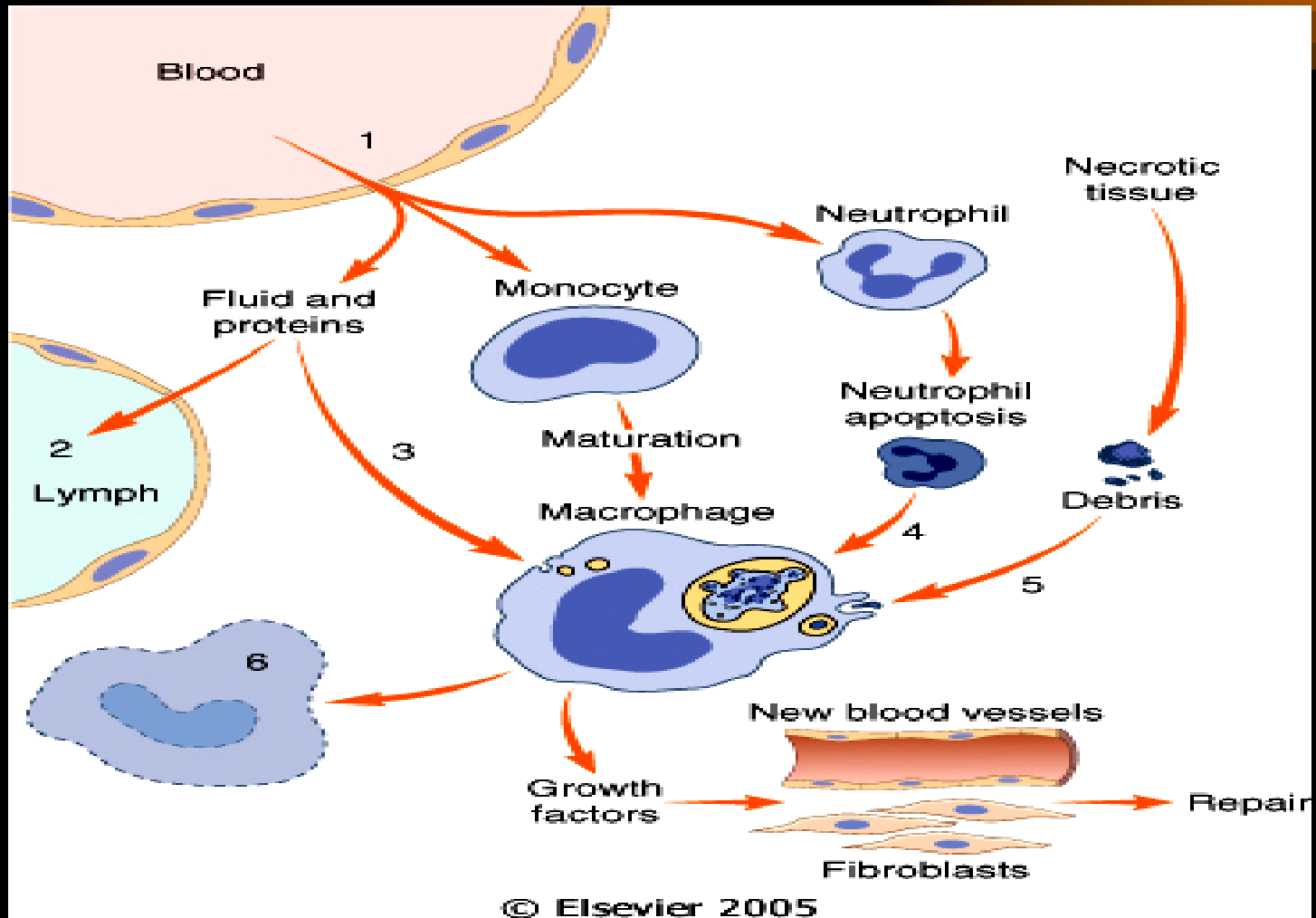
Role of Activated Macrophages in Chronic Inflammation



Macrophage-Lymphocyte Interactions



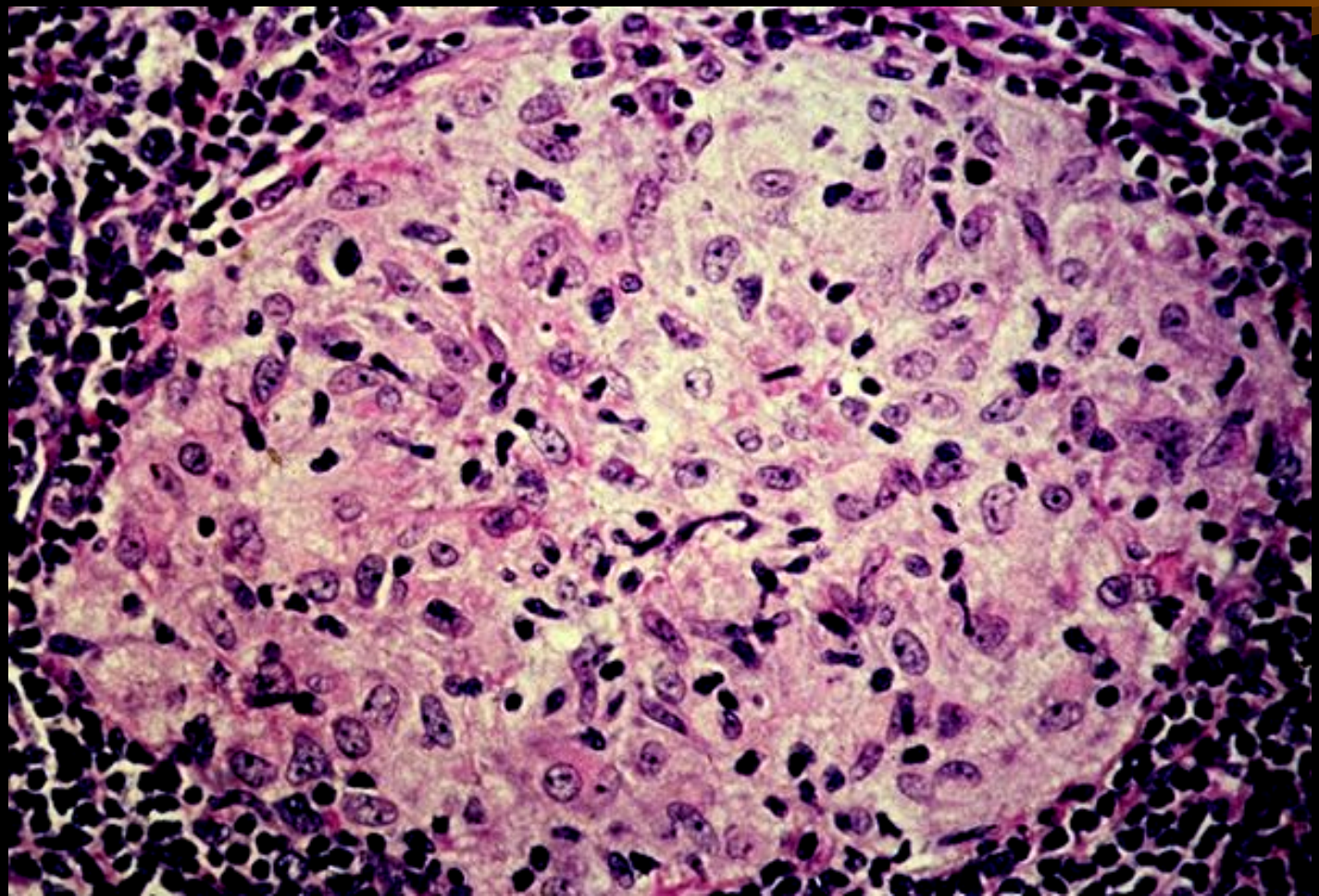
Complete Resolution of Inflammation



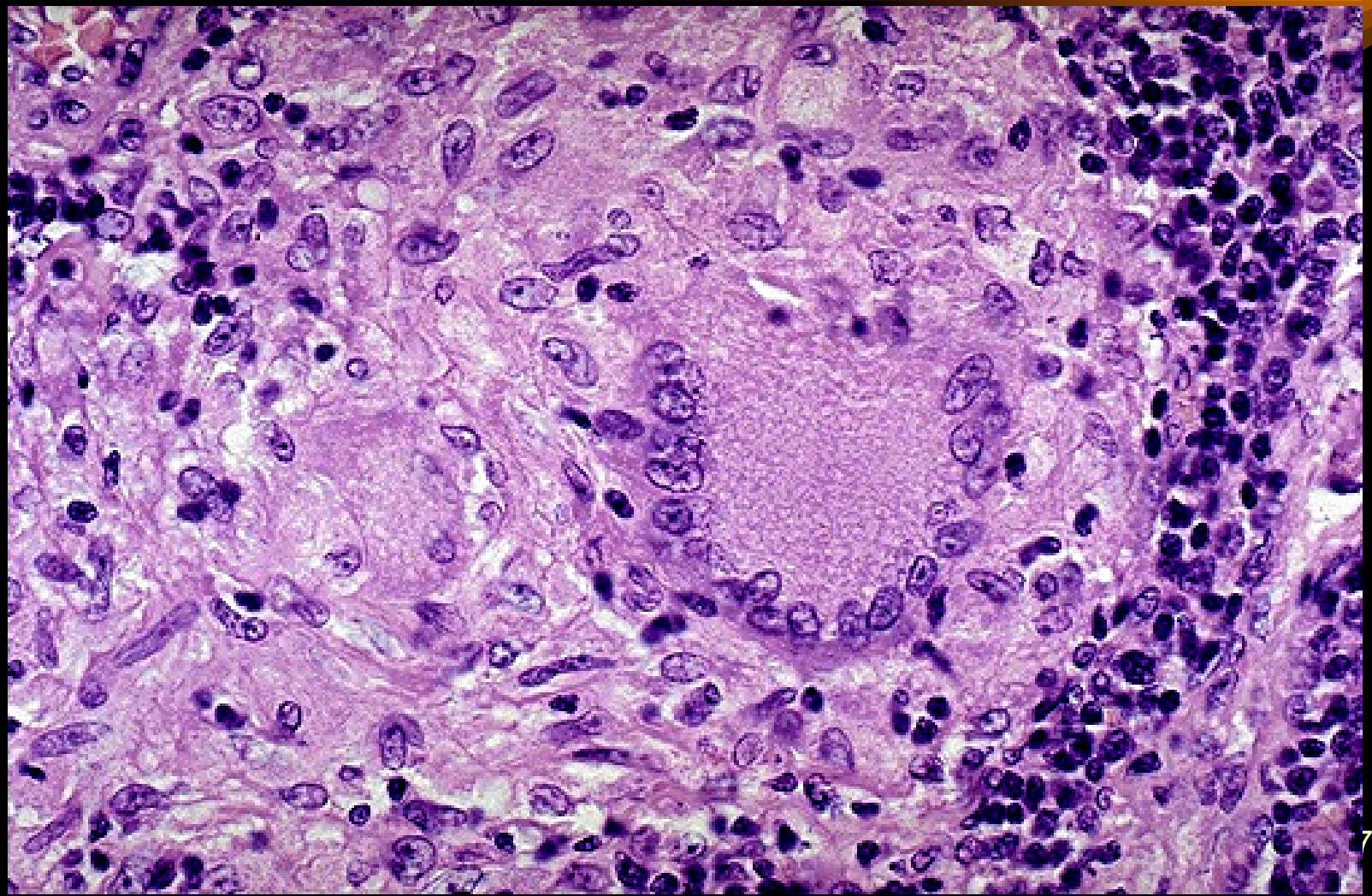
Granuolmatous Inflammation

- A distinctive form of chronic inflammation characterized by collections of epithelioid macrophages
- Granuloma, in addition to epithelioid macrophages, may have one or more of the following:
  - a surrounding rim lymphocytes & plasma cells
  - a surrounding rim of fibroblasts & fibrosis
  - giant cells
  - central necrosis e.g. caseating granulomas in TB

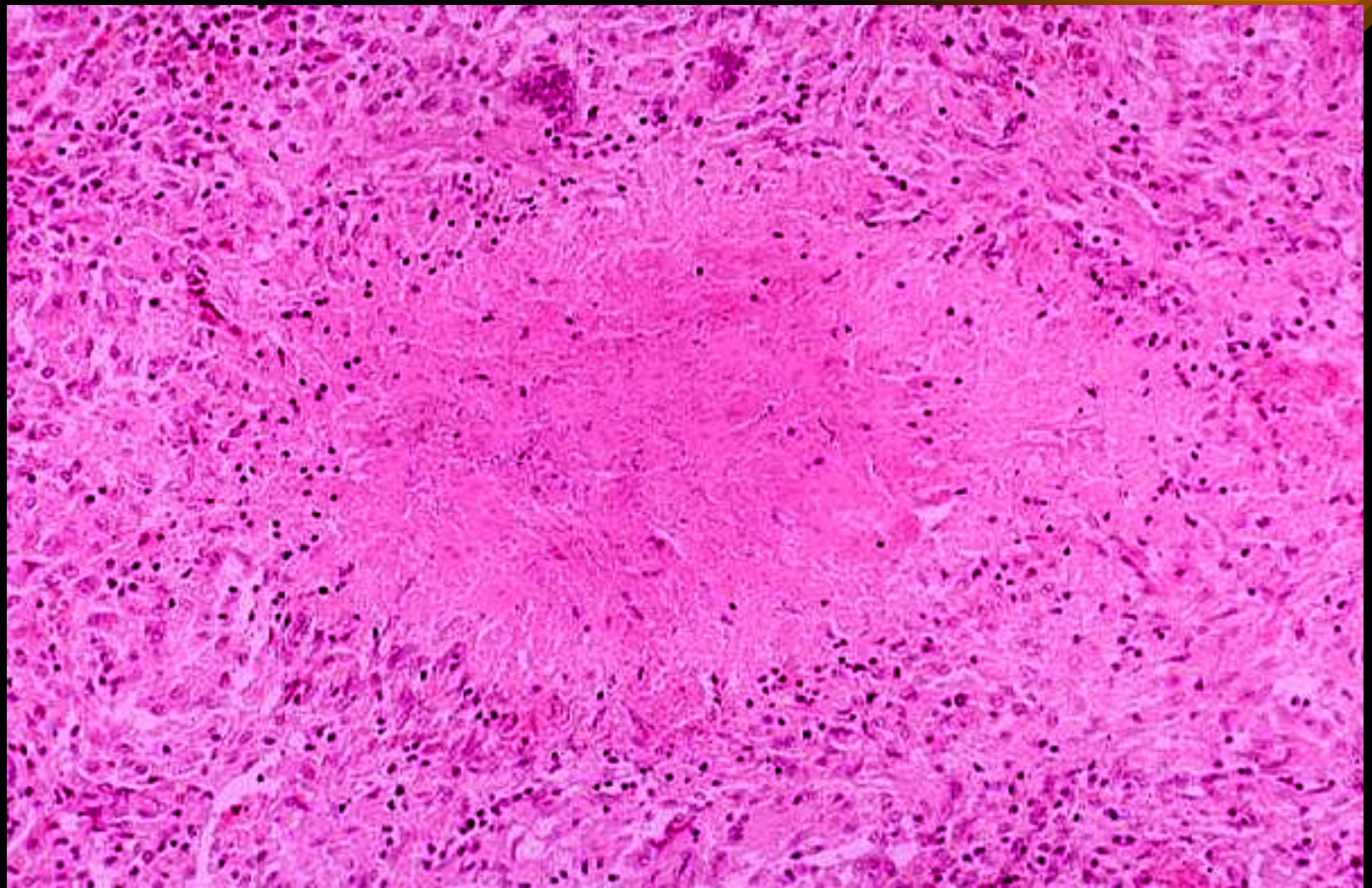
Histopathology of Granuloma



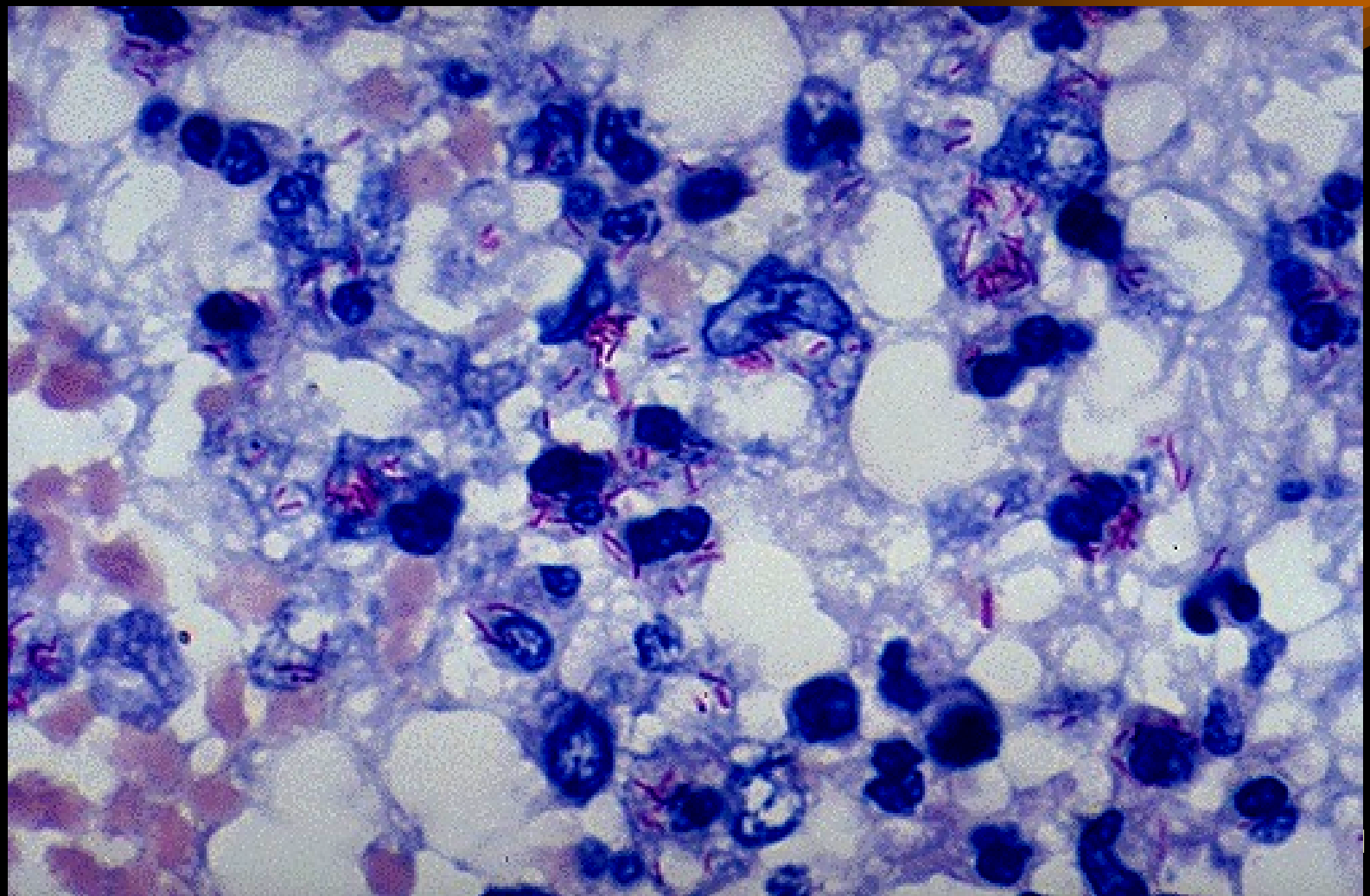
Histopathology of Granuloma



Caseating Granuloma



AFB Stain in Caseating Granuloma



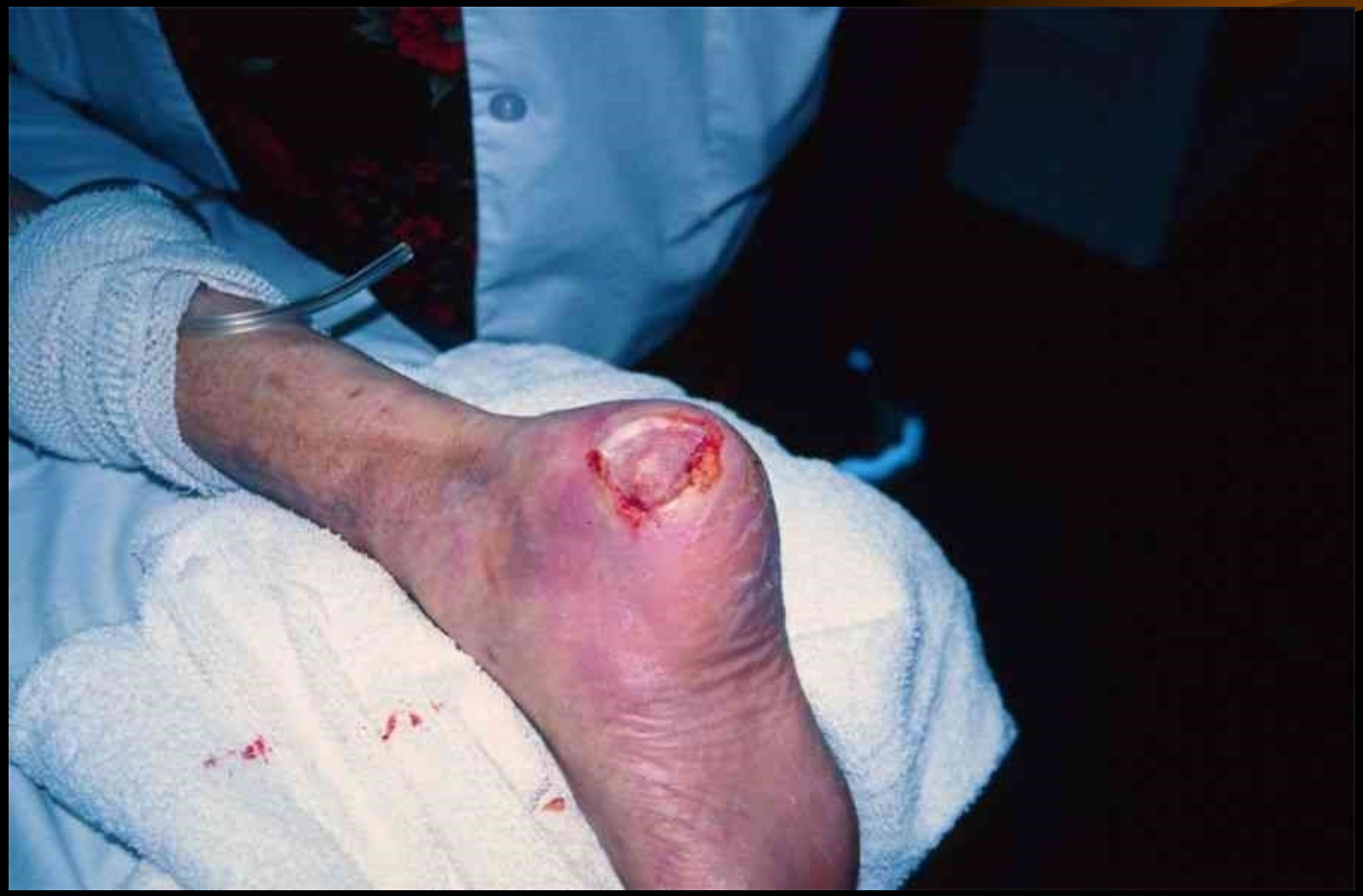
Examples of Granulomatous Inflammation

Bacterial	Mycobacterium tuberculosis Mycobacterium Leprae Trepnema pallidum Bartonella henslae
Parasitic	Scistosomiasis
Fungal	Histoplasma capsulatum Balsomycosis Cryptococcus neoformans Coccidioides immitis
Inorganic metals	Silicosis, Byrelliosis
Foreign body	Suture, other prosthesis, keratin
Unknown	Sarcoidosis

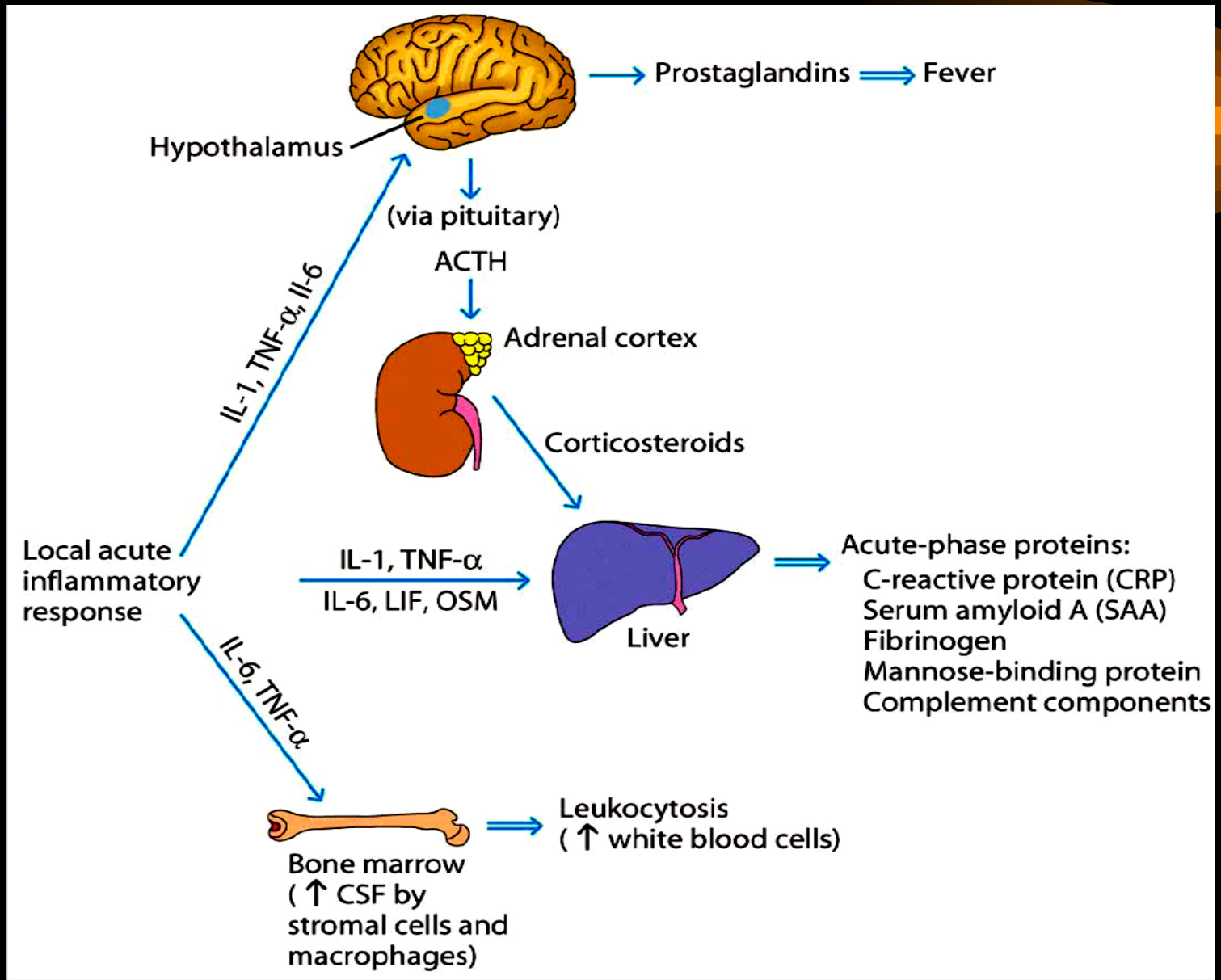
Morphologic Appearance of Chronic Inflammation

- Ulceration
  - Ulcer: Local defect or loss of continuity in surface epithelia
- Chronic abscess cavity
- Induration & fibrosis
- Thickening of the wall of a hollow viscus
- Caseous necrosis

Ulcer



Systemic Acute-phase Reactions



Systemic Effects of Inflammation (Acute phase reactions)

- Mediated by IL-1, IL6, TNF, which interact with vascular receptors in the thermoregulatory center of hypothalamus via local PGE production
- Systemic manifestations include:
  - Fever
  - Catabolism
  - Increased slow wave sleep, decreased appetite
  - Hypotension & other hemodynamic changes
  - Synthesis of acute-phase proteins by liver, e.g. CRP, fibrinogen, serum amyloid A protein (SAA)
  - Leukocytosis: neutrophilia, lymphocytosis, eosinophilia
  - Leukopenia
  - Increased ESR

Consequences of Defective Inflammation

- Susceptibility to infections
  - Defective innate immunity
- Delayed repair
  - Delayed clearance of debris and necrotic tissue
  - Lack of stimuli for repair

Consequences of Excessive Inflammation

- Allergic reactions
- Autoimmune disorders
- Atherosclerosis
- Ischemic heart disease

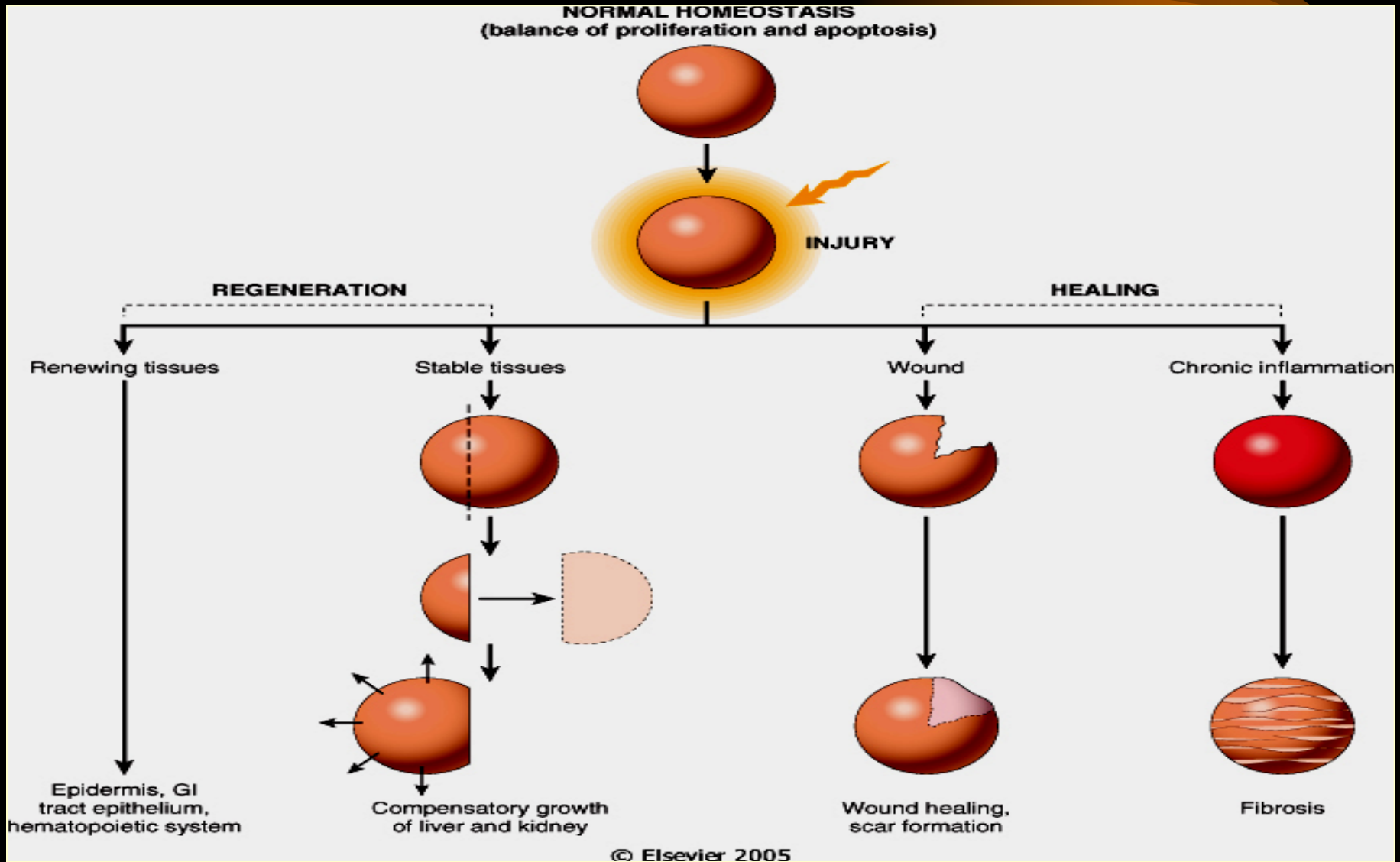
Tissue Repair

The two processes of repair

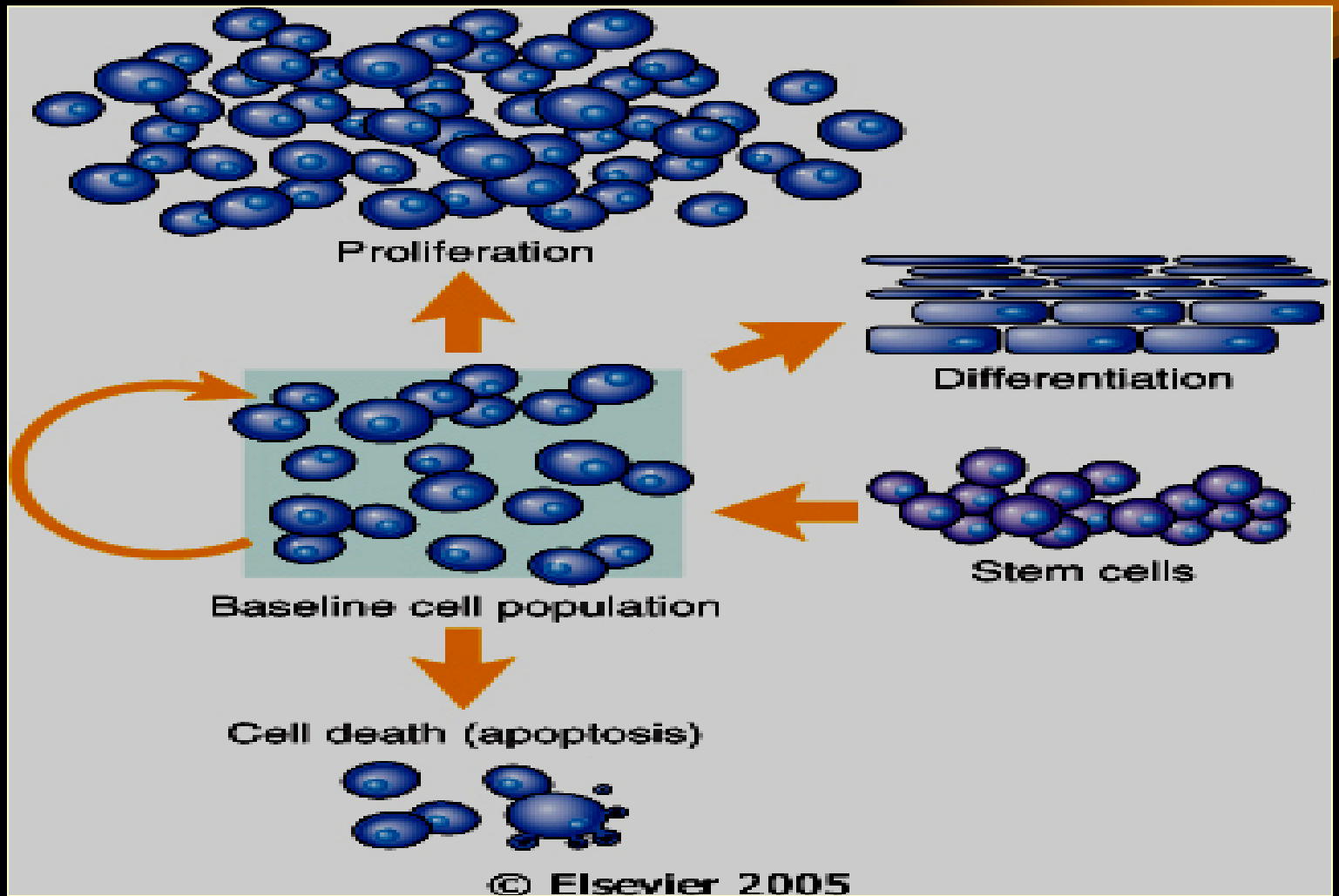
- Regeneration
  - Replacement of damaged cells by similar parenchymal cells, e.g. liver regeneration
  - Requires intact connective tissue scaffold
- Fibrosis
  - Replacement by connective tissue
  - ECM framework is damaged

Healing is a combination of regenerative and fibrotic processes

Regeneration and Healing



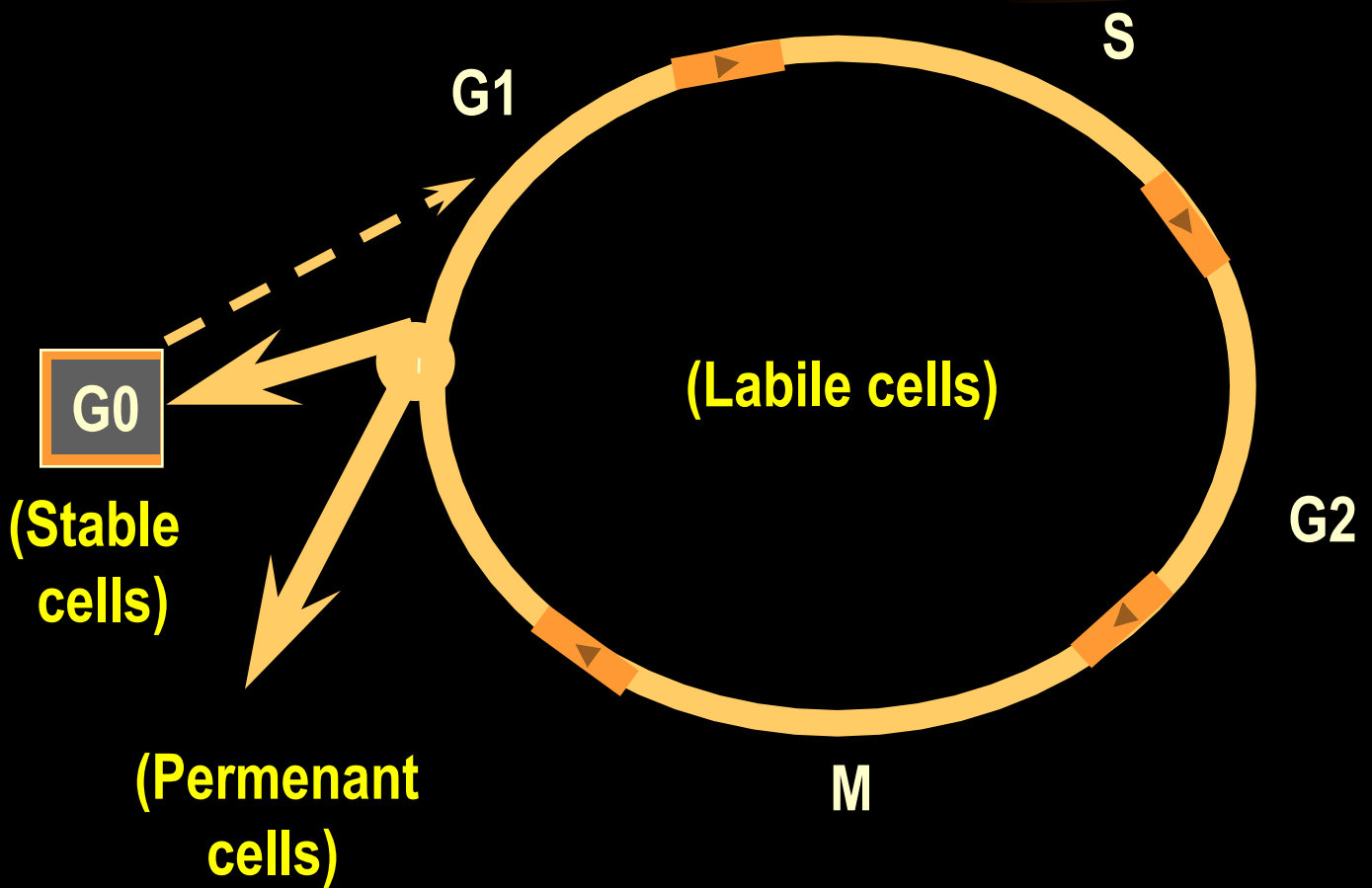
Regulation of Cell Populations



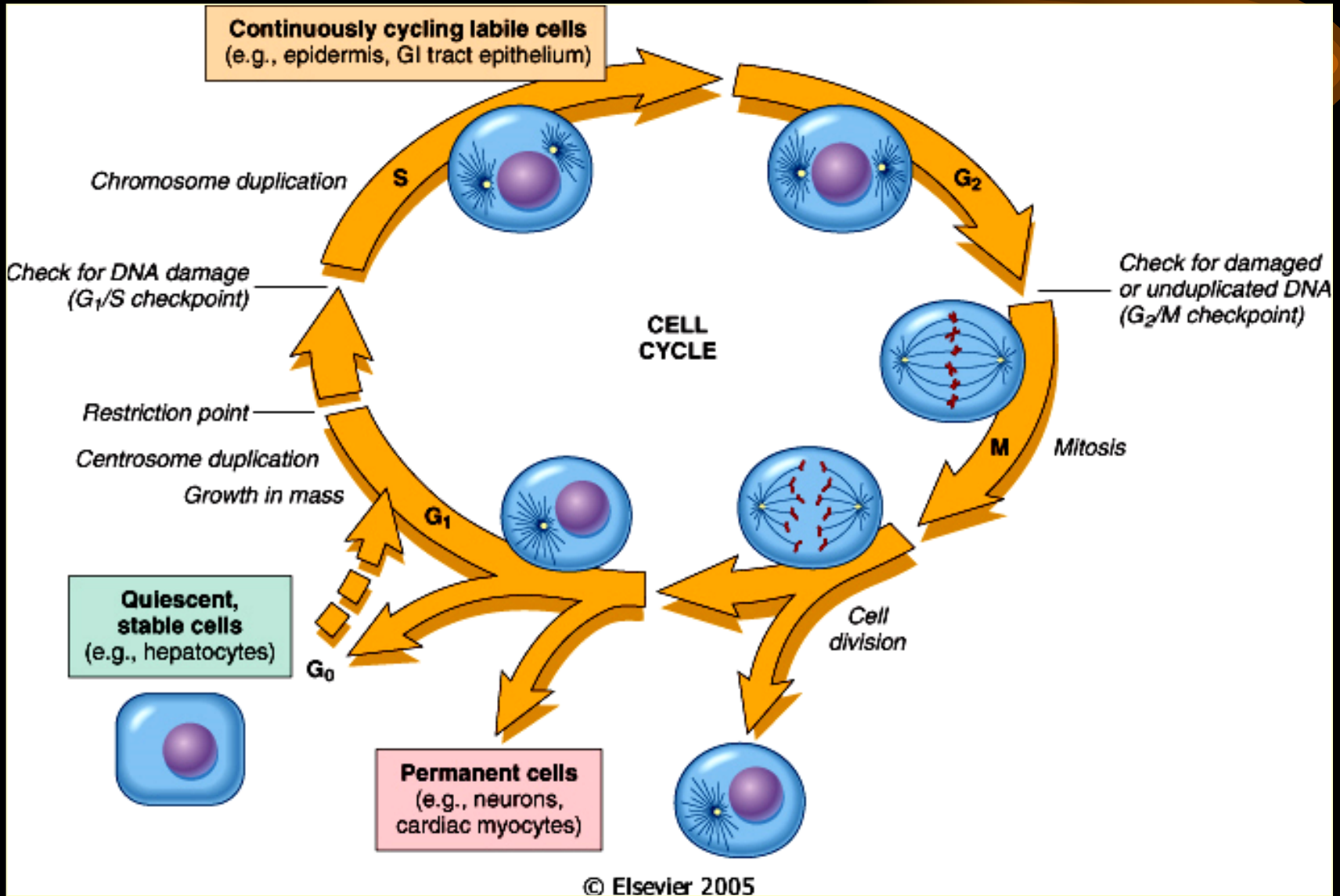
The Proliferative Potential of Different Cell Types

- Labile cells (continuously dividing & continuously dying)
  - Stem cells divide: self renewal and differentiation
  - Examples:
    - Skin epidermis
    - GIT epithelium
    - Bone marrow cells
- Stable cells (quiescent)
  - Examples:
    - Liver
    - Kidney,
    - Smooth muscles.
- Permanent (nondivivding),
  - Examples:
    - Cardiac muscle
    - Neurones

Cell Cycle Phases



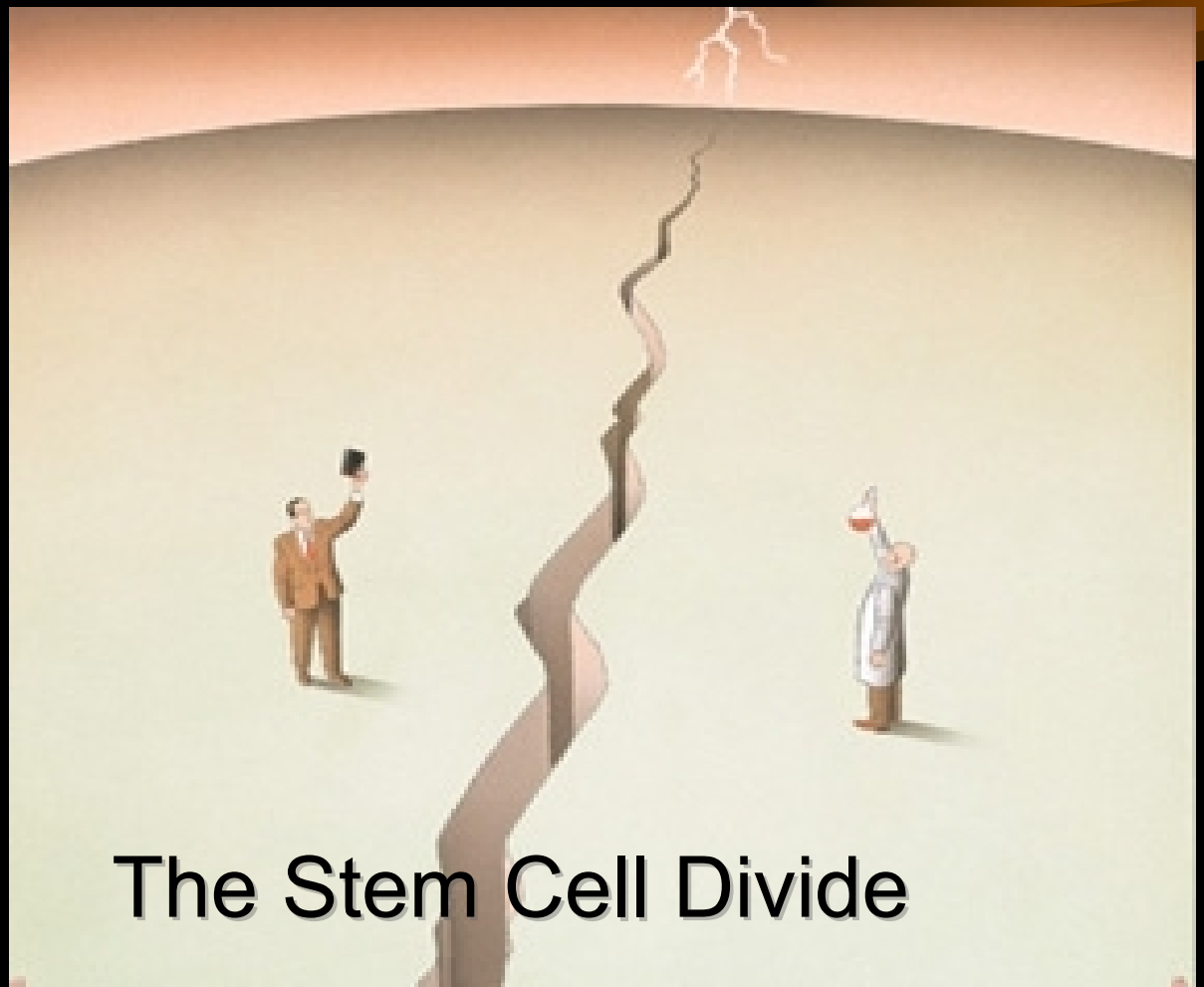
Cell-cycle Landmarks




SHAME
SHAME
SHAME
SHAME
START
Peace
START
Peace
with
OUR FAMILY VALUES
STEM CELLS WILL SAVE LIVES
BUSH
NEED

"the most promising research in health care, perhaps in the history of the world;" A U.S. senator

"morally unacceptable." the U.S. Conference of Bishops



Stem Cells

- Self renewal capacity
- Asymmetric replication
- Capacity to develop into multiple lineages
- Extensive proliferative potential

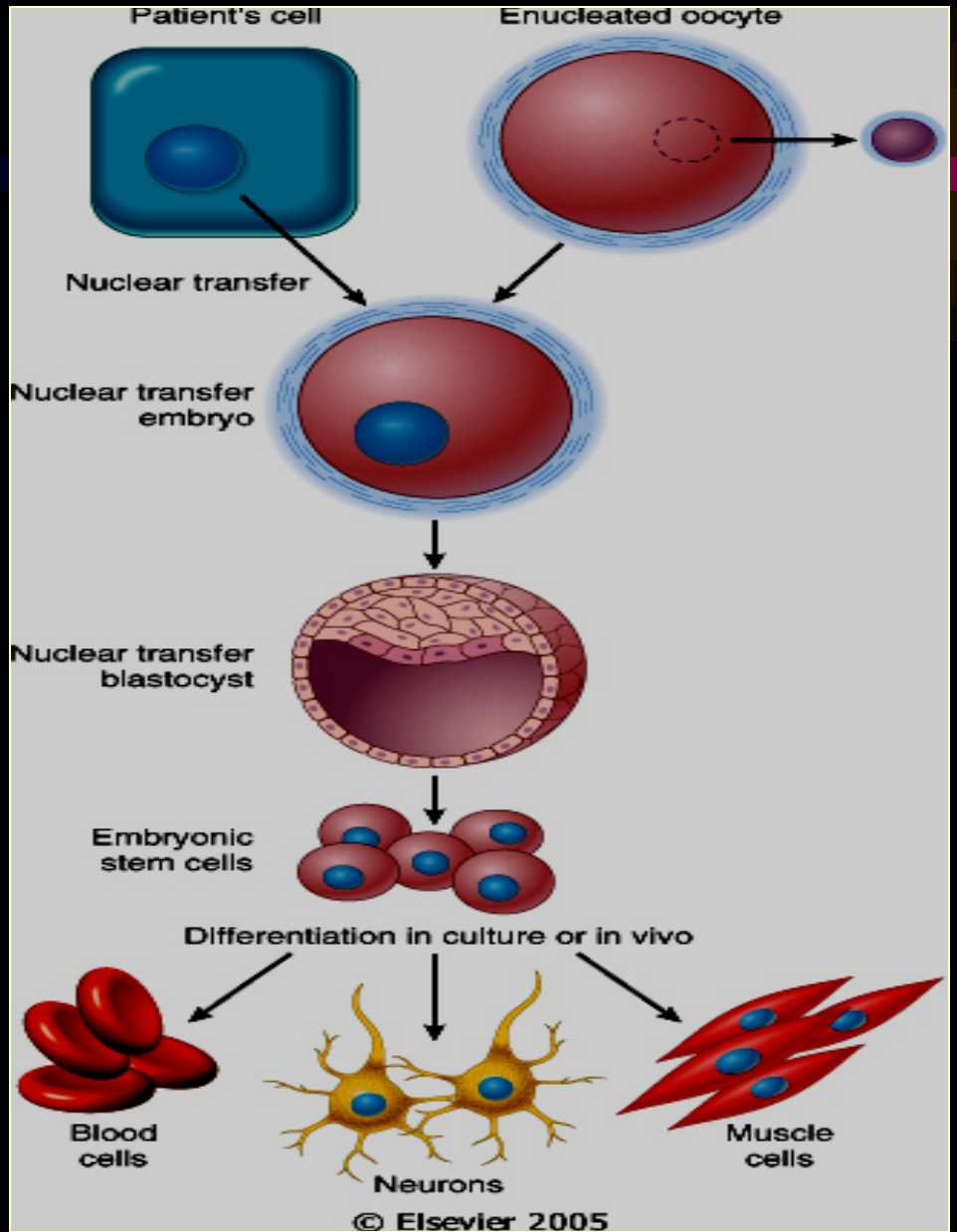
Embryonic stem cells: Pluripotent cells that can give rise to all tissues of the body

Adult stem cells: Restricted differentiation capacity (lineage specific)

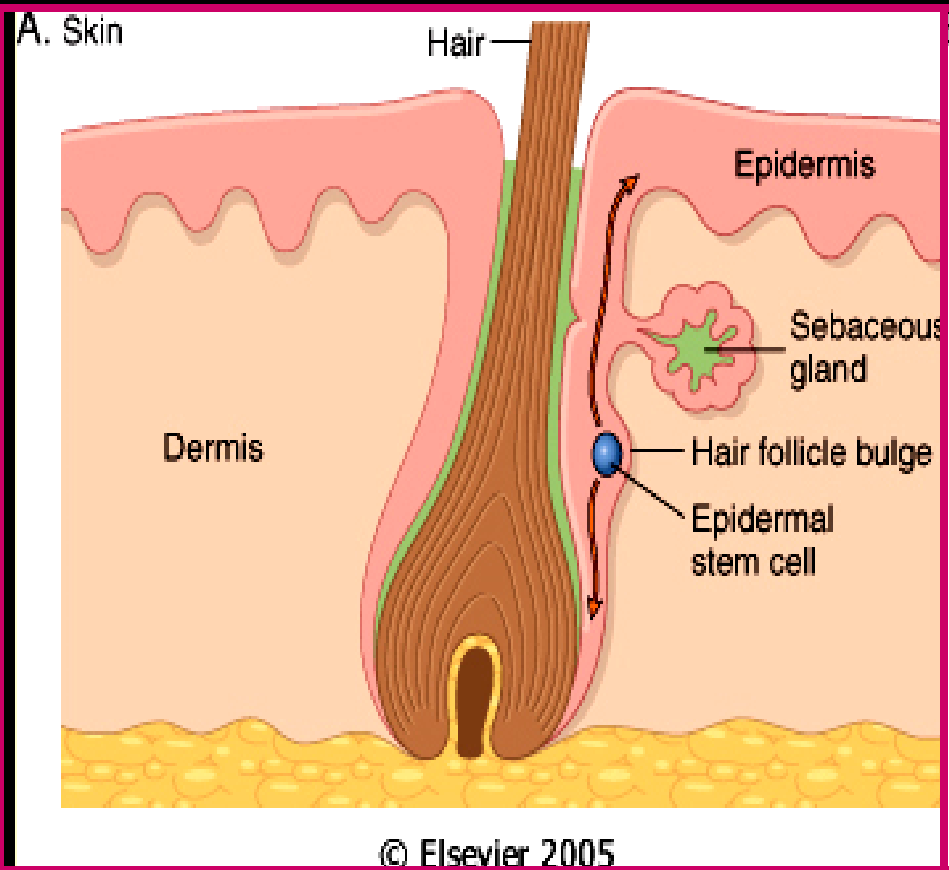
Impact of Embryonic Stem Cells on Medicine

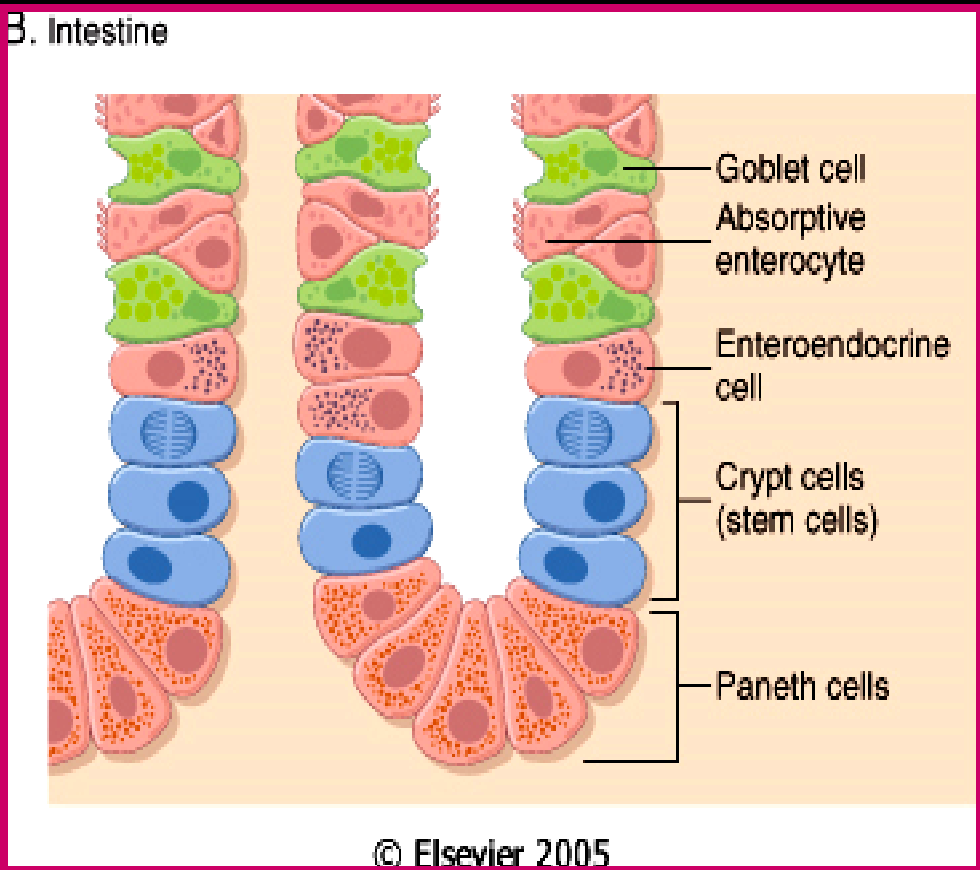
- Study of specific cell signaling and differentiation steps
- Production of knockout mice
- Potentially, generation of specific cell types to regenerate damaged tissue (therapeutic cloning)

Steps Involved in Therapeutic Cloning

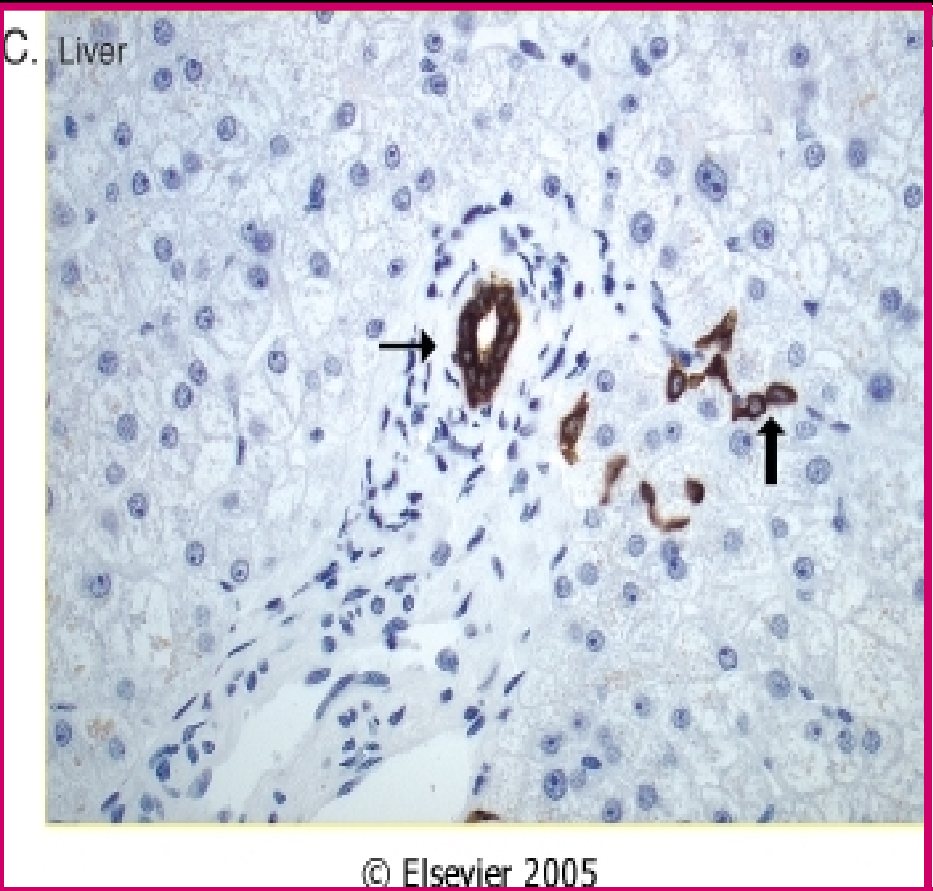


Examples of Adult Stem Cells Locations (1)





Examples of Adult Stem Cells Locations (2)



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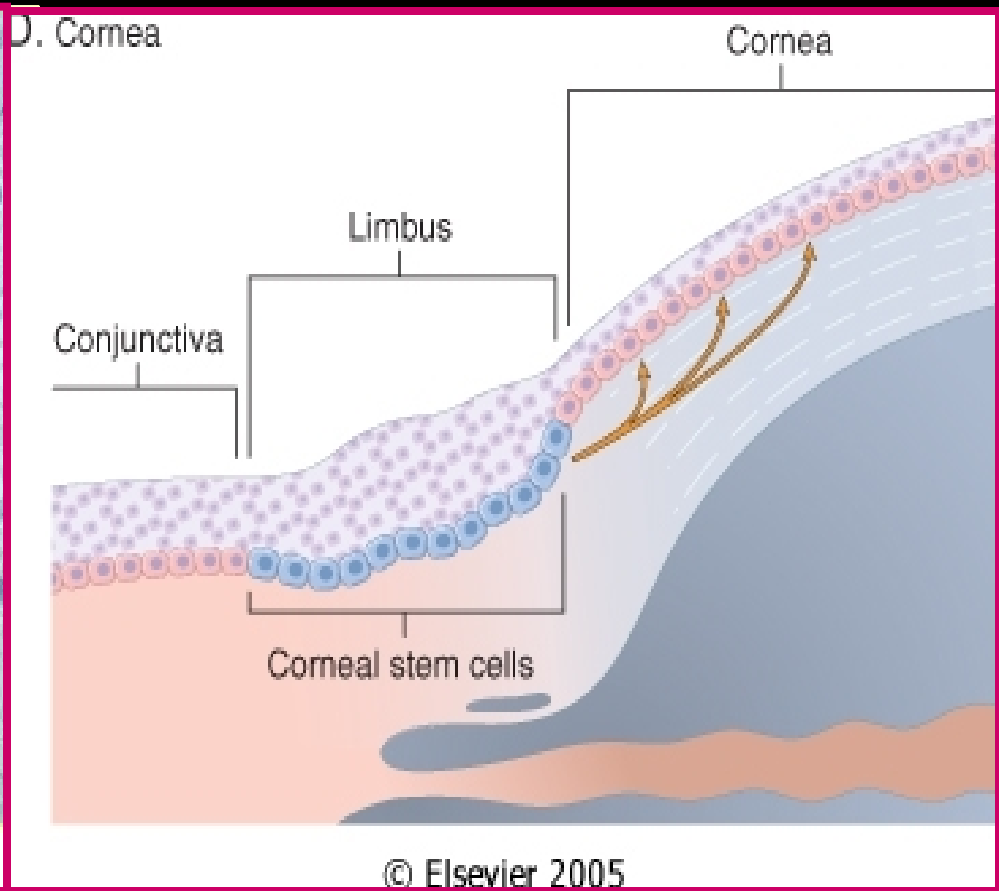

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Examples of Adult Stem Cells

• Bone marrow	Hemetopoietic stem cells
• Liver	Hering canal
• Skeletal muscle	Satellite cells
• Intestine	Base of crypts
• Skin	Hair follicle bulge

Cyclins

- Family of proteins that control entry of the cells at specific stages of cell cycle
- Level of a specific cyclin increases at a specific stage, then decreases rapidly after the cell departs that stage
- In order to accomplish their function, they have to bind to CDKs
- Different combinations are associated with each phase of the cell cycle
- They exert their function by phosphorylating certain proteins (kinase phosphorylate proteins)
- Examples:
  - Cyclin B-CDK1 activate G2 to M transition
  - Cyclin D-CDK4,6 activate G1 to S phase

Cyclins and Retinoblastoma Gene

- Hypophosphorylated RB, forms a complex with E2F transcription factor and DP1, blocking the effect of E2F.
- Blocking is mediated by histone deacetylase causing chromatin compaction.
- CyclinD/CDK4, and cyclinE/CDK2 phosphorylate RB.
- Phosphorylated RB dissociated from the complex, leading to activation of E2F.
- Target genes for E2F include: cyclin E, DNA polymerase, thymidine kinase, dihydrofolate reductase, and others.

CDK inhibitors

- Regulate cell cycle checkpoints (G1-S, & G2-M)
- Cip/Kip family: p21, p27 and p57
- INK4/ARF family: p16NK4A, p14ARF

P53 and the cell cycle

- If mutation occurs, TP53 is stabilized
- TP53 induce p21(CDKN1A) transcription
- P21 is a CDK inhibitor, thus arresting the cell at G1 until DNA is repaired
- If DNA damage cannot be repaired, TP53 induces apoptosis

Signals for Cell Growth and Differentiation

- Soluble polypeptide growth factors
- Insoluble elements of ECM interacting with integrins

Polypeptide Growth Factors

- Chemical mediators that affect cell growth by binding to specific receptors on the cell surface or intracellularly. They are the most important mediators affecting cell growth
- Present in serum or produced locally
- Exert pleiotropic effects; proliferation, cell migration, differentiation, tissue remodeling
- Regulate growth of cells by controlling expression of genes that regulate cell proliferation (protooncogenes)

Examples of Growth Factors (1)

- *EGF* (epidermal growth factor) & *TGF-*α
  - Binds to its receptor ERB B1
  - Mitogenic for epithelial cells & fibroblasts; migration of epithelial cells
- *PDGF* (platelet-derived growth factor)
  - Migration & proliferation of fibroblast, smooth muscle cell & monocyte; chemotactic

Examples of Growth Factors (2)

- *FGFs* (fibroblast growth factors)
  - Mitogenic for fibroblast & epithelial cells; angiogenesis; chemotactic for fibroblasts
  - Wound healing
- *VEGF* (vascular endothelial growth factor)
  - Angiogenesis
  - Increased vascular permeability
- HGF/scatter factor (hepatocyte growth factor)
  - Mitogenic to most epithelial cells including hepatocytes
  - Promotes scattering and migration of cells

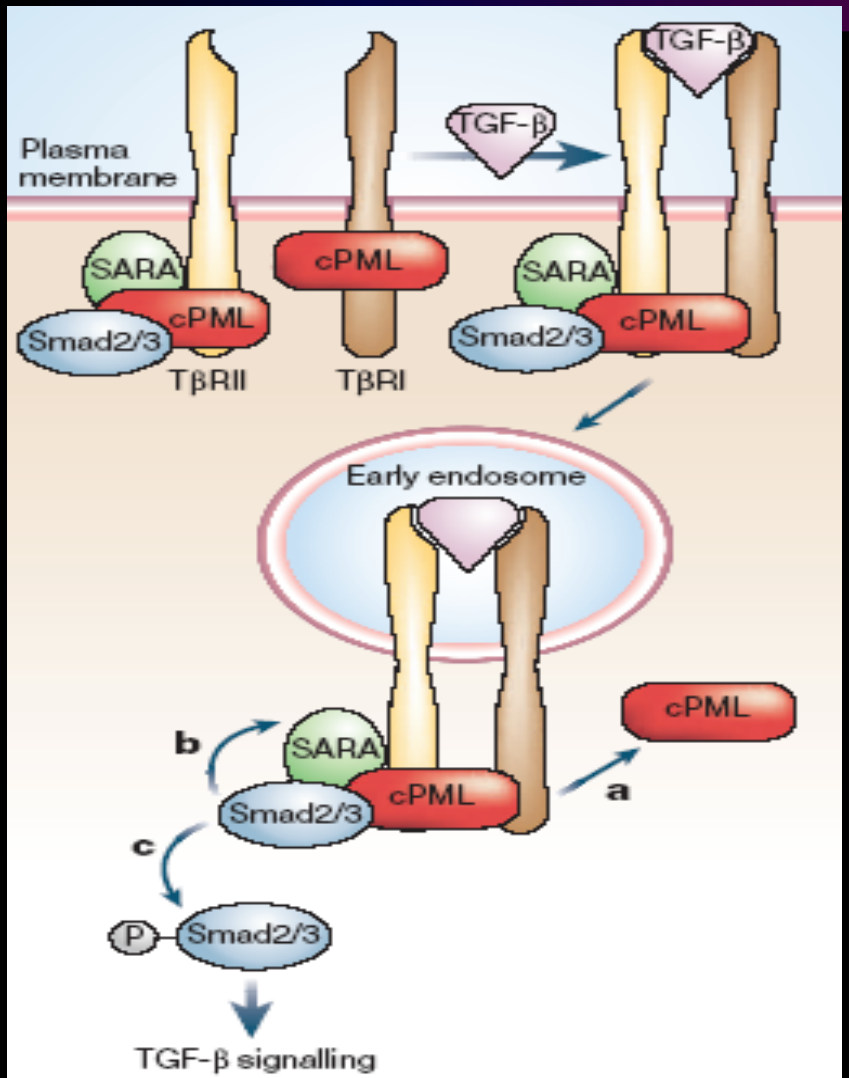
Transforming Growth Factor Beta (TGF-β):

- TGF-β binds to 2 receptors (types I &II) with serine/threonine kinase activity
- Rectors phosphorylates cytoplasmic transcription factors smads
- Smads enter the nucleus and associate with other DNA binding proteins activating or inhibiting gene transcription

Transforming Growth Factor Beta (TGF-β):

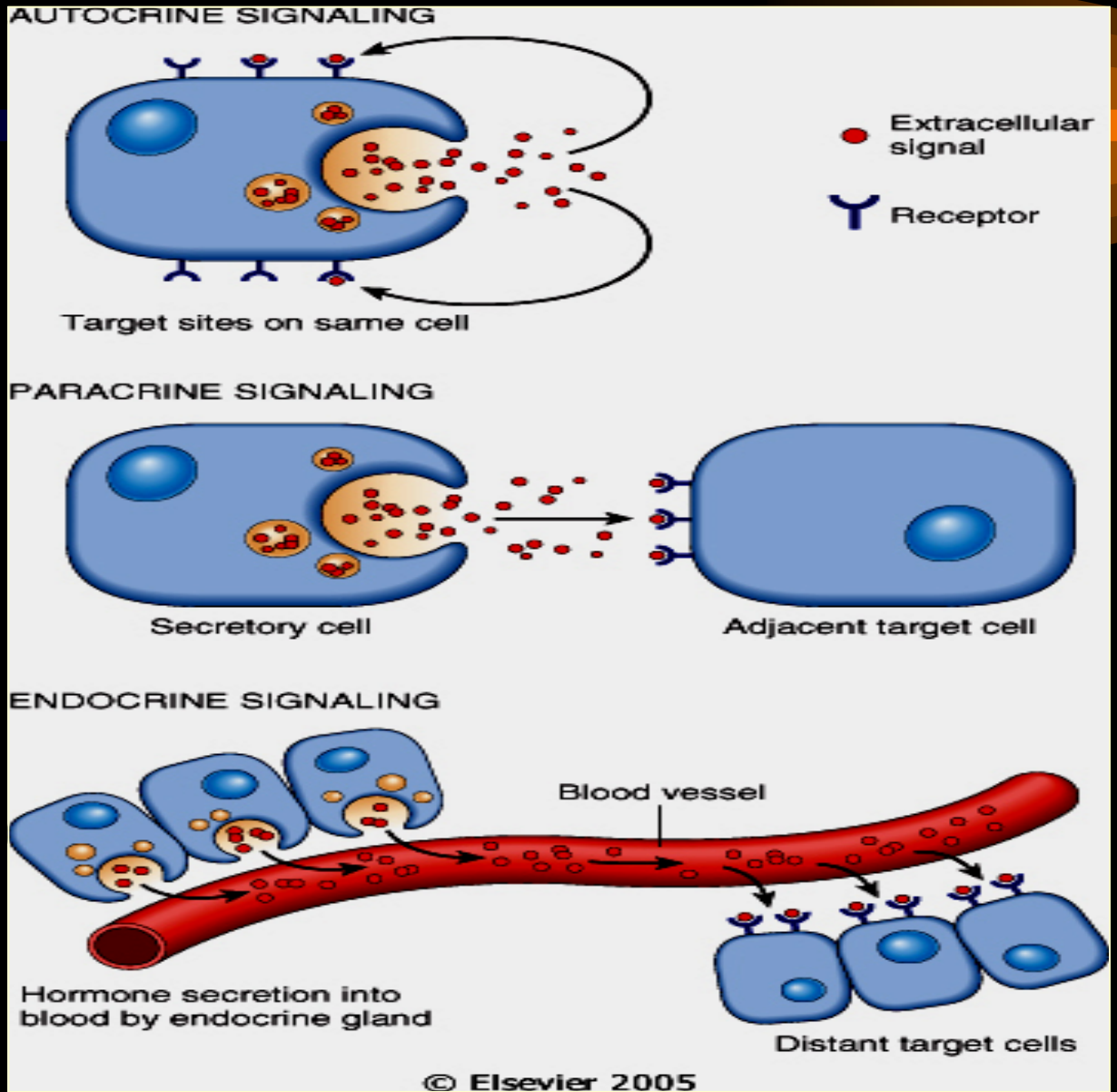
- Inhibitor of most epithelial cells and leukocytes. Increases expression of cell cycle inhibitors (Cip/Kip, INK4/ARF)
- Stimulates proliferation of fibroblasts & smooth muscles
- Stimulates fibrosis (fibroblasts chemotaxis, production of ECM, ↓ proteases, ↑ protease inhibitors)
- Strong anti-inflammatory effect

TGF-β Signaling



The role for the cytoplasmic form of promyelocytic leukaemia protein (cPML).At the cell surface, cPML might interact with the two TGF- receptors (TRI and TRII) and act as a bridging factor between SARA and Smad2/3. Upon stimulation with TGF-, cPML promotes the transfer of the complex containing TRI, TRII, SARA and Smad2 into early endosomes. There, cPML might dissociate from the complex (a), allowing Smad2/3 to interact with SARA (b) and to be phosphorylated (c) by TRI. Phosphorylated Smad2/3 moves into the nucleus to propagate TGF- signalling.

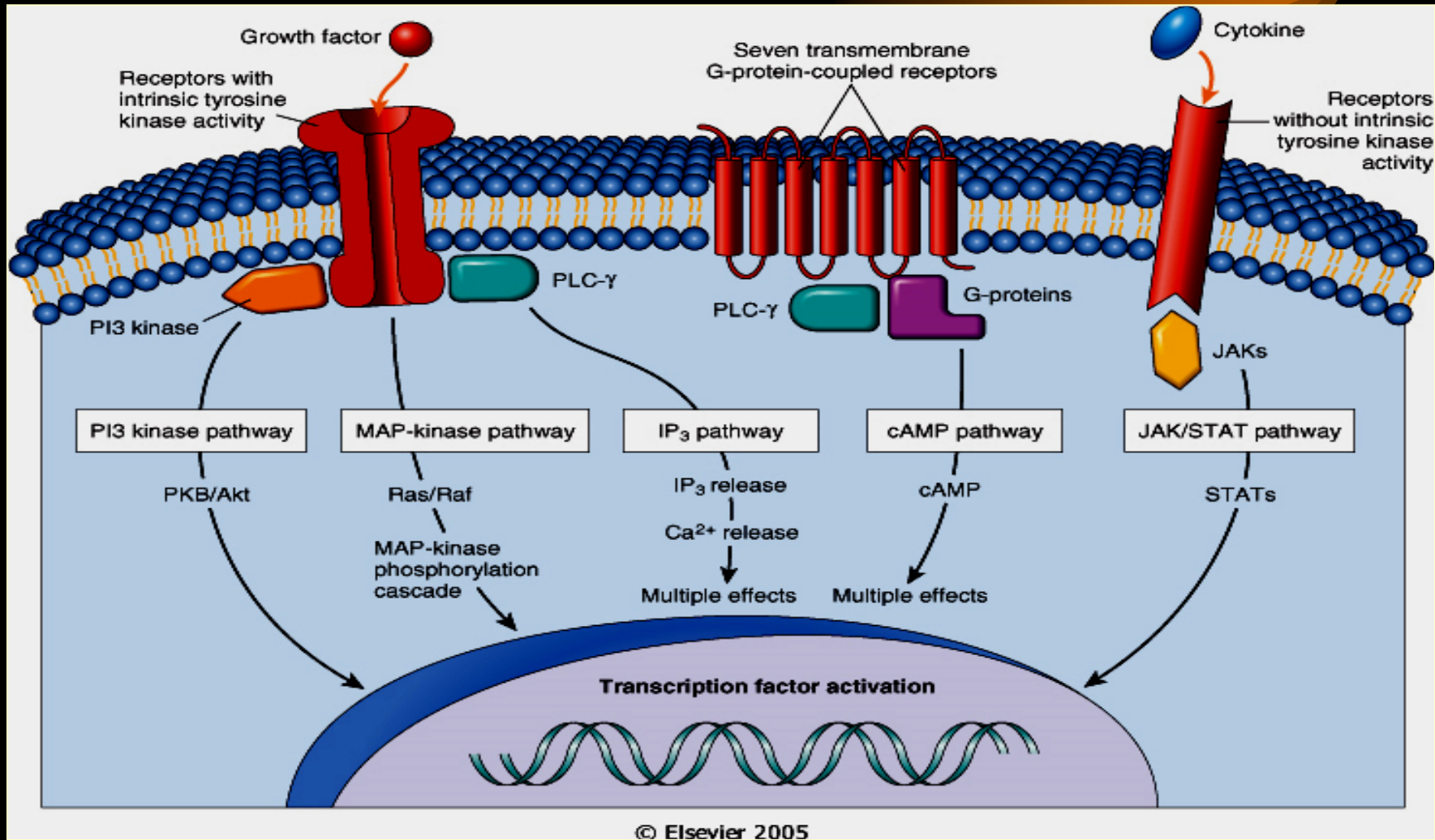
Patterns of Intercellular Signaling



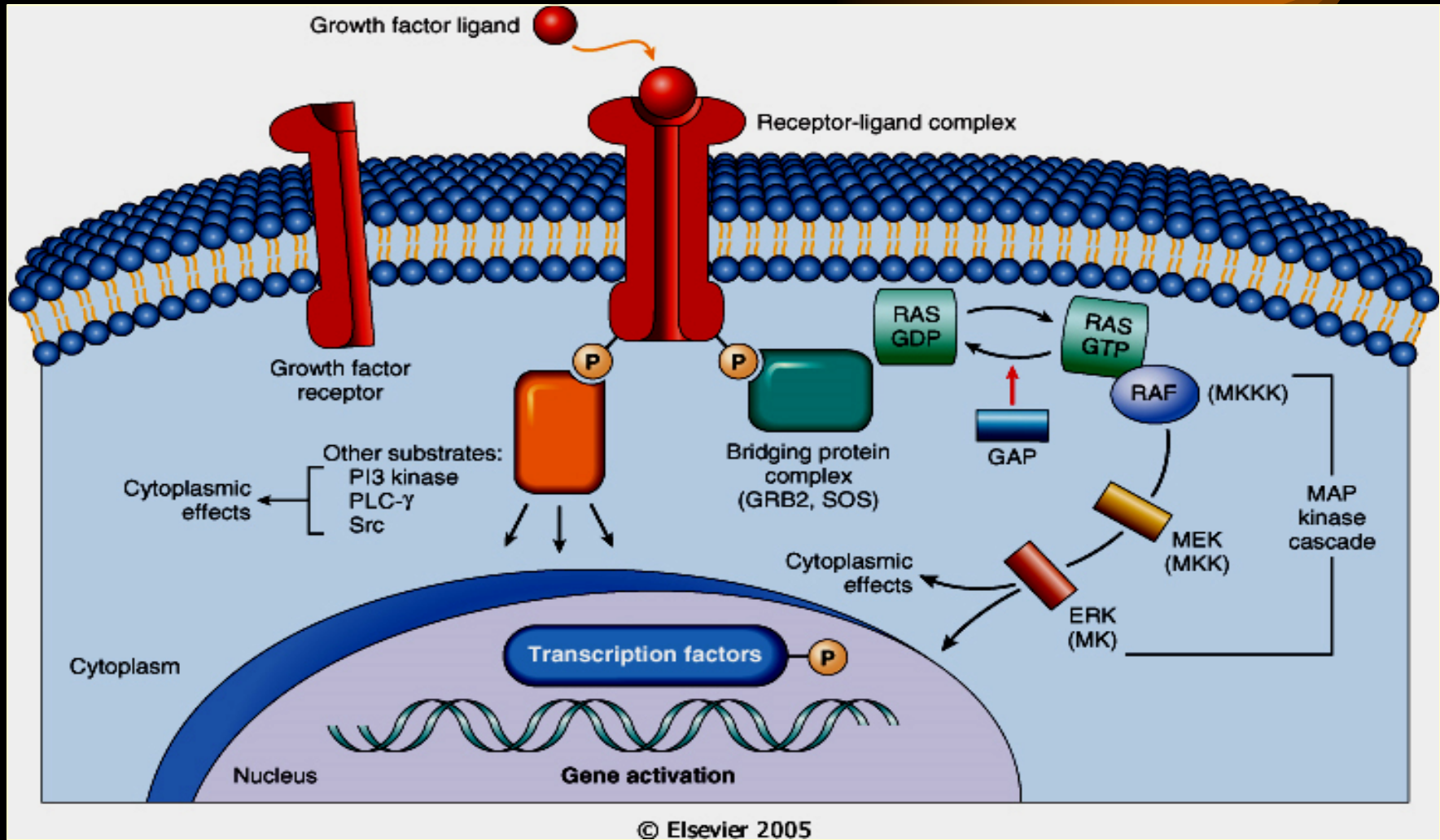
Receptors for Growth Factors

- Receptors with intrinsic tyrosine kinase activity
- Receptors lacking intrinsic tyrosine kinase activity that recruit kinases
- Seven transmembrane G-protein coupled receptors
- Steroid hormone receptors

Examples of Signal Transduction Systems



Signals from Tyrosinase Kinase Receptors



Extracellular Matrix

A major component of all tissues, provides the backbone & support. It regulates growth, movement and differentiation of cells.

- Basement membrane:
  - Type IV collagen
  - Adhesive glycoproteins
  - Laminin
- Interstitial matrix:
  - Fibrillary and nonfibrillar collagens
  - Elastin
  - Proteoglycans
  - Fibronectin

Major Components of the ECM

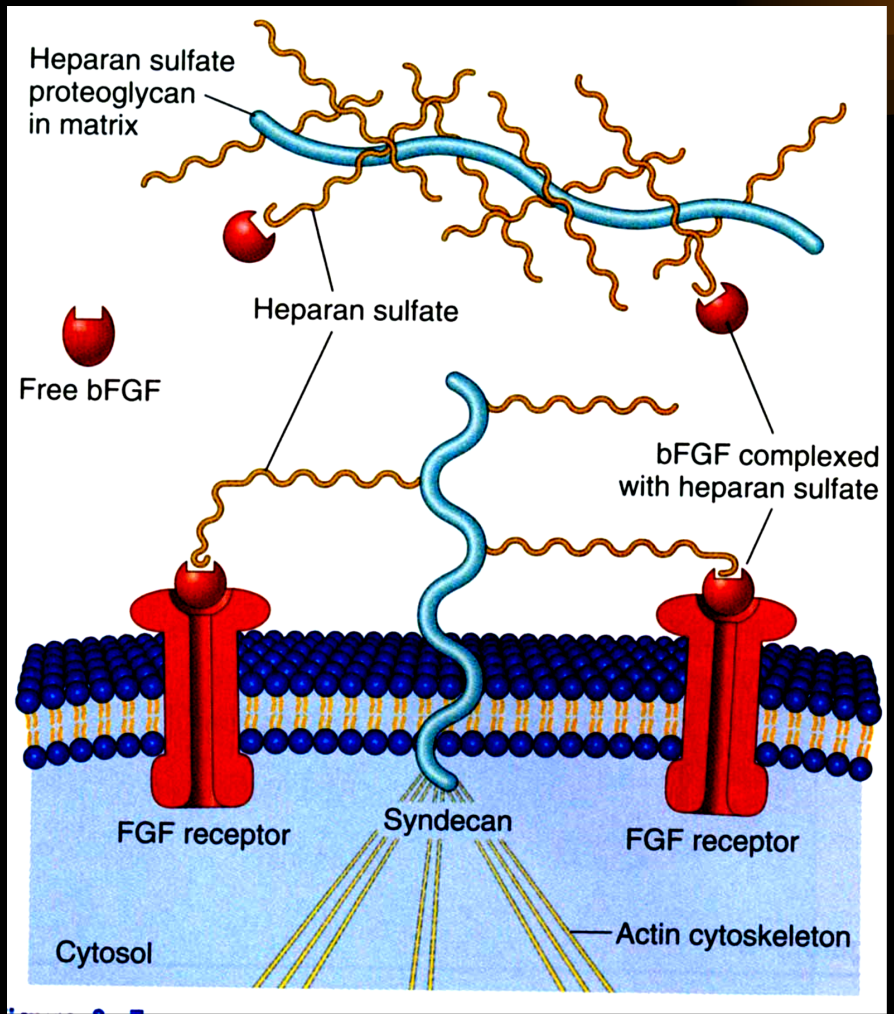
Components of the Extracellular Matrix (1)

- Collagen
  - The most common protein in animals
  - Fibrillar & nonfibrillar
  - Hydroxylation, mediated by vit C, provides strength
  - Fibrillar collagens form most of CT in wounds & scars
  - Non-fibrillar (type IV) main component of BM
- Elastin
  - Provides elasticity
  - Surrounded by a meshlike network of fibrillin which supports elastin deposition
  - Defective fibrillin leads to Marfan syndrome

Components of the Extracellular Matrix (2)

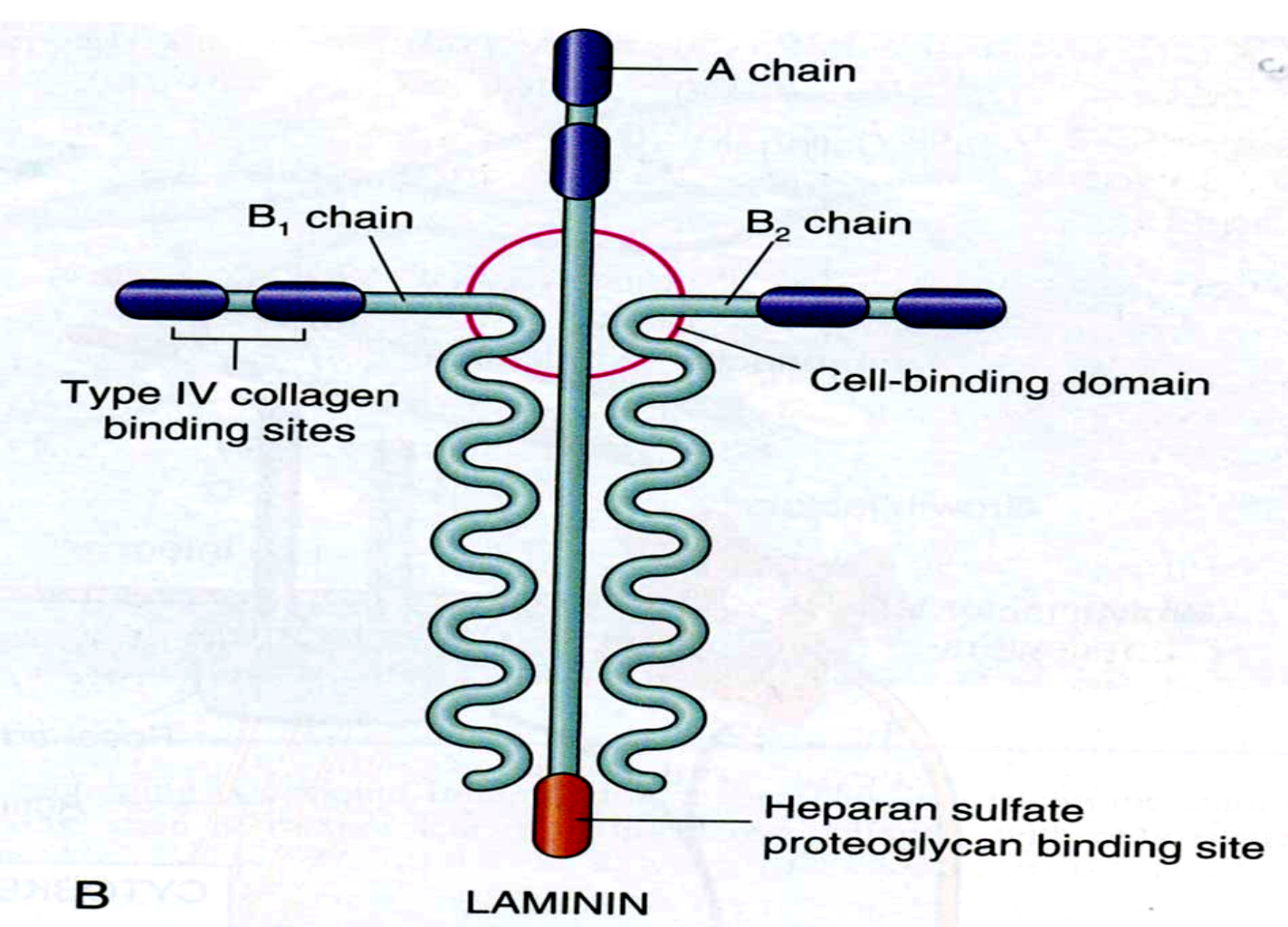
- Proteoglycans
  - Form highly hydrated gel like material
  - Protein core with many attached long polysaccharides (glycosaminoglycans)
  - Act as a reservoir for bFGF
  - Integral cell membrane proteins (e.g. syndecan)
- Adhesive glycoproteins
  - Fibronectin
    - Domains bind collagen, elastin, proteoglycans, etc.
    - Bind to integrins via RGD domains
  - Laminin
    - Connects cells to collagen and heparan sulfate

Proteoglycan



Fibronectin

Laminin


A chain
B1 chain
B2 chain
Type IV collagen binding sites
Cell-binding domain
Heparan sulfate proteoglycan binding site
B
LAMININ

Cadherins (Calcium Dependent Adherence Proteins)

- Homotypic interactions between cells
- Involved in 2 types of junctions:
  - Zonula adherens (apical)
  - Desmosomes
- β-catenin links cadherins with a catenin, which connects them with actin and cytoskeleton
- Regulate cell motility, proliferation, differentiation, and contact inhibition
- Free β-catenin regulates nuclear transcription factors through Wnt signaling pathway
- Abnormalities of the β-catenin pathway is involved in GI carcinomas

Interaction Between GF, ECM and Cells

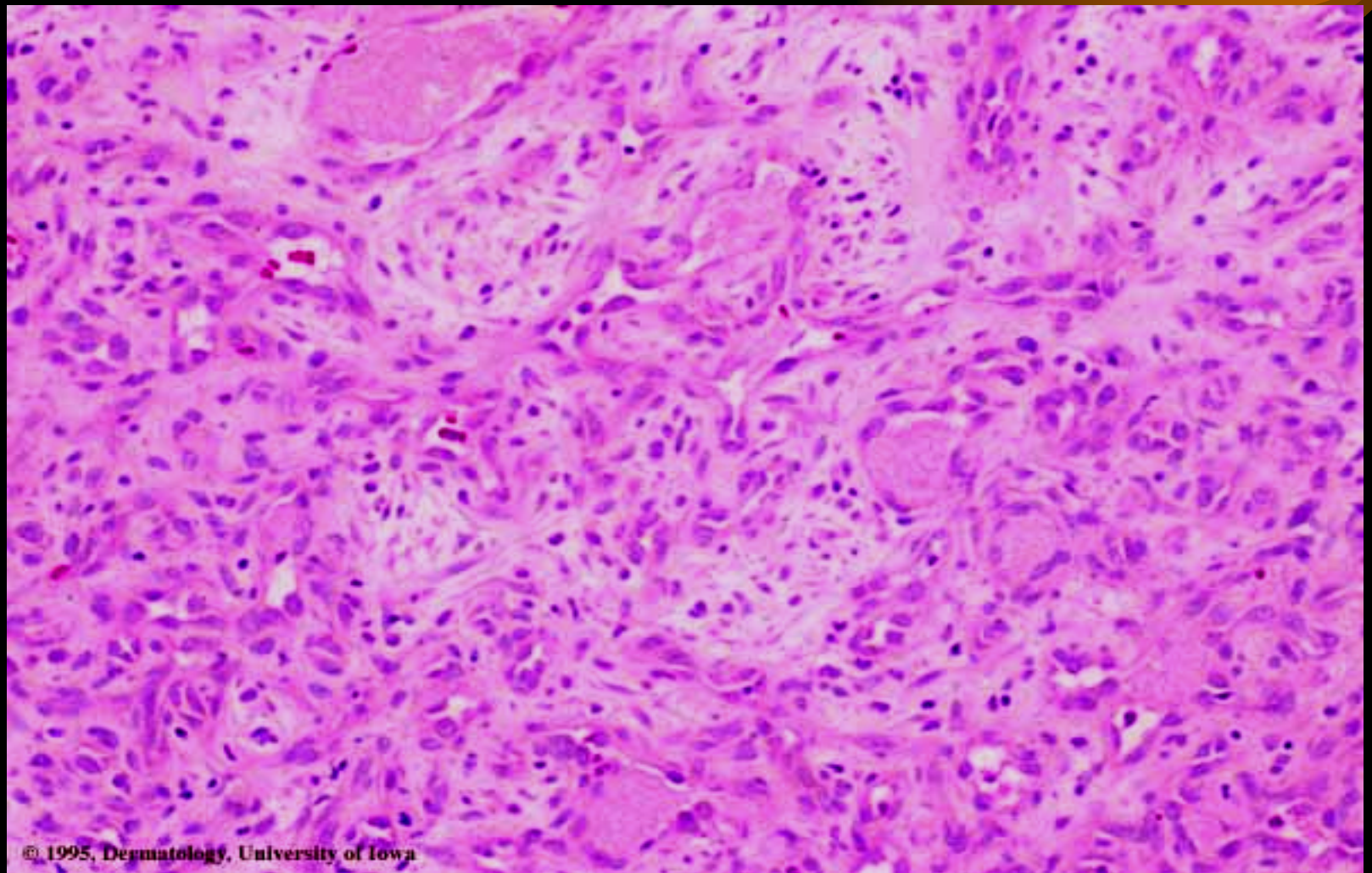
Repair by Regeneration

- Replacing injured tissue by same type of original tissue cells.
- Labile & stable cells
- Involves two tissue components:
  - Cellular proliferation, regulated by growth factors & growth inhibitors.
  - Extracellular matrix (ECM) & cell-matrix interaction
- An intact basement membrane directs epithelial cell polarity & is essential for its orderly regeneration

Repair by Connective Tissue

- Severe injury with damage to parencymal cells and stroma precludes parenchymal regeneration
- Repair occurs by CT
- Components of CT repair:
  - Neovascularization (angiogenesis)
  - Proliferation of fibroblasts
  - Deposition of ECM
  - Remodeling

Granulation Tissue



Angiogenesis

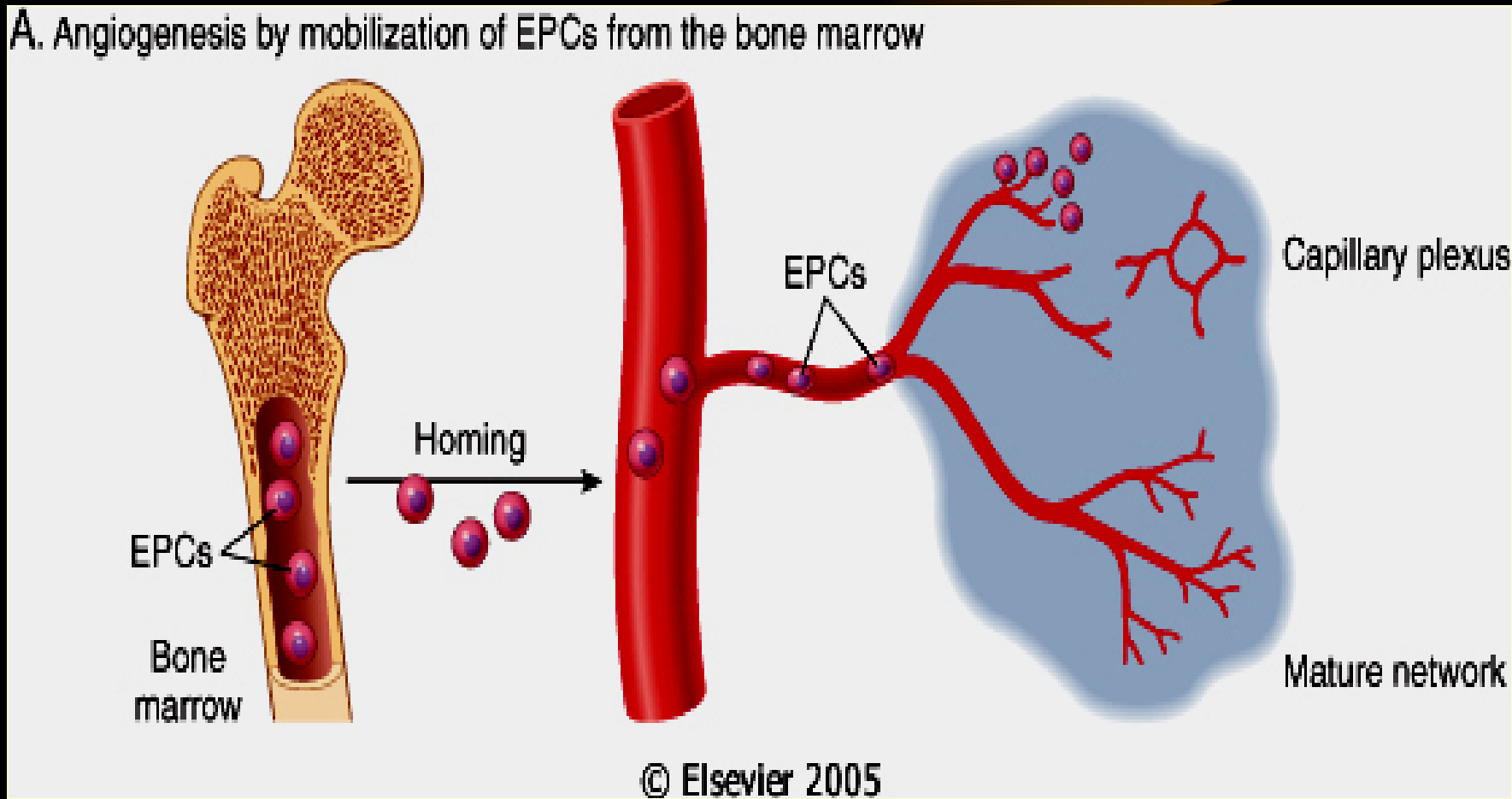
- From Endothelial precursor cells
- From pre-existing vessels

VEGF effects on endothelial cells :

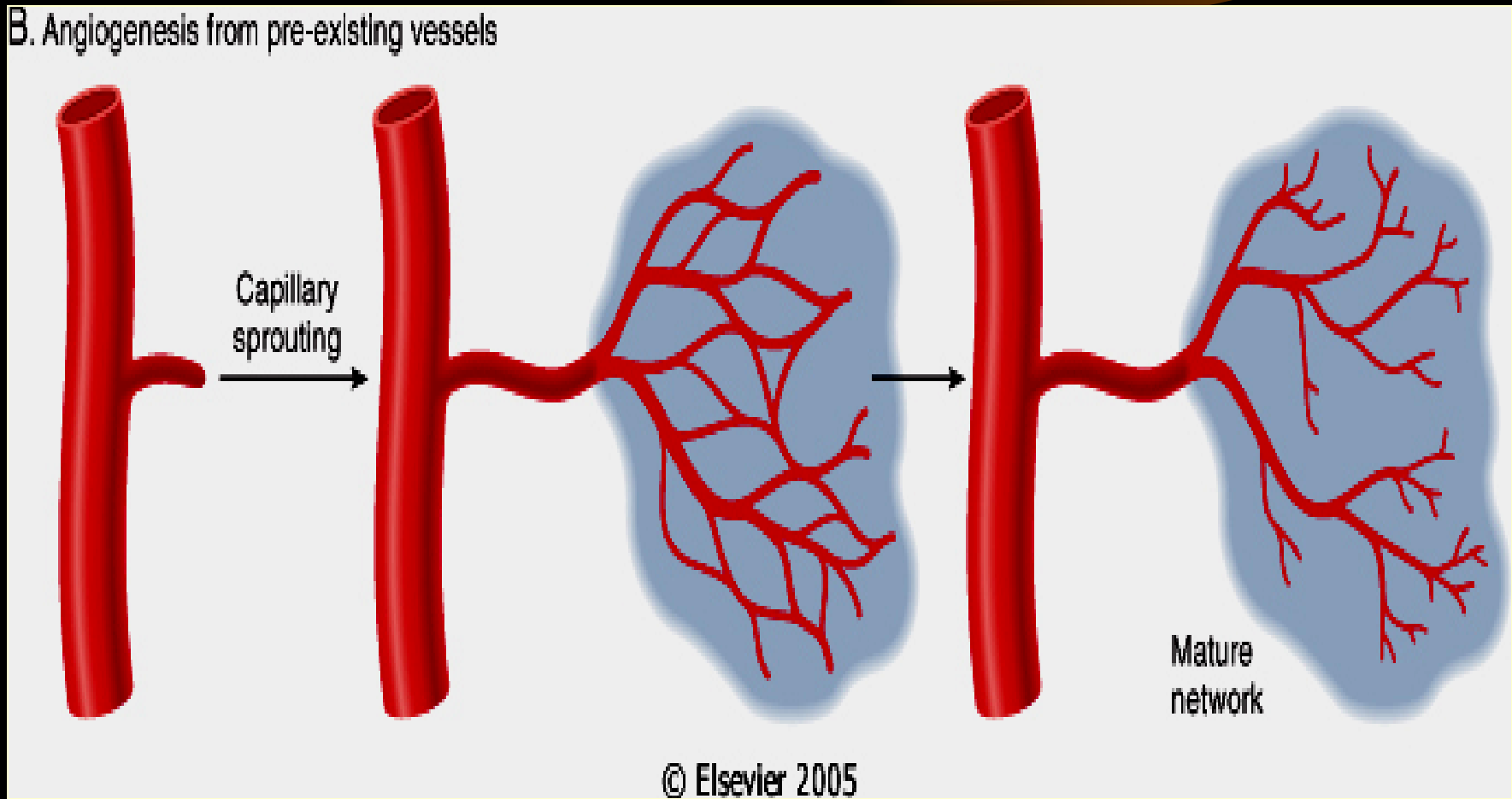
- ↑ migration
- ↑ proliferation
- ↑ Differentiation
- ↑ permeability

Angioipoietins 1 and 2, PDGF, and TGF-β stabilize the newly formed vessels.

Angiogenesis from Endothelial Precursor Cells



Angiogenesis from Pre-existing Vessels



Angiogenesis from Pre-existing Vessels

- A parent vessel sends out capillary sprouts to produce new vessels
- Steps involned:
  - Degradation of the parent vessel BM
  - Migration of endothelial cells (EC)
  - Proliferation of endothelial cells
  - Maturation of EC and organization into capillary tubes
- Growth factors involved:
  - Basic fibroblast growth factor (βFGF)
  - Vascular endothelial growth factor (VEGF)

Angiogenesis

Angiogenesis from Endothelial Precursor Cells (EPCs)

- Hemangioblast → Hematopoietic stem cells and angioblasts (EPCs)
- EPCs are stored in bone marrow
- EPCs express markers of hematopoietic stem cells and of endothelial cells
- EPCs play a role in neovascularization, replacement of endothelial cells, re-endothelialization of vascular implants.

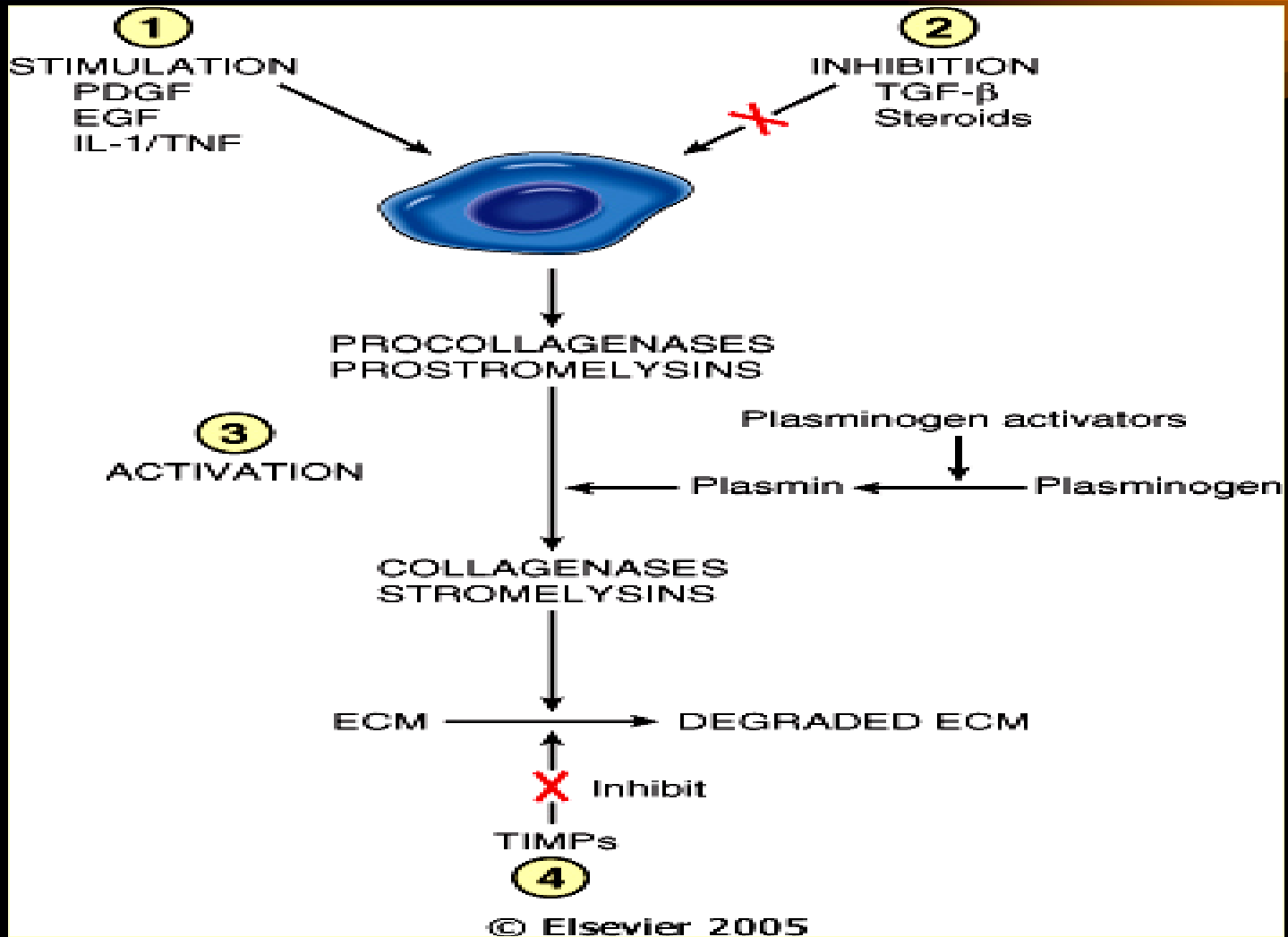
Fibrosis

- Emigration and proliferation of fibroblasts
  - Growth factors: PDGF, FGF, EGF, TGF-β
- Deposition of ECM
  - Growth factors: PDGF, FGF, TGF-β and cytokines (IL-1 &TNF)

Scar Remodeling

- Shift and change of the composition of the ECM of the scar as a result of synthesis and degradation
- Metalloproteinases: Enzymes produced by many cells and capable of degrading different ECM constituents
  - Interstitial collagenases
  - Gelatinases
  - Stromelysins
- Metalloproteinases (Zn dependent) activated by HOCl or proteases (plasmin). Inactivated by tissue inhibitors of metalloproteinases (TIMP) and steroids.

Matrix Metalloproteinase Regulation

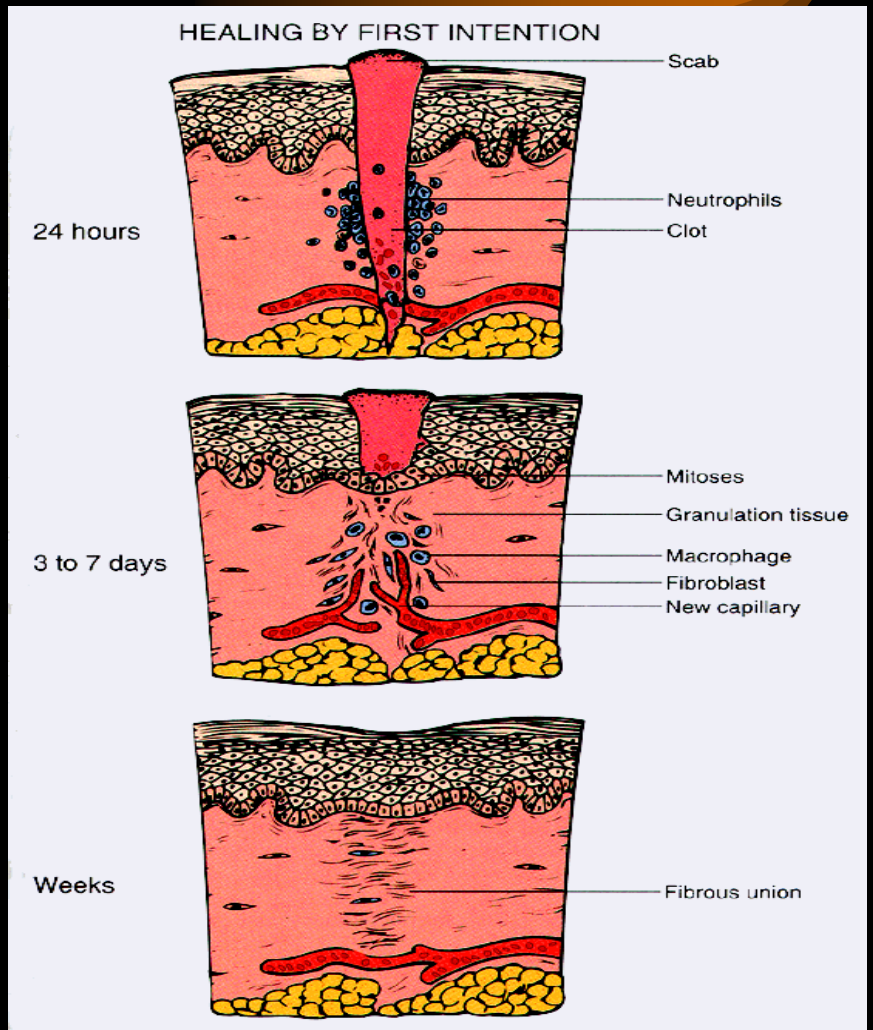


Wound Healing

- Fibrin clot formation → filling the gap
- Induction of acute inflammatory response by an initial injury
  - Neutrophils (1st 24 h), Monocytes by 3rd day
- Parenchymal cell regeneration
- Migration and proliferation of parenchymal and connective tissue cells and granulation tissue
- Synthesis of ECM proteins
- Remodeling of parenchymal elements to restore tissue function
- Remodeling of connective tissue to achieve wound strength

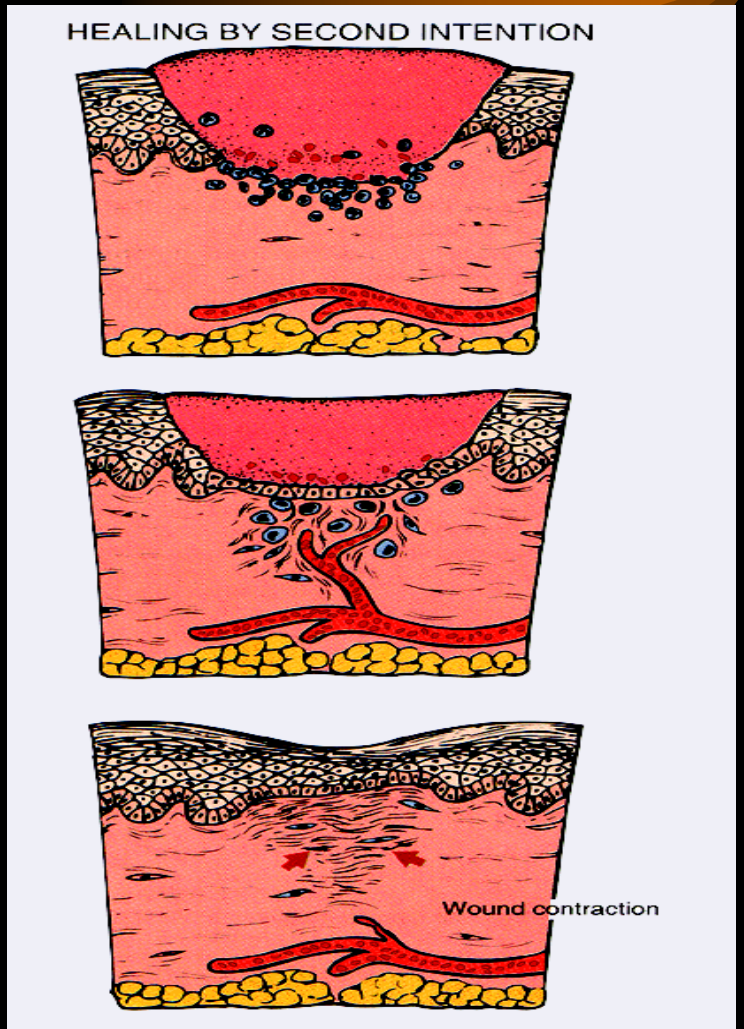
Healing by First Intention

Focal Disruption of Basement Membrane and loss of only a few epithelial cells
e.g. Surgical Incision

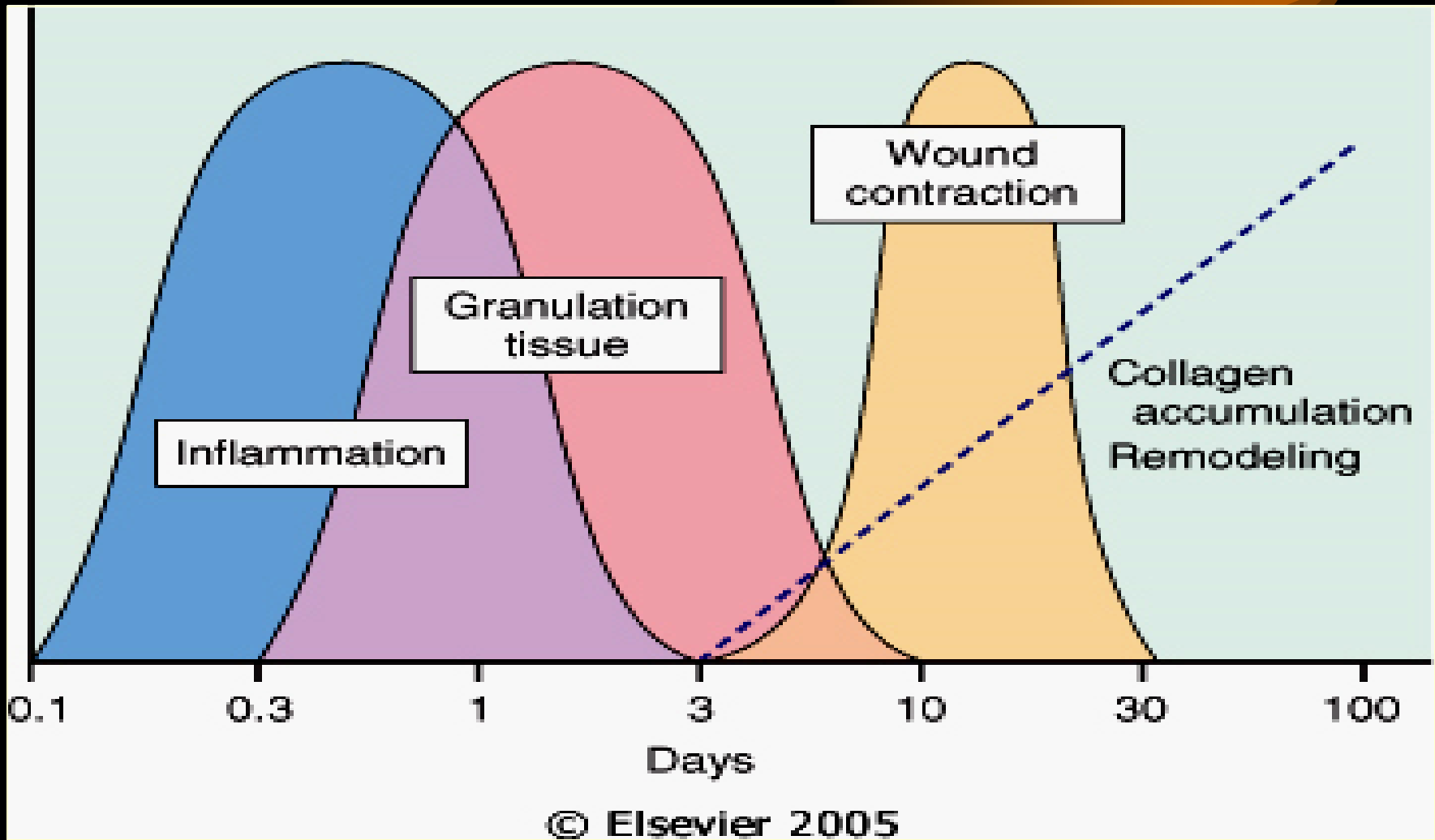


Healing by Second Intention

- Larger injury, abscess, infarction Results in much larger Scar and then CONTRACTION



Phases of Wound Healing



Wound Strength

- Sutured wounds have 70% of the strength of unwounded skin
- After sutures are removed at one week, wound strength is only 10% of unwounded skin
- By 3-4 months, wound strength is about 80% of unwounded skin

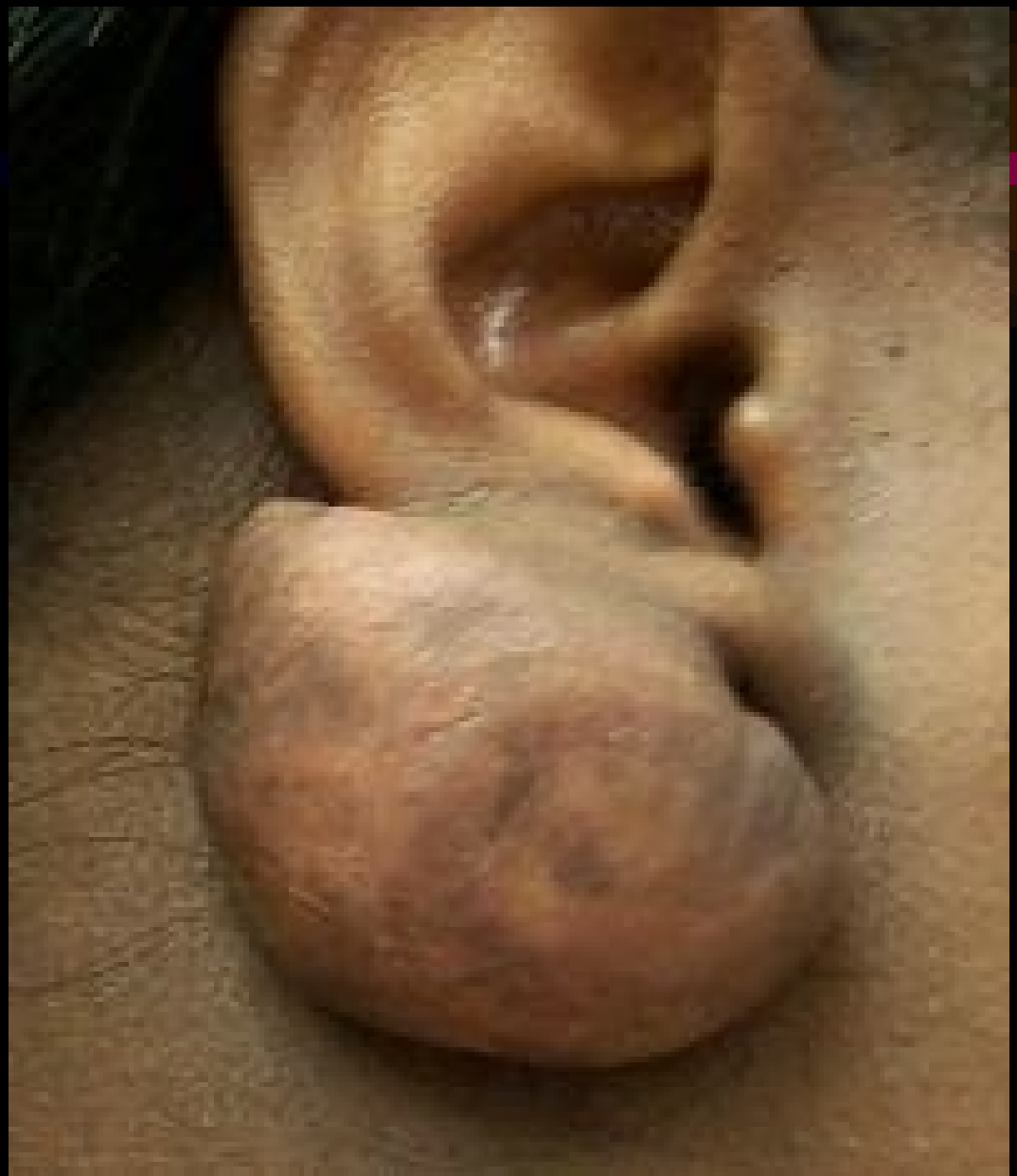
Factors affecting Healing:

- SYSTEMIC
  - Nutritional
    - Protein deficiency
    - Vitamin C deficiency
    - Zinc deficiency
  - Systemic diseases
    - Diabetes mellitus
    - Arteriosclerosis
    - Renal failure
    - Infections (systemic)
  - Corticosteroid treatment
  - Age
  - Immune status
- LOCAL
  - Infection
  - Poor blood supply
  - Type of tissue
  - Presence of foreign body material
  - Ionizing irradiation
  - Mechanical factors
    - Excessive movement
    - Hematoma
    - Apposition

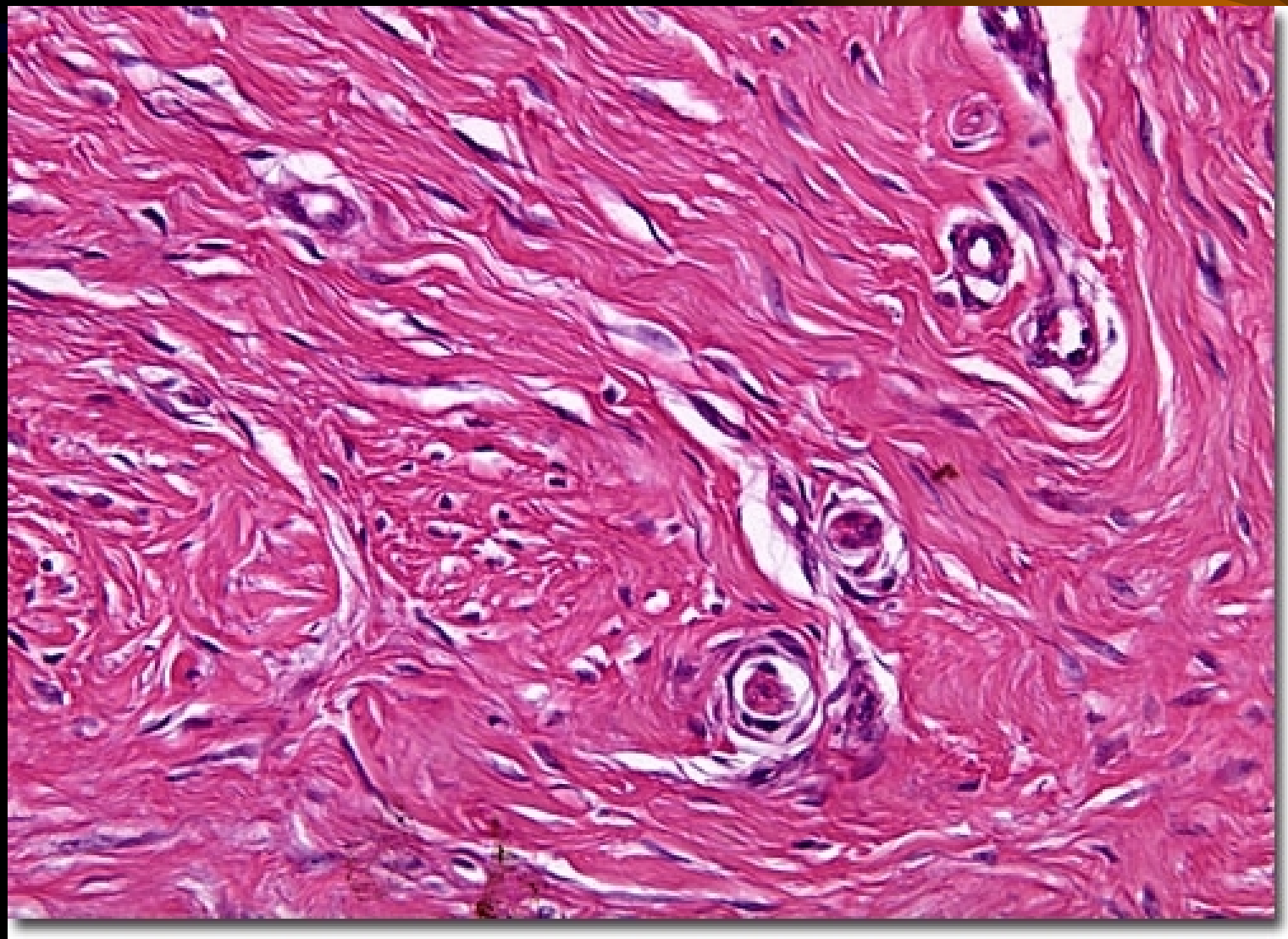
Pathologic Aspects of Repair

- Aberrations of growth may occur
  - Exuberant granulation:
    - Excessive amount of granulation tissue during wound healing
  - Keloid:
    - Excessive collagen accumulation during wound healing resulting in raised tumorous scar
  - Excessive fibrosis:
    - Cirrhosis, pulmonary fibrosis, rheumatoid arthritis (RA)
- Tissue damage
  - Collagen destruction by collagenases in RA

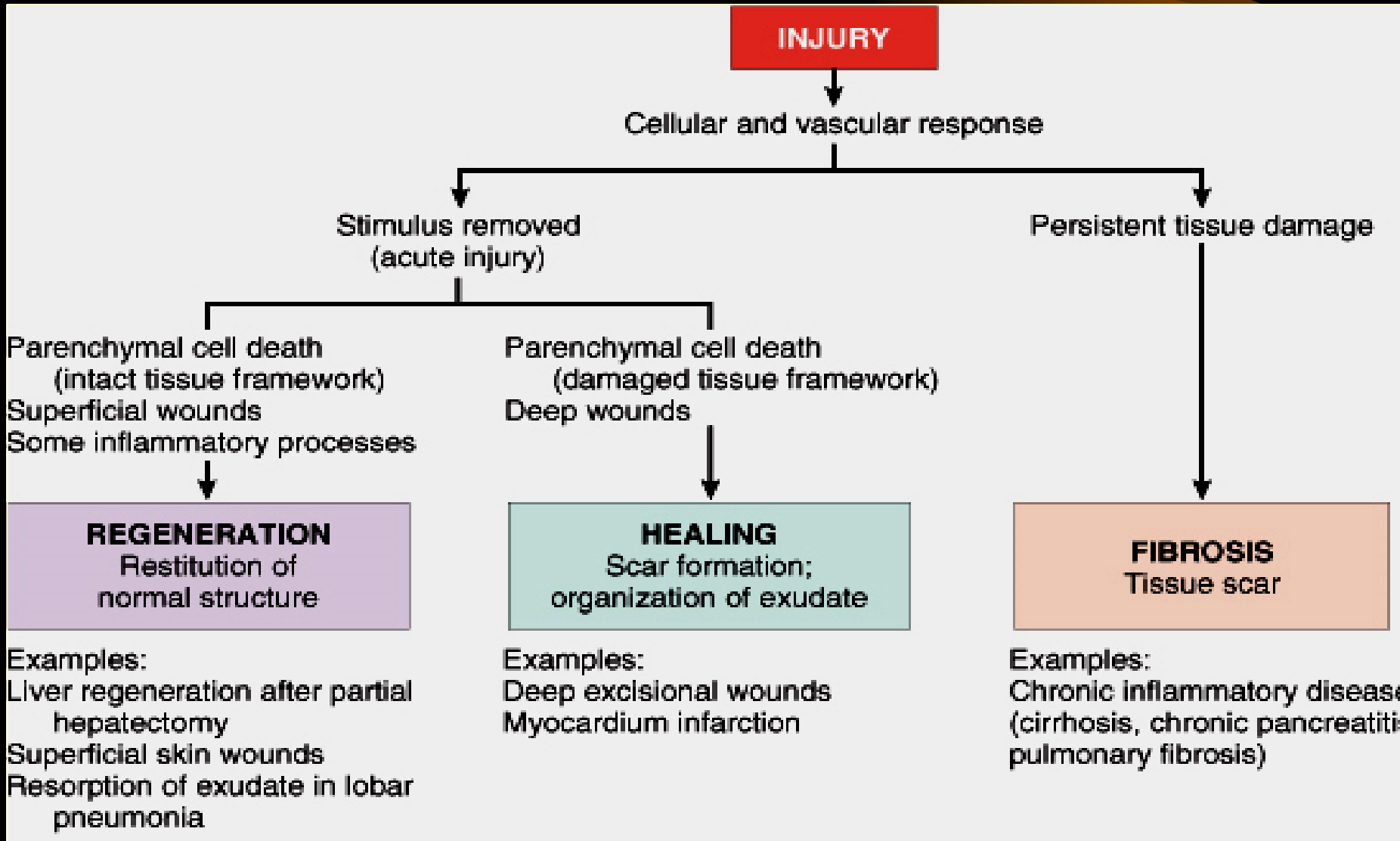
Keloid



Keloid



Repair Outcomes After Injury




THANK
YOU!