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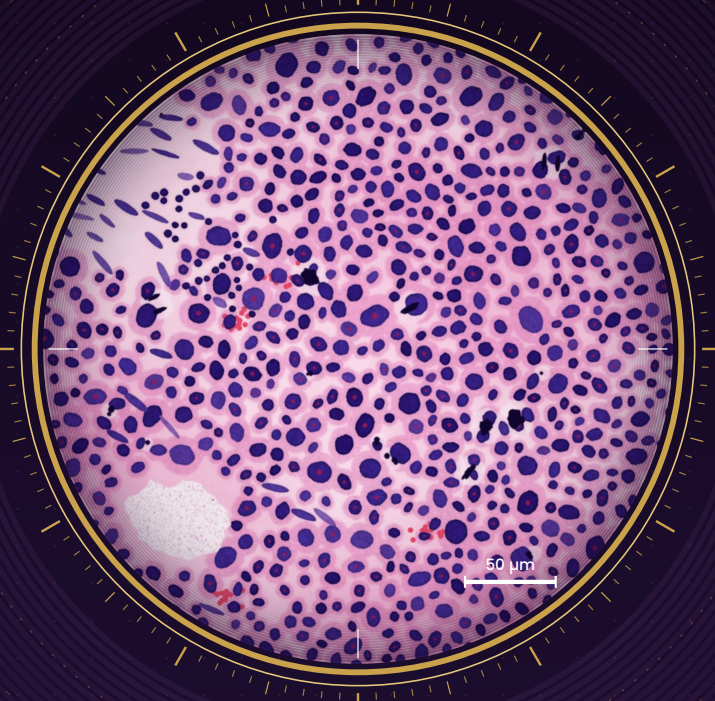

Neoplastic cell sheets with mitoses, apoptosis and necrosis · H&E ×400 · illustrative

DNB Pathology

Solved Previous-Year Questions

VOLUME I

General Pathology, Immunology, Neoplasia, Infection and the Heart

Part 1 · Cell Biology, Cell Injury and Cell Death
Part 2 · Inflammation, Repair and Haemodynamic Disorders
Part 3 · Genetics, Epigenetics and Non-coding RNA
Part 4 · Immunopathology
Part 5 · Neoplasia
Part 6 · Infectious, Environmental and Nutritional Diseases
Part 7 · Blood Vessels and Heart

COMPILED BY

Dr Akshay Gupta
DNB Pathology

Dr Sameer Maheshwari
DNB Pathology

Based on Robbins & Cotran 11e, Rosai and Ackerman 11e, Blaustein 7e, Orell and Sterrett 5e, Dacie and Lewis 12e, Kawthalkar and the current WHO classifications and guidelines

UPDATED TILL OCTOBER 2026

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Answers are written from the reference books — Robbins & Cotran 11e, Rosai and Ackerman 11e, Blaustein 7e, Orell and Sterrett 5e, Diagnostic Cytology 3e, the Bethesda Systems for thyroid and cervical cytology, Dacie and Lewis 12e and Kawthalkar — with the book and printed pages named in the SOURCE box of each answer, and from the current WHO classifications, reporting systems and Indian/international guidance found on official sites, listed in the Web-Sourced Facts Register.

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HOW TO USE

Repeats and near-repeats of a question are answered once; "Asked in" lists every paper and year, and the badge shows how often it was asked (1×, 2×, 3×, 4×+). Each answer covers exactly what the grouped versions asked, at the length the marks allow. The SOURCE box at the start names the book(s) and printed pages, and any current classification or guideline used (W#). Book figures and tables are reproduced from the books with their page. Green boxes are exam pearls; blue boxes name the diagram worth drawing. A lists the parts; B this volume question by question; C the high-yield topics (asked 3× or more).

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PART 1

Cell Biology, Cell Injury and Cell Death

1. Telomeres, telomerase, telomeropathies, cellular ageing & senescence

ASKED 4×+ · 5-mark answer

Asked in: Paper I · Apr 2023 · Q6(b) · Paper I · Dec 2020 · Q6(a) · Paper I · Dec 2021 · Q2(a) · Paper I · Dec 2025 · Q2(a)

Q: Telomeropathies. [5]
Q: Telomerase and its defects. [5]
Q: Cellular ageing and telomerase. [5]
Q: Cellular senescence mechanism. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Metastatic Calcification; §Dysregulated Nutrient Sensing — pp. 60–62
- Robbins — ch. 7 Neoplasia — §Limitless Replicative Potential: Stem Cell–Like Properties of Cancer Cells — p. 271

Telomeres and telomerase

- **Telomeres** are short repeated DNA sequences (TTAGGG) at the ends of linear chromosomes. They ensure complete replication of chromosome ends and protect the ends from fusion and degradation.
- With each somatic cell division a small section of the telomere is not duplicated, so telomeres shorten progressively.
- **Telomerase** is a specialised RNA-protein complex. It uses its own RNA as a template to add nucleotides to chromosome ends and so maintains telomere length.
- Telomerase expression differs by cell type:
  - Germ cells express it, and only germ cells have enough to stabilise telomere length completely.
  - Stem cells have low levels.
  - Most somatic tissues lack it.
  - Cancer cells usually reactivate it.

Mechanism of cellular senescence

- Normal cells have a limited replicative capacity. After a fixed number of divisions (60–70 for most human cells) they enter a terminally non-dividing state, **replicative senescence**.
- Cells from children can divide more often than cells from older people.
- Two mechanisms underlie senescence:
  - **Telomere attrition:**
  - Shortened telomeres leave chromosome ends unprotected.
  - The ends are sensed as broken DNA, which signals cell cycle arrest.
  - Somatic cells therefore exit the cycle and cannot replace damaged cells.
  - **Activation of tumour suppressor genes:**
  - The **CDKN2A** locus encodes p16 (INK4a).
  - p16 expression correlates with chronological age in virtually all human and mouse tissues examined.
  - p16 controls G1 → S progression and pushes cells along the senescence pathway.
- Senescence is associated with upregulation of p53 and INK4a/p16. These keep RB hypophosphorylated, which favours cell cycle arrest.

Flowchart 1: Telomere-driven senescence

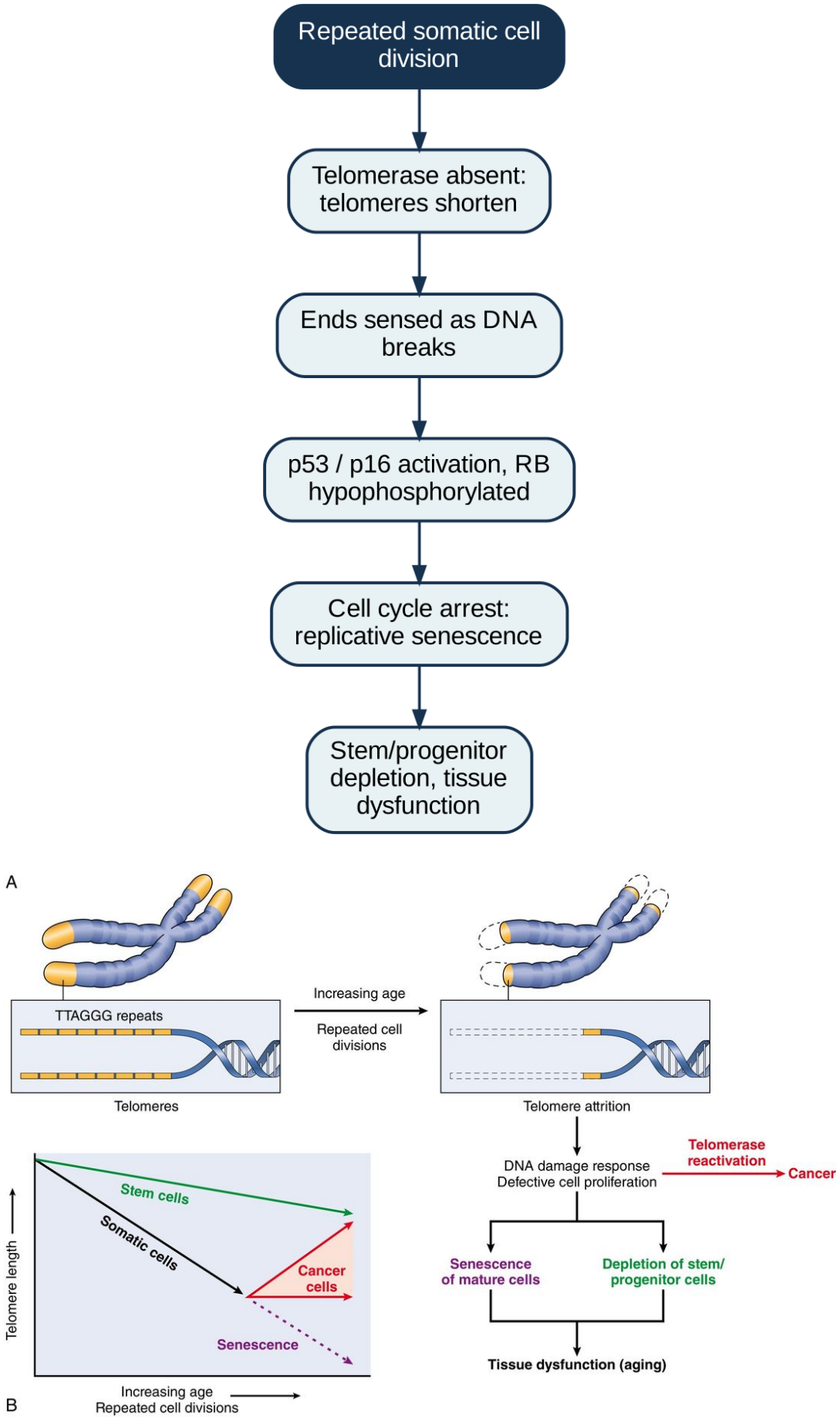


Fig. 2.36 *Role of telomeres and telomerase in replicative senescence of cells. (A) Mechanisms and conse-* — *Robbins 11e, p. 62*

Telomerase defects and telomeropathies

- Telomere shortening in tissues is linked to premature development of disease. The pages name **pulmonary fibrosis** and **aplastic anaemia** as examples.
- In mouse models, genetically engineered mice with shortened telomeres have reduced life spans. Telomerase activation restores normal life span, which establishes the causal link.
- Consequences of excessive attrition are depletion of the stem/progenitor pool, a DNA damage response, defective proliferation and tissue dysfunction.
- Telomerase **loss** therefore gives premature senescence and tissue failure. Telomerase **reactivation** gives immortality and cancer.

Telomere crisis and telomerase in cancer

- Cells that evade senescence (for example through loss of RB checkpoint control) are not immortal. They enter **mitotic crisis** and die.
- The sequence of events in crisis:
  - Completely eroded telomeres are sensed as double-strand breaks.
  - With functional p53, cells arrest or undergo apoptosis.
  - With dysfunctional p53, the non-homologous end-joining (NHEJ) pathway joins chromosome ends and forms dicentric chromosomes.
  - These chromosomes break at anaphase, producing "breakage-fusion-bridge" cycles that end in mitotic catastrophe.
- Cells in crisis that reactivate telomerase restore their telomeres and survive. They may carry oncogene and tumour suppressor damage and are at high risk of malignant transformation.
- Telomere maintenance is seen in virtually all cancers:
  - In **85–95%** it is due to telomerase expression.
  - The rest use **alternative lengthening of telomeres (ALT)**, which depends on DNA recombination.
- Tissue stem cells and germ cells express telomerase, which makes them resistant to mitotic crisis. They also have the capacity for self-renewal.

Other mechanisms of cellular ageing

- Cellular ageing is regulated by evolutionarily conserved genes. It results from accumulated cellular and molecular damage together with reduced replicative capacity.
- **DNA damage:**
  - Damage accumulates as repair fails.
  - The average haematopoietic stem cell acquires 14 new mutations per year.
  - Werner syndrome (DNA helicase defect), Bloom syndrome and ataxia-telangiectasia show premature ageing from defective DNA repair.
- **Defective protein homeostasis:**
  - Chaperone function and autophagy/proteasomal degradation are impaired.
  - Misfolded proteins accumulate and may trigger apoptosis.
- **Dysregulated nutrient sensing:**
  - Caloric restriction increases longevity by reducing insulin/IGF-1 signalling and increasing sirtuins.
  - IGF-1 acts through mTOR, and rapamycin (an mTOR inhibitor) extends lifespan in animals.
  - Sirtuins are NAD-dependent deacetylases. Sirtuin-6 activates DNA repair enzymes.

DRAW IN THE EXAM

Telomere length versus number of cell divisions for somatic cells, stem cells, germ cells and cancer cells, ending in senescence or telomerase reactivation (Fig. 2.36).

PEARLS

- Telomerase is an RNA-protein complex that adds TTAGGG repeats. It is present in germ cells, low in stem cells, absent in most somatic cells, and reactivated in 85–95% of cancers.
- Replicative senescence has two arms: telomere attrition and p16/INK4a (CDKN2A) activation. The p53/RB pathway enforces the arrest.
- Short telomeres are associated with pulmonary fibrosis and aplastic anaemia.
- Loss of p53 lets cells enter crisis, with NHEJ fusion and breakage-fusion-bridge cycles. Survivors that reactivate telomerase are at high risk of cancer.
- ALT is the telomerase-independent route to telomere maintenance in the remaining tumours.
- Werner syndrome is a DNA helicase defect. Caloric restriction acts through reduced IGF-1 and increased sirtuins.

2. Pyroptosis and its role in health and disease

ASKED 1× · 5-mark answer

Asked in: Paper I · Dec 2020 · Q8(b)

Q: Pyroptosis and its role in health and disease. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Pyroptosis — p. 45
- Robbins — ch. 6 Diseases of the Immune System — §Cellular Receptors for Microbes, Products of Damaged Cells, and Foreign Substances; §Natural Killer Cells — pp. 169–170

Definition

- **Pyroptosis** ("fiery death") is a **lytic**, genetically regulated form of cell death. It is accompanied by release of inflammatory mediators, including the fever-inducing cytokine **IL-1** ("pyro" refers to fever).
- It is thought to be the mechanism by which some microbes kill infected cells while triggering local inflammation.
- It is a form of cell death that, unlike apoptosis, releases cell contents and evokes inflammation, as in necrosis.
- Its effector is a family of **pore-forming proteins, the gasdermins**. The best characterised is **gasdermin D (GSDMD)**.

Mechanism

- Pyroptosis is activated by two pathways, **canonical** and **noncanonical**. Both converge on gasdermin activation.
- Canonical pathway:
  - Microbial products entering infected cells are recognised by **cytoplasmic innate immune receptors**.
  - These receptors activate the **inflammasome**, a multiprotein complex.
  - The inflammasome activates **caspase-1** (IL-1β-converting enzyme). Caspase-1 cleaves pro-IL-1β into its active form.
  - The inflammasome sensor example is **NLRP3**, with an adapter and caspase-1.
  - NLRP3 is triggered by PAMPs and DAMPs such as extracellular ATP, K^+ efflux, reactive oxygen species and crystals (uric acid, cholesterol).
- Noncanonical pathway:
  - Intracellular **LPS** from gram-negative bacteria activates **caspases-4/5**.
- Common final steps:
  - Caspase-1 or caspases-4/5 cleave GSDMD. GSDMD **oligomerises and forms pores** in the plasma membrane.
  - The pores release IL-1β and other proinflammatory mediators.
  - They also disrupt local osmotic potential. This leads to **pyroptotic bodies**, membrane rupture and lytic death.

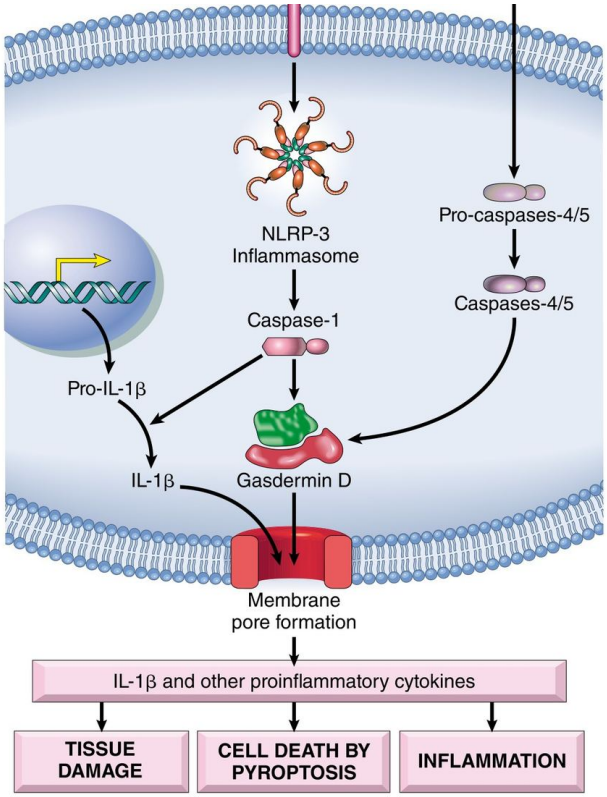
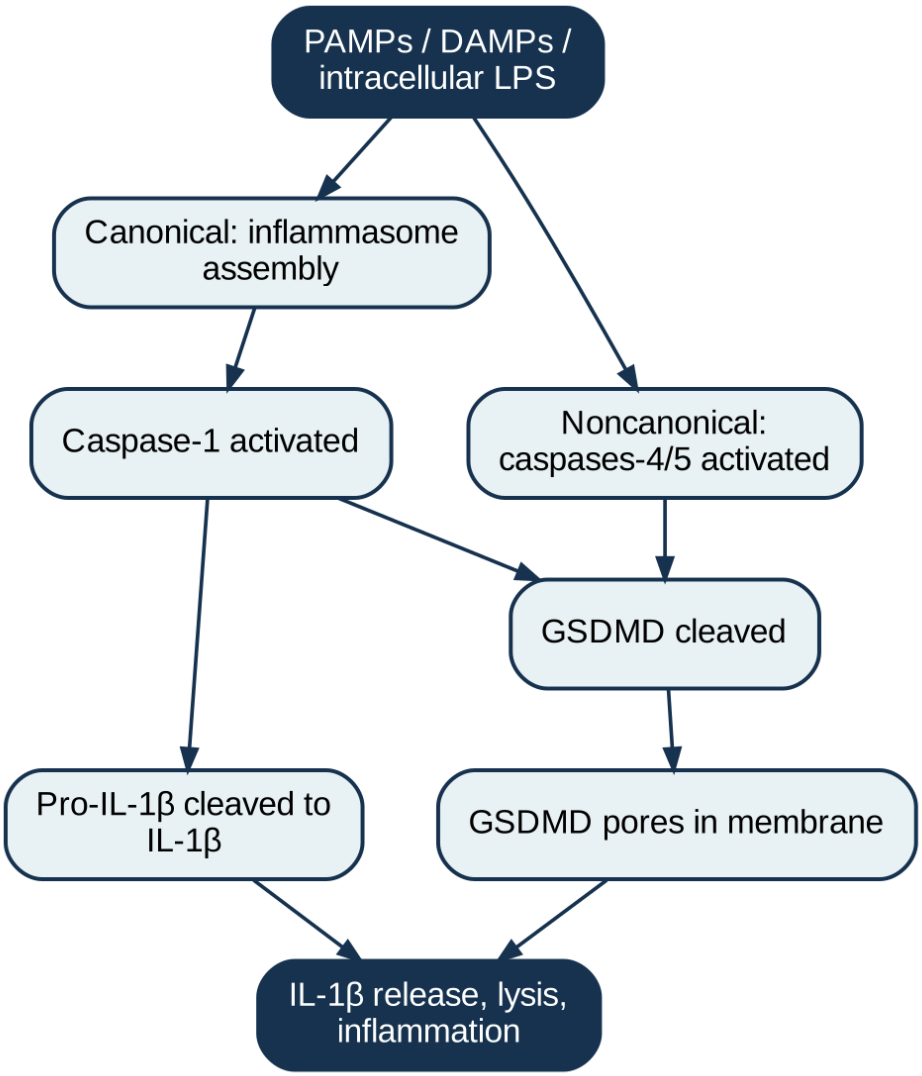


Fig. 2.18 *Canonical and noncanonical pyroptosis. Canonical pyroptosis — Robbins 11e, p. 45*

Flowchart 1: Canonical and noncanonical pyroptosis



DRAW IN THE EXAM

Fig. 2.18-type diagram: canonical (inflammasome → caspase-1) and noncanonical (LPS → caspases-4/5) pathways converging on GSDMD pores, IL-1β release and lytic death.

Role in health and disease

- Health (protective role):
  - Pyroptosis is part of the **host defence against intracellular microbes**. It kills infected cells and triggers local inflammation.
  - The inflammasome-generated IL-1 recruits leukocytes, which eliminate damaged cells, and induces fever.
  - Pattern recognition receptors (TLRs, NOD-like receptors) feed this response. TLRs signal via NF-κB to stimulate cytokine synthesis and adhesion molecule expression.
- Disease (excess or dysregulated activation):
  - **Excessive pyroptosis** may contribute to inflammatory disorders, including **sepsis** and **autoimmune disease**.
  - **Autoinflammatory syndromes** present with periodic fever. They result from gain-of-function mutations in NLRs and related proteins, or loss-of-function mutations in inflammasome regulators.
  - They are distinct from autoimmune diseases, which result from T and B lymphocyte reactions against self antigens.
  - They respond very well to **IL-1 antagonists**.
  - **Gout:** NLRs recognise urate crystals, and this underlies the inflammation.
  - **Atherosclerosis:** NLRs detect cholesterol crystals deposited in abnormally large amounts, and the inflammation may contribute to atherosclerosis.

Comparison with other regulated necrosis-like deaths

Table 1: Pyroptosis versus necroptosis and ferroptosis

Feature	Pyroptosis	Necroptosis	Ferroptosis
Trigger	Infected cells; microbial products, LPS	TNFR1 ligation; viral proteins	Excess iron or ROS
Key mediators	Inflammasome, caspase-1, caspases-4/5, GSDMD	RIPK1–RIPK3, MLKL; caspase independent	Lipid peroxidation; glutathione-dependent defences overwhelmed
Effector	GSDMD pores	MLKL pores	Membrane permeability loss
Inflammation	Yes, IL-1β release	Yes	Necrosis-like

PEARLS

- Pyroptosis = **inflammatory lytic death** of microbe-infected cells via **gasdermin D pores**.
- Canonical: **inflammasome → caspase-1**. Noncanonical: **intracellular LPS → caspases-4/5**.
- Caspase-1 also activates **IL-1β**, the mediator of leukocyte recruitment and fever.
- Excess pyroptosis is linked to sepsis and autoimmune disease; inflammasome activation is linked to gout and atherosclerosis.
- Autoinflammatory syndromes (inflammasome dysregulation) respond to **IL-1 antagonists**.
- Exam trap: necroptosis is caspase independent (RIPK1–RIPK3–MLKL), whereas pyroptosis is caspase dependent.

3. Necroptosis

ASKED 1× · 5-mark answer

Asked in: Paper I · Jun 2022 · Q10(a)

Q: Necroptosis. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Necroptosis, §Pyroptosis — pp. 44–45

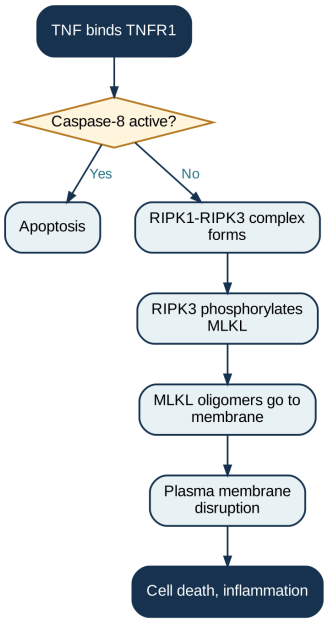
Definition

- **Necroptosis** is a regulated, genetically programmed form of cell death. It is a hybrid that shares features of necrosis and apoptosis.
- It is also called **programmed necrosis**. This distinguishes it from necrosis driven passively by toxic or ischaemic injury.
- It is **caspase-independent**. For this reason it is also called "caspase-independent" programmed cell death.
- Morphologically, and partly biochemically, it resembles necrosis.
  - Both show loss of ATP.
  - Both show swelling of the cell and its organelles.
  - Both show generation of reactive oxygen species.
  - Both show release of lysosomal enzymes.
  - Both end in rupture of the plasma membrane.
- Mechanistically it resembles apoptosis, because it is triggered by signal transduction pathways that culminate in cell death.

Pathogenesis (mechanism)

- Initiation resembles the **extrinsic pathway of apoptosis**: a receptor is ligated by its ligand.
- **TNFR1 ligation** is the most widely studied model.
- Other triggers:
  - ligation of Fas
  - as yet unidentified sensors of viral DNA and RNA
- TNF can cause either apoptosis or necroptosis. The outcome depends on caspase-8 status.
- Necroptosis occurs when **caspase-8 is inactive**.
- The key kinases are **RIPK1 and RIPK3** (receptor-interacting protein kinases 1 and 3).
- TNFR1 ligation recruits these kinases into a multiprotein complex.
- **RIPK3 phosphorylates MLKL**, a cytoplasmic protein.
- Phosphorylated MLKL monomers assemble into **oligomers**.
- The oligomers translocate from the cytosol to the plasma membrane.
- This causes plasma membrane disruption, the characteristic event of necrosis. It explains the morphological similarity to necrosis from other injuries.
- The cell swells and its contents are released. This causes tissue damage and **inflammation**, as in necrosis.

Flowchart 1: Molecular pathway of TNF-mediated necroptosis



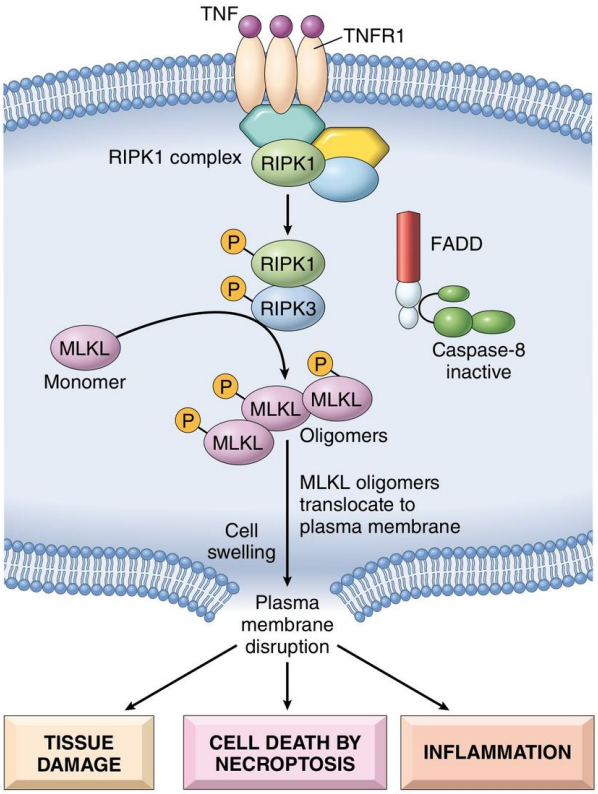


Fig. 2.17 Molecular mechanism of TNF-mediated necroptosis. When — Robbins 11e, p. 44

DRAW IN THE EXAM

Flowchart of TNF–TNFR1 → RIPK1/RIPK3 → MLKL phosphorylation → oligomerisation → membrane pore → necroptosis (Fig. 2.17).

Physiological and pathological roles

- **Physiological:** it occurs during formation of the mammalian bone growth plate.
- **Pathological:** it is associated with cell death in the following conditions.
  - steatohepatitis
  - acute pancreatitis
  - ischaemia–reperfusion injury
  - neurodegenerative diseases such as Parkinson disease
- **Host defence:** it acts as a backup mechanism against viruses that encode caspase inhibitors, e.g. cytomegalovirus.

Comparison with apoptosis and necrosis

Table 1: Necroptosis compared with apoptosis and necrosis

Feature	Apoptosis	Necroptosis	Necrosis
Regulation	Programmed	Programmed (genetically controlled)	Passive, from injury
Caspases	Caspase-dependent	Caspase-independent	Not involved
Key mediators	Caspases	RIPK1, RIPK3, MLKL	ATP loss, ROS, lysosomal enzymes
Cell swelling	Cell shrinkage with bodies	Swelling	Swelling
Membrane	Intact until cleared	Ruptured (MLKL)	Ruptured
Inflammation	Minimal	Present	Present

- Apoptosis is cleared by efferocytosis with reduced proinflammatory cytokines. Necroptosis releases cell contents and evokes inflammation.

PEARLS

- Necroptosis = **programmed necrosis**: it looks like necrosis, but is regulated like apoptosis.
- It is **caspase-independent** and occurs when **caspase-8 is inactive**.
- Key trio: **RIPK1 – RIPK3 – MLKL**. MLKL oligomers form pores in the plasma membrane.
- The best-studied trigger is **TNFR1**. Fas and viral nucleic acid sensors can also trigger it.
- Its contents are released, so it is **inflammatory**, unlike apoptosis.
- It acts as a backup defence against viruses with caspase inhibitors, e.g. CMV.
- Pyroptosis uses gasdermin D and ferroptosis is iron/lipid-peroxidation dependent. Do not confuse these with necroptosis.

4. Ferroptosis

ASKED 2× · 10-mark answer

Asked in: Paper I · May 2024 · Q8(a) · Paper I · Oct 2024 · Q3

Q: Ferroptosis. [5]
Q: Ferroptosis. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Pyroptosis; §Generation of Free Radicals; §Removal of Free Radicals; §Chemical (Toxic) Injury; §Exogenous Pigments — pp. 45, 48, 49, 51, 58
- W1 · PMC (PubMed Central) — Mechanisms and pharmacological applications of ferroptosis: a narrative review
- W2 · PMC (PubMed Central) — Ferroptosis turns 10: Emerging Mechanisms, Physiological Functions, and Therapeutic Applications
- W3 · PMC (PubMed Central) — Harnessing ferroptosis for cancer therapy: Mechanisms and therapeutic strategies (Review)

Definition

- **Ferroptosis** is a distinct, regulated form of cell death. It is triggered when excessive intracellular **iron or ROS** overwhelm the **glutathione-dependent antioxidant defences**, causing unchecked **membrane lipid peroxidation**.
- Name: it is **iron-dependent**, and it can be prevented by reducing iron levels.
- It resembles necrosis in outcome. Unlike necrosis, it is controlled by specific signals.
- It is one of the newer regulated non-apoptotic death pathways, along with necroptosis and pyroptosis.
- Its role in normal development and physiology remains controversial.

Pathogenesis

Prerequisite concepts: free radicals and lipid peroxidation

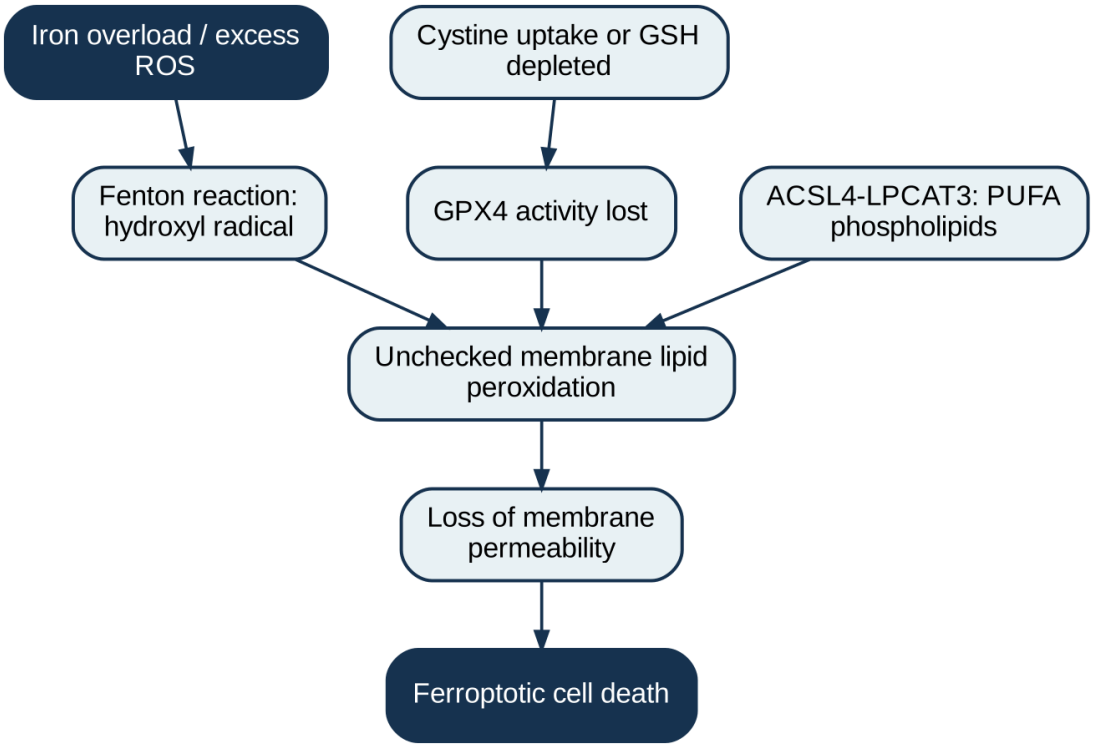
- **Free radicals** are chemical species with a single unpaired electron in the outer orbit. They are highly reactive and attack lipids, proteins and nucleic acids.
- Some reactions are **autocatalytic**: the attacked molecule becomes a free radical itself, so the chain of damage propagates.
- **Oxidative stress** is increased ROS production or decreased scavenging.
- **Iron and copper** are transition metals that catalyse radical formation by the **Fenton reaction**.
  - Reaction: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^-$.
  - Most free intracellular iron is ferric (Fe^{3+}). It must be reduced to Fe^{2+} to join the reaction.
  - Superoxide ($O_2^{\bullet}$) enhances this reduction, so iron and $O_2^{\bullet}$ cooperate in oxidative damage.
- The **hydroxyl radical (˙OH)** is the most reactive oxygen-derived radical. It is the principal ROS damaging lipids, proteins and DNA, and it has **no enzymatic inactivation**.

- **Lipid peroxidation** begins when O2-derived radicals, especially O2•, attack the double bonds of unsaturated fatty acids in membrane lipids.
  - This yields unstable peroxides.
  - An autocatalytic **propagation** chain follows, causing extensive membrane damage.

Steps leading to ferroptosis

- **Trigger 1: iron overload.** Excess labile iron drives Fenton chemistry and lipid peroxidation.
  - In the labile iron pool, Fe2+ drives lipid peroxidation through Fenton reactions.
- **Trigger 2: failure of antioxidant defence.**
  - Normally, **GSH** is a cofactor for **glutathione peroxidase**.
  - The GSSG:GSH ratio reflects the cell's ability to detoxify ROS.
  - In ferroptosis, glutathione-dependent defences are overwhelmed.
- **System Xc−–GSH–GPX4 axis (the brake)**.
  - System Xc− is a cystine–glutamate antiporter made of **SLC7A11 + SLC3A2**. It imports cystine, which is converted to cysteine for **GSH synthesis**.
  - **GPX4** uses GSH to reduce toxic lipid hydroperoxides (L-OOH) to non-toxic lipid alcohols (L-OH).
  - Inhibiting system Xc− lowers GSH. GPX4 activity falls, lipid ROS accumulate and ferroptosis follows.
  - p53 down-regulates SLC7A11, so cystine uptake falls and ferroptosis is favoured.
  - Experimentally, **erastin** blocks cystine uptake through system Xc−, depleting cysteine and GSH. **RSL3** targets GPX4 directly.
- **Lipid substrate (the accelerator)**.
  - **ACSL4 and LPCAT3** incorporate **polyunsaturated fatty acids (PUFAs)** into membrane phospholipids.
  - This makes oxidation-prone membrane lipids.
  - They are the first identified **pro-ferroptotic** gene products.
  - Lipoxygenases (LOXs) also promote phospholipid peroxidation.
- **Execution.**
  - Widespread peroxidation disrupts membrane fluidity, lipid–protein interactions, ion and nutrient transport, and signalling.
  - The result is loss of plasma membrane permeability and necrosis-like death.

Flowchart 1: Pathogenesis of ferroptosis



DRAW IN THE EXAM
Labelled diagram of ferroptosis: iron/Fenton, system Xc−–GSH–GPX4 axis, ACSL4–PUFA lipid peroxidation, membrane rupture.

Morphology

- **Ultrastructure (electron microscopy)** shows two prominent features:
  - loss of **mitochondrial cristae**;
  - ruptured **outer mitochondrial membrane**.
- Overall, the cell death resembles **necrosis**.
- The standard routine-stain features of apoptosis are not described for ferroptosis. In the book, apoptosis is the contrast case, and ferroptosis is regulated unlike necrosis.
- **Lipofuscin** is the tissue-level sign of lipid peroxidation. It is a yellow-brown, finely granular, often perinuclear pigment derived from lipid peroxidation of intracellular membranes.
  - It is not injurious in itself, but it is a telltale sign of free-radical injury.
  - It is not specific for ferroptosis.

Markers and diagnosis

- There is no single routine IHC test for ferroptosis. It is identified by features such as lipid peroxidation, iron dependence and the regulators below.
- The relevant regulators are:
  - **GPX4**: suppressor; low or inhibited activity marks susceptibility.
  - **SLC7A11 / system Xc−**: suppressor; reduced expression impairs the antioxidant pathway and induces ferroptosis.
  - **ACSL4 / LPCAT3**: drivers; they supply PUFA phospholipids.
- A defining test is **rescue by iron reduction**. Reducing iron levels prevents ferroptosis.
  - Experimentally, ferrostatin-1 (lipid-peroxidation inhibitor) and deferoxamine (iron chelator) block it.
- Caspase inhibition does not rescue it. In the book, ferroptosis has no caspase or RIPK–MLKL requirement, in contrast to pyroptosis and necroptosis.

Table 1: Ferroptosis compared with other regulated necrosis-like deaths

Feature	Ferroptosis	Necroptosis	Pyroptosis
Trigger	Excess iron or ROS, GSH-defence failure	TNFR1 ligation, viral proteins	Microbial products via inflammasome; LPS
Key mediators	Lipid peroxidation (GPX4 loss, ACSL4)	RIPK1–RIPK3, MLKL	Caspase-1, caspases-4/5, GSDMD
Caspases	Not the executioner	Independent	Required
Execution	Membrane lipid peroxidation, permeability loss	MLKL pores	GSDMD pores, IL-1β release
Morphology	Necrosis-like; shrunken mitochondria, lost cristae	Necrosis-like	Lytic; pyroptotic bodies
Prevented by	Reducing iron	Blocking RIPK1/RIPK3	Blocking inflammasome/GSDMD

Disease relevance and clinical correlation

- Ferroptosis is linked to cell death in **cancer, neurodegenerative diseases and stroke**.
- **Ischemia–reperfusion injury** is relevant because it involves oxidative stress. Reperfusion raises reactive oxygen and nitrogen species, and antioxidant defences are compromised during ischemia.
  - Ferroptosis regulators (GPX4, ACSL4) are being studied in myocardial ischemia–reperfusion.
- Free-radical injury is also relevant to chemical injury, for example $CCl_4 \rightarrow {}^{\cdot}CCl_3$ causing lipid peroxidation.

Therapeutic and prognostic relevance

- **Cancer therapy:** inducing ferroptosis is being explored as a treatment.
  - GPX4 inhibitors (RSL3, ML162) bind the GPX4 active site and block lipid peroxide reduction. This is an option when cells resist system Xc− inhibitors.
  - Radiation therapy may also induce ferroptosis.
- **Degenerative and ischemic disease:** the opposite approach is to inhibit ferroptosis. Lowering iron prevents it.
- Prognostic biomarkers for ferroptosis are not yet established in routine pathology.

PEARLS

- **Ferroptosis** is iron-dependent, regulated cell death from membrane **lipid peroxidation** that overwhelms glutathione-dependent defences.
- Hallmark EM change: **loss of mitochondrial cristae** with ruptured outer mitochondrial membrane.
- Core axis: **System Xc− (SLC7A11/SLC3A2) → GSH → GPX4**. ACSL4/LPCAT3 are pro-ferroptotic.
- Erastin blocks system Xc−. RSL3 inhibits GPX4.
- Rescue by **iron reduction** distinguishes it from necrosis.
- Exam trap: ferroptosis is **not** caspase-driven like pyroptosis, and it is **not** RIPK1–RIPK3–MLKL-driven like necroptosis.
- The Fenton reaction yields ˙OH, which has no enzymatic inactivation.

5. Cytosolic calcium and cell injury

ASKED 1× · 5-mark answer
Asked in: Paper I · Jun 2020 · Q9(b)
Q: Cytosolic calcium and cell injury. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Removal of Free Radicals; Mechanisms of Ischemic Cell Injury; Chemical (Toxic) Injury — pp. 49–51

Normal calcium homeostasis

- Calcium ions normally act as **second messengers** in several signalling pathways. Released into the cytoplasm in excess, they become an important source of cell injury.
- Cytosolic free Ca^{2+} is normally kept very low, **<0.1 µmol**, against extracellular levels of **1.3 mmol**.
- Most intracellular Ca^{2+} is sequestered in **mitochondria and the endoplasmic reticulum (ER)**.
- Calcium depletion protects cultured cells from injury by a variety of harmful stimuli. This shows that calcium is central to injury.

Causes of increased cytosolic calcium

- **Ischemia** and certain **toxins** cause an excessive rise in cytosolic Ca^{2+}.
- The rise happens in two phases:
  - Early: release from intracellular stores (mitochondria, ER).
  - Late: increased influx across the plasma membrane.
- In reperfusion, calcium overload is worsened by influx through damaged cell membranes. ROS-mediated injury to the sarcoplasmic reticulum adds to it.

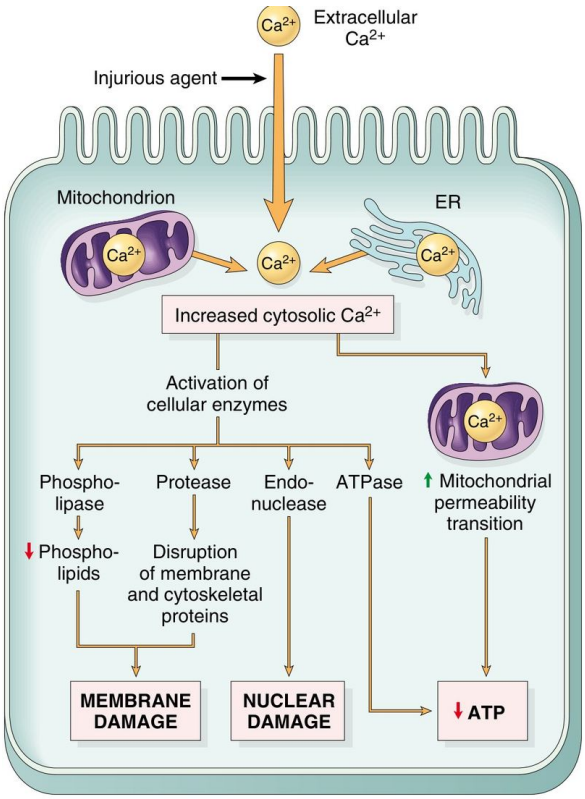


Fig. 2.23 Role of increased cytosolic calcium in cell injury. ER, Endo- — Robbins 11e, p. 50

Mechanisms of cell injury by excess cytosolic calcium

- The significance of these mechanisms in cell injury in vivo is not firmly established.
- **Mitochondrial effect**
  - Ca^{2+} accumulation in mitochondria opens the **mitochondrial permeability transition pore**.
  - This causes failure of ATP generation.
- **Activation of cellular enzymes**
  - Increased cytosolic Ca^{2+} activates several enzymes with potentially deleterious effects.

Table 1: Calcium-activated enzymes and their injurious effects

Enzyme	Effect	Result
Phospholipases	Break down membrane phospholipids	Membrane damage
Proteases	Break down membrane and cytoskeletal proteins	Disruption of membrane and cytoskeleton
Endonucleases	Fragment DNA and chromatin	Nuclear damage
ATPases	Hasten consumption of ATP	Faster ATP depletion

- Source for the table:.
- **Overall outcome**
  - Calcium influx activates enzymes that damage cellular components.
  - It may also trigger **apoptosis**.
  - Membrane permeability changes typically culminate in **necrosis**.

Role in ischemia–reperfusion injury

- Restoring blood flow to ischemic tissue can paradoxically **exacerbate injury** and cause cell death. This is called ischemia-reperfusion injury.
- It is clinically important in myocardial and cerebral infarction after therapies that restore blood flow.
- **Intracellular calcium overload** is one of the proposed mechanisms:
  - It begins during acute ischemia.

  - It is exacerbated during reperfusion by influx through damaged membranes and ROS-mediated injury to the sarcoplasmic reticulum.
  - It favours opening of the mitochondrial permeability transition pore.
  - The resulting ATP depletion causes further cell injury.
- Other proposed mechanisms are oxidative stress, inflammation and complement activation.

Link with other injury pathways

- ATP depletion leads to failure of energy-dependent functions, then reversible injury, then necrosis.
- Calcium influx is one of the key mechanisms of cell injury, alongside:
  - ATP depletion
  - mitochondrial damage
  - increased membrane permeability
  - accumulation of damaged DNA and misfolded proteins
  - accumulation of ROS

DRAW IN THE EXAM

Flow diagram (Fig. 2.23): injurious agent → ↑ cytosolic Ca^{2+} (from ER, mitochondria, extracellular influx) → mitochondrial permeability transition, phospholipase, protease, endonuclease and ATPase activation → membrane and nuclear damage.

PEARLS

- Cytosolic Ca^{2+} is normally **<0.1 µmol**, against **1.3 mmol** outside the cell. Most intracellular Ca^{2+} is stored in mitochondria and ER.
- Four calcium-activated enzymes cause injury: **phospholipases, proteases, endonucleases, ATPases**.
- Ca^{2+} overload opens the **mitochondrial permeability transition pore**, so ATP generation fails.
- Calcium influx can trigger apoptosis as well as necrosis.
- Calcium overload is a mechanism of **reperfusion injury**, together with oxidative stress, inflammation and complement.
- Exam trap: ischemia releases Ca^{2+} first from stores, and influx follows later.

6. Mechanism of cell death in ischemic & hypoxic injury

ASKED 1× · 5-mark answer

Asked in: Paper I · May 2024 · Q2(a)

Q: Mechanism of cell death in ischemic & hypoxic injury. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Mitochondrial Damage, §Membrane Damage, §Mechanisms of Ischemic Cell Injury, §Chemical (Toxic) Injury — pp. 46–47, 50–51

Definition and difference between hypoxia and ischemia

- **Hypoxia**: oxygen deficiency with blood flow maintained, so anaerobic glycolysis can continue to make ATP.
- **Ischemia**: hypoxia from reduced blood flow, most often mechanical arterial obstruction, or from reduced venous drainage.
- Ischemia also cuts the supply of glycolytic substrates, and metabolites that would otherwise be washed out accumulate and inhibit glycolysis.
- Ischemia therefore causes more rapid and severe cell and tissue injury than hypoxia.

Mechanism of cell death in ischemic and hypoxic injury

- As intracellular oxygen tension falls, oxidative phosphorylation fails and ATP generation decreases.
- Loss of ATP first causes reversible injury (cell and organelle swelling). It later causes cell death by **necrosis**.

Consequences of ATP depletion

- Depletion of ATP to **5–10% of normal** has widespread effects on critical cellular systems.
- **Na+,K+-ATPase pump failure**: sodium and water enter the cell and potassium falls. This causes cell swelling and ER dilation.
- **Altered energy metabolism**: AMP rises and stimulates phosphorylase and phosphofructokinase. Glycogenolysis and glycolysis increase, and glycogen stores are rapidly depleted.
- Anaerobic glycolysis produces lactic acid and inorganic phosphates, which lower intracellular pH and reduce the activity of cytosolic enzymes.
- **Protein synthesis fails**: ribosomes detach from the rough ER and polysomes dissociate. Protein misfolding may increase.
- Ultimately there is irreversible damage to mitochondrial and lysosomal membranes, and the cell undergoes necrosis.
- Incomplete oxidative phosphorylation forms **ROS**.

Mitochondrial damage

- Mitochondria are damaged by raised cytosolic Ca2+, ROS and oxygen deprivation.
- Damage opens the **mitochondrial permeability transition pore (MPTP)**. This causes loss of membrane potential, failure of oxidative phosphorylation and progressive ATP depletion, ending in necrosis.
- Leakage of mitochondrial proteins through BAX/BAK channels is the initial step of apoptosis by the intrinsic pathway.

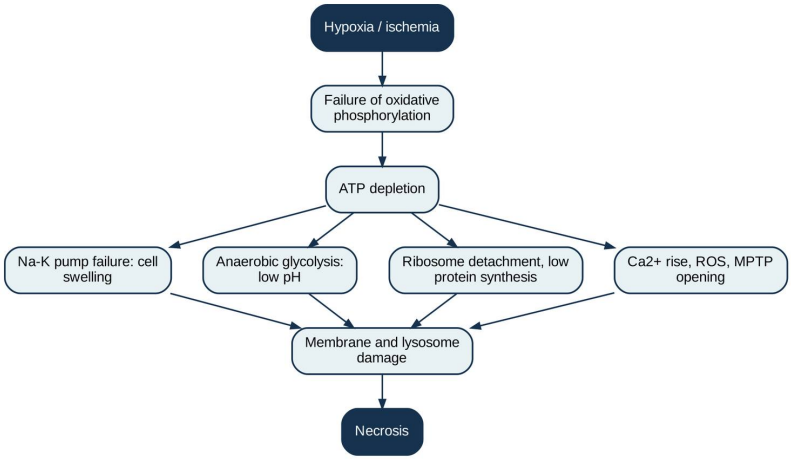
Membrane damage

- Early loss of selective membrane permeability is a consistent feature of injury (except apoptosis). In ischemia it results from ATP depletion and Ca2+-mediated phospholipase activation.
- Mechanisms of membrane damage:
  - ROS cause lipid peroxidation.
  - Phospholipid synthesis decreases.
  - Phospholipid breakdown increases. The products (free fatty acids, acyl carnitine, lysophospholipids) have a detergent effect.
  - Cytoskeletal damage detaches the membrane from the cytoskeleton, making it prone to rupture.

Calcium influx

- Cytosolic Ca2+ rises through release from stores and increased influx.
- Ca2+ in mitochondria opens the MPTP, so ATP generation fails.
- Ca2+ activates phospholipases (membrane damage), proteases (membrane and cytoskeletal proteins), endonucleases (DNA and chromatin fragmentation) and ATPases (hastening ATP depletion).

Flowchart 1: Mechanism of cell death in ischemic and hypoxic injury



Protective response

- **HIF-1** is induced. It promotes new blood vessel formation, stimulates cell survival pathways and enhances glycolysis.

Reperfusion injury (extension of ischemic death)

- Restoring blood flow can paradoxically worsen injury and cause cell death. This is called **ischemia-reperfusion injury**.
- Mechanisms:
  - **Oxidative stress**: increased reactive oxygen and nitrogen species on reoxygenation.
  - **Calcium overload**: this favours MPTP opening.
  - **Inflammation**: neutrophils are recruited.
  - **Complement activation**: IgM deposited in ischemic tissue binds complement.

Table 1: Reversible vs irreversible ischemic injury

Feature	Reversible	Irreversible
ATP	Decreased	Further depleted
Morphology	Cell and organelle swelling	Necrosis
Membranes	Increased permeability	Mitochondrial and lysosomal membrane damage

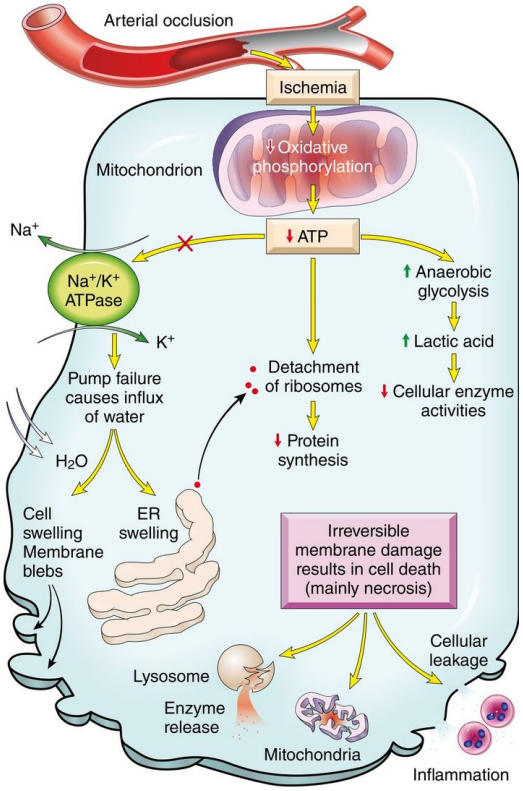


Fig. 2.24 Functional and morphologic consequences of decreased in- — Robbins 11e, p. 51

DRAW IN THE EXAM

Flowchart of ATP depletion in ischemic cell injury, with its functional and morphologic consequences (as in Fig. 2.24).

PEARLS

- Ischemia is more injurious than hypoxia because glycolytic substrate delivery also fails.
- The final common pathway is ATP depletion followed by **necrosis**.
- MPTP opening is the key mitochondrial event.
- Raised cytosolic Ca2+ activates phospholipases, proteases, endonucleases and ATPases.
- **Reperfusion** adds injury through ROS, calcium overload, neutrophils and complement.
- HIF-1 is the protective response; hypothermia is useful in brain and spinal cord ischemia.

7. Pathological effects of free radicals

ASKED 1× · 5-mark answer

Asked in: Paper I · Jun 2025 · Q4(a)

Q: Pathological effects of free radicals. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Generation of Free Radicals; Removal of Free Radicals — pp. 48–49

Free radicals: definition

- **Free radicals** are chemical species with a single unpaired electron in an outer orbit. They are highly reactive and attack adjacent molecules (proteins, lipids, carbohydrates, nucleic acids).
- Some reactions are **autocatalytic**. The attacked molecule is itself converted into a free radical, so the chain of damage propagates.
- **Reactive oxygen species (ROS)** are the oxygen-derived free radicals whose role in cell injury is best established.
- **Oxidative stress** is an excess of free radicals, caused by increased production or decreased scavenging of ROS.
- Oxidative stress is implicated in cell injury, cancer, aging and degenerative diseases such as Alzheimer disease.

Pathological effects of free radicals

1. Lipid peroxidation of membranes

- In the presence of O2, free radicals peroxidise lipids in plasma and organellar membranes.
- Damage starts when O2-derived radicals, particularly **O2•**, attack the **double bonds of unsaturated fatty acids** in membrane lipids.
- The lipid–radical interaction yields unstable, reactive peroxides. An autocatalytic chain reaction (**propagation**) follows and causes extensive membrane damage.

2. Oxidative modification of proteins

- Oxidation of amino acid side chains.
- Formation of covalent protein–protein cross-links, e.g. **disulfide bonds**.
- Oxidation of the protein backbone.
- Damage to the active sites of enzymes.
- Disruption of the conformation of structural proteins.
- Enhanced **proteasomal degradation** of unfolded or misfolded proteins.

3. Lesions in DNA

- Single- and double-strand breaks.
- Cross-linking of DNA strands.
- Formation of adducts.
- Oxidative DNA damage is implicated in **cell aging** and **malignant transformation**.

4. Mode of cell death and other effects

- Free radicals were traditionally thought to cause cell injury and death by **necrosis**. They can also trigger **apoptosis**.
- Under controlled conditions and at the "right" dose, they may serve physiological signalling functions.

Settings in which these effects are important

- Chemical and radiation injury.
- Ischemia–reperfusion injury.
- Cellular aging.
- Microbial killing by phagocytes.

Properties of the principal free radicals

Table 1: Principal free radicals and their pathological effects

Radical	Production	Inactivation	Pathological effect
Superoxide ($O_2^{\bullet}$)	Incomplete reduction of O2 in oxidative phosphorylation; phagocyte oxidase	SOD converts it to H2O2 and O2	Stimulates degradative enzymes; may damage lipids, proteins and DNA; acts close to its site of production
Hydrogen peroxide (H_2O_2)	SOD action on $O_2^{\bullet}$; peroxisomal oxidases	Catalase (peroxisomes); glutathione peroxidase (cytosol, mitochondria)	Converted to $OH^{\bullet}$ and OCl^-, which destroy microbes and cells; can act distant from its site of production
Hydroxyl radical ($^{\bullet}OH$)	Radiolysis of water; Fenton reaction; from $O_2^{\bullet}$	No enzymatic inactivation	Most reactive; principal ROS damaging lipids, proteins and DNA
Peroxynitrite ($ONOO^-$)	$O_2^{\bullet}$ + NO (NO synthase)	Peroxiredoxins convert it to HNO2	Damages lipids, proteins and DNA

Generation and removal (brief, for context)

- **Generation:**
  - Normal redox reactions: O2 is partially reduced to $O_2^{\bullet}$, H2O2 and $^{\bullet}OH$.
  - Radiant energy (UV, x-rays).
  - NADPH oxidase in activated leukocytes. Defects cause chronic granulomatous disease.
  - Metabolism of drugs and chemicals, e.g. $CCl_4 \rightarrow {}^{\bullet}CCl_3$.
  - Transition metals (Fenton reaction): $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$.
  - Nitric oxide.
- **Removal:**
  - Spontaneous decay.
  - Antioxidants: vitamins E and A, ascorbic acid, glutathione.
  - Metal-binding proteins: transferrin, ferritin, lactoferrin, ceruloplasmin.
  - Enzymes: catalase, SOD (Mn-SOD in mitochondria, Cu-Zn-SOD in cytoplasm), glutathione peroxidase.
  - The **GSSG:GSH ratio** indicates the oxidative state of the cell.

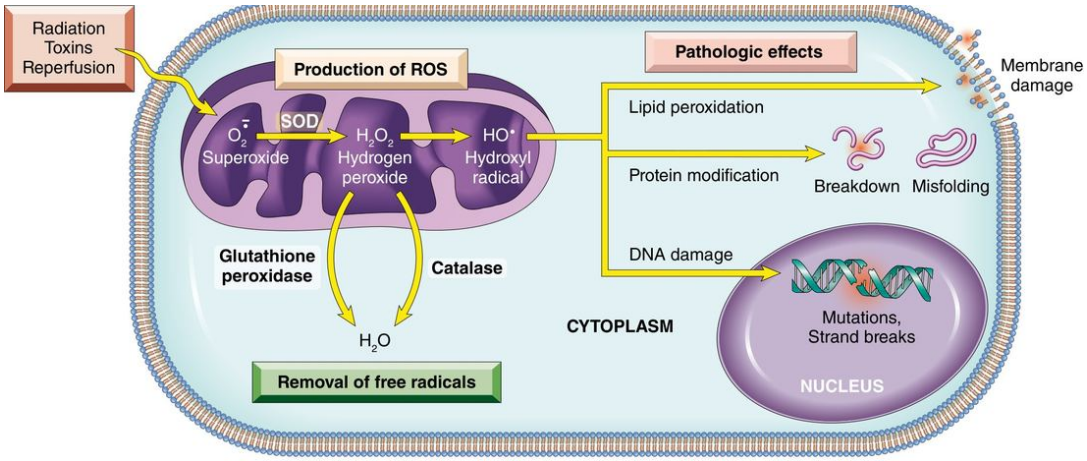


Fig. 2.22 The generation, removal, and role of reactive oxygen species (ROS) in cell injury. The production of — Robbins 11e, p. 49

DRAW IN THE EXAM

Fig. 2.22 of Robbins: production of ROS (O2 → superoxide → H2O2 → hydroxyl radical), removal by SOD, catalase and glutathione peroxidase, and effects (lipid peroxidation, protein modification, DNA damage).

PEARLS

- Free radical = a species with an unpaired outer-orbit electron. Excess ROS = oxidative stress.
- The three key effects are lipid peroxidation, protein oxidation/cross-linking and DNA strand breaks.
- **•OH** is the most reactive ROS and has no enzymatic inactivation.
- Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow$ •OH.
- Free radicals cause necrosis and can also trigger apoptosis.
- Defects in leukocyte superoxide production cause chronic granulomatous disease.

8. Role of "unfolded protein response in cell injury"

ASKED 2× · 10-mark answer

Asked in: Paper I · Jun 2021 · Q4(a) · Paper I · Jun 2021 · Q4(b) · Paper I · Jun 2025 · Q2

Q: Role of "unfolded protein response in cell injury". [5]
Q: Enumerate diseases caused by misfolding of proteins. [5]
Q: What is protein misfolding disease? Give four examples. Describe the pathobiology of any one of the protein misfolding diseases. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Nutritional Imbalances; §Unfolded Protein Response and Endoplasmic Reticulum Stress — pp. 32–33
- W1 · PubMed Central (Perlmutter D.) — Disorders of Protein Misfolding: Alpha-1-antitrypsin Deficiency as Prototype
- W2 · PubMed Central — Chemical Chaperone and Inhibitor Discovery: Potential Treatments for Protein Conformational Diseases
- W3 · PubMed Central — Protein Misfolding in the Pathogenesis and Diagnosis of Neurodegenerative Diseases (Review)
- W4 · PubMed Central — Susceptibility of alpha-1 antitrypsin deficiency variants to polymer-blocking therapy
- W5 · PubMed Central — Z α-1 antitrypsin deficiency and the endoplasmic reticulum stress response
- W6 · PubMed Central — Protein Misfolding in the Pathogenesis and Diagnosis of Neurodegenerative Diseases (Review)
- W7 · PubMed Central — Molecular Mechanism of Z α1-Antitrypsin Deficiency
- W8 · PubMed Central — The pathological Trento variant of alpha-1-antitrypsin (E75V) shows nonclassical behaviour during polymerization
- W9 · PubMed Central — The polymerized protein response (PPR) is activated by genetic variants that polymerize in the ER and is mediated by NFκBp50
- W10 · PubMed Central — Z variant heterozygosity in alpha-1 antitrypsin deficiency: disease risk and treatment implications

Definition of protein misfolding disease

- Protein misfolding disease is a disease in which a protein fails to fold into its native conformation. Abnormal proteins accumulate, or the protein is lost from its site of action, and the cell is injured as a result.
- Other names are proteinopathy and conformational disease. The shared feature is the accumulation of misfolded protein.
- **ER stress** is the cell state in which protein-folding demand exceeds the folding capacity of the ER.
- Misfolded proteins that cannot be corrected are ubiquitinated and degraded in proteasomes.
- Misfolded proteins that accumulate trigger the unfolded protein response (UPR) and, if unchecked, apoptosis.
- More than twenty human proteins undergo aberrant aggregation, including Aβ, prion, β2-microglobulin, tau and α-synuclein.
- Mutations accelerate misfolding but are not an absolute requirement.

Mechanisms of tissue injury

- **Loss of function:** the mutation alters folding so that the protein is non-functional or never reaches its destination. Cystic fibrosis is the prototype, because all of its manifestations arise from lack of chloride transport.
- **Toxic gain of function:** the mutant protein or its mislocalisation is itself pathogenic. Huntington's disease and early-onset Alzheimer's disease are prototypes, with neuronal degeneration.
- Some diseases combine both. In α1-antitrypsin deficiency, lung disease (COPD) arises by loss of function and liver disease by gain of function.

Role of the unfolded protein response in cell injury

Why misfolded protein accumulates

- Increased misfolding occurs with deleterious mutations, or with a decreased capacity to correct misfolding, as in ageing.
- Increased or altered protein synthesis, as in plasma cell tumours, also increases misfolding.
- Viral infection can overwhelm the folding quality control system when viral proteins are made in large amounts.
- Increased demand for secretory proteins such as insulin in insulin-resistant states, and changes in intracellular pH and redox state, are further stressors.
- Many foldases need ATP, so glucose and oxygen deprivation in ischaemia and hypoxia increase the burden of misfolded protein.

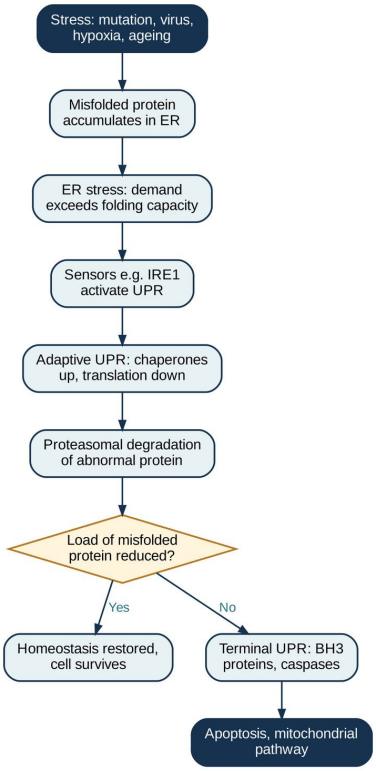
Adaptive (cytoprotective) UPR

- Chaperones in the ER control folding of newly synthesised proteins.
- When unfolded protein accumulates, the UPR activates signalling pathways, for example through the sensor IRE1.
- These pathways **increase chaperone production**.
- They **enhance proteasomal degradation** of abnormal proteins.
- They **slow protein translation**. The net effect is a reduced load of misfolded protein.

Terminal (pro-apoptotic) UPR

- If the cytoprotective response cannot cope, the cell activates caspases and dies by apoptosis.
- In severe ER stress the UPR activates BH3 proteins and caspases. Death occurs by the mitochondrial pathway.
- Cell loss from this terminal UPR is the injury seen in protein misfolding diseases.

Flowchart 1: Unfolded protein response in cell injury



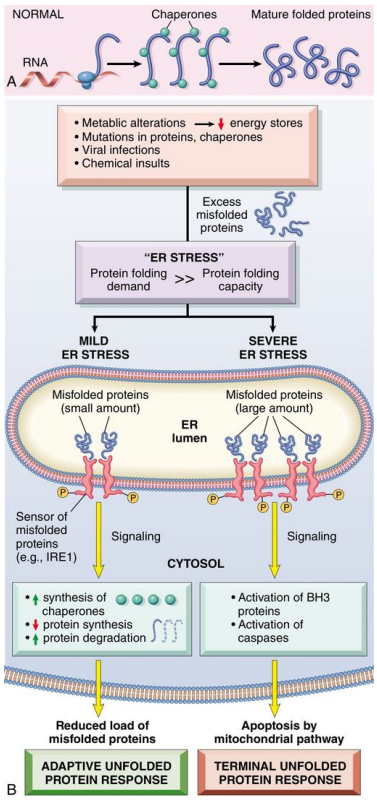


Fig. 2.4 The unfolded protein response and endoplasmic reticulum (ER) — Robbins 11e, p. 33

Related integrated stress response (ISR)

- The ISR is activated by four kinases: PKR, PERK, GCN2 and HRI.
- All four phosphorylate eIF2α on serine 51, the core ISR event.
- This causes global repression of translation, which conserves energy.
- It also promotes translation of select transcription factors such as **ATF4**. ATF4 controls genes for the antioxidant response and protein folding.
- Through PERK, the ISR can activate the UPR.
- Dysfunctional ISR and UPR are linked to cancer, type 2 diabetes and age-related neurodegeneration.

> **DRAW IN THE EXAM**
> Flow diagram of ER stress: mild stress leads to the adaptive UPR and survival, severe stress leads to the terminal UPR and apoptosis.

Diseases caused by misfolding of proteins

Table 1: Protein misfolding diseases with the protein involved and the mechanism

Disease	Protein	Mechanism
Cystic fibrosis	CFTR	Loss of function
α1-antitrypsin deficiency	Mutant α1-antitrypsin (Z)	Loss of function (lung) and gain of function (liver)
Huntington's disease	Huntingtin	Toxic gain of function
Alzheimer's disease	Aβ and tau	Extracellular Aβ and tau accumulation
Parkinson's disease	α-synuclein	Synucleinopathy
Prion disease	Prion protein	Aberrant aggregation
Frontotemporal dementia	Tau	Tau aggregation
Amyloidosis (familial, dialysis-related)	Transthyretin, β2-microglobulin	Aberrant aggregation

- Neurodegenerative diseases as a group are thought to have protein misfolding as the causative cellular abnormality.

- Plasma cell tumours (altered protein synthesis), type 2 diabetes (secretory demand and ISR/UPR dysfunction) and viral infections show misfolding stress.
- Neurodegenerative proteins share a pathway of oligomerisation and aggregation into amyloid fibrils. The oligomeric intermediates, rather than the final aggregates, are thought to be the toxic species that evoke ER stress.

Pathobiology of α1-antitrypsin deficiency (one example)

Molecular basis

- The classic variant is the **Z allele (Glu342Lys)**.
- It is carried by about 4% of Northern Europeans.
- The Z mutation alters the reactive centre loop, so one Z molecule can interact with another until large polymers form.
- The Z protein has delayed folding and compromised secretion.
- Because the liver is the main site of synthesis, mutant α1-antitrypsin accumulates in the ER of hepatocytes.

Two pathways of injury

- **Loss of function (lung):**
  - Retention in hepatocytes means little circulating α1-antitrypsin.
  - Neutrophil elastase activity in the lung is then uncontrolled. This predisposes to early-onset emphysema.
- **Gain of function (liver):**
  - Polymers accumulate in the hepatocyte ER and form **PAS-positive inclusions**, which are characteristic.
  - Polymers do not trigger the classical UPR. They induce an ER overload response, which with obesity, alcohol and other genetic factors leads to cirrhosis.
  - ATZ causes liver fibrosis and carcinogenesis by gain of function.

Disposal of mutant protein

- Soluble Z protein is removed by ER-associated degradation (ERAD) through the proteasome.
- Polymerised Z protein is cleared by autophagy.
- When the burden exceeds degradative capacity, ER accumulation and ER stress follow.

Modifiers

- Heterozygous MZ and SZ individuals have a risk of liver disease. Z polymers can co-polymerise with S and rare variants, and alcohol and fat act as a second hit.
- Plasma deficiency is proportional to the rate of polymerisation in all but null mutants.

Therapeutic principle

- Polymer-blocking small molecules such as GSK716, and siRNA, are being developed.

PEARLS

- Protein misfolding disease results from loss of function (cystic fibrosis) or toxic gain of function (Huntington's disease, Alzheimer's disease).
- ER stress is present when protein-folding demand exceeds folding capacity.
- The UPR is first adaptive (more chaperones, more proteasomal degradation, less translation) and terminally apoptotic through caspases.
- ISR core event: phosphorylation of eIF2α on serine 51 by PKR, PERK, GCN2 or HRI. The key effector is ATF4.
- Plasma cell tumours, viral infection, ageing, ischaemia and insulin resistance are all stressors that raise misfolded protein load.
- α1-antitrypsin deficiency is the prototype: Z allele Glu342Lys, PAS-positive hepatocyte ER inclusions, emphysema (loss of function) and cirrhosis (gain of function).

9. Proteasomes

ASKED 1× · 5-mark answer
Asked in: Paper I · Jun 2021 · Q5(a)
Q: Proteasomes. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Unfolded Protein Response and Endoplasmic Reticulum Stress; §Mechanisms of Metaplasia (Mechanisms of Atrophy); §Steatosis (Fatty Change); §Dysregulated Nutrient Sensing (Defective Protein Homeostasis) — pp. 33, 55, 56, 61

Definition

- **Proteasomes** are the cytoplasmic proteolytic complexes that degrade proteins tagged with ubiquitin.
- Cellular proteins are degraded mainly by the **ubiquitin-proteasome pathway**.
- The pathway works with the **autophagy-lysosome system** to maintain protein homeostasis.
- Protein homeostasis has two arms:
  - Chaperone-mediated maintenance of the correct folded conformation.
  - Degradation of misfolded, damaged or unneeded proteins.

Mechanism of ubiquitin-proteasome degradation

- **Ubiquitin ligases** attach the small peptide ubiquitin to cellular proteins.
- Ubiquitination marks the protein for degradation in proteasomes.
- Nutrient deficiency and disuse activate the ubiquitin ligases.
- Misfolded polypeptides in the ER are shuttled into the cytoplasm. They are ubiquitinated and targeted for proteolysis in proteasomes.

Role in the unfolded protein response (ER stress)

- **ER chaperones** control the folding of newly synthesised proteins.
- **ER stress** is the state in which protein-folding demand exceeds the folding capacity of the ER.
- Misfolded proteins accumulating in the ER activate the **unfolded protein response (UPR)**.
- The adaptive UPR has three actions that reduce the load of misfolded proteins:
  - Increased production of chaperones.
  - **Enhanced proteasomal degradation** of abnormal proteins.
  - Slowed protein translation.
- If the UPR cannot cope, caspases are activated and the cell dies by **apoptosis** (mitochondrial pathway).
- Sensors of misfolded proteins, such as **IRE1**, start the signalling.

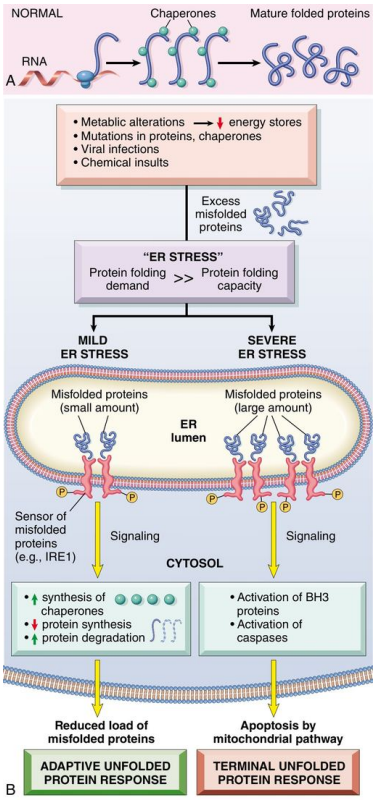


Fig. 2.4 The unfolded protein response and endoplasmic reticulum (ER) — Robbins 11e, p. 33

- Causes of increased misfolding that raise the proteasomal load:
  - Deleterious mutations.
  - Ageing.
  - Increased or altered protein synthesis, as in plasma cell tumours.
  - Viral infections.
  - Increased demand for secretory proteins such as insulin.
  - ATP depletion in ischaemia and hypoxia, because many foldases need ATP.

Role in atrophy

- Atrophy results from **decreased protein synthesis** and **increased protein degradation**.
- Degradation occurs mainly through the ubiquitin-proteasome pathway.
- The same pathway is thought to cause the accelerated proteolysis of catabolic states, including **cancer-related cachexia**.
- Autophagy often accompanies atrophy. Undigested debris persists as **residual bodies** such as lipofuscin, which cause brown atrophy.

Role in ageing

- Both normal folding and the degradation of misfolded proteins are impaired with ageing.
- Mice deficient in heat shock protein chaperones age rapidly. Mice that overexpress them are long lived.
- Similar data exist for autophagy and proteasomal degradation.
- Rapamycin inhibits the **mTOR** pathway and increases life span in middle-aged mice. It also promotes autophagy.
- Abnormal protein homeostasis can lead to accumulation of misfolded proteins, which can trigger apoptosis.

Disease relevance (pathological correlation)

- Failure of protein quality control causes intracellular accumulation of abnormal proteins.
- Examples are mutant **α1-antitrypsin**, and defects in protein folding, transport or secretion.
- Protein misfolding is thought to be the causative abnormality in several **neurodegenerative diseases**.

- A dysfunctional integrated stress response (ISR) and UPR are linked to type 2 diabetes, cancer and age-related degenerative disease.
- Accumulation of misfolded proteins is reversible if the overload is controlled.

PEARLS

- Proteasome = cytoplasmic protease complex that degrades **ubiquitin-tagged** proteins.
- Ubiquitin ligases tag the protein, and the proteasome then degrades it.
- Adaptive UPR = more chaperones + more proteasomal degradation + slowed translation. Failed UPR = **apoptosis**.
- The ubiquitin-proteasome pathway drives atrophy and cancer cachexia.
- Autophagy leaves residual bodies (lipofuscin = brown atrophy), whereas proteasomes degrade ubiquitinated proteins.
- Impaired proteostasis (chaperones, proteasome, autophagy) contributes to ageing and neurodegeneration.

10. Cellular Housekeeping

ASKED 1× · 5-mark answer
Asked in: Paper I · Dec 2020 · Q9(a)
Q: Cellular Housekeeping. [5]

SOURCE

- Robbins — ch. 1 The Cell as a Unit of Health and Disease — §CELLULAR HOUSEKEEPING; Intracellular Organelles; Plasma Membrane; Biosynthesis and Secretion; Waste Disposal — pp. 5–7, 12–13

Definition

- **Cellular housekeeping** means the fundamental functions that all differentiated cells must perform to maintain viability and normal activity.
- These functions include nutrient acquisition, metabolism, communication, movement, renewal of senescent molecules, molecular catabolism, energy generation and protection from environmental stressors.

Compartmentalization by organelles

- Many housekeeping functions are compartmentalized in membrane-bound organelles.
- Isolation keeps injurious degradative enzymes and toxic metabolites at usefully high concentrations without damaging delicate constituents.
- It also creates unique environments (e.g., low pH or high calcium) in which certain enzymes or pathways work more efficiently.
- **Biomolecular condensates** are dynamic, membraneless compartments formed by phase separation of proteins and nucleic acids into droplets. Examples are stress granules, P-bodies and nucleoli.

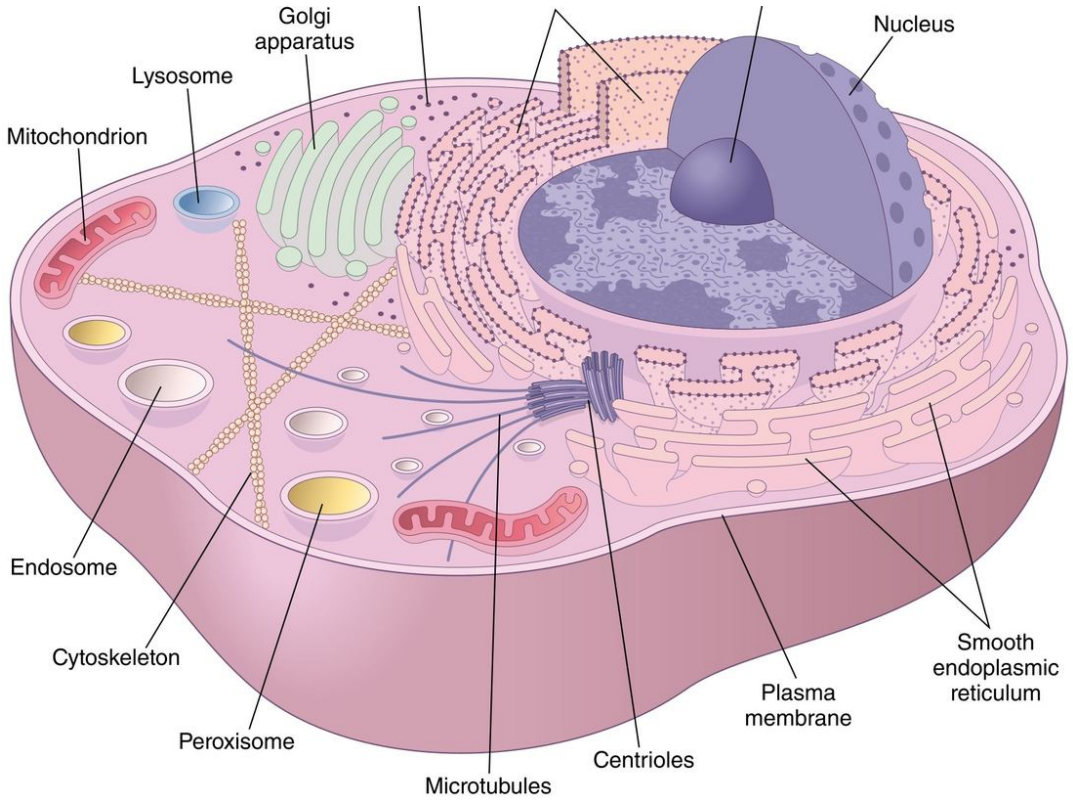


Fig. 1.6 Basic subcellular constituents of cells. ER, Endoplasmic reticulum. (Modified from Weibel ER, Stäubli — Robbins 11e, p. 7

Components and their housekeeping roles

Table 1: Housekeeping functions of the cell components

Component	Housekeeping role
Plasma membrane	Fluid bilayer of amphipathic phospholipids; barrier to passive diffusion of large or charged molecules
Mitochondria	Most ATP via oxidative phosphorylation; synthesis of heme; sensors of damage that initiate apoptosis; life span about 10 days
RER and Golgi	Assembly of proteins for the plasma membrane or secretion
Free ribosomes	Synthesis of cytosolic proteins
SER	Steroid hormone and lipoprotein synthesis; converts hydrophobic compounds into water-soluble ones
Proteasomes	Degrade denatured or "tagged" cytosolic proteins
Lysosomes	Digest proteins, polysaccharides, lipids and nucleic acids; autophagy; kill phagocytosed microbes
Peroxisomes	Catalase, peroxidase and oxidative enzymes; break down very long-chain fatty acids, generating H2O2
Cytoskeleton	Movement, cell shape, intracellular organization and polarity

Plasma membrane lipids

- **Phosphatidylinositol** in the inner leaflet can be phosphorylated to act as a scaffold. Phospholipase C hydrolyses it to diacylglycerol and inositol trisphosphate.
- **Phosphatidylserine** is normally on the inner face. When flipped outward it is an "eat me" signal in apoptosis. In platelets it is a clotting cofactor.
- **Glycolipids and sphingomyelin** lie on the extracellular face. They support inflammatory cell recruitment and sperm–egg fusion.

Biosynthesis and secretion

- The **ER** synthesizes all transmembrane proteins and lipids of the plasma membrane and organelles, and is the initial site of synthesis of secreted proteins.
- **RER**: signal sequences at the N-terminus direct nascent proteins into the ER lumen or membrane. Proteins without a signal sequence stay on free ribosomes in the cytosol.

- Folding in the ER involves disulfide bonds and N-linked oligosaccharides, assisted by chaperones.
- Misfolded proteins are retained and degraded. The example is CFTR in cystic fibrosis, with deletion of Phe508.
- Excess misfolded protein causes the **ER stress response (UPR)**. Protein synthesis falls and chaperones rise. Failure to correct the overload triggers apoptosis.
- **Golgi**: stacked cisternae modify proteins from cis (near ER) to trans (near plasma membrane). N-linked oligosaccharides are pruned and extended, and O-linked sugars are added.
- Mannose-6-phosphate sorts proteins to lysosomes. The trans-Golgi network dispatches proteins and lipids to organelles, the membrane or secretory vesicles.
- The Golgi is prominent in goblet cells. In plasma cells it forms the perinuclear hof.
- **SER** is conspicuous in steroid-synthesizing cells and in hepatocytes. Phenobarbital causes SER hyperplasia. SER sequesters calcium, and in muscle it forms the sarcoplasmic reticulum.
- **Unconventional secretion** includes IL-1β and α-synuclein. It also includes extracellular vesicles (exosomes, microvesicles), which are a source of liquid-biopsy biomarkers.

Cell junctions

- **Adherens junctions** link cadherins to actin. Loss of E-cadherin explains the discohesive pattern of gastric cancer and lobular breast carcinoma.
- **Desmosomes** link cadherins to intermediate filaments.
- **Hemidesmosomes** use integrins to link intermediate filaments to the ECM.
- **Gap junctions** are connexon pores of 1.5–2 nm. Permeability falls with low pH or raised calcium.

Catabolism: waste disposal

- **Lysosomes** hold about 40 acid hydrolases that function best at pH ≤5. The enzymes are tagged with M6P in the Golgi and delivered via M6P receptors.
- Three pathways deliver material to lysosomes:
  - Endocytosis, via early then late endosomes.
  - **Autophagy**, which uses Atg genes. A double membrane forms the autophagosome, which fuses with a lysosome. It also aids nutrient salvage in starvation.
  - Phagocytosis, in macrophages and neutrophils, forms a phagosome that fuses with lysosomes.
- **Proteasomes** degrade denatured or regulatory cytosolic proteins. These are tagged by multiple ubiquitins through E1, E2 and E3 ligases.

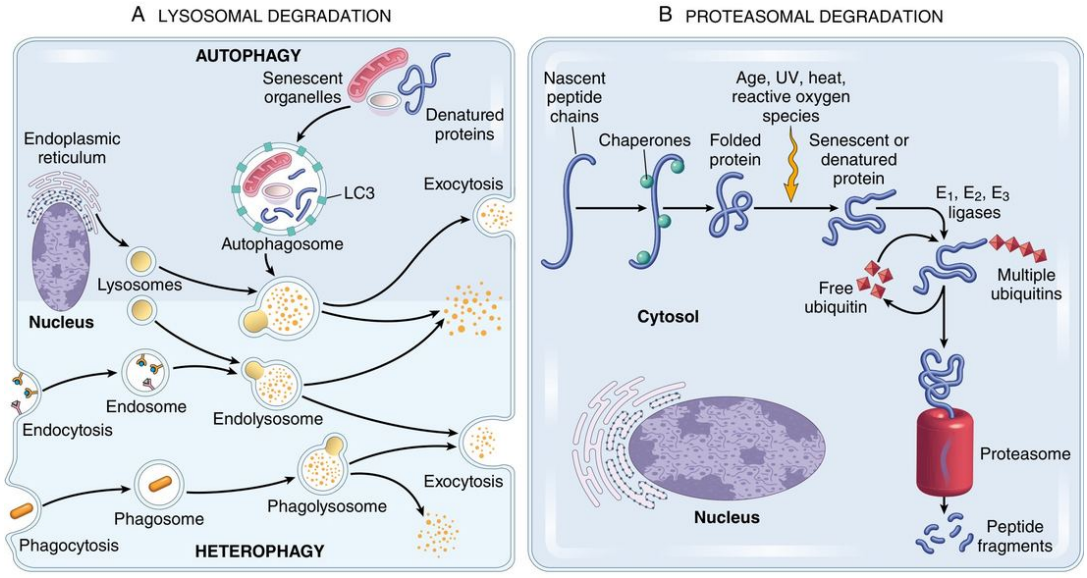


Fig. 1.10 Intracellular catabolism. (A) Lysosomal degradation. In autophagy (top of panel A), senescent organ- — Robbins 11e, p. 13

Regulation by non-coding RNA

- **miRNA**: Dicer trims pre-miRNA to mature miRNAs of 21–30 nucleotides, which enter RISC. A perfect match causes mRNA cleavage and an imperfect match causes translational repression.
- **lncRNAs** can activate or suppress genes. They also direct chromatin modification and act as scaffolds for protein complexes.

DRAW IN THE EXAM

Labelled diagram of a cell showing organelles with their housekeeping functions (Fig. 1.6), plus intracellular catabolism by autophagy, heterophagy and the proteasome (Fig. 1.10).

PEARLS

- Housekeeping means the basic functions every differentiated cell needs to stay viable.
- Compartmentalization keeps degradative enzymes and toxic metabolites away from delicate cell parts.
- The lysosome works at pH ≤5 and is tagged by mannose-6-phosphate. The proteasome degrades ubiquitinated cytosolic proteins.
- CFTR Phe508 deletion is the classic misfolding example. It causes ER retention and then the UPR.
- Phosphatidylserine flipped to the outer leaflet is an "eat me" signal.
- Mitochondria produce most ATP and last about 10 days. Loss of E-cadherin gives discohesive invasion.

11. Cell-cell interactions

ASKED 1× · 5-mark answer

Asked in: Paper I · Jun 2021 · Q5(b)

Q: Cell-cell interactions. [5]

SOURCE

- Robbins — ch. 1 The Cell as a Unit of Health and Disease — §Cell-Cell Interactions — pp. 11–12

Definition and types of cell-cell junctions

- Cells connect and communicate through **junctional complexes** that form mechanical links and facilitate receptor-ligand interactions; similar complexes mediate interaction with the ECM.
- Three basic types: **occluding (tight) junctions**, **anchoring junctions** (adherens junctions, desmosomes, hemidesmosomes), and **communicating (gap) junctions**.

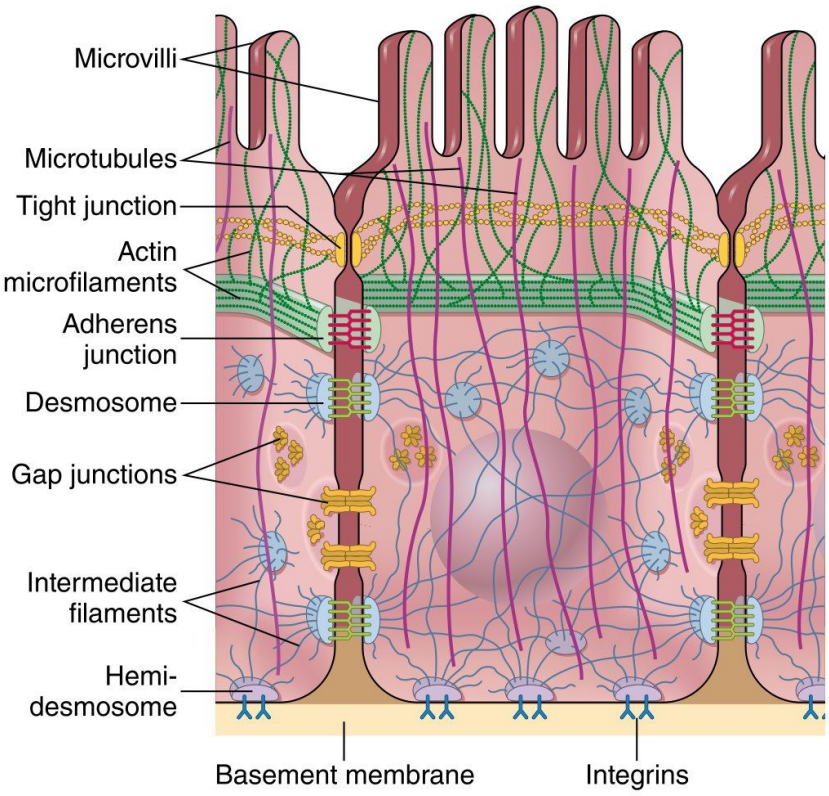


Fig. 1.9 *Cytoskeletal elements and cell-cell interactions. Interepithelial — Robbins 11e, p. 11*

Occluding (tight) junctions

- Seal adjacent epithelial cells into a continuous barrier that restricts **paracellular** movement of ions and other molecules.
- Form a tight meshlike network of contacts between neighbouring cells when viewed en face by freeze-fracture EM.
- Transmembrane proteins: **claudins** and tight junction–associated MARVEL proteins (TAMP).
- Intracellular adaptors and scaffolds: **ZO-1, ZO-2, ZO-3** (zonula occludens family) and **cingulin**.
- Functions:
  - Form a selectively permeable barrier sealing the paracellular space.
  - Mark the boundary between **apical and basolateral** membrane domains, maintaining cell polarity.
  - They are dynamic and can be modified to allow epithelial healing and inflammatory cell migration across mucosal surfaces.

Anchoring junctions

- Mechanically attach cells, and their cytoskeletons, to other cells or to the ECM.
- **Adherens junctions** lie close to and beneath tight junctions. **Desmosomes** are more basal. Desmosomes attaching a cell to the ECM are called **hemidesmosomes**.
- Adherens junctions and desmosomes are formed by homotypic extracellular interactions between transmembrane glycoproteins called **cadherins** on adjacent cells.
- **Adherens junctions:**
  - Cadherins are associated with intracellular **actin microfilaments**, so they can influence cell shape and motility.
  - Loss of **E-cadherin** explains the discohesive invasion pattern of some gastric cancers and lobular carcinomas of the breast.
- **Desmosomes:**
  - Cadherins are linked to intracellular **intermediate filaments**. This lets extracellular forces be communicated and dissipated over multiple cells.
- **Hemidesmosomes:**
  - Transmembrane connectors are **integrins**, which attach to intermediate filaments and link the cytoskeleton to the ECM.

  - Focal adhesion complexes (>100 proteins) localize here. They generate intracellular signals under shear stress, e.g. in endothelium or in cardiac myocytes of a failing heart.
- Intermediate filaments (10 nm) link desmosomes and hemidesmosomes, giving tensile strength to epithelia.

Communicating (gap) junctions

- Permit diffusion of chemical or electrical signals from one cell to another.
- Structure: a dense planar array of **1.5–2 nm pores (connexons)**. Each connexon is a hexamer of transmembrane **connexin** proteins, and a pair of hexamers (one from each cell) forms the pore.
- Pores pass ions, nucleotides, sugars, amino acids, vitamins and other small molecules.
- Permeability falls rapidly with **lowered intracellular pH** or **increased intracellular calcium**.
- They do not impart structural integrity; they serve only cell-cell communication.
- Example: in cardiac myocytes they allow cell-to-cell calcium flux, so the myocardium acts as a **functional syncytium** with coordinated contraction.

Comparison of the junction types

Table 1: Cell-cell junctions

Junction	Key proteins	Cytoskeletal link	Function
Tight (occluding)	Claudins, TAMP, ZO-1/2/3, cingulin	Not stated	Paracellular barrier, polarity
Adherens	Cadherins (E-cadherin)	Actin microfilaments	Cell-cell adhesion, shape/motility
Desmosome	Cadherins	Intermediate filaments	Mechanical strength
Hemidesmosome	Integrins	Intermediate filaments	Cell-ECM attachment
Gap	Connexins (connexons)	None	Passage of small molecules and ions

DRAW IN THE EXAM

Labelled diagram of an epithelial cell pair showing tight junction, adherens junction, desmosome, hemidesmosome and gap junction with their cytoskeletal links.

PEARLS

- Three junction classes: occluding, anchoring, communicating.
- Tight junctions: claudins plus ZO proteins. They maintain apical-basolateral polarity.
- Cadherins form adherens junctions (actin) and desmosomes (intermediate filaments).
- Integrins form hemidesmosomes and attach the cell to the ECM.
- Loss of E-cadherin gives discohesive growth in lobular breast carcinoma and some gastric cancers.
- Gap junctions: connexin hexamers (connexons). Closed by low pH or high Ca^{2+}.

12. Describe the role of tensins in health and diseases

ASKED 1× · 5-mark answer

Asked in: Paper I · Dec 2021 · Q6(a)

Q: Describe the role of tensins in health and diseases. [5]

SOURCE

- Robbins — ch. 1 The Cell as a Unit of Health and Disease — §Components of the Extracellular Matrix; §Proliferation and the Cell Cycle — pp. 22, 24
- W1 · PMC (MDPI review) — Tensins in Cancer: Integration of Their Domain Functions, Context-Dependent Regulation and Biomarker Potential — 2025
- W2 · PMC — The role of tensins in malignant neoplasms
- W3 · J Cell Mol Med (PMC) — The molecular and clinical role of Tensin 1/2/3 in cancer — 2023
- W4 · PMC — Molecules in focus: C-terminal tensin-like (CTEN): a promising biomarker and target for cancer
- W5 · PMC — Down-regulation of tensin2 enhances tumorigenicity and is associated with a variety of cancers
- W6 · PMC — The tensin-3 protein, including its SH2 domain, is phosphorylated by Src and contributes to tumorigenesis and metastasis
- W7 · PMC — Tensin4 is up-regulated by EGF-induced ERK1/2 activity and promotes cell proliferation and migration in hepatocellular carcinoma

NOT IN THE REFERENCE BOOKS

Searched: the supplied Robbins 11e pages on growth factors, extracellular matrix, integrins, focal adhesions and the cell cycle (ROB pr.19–24). Tensin appears there only as a named component of focal adhesion complexes. The structure, functions and disease roles below come from PubMed Central review articles.

Definition and family

- Tensins are **focal adhesion adaptor proteins** that bridge the extracellular matrix (ECM) and the intracellular actin skeleton.
- The family has **four members, tensin-1 to -4**. They are widely expressed in normal and cancerous tissues.
- Tensin-4 is also called **CTEN (C-terminal tensin-like)**.
- Focal adhesion complexes contain linking molecules such as vinculin, talin and tensin. Integrins connect the ECM to the cytoskeleton at these sites.
- Integrins are transmembrane α–β heterodimers that attach to laminin and fibronectin through the RGD motif.

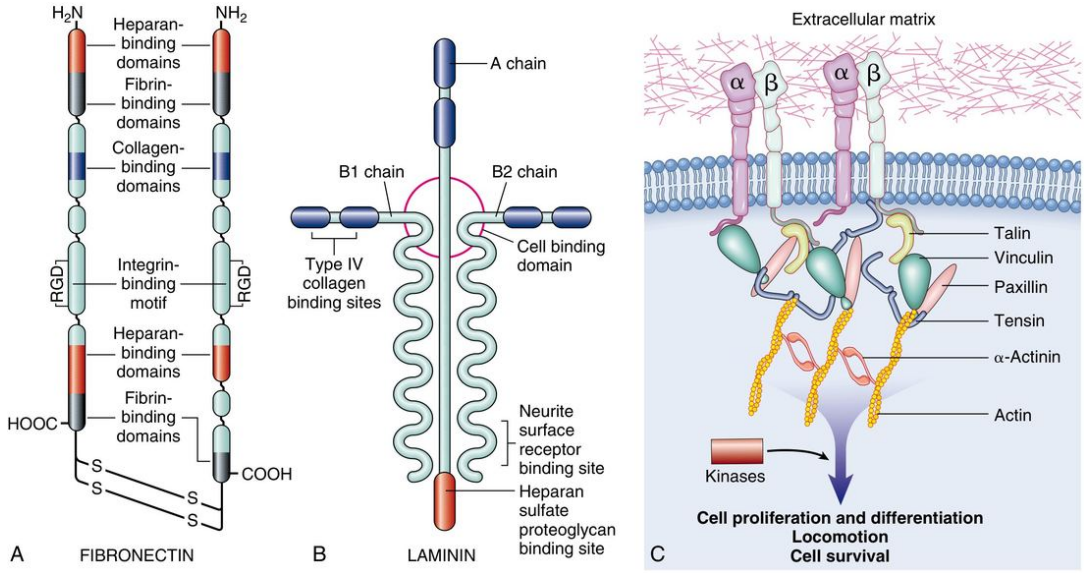


Fig. 1.18 Cell and extracellular matrix (ECM) interactions: adhesive glycoproteins and integrin signaling. — Robbins 11e, p. 24

Structure

- All four tensins localise to focal adhesions through **focal adhesion binding (FAB) domains**.
- **TNS1–3** also have an N-terminal **actin-binding domain (ABD)**, which TNS4 lacks.
- The N-terminal FAB domain of TNS1–3 lies within the ABD and includes conserved **PTP and C2 domains**.

- The **C-terminal SH2–PTB tandem** is conserved in all four tensins.
  - The **PTB domain** binds the NPXY sites of β-integrin.
  - The **SH2 domain** binds partners such as DLC1 and phosphorylated c-Cbl.
  - The SH2–PTB tandem scaffolds integrin binding and interaction with EGFR, MET, Axl, Src, FAK and p130Cas.
- TNS2 has an additional **C1 region**, which the other tensins lack.
- CTEN is shorter than the others and has no PTP domain.

Table 1: Tensin domains and their function

Domain	Present in	Function
N-terminal ABD / FAB	TNS1–3	Actin linkage, mechanotransduction
PTP, C2	TNS1–3	Part of the N-terminal FAB region
C1	TNS2 only	Unique region
SH2	All four	Binds DLC1, c-Cbl, phosphoproteins
PTB	All four	Binds β-integrin NPXY motif

Role in health (normal function)

- Tensins act as a **structural link** between ECM-bound integrins and the actin cytoskeleton.
- They have a **signal transduction role** as well as a structural one.
- They regulate fundamental processes through focal adhesion dynamics: **cell adhesion, migration/invasion, proliferation and mechanotransduction**.
- Focal adhesions organise the cytoskeletal network. They transmit signals for adhesion, migration, proliferation, differentiation and tissue development.
- Tensins maintain cytoskeletal structure. They interact with **FAK, p130Cas and PI3K** and regulate **Rho GTPase, EGFR and c-Met** signalling.
- Focal adhesion proteins are involved in normal events such as adhesion, movement, cytoskeletal structure and intracellular signalling.
- TNS2 is critical for cells to contract collagen gels, and it regulates proliferation and migration.
- CTEN has roles in cell motility, apoptosis and growth factor receptor homeostasis.
  - CTEN reduces ligand-induced EGFR degradation by binding c-Cbl.
  - Caspase-3 cleaves CTEN between the SH2 and PTB domains. The PTB fragment promotes apoptosis.

Role in disease

- **General principle:** dysregulated focal adhesions are one mechanism by which cancer cells metastasise.
- The tensin effect is **tumour-type specific**, and a clear oncogenic mechanism is not established.
- Dysregulated focal adhesion proteins contribute to adverse tumour behaviour.

Tensin-wise involvement in cancer

- **TNS2:** it acts mainly as a **tumour suppressor**. Down-regulation enhances tumorigenicity and is associated with a variety of cancers.
- **TNS1–3:** an oncogenic role is associated with **colorectal carcinoma and pancreatic ductal adenocarcinoma**, which may be clinically relevant.
- **TNS3:** it is phosphorylated by Src, including at its SH2 domain, and it contributes to tumorigenesis and metastasis.
  - Tensin-3 siRNA caused a 5-fold fall in Src-induced foci.
  - Phosphorylated p130Cas co-localises with tensin-3 at focal adhesions.
- **TNS4 (CTEN):** it lacks the N-terminal domains, so it amplifies signal transduction at focal adhesions. This is a mechanism often dysregulated in cancer.
  - CTEN may serve as a biomarker and target in cancer.
  - In hepatocellular carcinoma, tensin-4 is up-regulated by EGF-induced ERK1/2 activity. It promotes proliferation and migration, probably as a downstream effector of Ras/MAPK signalling.

Wider relevance

- Tensins are proposed to have **diagnostic, prognostic and potential therapeutic** roles.
- Disordered tensin function disturbs adhesion, migration, proliferation and mechanotransduction, which drives tumour progression and metastasis.

PEARLS

- Tensins are **focal adhesion adaptors** linking β-integrin to actin. The four members are TNS1, TNS2, TNS3 and TNS4 (CTEN).
- Domains: **SH2–PTB** at the C-terminus in all four. **ABD** at the N-terminus in TNS1–3 only. CTEN lacks the PTP domain.
- **TNS2 is a tumour suppressor** (down-regulated in cancers). **CTEN and TNS3 are mainly pro-oncogenic**.
- TNS1–3 are linked to colorectal and pancreatic ductal carcinoma. CTEN is linked to hepatocellular carcinoma.
- Tensin partners: FAK, p130Cas, Src, EGFR, MET, DLC1 and c-Cbl.
- Exam trap: the role of tensins in cancer is **context- and tumour-type dependent**, so do not call them uniformly oncogenic.

13. Cell cycle regulators & cyclin-dependent kinase inhibitors (CDKIs)

ASKED 2× · 5-mark answer

Asked in: Paper I · Dec 2020 · Q9(b) · Paper I · Dec 2025 · Q8(a)

Q: Cyclin Dependent Kinase Inhibitors (CDKIs). [5]

Q: Cell cycle inhibitors. [5]

SOURCE

- Robbins — ch. 7 Neoplasia — §Transcription Factors; §TP53: Guardian of the Genome; §Other Tumor Suppressor Genes — pp. 257–266

Definition

- **CDK inhibitors (CDKIs)** are proteins that bind cyclin–CDK complexes and silence them, exerting negative control over the cell cycle.
- Cyclins activate CDKs, whereas CDKIs inhibit them. Mitogenic signalling downregulates CDKI expression, so the cell cycle can progress.
- Loss or silencing of CDKIs is common in human malignancy. They act as tumour suppressors.

Classification and function of cell cycle inhibitors

Table 1: Cell cycle inhibitors frequently mutated in cancer

Family / protein	Target and action	Regulator / significance
CIP/KIP: p21 (CDKN1A)	Binds cyclin–CDK complexes and blocks the cell cycle	Induced by p53
CIP/KIP: p27 (CDKN1B)	Binds cyclin–CDK complexes and blocks the cell cycle	Responds to growth suppressors such as TGF-β
INK4/ARF: p16/INK4a (CDKN2A)	Binds cyclin D–CDK4 and promotes the inhibitory effect of RB	Reinforces the G1/S checkpoint
INK4/ARF: p14/ARF (CDKN2A)	Inhibits MDM2, so p53 levels rise	Activates the p53 pathway
RB (checkpoint component)	Binds E2F when hypophosphorylated and prevents G1/S transition	Governor of the cell cycle
p53 (checkpoint component)	Causes arrest by upregulating p21; induces apoptosis via BAX	Mutated in the majority of cancers

- The CDKN2A gene encodes two products, **p16/INK4a** and **p14/ARF**.

Mechanism of action

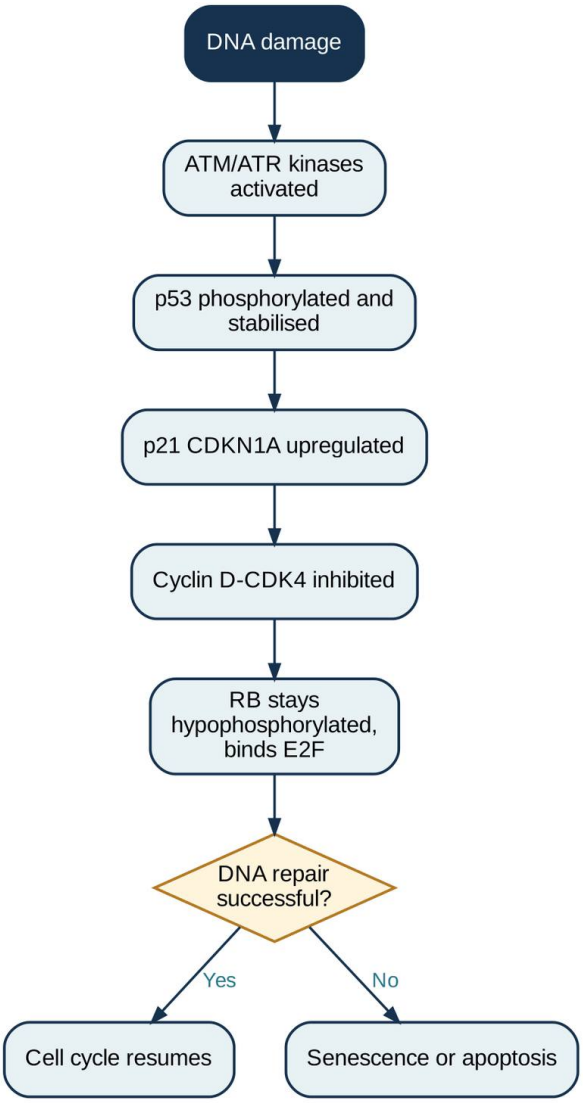
G1/S checkpoint

- The cyclin D–CDK4 complex phosphorylates RB, letting the cell pass the G1 restriction point.
- **Hypophosphorylated RB** binds and inhibits E2F transcription factors, which are needed for G1/S passage.
- Normal growth-factor signalling hyperphosphorylates and inactivates RB, so the cell cycle progresses.
- p16 and p21 inhibit cyclin D–CDK4 complexes, which keeps RB active and hypophosphorylated.

p53–p21 axis

- DNA damage or hypoxia activates ATM/ATR kinases. These phosphorylate p53 and MDM2, so p53 accumulates.
- p53 transcribes **CDKN1A (p21)**, which causes G1 arrest late in G1.
- The pause allows DNA repair, helped by proteins such as GADD45.
- If repair fails, sustained p53 triggers senescence or apoptosis.

Flowchart 1: p53–p21 mediated G1 arrest



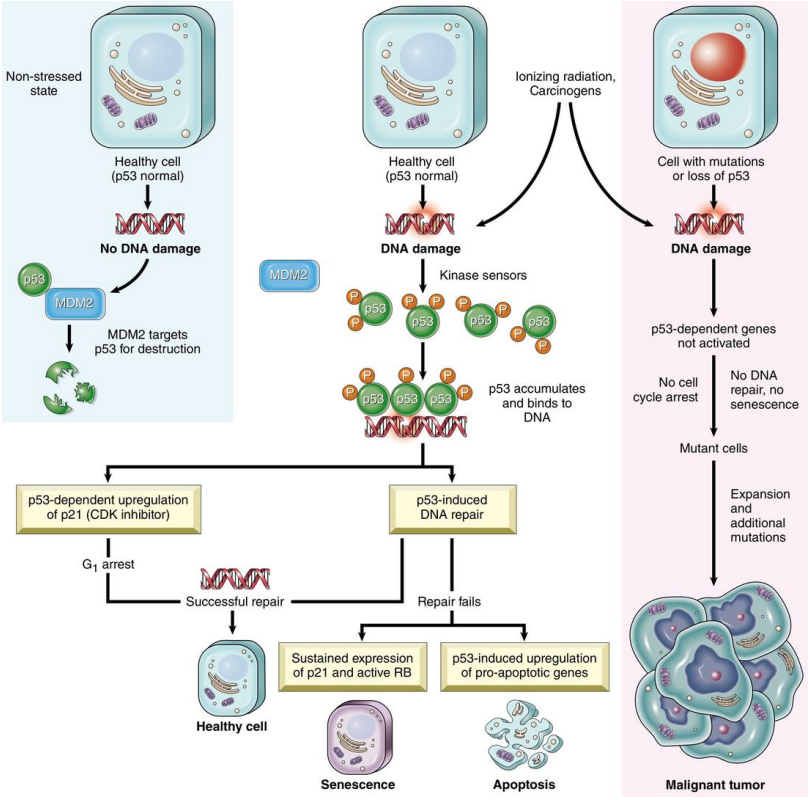


Fig. 7.27 The role of p53 in maintaining the integrity of the genome. Levels of p53 protein are low in non- — Robbins 11e, p. 263

Alterations of CDKIs in cancer

- Loss-of-function mutations or silencing of CDKIs, which normally inhibit G1/S progression, occur in many malignancies.
- Germline **CDKN2A** mutations are associated with familial melanoma.
- Sporadic CDKN2A mutations occur in bladder cancer, head and neck tumours, acute lymphoblastic leukaemia and cholangiocarcinoma.
- In some tumours, such as **cervical cancer**, p16 is silenced by hypermethylation rather than mutation.
- The p16/cyclin D/CDK4/RB pathway is dysregulated in most human cancers. RB may be functionally inactivated even if the RB gene is intact.
- Gain-of-function mutations of D cyclins and CDK4 act as oncogenes. CDK4 is amplified in melanomas, sarcomas and aggressive glial tumours.
- CDK4/CDK6 kinase inhibitors are effective in certain breast cancers.
- Viral oncoproteins bypass the pathway. The HPV E7 protein and polyomavirus large T antigen bind hypophosphorylated RB and release E2F.
- HPV E6 and MDM2 amplification (33% of sarcomas) degrade p53, which removes p21 induction.
- In the TGF-β pathway, loss of p21 function or persistent MYC expression abolishes TGF-β growth inhibition.

DRAW IN THE EXAM

Cell cycle diagram with cyclin D–CDK4 and cyclin E–CDK2 driving G1/S, and p16, p21 and p27 blocking them, with RB–E2F.

PEARLS

- CDKIs bind cyclin–CDK complexes and are negative regulators. The two families are **CIP/KIP (p21, p27)** and **INK4/ARF (CDKN2A–D)**.
- p21 is a p53 target, p27 responds to TGF-β, and p16 specifically inhibits cyclin D–CDK4.
- **CDKN2A** encodes both p16/INK4a and p14/ARF, so one locus affects both the RB and p53 pathways.
- Hypophosphorylated RB binds E2F and blocks G1/S. HPV E7 inactivates RB and E6 degrades p53.
- p16 is silenced by hypermethylation in cervical cancer.
- Germline CDKN2A mutation is linked to familial melanoma.

14. Radiation injury — mechanism of cell death & systemic changes

ASKED 2× · 5-mark answer

Asked in: Paper I · Dec 2022 · Q2(a) · Paper I · Jun 2025 · Q9(a)

Q: Mechanism of cell death in ionizing radiation. [5]
Q: Radiation induced systemic changes. [5]

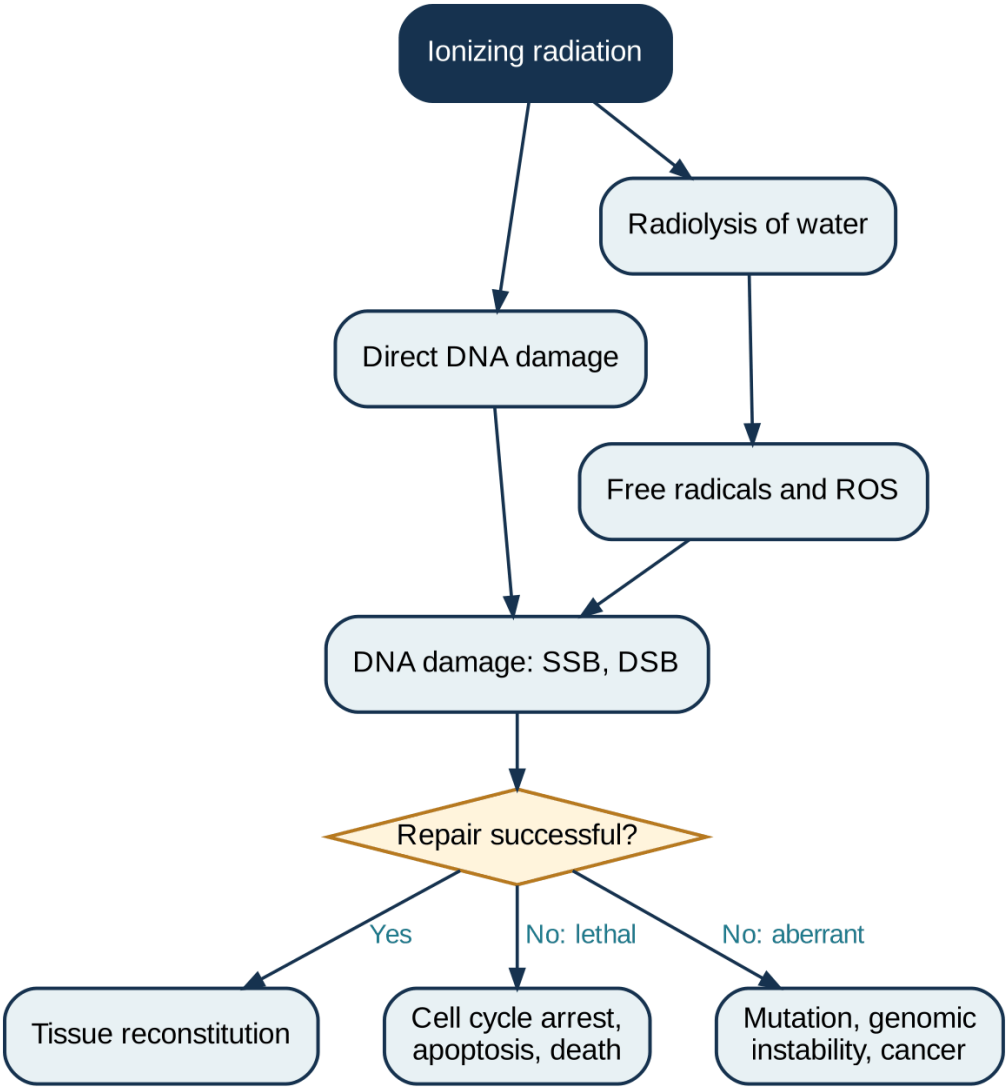
SOURCE

- Robbins — ch. 9 Environmental and Nutritional Diseases — §Radiation Units; Total-Body Irradiation; Fibrosis; Cancer Risks From Exposures to Radiation — pp. 391–394

Mechanism of cell death in ionizing radiation

- **Ionization**: collision of free electrons with other molecules releases more electrons in a cascade; sources are x-rays, gamma rays, neutrons, alpha and beta particles.
- **Direct effect**: radiation damages DNA directly.
- **Indirect effect** (the more prominent route): radiolysis of water produces free radicals and ROS, which are the major mechanism of DNA damage.
- **Oxygen effect**: oxygen enhances the damage. Hypoxic tissue, such as the centre of a rapidly growing tumour, is less radiosensitive.
- **DNA lesions**: single-base damage, single- and double-strand breaks (DSBs), and DNA-protein crosslinks.
- **DSBs** are the most serious lesion. They are repaired by homologous recombination or non-homologous end joining (NHEJ), and NHEJ is the commoner pathway.
- **Outcomes of DNA damage**:
  - Successful repair restores the tissue.
  - Inhibition of cell division and cell death.
  - Failed or aberrant repair leads to genomic instability and carcinogenesis, or to teratogenesis in the fetus or germ cells.
- NHEJ often produces short deletions or duplications, or translocations and inversions. Cells with DSBs that escape checkpoint control persist and may initiate cancer years later.
- **Cell death modes**: apoptosis, and nuclear pyknosis and lysis at extremely high doses.
- **Vascular route**: endothelial injury causes ischaemia, and stem cell death and cytokine release promote fibrosis.

Flowchart 1: Mechanism of radiation-induced cell injury and death



Determinants of injury

- **Rate of delivery**: fractionated doses allow repair between exposures. Radiotherapy exploits the faster recovery of normal cells compared with tumour cells.
- **Field size**: small shielded fields tolerate high doses, whereas smaller doses to large fields may be lethal.
- **Cell proliferation**: rapidly dividing cells are more vulnerable. Non-dividing cells (neurons, muscle) are not killed except at extremely high doses.

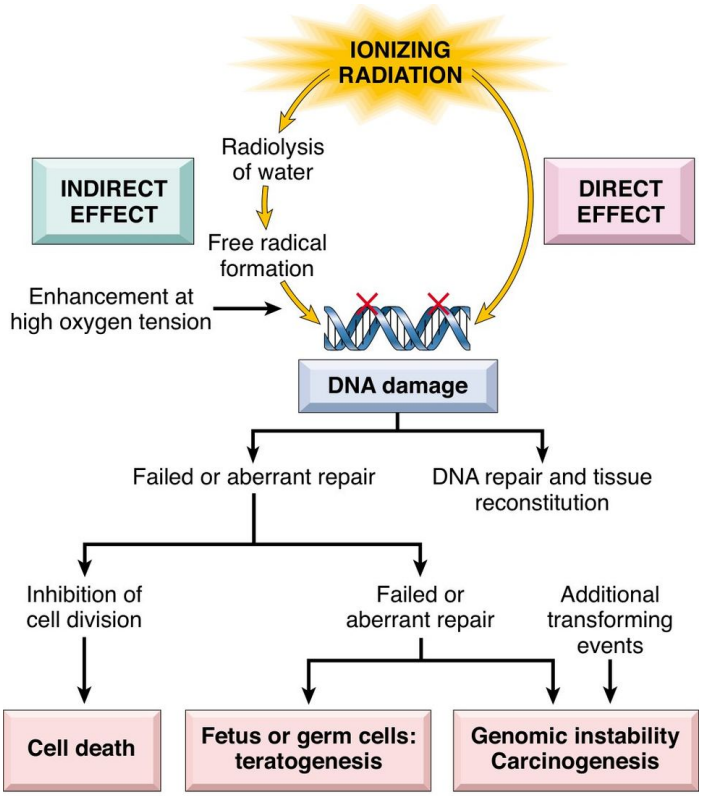


Fig. 9.15 Effects of ionizing radiation on DNA and downstream conse- — Robbins 11e, p. 391

Radiation-induced systemic changes

- **Radiosensitive tissues**: gonads, bone marrow, lymphoid tissue and GI mucosa show early injury.
- **Units**: 1 Gy is energy absorbed per unit mass. The Sievert is the equivalent dose, equal to Gy × relative biologic effectiveness.

Blood, bone marrow and lymphoid system

- **Lymphopenia** appears within hours, with shrinkage of nodes and spleen. Lymphocytes are killed directly, and sublethal doses allow recovery within weeks to months.
- **Marrow**: dose-dependent aplasia.
- **Neutrophils**: a brief rise, then neutropenia within days, with a nadir near zero in week 2. Recovery takes 2–3 months.
- **Platelets**: thrombocytopenia appears by the end of week 1. The nadir is later than that of granulocytes.
- **Anaemia** appears after 2–3 weeks.
- Very high doses kill stem cells and cause permanent aplastic anaemia.

Other organs

- **Skin**: early erythema and oedema, later dyspigmentation, then atrophy and cancer.
- **Lung**: oedema, ARDS and interstitial fibrosis.
- **GI tract**: early mucosal injury and ulceration, late fibrosis of the wall.
- **Gonads**: destruction of germ cells and granulosa cells, then atrophy and fibrosis.
- **Brain**: adult brain is resistant. The embryonic brain shows neuronal and glial destruction.
- **Fibrosis**: common in irradiated fields (lungs, salivary glands, pelvis). It results from vascular damage, stem cell death and cytokine release.
- **Vessels**: early dilation, then endothelial swelling and necrosis, then intimal hyaline thickening with luminal obliteration.
- **Cancer**: leukaemias and thyroid, breast and lung tumours. Second cancers (AML, MDS) follow radiotherapy.
- **Radon**: its alpha-emitting decay products cause lung carcinoma in uranium miners.

Table 1: Effects of total-body ionizing radiation

Dose	Main site	Features	Lethality
0–1 Sv	None	None	None
1–2 Sv	Lymphocytes	Moderate granulocytopenia, lymphopenia	None
2–10 Sv	Bone marrow	Leukopenia, haemorrhage, hair loss, vomiting	Variable (0–80%)
10–20 Sv	Small bowel	Diarrhoea, fever, electrolyte imbalance	100%
>50 Sv	Brain	Ataxia, coma, convulsions	100%

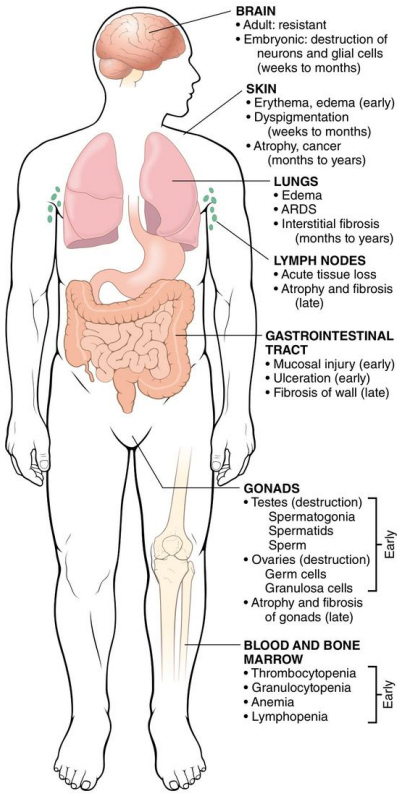


Fig. 9.16 Overview of the major morphologic consequences of radia- — Robbins 11e, p. 392

Cellular morphology

- Chromosomal deletions, translocations and fragmentation; polyploidy and aneuploidy.
- Giant cells with pleomorphic nuclei and abnormal mitoses may mimic tumour cells.

DRAW IN THE EXAM

Flowchart of direct and indirect DNA damage by ionizing radiation, with the downstream outcomes, plus a body outline listing organ-wise radiation effects.

PEARLS

- Free radicals from radiolysis of water are the main route of DNA damage. DSBs are the most serious lesion, repaired mainly by NHEJ.
- Rapidly dividing tissues (marrow, gut mucosa, gonads, lymphoid) are the most radiosensitive. Hypoxic tissue is less sensitive.
- Lymphopenia is the earliest haematological change. The neutrophil nadir is in week 2, and anaemia appears after 2–3 weeks.
- Total-body dose 2–10 Sv causes marrow syndrome, 10–20 Sv GI syndrome, and >50 Sv CNS syndrome.
- Irradiated cells show pleomorphism and giant forms that mimic cancer cells. Late vascular sclerosis leads to fibrosis.

15. Intermediate filament proteins — types, diseases, role in determining cell of origin of tumours

ASKED 2× · 10-mark answer

Asked in: Paper I · Dec 2022 · Q5 · Paper I · May 2024 · Q5

Q: Types of intermediate filament proteins and associated diseases. [10]

Q: Discuss role of expression of intermediate filament proteins in determining cell of origin of poorly differentiated tumours. [10]

SOURCE

- Robbins — ch. 1 The Cell as a Unit of Health and Disease — §Cell-Cell Interactions — p. 11
- W1 · PMC — Skeletal and Cardiac Muscle Disorders Caused by Mutations in Genes Encoding Intermediate Filament Proteins
- W2 · PMC — Intermediate filaments: primary determinants of cell architecture and plasticity
- W3 · PMC — Type III Intermediate Filaments Desmin, GFAP, Vimentin, and Peripherin
- W4 · PMC — Dysfunctions of neuronal and glial intermediate filaments in disease
- W5 · PMC — Desminopathies: pathology and mechanisms
- W6 · PMC — Desminopathies: pathology and mechanisms (conclusion)
- W7 · PMC — Filaments and phenotypes: cellular roles and orphan effects associated with mutations in cytoplasmic intermediate filament proteins
- W8 · PMC — Skeletal and Cardiac Muscle Disorders Caused by Mutations in Genes Encoding Intermediate Filament Proteins (abstract)
- W9 · PMC — Immunophenotyping of Tumours
- W10 · PMC — Immunohistochemistry for Diagnosis of Metastatic Carcinomas of Unknown Primary Site
- W11 · PMC — An Algorithmic Immunohistochemical Approach to Define Tumor Type and Assign Site of Origin
- W12 · PubMed — Value of immunohistochemistry in confirming undifferentiated ovarian carcinomas — Rom J Morphol Embryol — 1999
- W13 · PubMed — Role of immunohistochemistry in the diagnosis of undifferentiated tumors involving the skin
- W14 · PMC — An Unusual Presentation of Desmoplastic Small Round Cell Tumour of the Abdomen
- W15 · PMC — Immunohistochemical analysis of undifferentiated and poorly-differentiated head and neck malignancies at a tertiary hospital in Nigeria

NOT IN THE REFERENCE BOOKS

The Robbins page supplied (ch. 1, Cell-Cell Interactions, pr.11) covers only the general classes and tissue distribution of intermediate filaments. It has nothing on associated diseases or on tumour immunohistochemistry. A web search was run on intermediate filament gene mutations and diseases, and on cytokeratin, vimentin, desmin and GFAP immunohistochemistry in undifferentiated tumours. The disease and tumour-marker points below come from PMC and PubMed sources.

Intermediate filament proteins: general features

- Intermediate filaments are **10-nm** fibrils forming a large, heterogeneous family. It includes keratins and nuclear lamins.
- They form **ropelike polymers** and do not usually reorganize actively, unlike actin and microtubules.
- This gives **tensile strength** so that cells can bear mechanical stress. In epithelia they link desmosomes and hemidesmosomes.
- Each protein has a **tissue-specific expression pattern**. This can be used to assign a cell of origin to poorly differentiated tumours.
- The family comprises about 70 genes, expressed in a cell- and tissue-specific manner.

Types of intermediate filament proteins and associated diseases

Table 1: Types of intermediate filament proteins, tissue distribution and associated diseases

Type	Proteins	Distribution	Associated diseases
I	Acidic keratins	Epithelia	Epidermal blistering diseases (mutations in epidermal keratins)
II	Basic keratins	Epithelia	Epidermal blistering diseases
III	Vimentin	Mesenchymal cells: fibroblasts, endothelium	–
III	Desmin	Muscle cells	Desminopathy: desmin-related myopathy and cardiomyopathy
III	GFAP	Glial cells	Alexander disease
III	Peripherin, syncoilin	Peripheral nervous system (peripherin)	–
IV	Neurofilaments	Neurons	Charcot–Marie–Tooth disease; inherited neuropathies
IV	Nestin, synemins, internexin	Neural and muscle cells	Synemin (SYNM) mutations: cardiomyopathy
V	Lamins	Nuclear lamina	Striated muscle disease, cardiomyopathy with conduction defects (CMD1A)
VI	Phakinin, filensin	Lens	–

- Acidic keratins are type I, basic keratins type II, and desmin, GFAP, peripherin, vimentin and syncoilin type III. Nestin, neurofilament, synemins and internexin are type IV, lamins type V, and lens-specific phakinin and filensin type VI.
- The intermediate filament gene family is one of the most highly mutated groups of related genes in the human genome. It accounts for at least **94 disease entities**.

Cytoplasmic proteins in detail

- **Cytokeratins**: epithelial cells express at least **30** distinct cytokeratins, in different lineages (e.g., lung vs. gastrointestinal epithelium).
- **Vimentin**: it marks mesenchymal cells such as fibroblasts and endothelium.
- **Desmin**: it forms the scaffold on which actin and myosin contract in muscle.
- **Neurofilaments**: they give neuronal axons both strength and rigidity.
- **GFAP**: it is the major intermediate filament protein of astrocytes.
  - Astrocytes also contain lower levels of nestin, vimentin and synemin.
- **Peripherin**: it is expressed along with neurofilaments in the peripheral nervous system.

Keratin diseases

- Mutations of the conserved inter-helical salt-bridge residues in epidermal keratins cause **blistering diseases**.
- Mutations at the homologous positions in desmin, GFAP and lamins also cause disease, according to where each protein is expressed.

Desminopathy

- Mutations in desmin lead to **muscular dystrophy**.
- About **1–2%** of patients with a dilated cardiomyopathy phenotype carry desmin mutations.
- Nearly **70** *DES* mutations are reported. Most lie in **coil 2B**, an important domain for filament assembly.
- Most mutants cannot form a normal desmin network. They form non-filament structures and **protein aggregates**.
- This causes loss of function of desmin and its binding partners, plus possible toxic effects of the aggregates.
- Symptoms of muscle weakness appear only after decades.

Alexander disease (GFAP)

- It is a progressive, fatal neurodegenerative **leukodystrophy**.
- It nearly always results from dominantly acting mutations in the coding region of *GFAP*.

- Most patients have first symptoms, such as seizures or developmental delay, before **2 years** of age.
- The mutations seem to cause **accumulation of GFAP protein**, which may be more harmful to astrocytes than the mutant protein itself.
- Mutant GFAP forms soluble oligomers that inhibit proteasome activity.

Neurofilament diseases

- Charcot–Marie–Tooth disease neurofilament mutations disrupt neurofilament assembly and **axonal transport**.

Nuclear lamins (type V)

- Lamins form the nuclear lamina, define nuclear shape and contribute to gene regulation.
- A-type lamins are encoded by *LMNA*, on chromosome 1q22. Lamins A and C are splice products.
- *LMNA* mutations are linked to striated muscle disorders, including **cardiomyopathy with conduction defects (CMD1A)**.
- In healthy muscle, desmin and lamin A/C take part in mechanosignalling, nuclear positioning and mitochondrial homeostasis.

Role of intermediate filament expression in determining cell of origin of poorly differentiated tumours

Principle

- Tumour cells usually retain the intermediate filament of their parent cell. This gives a broad lineage assignment even when morphology is undifferentiated.
- In poorly differentiated tumours, IHC can at least assign a broad category: **carcinoma** (epithelial markers), **sarcoma** (mesenchymal markers) or **lymphoma** (lymphoid markers).
- The first panel for an undifferentiated tumour is:
  - Pan-cytokeratin (AE1/AE3, KL1).
  - Lymphoid markers (CD45, or CD20 and CD3).
  - Melanocytic markers (S100, SOX10).

Table 2: Intermediate filament markers in assigning lineage

Marker	Normal lineage	Use in poorly differentiated tumours	Pitfall
Cytokeratin	Epithelium	Carcinoma; first-line broad-spectrum marker	Also in non-epithelial tumours (see Table 3)
Vimentin	Mesenchyme	Vimentin-negative tumour is unlikely to be sarcoma, lymphoma or melanoma	Non-specific; melanoma and Burkitt lymphoma are positive
Desmin	Muscle	Myogenic differentiation in undifferentiated sarcoma	Also in sarcomatoid carcinoma, with SMA >> desmin
GFAP	Glia	Astrocytic vs. non-astrocytic high-grade tumour, with CK/EMA	–
Neurofilament	Neurons	Neuronal differentiation	–

Cytokeratin

- Cytokeratin is the key marker for **carcinoma**.
- Its expression is **not restricted** to epithelial neoplasms.
- It is commonly expressed in some tumours with epithelial differentiation:
  - Synovial sarcoma.
  - Epithelioid sarcoma.
  - Desmoplastic small round cell tumour.
  - Chordoma.
  - Adamantinoma.
  - Myoepithelial carcinoma.
- Aberrant focal expression is reported in several tumours:

  - Angiosarcoma.
  - Epithelioid haemangioendothelioma.
  - Epithelioid leiomyosarcoma.
  - Meningioma.
  - Undifferentiated pleomorphic sarcoma.
  - Rhabdomyosarcoma.
  - Malignant rhabdoid tumour.
  - Peripheral nerve sheath tumour.
  - Clear cell sarcoma.
  - Plasmacytoma.
- Cytokeratin is a **broad-spectrum** panel marker, so it features in the small round cell, spindle cell and anaplastic panels (Table 3).
- Co-expression of cytokeratin and vimentin is possible. In one series of undifferentiated ovarian carcinomas, **30%** co-expressed both.
- Cytokeratin subtypes help with the site: the endometrial profile is ER+, PAX8+, vimentin+.
- In undifferentiated forms, CK20 may be only focally positive.

Vimentin

- Vimentin is a **non-specific** marker.
- A vimentin-negative tumour is unlikely to be a sarcoma, lymphoma or melanoma. The exception is alveolar soft part sarcoma.
- Melanoma and Burkitt lymphoma express vimentin, so its diagnostic utility is limited.
- In undifferentiated skin tumours, vimentin-positive tumours were subdivided with S-100 (melanoma), immunoglobulin (lymphoma) and other markers (mesenchymal tumours).

Desmin

- Undifferentiated sarcomas often show a degree of **myogenic differentiation**, so SMA and desmin help.
- Both can also be expressed by sarcomatoid carcinoma, with SMA >> desmin.
- A desmin-positive undifferentiated skin tumour was diagnosed as leiomyosarcoma.
- Desmoplastic small round cell tumour shows strong vimentin with patchy perinuclear desmin.
  - Intermediate filament whorls are seen by electron microscopy.
  - WT1 is positive in 91% and CD99 in only 23% of cases.

GFAP

- In high-grade pleomorphic CNS tumours, distinguishing an astrocytic tumour from a metastasis needs **GFAP** with epithelial markers (CK/EMA).

Table 3: Panel approach by morphological pattern in undifferentiated tumours

Pattern	Differential	Panel
Small round cell	Sarcoma, lymphoma, small cell carcinoma	CD99, NKX2.2, desmin, myogenin, CD45, TdT, chromogranin, synaptophysin, broad-spectrum keratin
Spindle cell	Sarcomatoid carcinoma, sarcoma, spindle cell/desmoplastic melanoma	Broad-spectrum keratin, p40, SMSA, desmin, SOX10
Anaplastic	Anything	Broad-spectrum keratin, CD45, SOX10

Workflow and pitfalls

- Check controls with each run: skin for cytokeratin, smooth muscle for desmin, skeletal muscle for vimentin.
- Do not use any single intermediate filament alone. Combine with lineage-specific markers (CD45, S100/SOX10, myogenin, CD99).
- In poorly differentiated neuroendocrine carcinoma, IHC has limited value for site of origin. TTF-1 and CK20 help separate visceral from Merkel cell (cutaneous) origin.

DRAW IN THE EXAM

Flowchart: undifferentiated tumour → pan-cytokeratin / CD45 / S100–SOX10 / vimentin / desmin / GFAP → carcinoma, lymphoma, melanoma, sarcoma or glial tumour.

PEARLS

- Intermediate filaments are **10 nm**. Their tissue-specific expression assigns the cell of origin: cytokeratin (epithelial), vimentin (mesenchymal), desmin (muscle), GFAP (glia), neurofilament (neurons), lamins (nuclear lamina).
- Acidic keratins are type I, basic keratins type II. Desmin, GFAP, vimentin and peripherin are type III. Neurofilaments are type IV, lamins type V.
- Key diseases: keratin gene mutations cause blistering; desmin mutations cause desminopathy and cardiomyopathy; GFAP mutations cause Alexander disease; neurofilament mutations cause CMT; *LMNA* mutations cause cardiomyopathy with conduction defects.
- Vimentin-negative tumour: unlikely to be sarcoma, lymphoma or melanoma (except alveolar soft part sarcoma).
- Cytokeratin positivity does not prove carcinoma. Synovial sarcoma, epithelioid sarcoma, desmoplastic small round cell tumour and chordoma are keratin-positive.
- Desmin is positive in myogenic tumours but also in sarcomatoid carcinoma; SMA is commoner than desmin in the latter.

16. Pigments in health & disease / endogenous pigment disorders

ASKED 2× · 10-mark answer

Asked in: Paper I · Jun 2022 · Q8 · Paper I · Oct 2023 · Q5

Q: Write briefly on pigments in health & diseases. [10]
Q: Endogenous pigment disorders. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Exogenous Pigments, Dystrophic Calcification — pp. 58–59
- Robbins — ch. 18 Liver and Gallbladder — §Wilson Disease, Bile Formation and Secretion, Pathophysiology of Hyperbilirubinemia, Physiologic Jaundice of the Newborn — pp. 773, 775–777

Classification of pigments

- **Pigments** are colored substances. Some are normal constituents of cells (e.g., melanin). Others are abnormal and accumulate only under special circumstances.
- **Exogenous** pigments come from outside the body. **Endogenous** pigments are synthesized within the body.
- Endogenous pigments in the notes below:
  - Lipofuscin
  - Melanin
  - Hemosiderin
  - Homogentisic acid (alkaptonuria)
  - Bilirubin and bile pigment

Exogenous pigments

- **Carbon (coal dust)** is the most common exogenous pigment. It is a ubiquitous urban air pollutant.
  - Inhaled carbon is picked up by alveolar macrophages.
  - The macrophages carry it through lymphatics to tracheobronchial lymph nodes.
  - The tissues of lung and draining nodes blacken, called **anthracosis**.

  - In coal miners, aggregates may induce a fibroblastic reaction or emphysema. This causes **coal worker's pneumoconiosis**.
- **Tattooing** injects indigestible pigments into the dermis.
  - Macrophages phagocytose the pigment and retain it for life.
  - There is usually no inflammatory response.

Endogenous pigments

Lipofuscin

- It is also called lipochrome or **wear-and-tear pigment**. It is insoluble.
- It is composed of polymers of lipids and phospholipids complexed with protein. This suggests origin from **lipid peroxidation** of polyunsaturated lipids of intracellular membranes.
- It is **not injurious** to the cell or its functions. Its importance is as a telltale sign of free radical injury and lipid peroxidation.
- Latin *fuscus* means brown.
- Microscopy: **yellow-brown, finely granular, often perinuclear** cytoplasmic pigment.
- Electron microscopy: perinuclear and **intralysosomal**.
- It is seen in cells undergoing slow, regressive change. It is prominent in the liver and heart of **aging** patients, and in severe malnutrition and cancer cachexia.

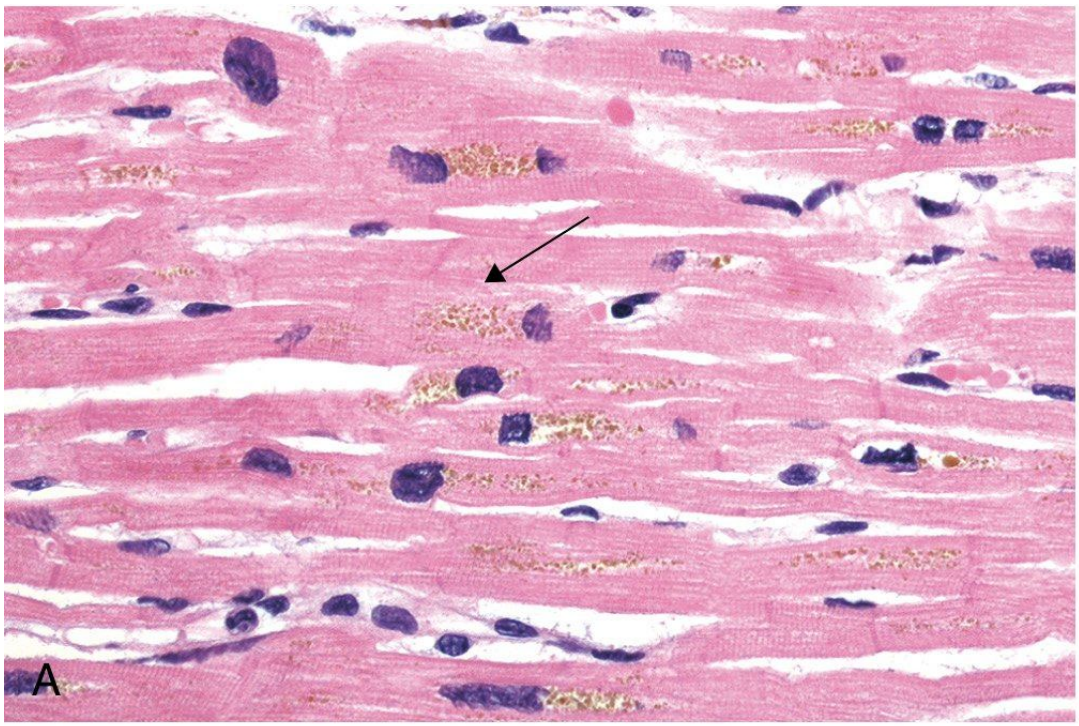


Fig. 2.33 Lipofuscin granules in cardiac myocytes. (A) Light microscopy (deposits indicated by arrow) and (B) — Robbins 11e, p. 59

Melanin

- It is a brown-black pigment.
- It forms when **tyrosinase** catalyzes oxidation of tyrosine to dihydroxyphenylalanine (DOPA) in melanocytes.
- For practical purposes, melanin is the **only endogenous brown-black pigment**.
- The only other candidate is homogentisic acid.
- Increased epidermal melanin production contributes to the skin pigmentation of hemochromatosis.
- Dubin-Johnson syndrome shows a melanin-like pigment in hepatocytes (see below).

Homogentisic acid (alkaptonuria)

- Homogentisic acid is a **black pigment** found only in patients with alkaptonuria, a rare metabolic disease.
- It is deposited in skin, connective tissue and cartilage. The pigmentation is called **ochronosis**.

Hemosiderin

- It is a hemoglobin-derived, **golden yellow-to-brown**, granular or crystalline pigment. It is one of the major storage forms of iron.
- Iron handling:
  - Iron is carried by the transport protein **transferrin**.
  - In cells it is stored with **apoferritin** as ferritin micelles.
  - With local or systemic iron excess, ferritin micelles aggregate to form **hemosiderin granules**, visible by light microscopy.
- Normal state: small amounts are seen in mononuclear phagocytes of bone marrow, spleen and liver. These cells recycle iron from effete red cells.
- **Local excess (localized hemosiderosis)** results from tissue hemorrhage. The best example is the common **bruise**.
  - Macrophages phagocytose extravasated red cells over several days.
  - Heme is converted to **biliverdin** ("green bile"), then to **bilirubin** ("red bile").
  - The released iron goes into ferritin and then hemosiderin.
  - The bruise colour therefore changes from red-blue to green-blue to golden-yellow.
- **Systemic overload** is called **hemosiderosis**, with deposition in many organs. Its main causes:
  - Increased dietary iron absorption due to hemochromatosis.
  - Hemolytic anemias.
  - Repeated blood transfusions, because transfused red cells are an exogenous iron load.
- Special stain: **Prussian blue** shows hepatocellular iron as blue.

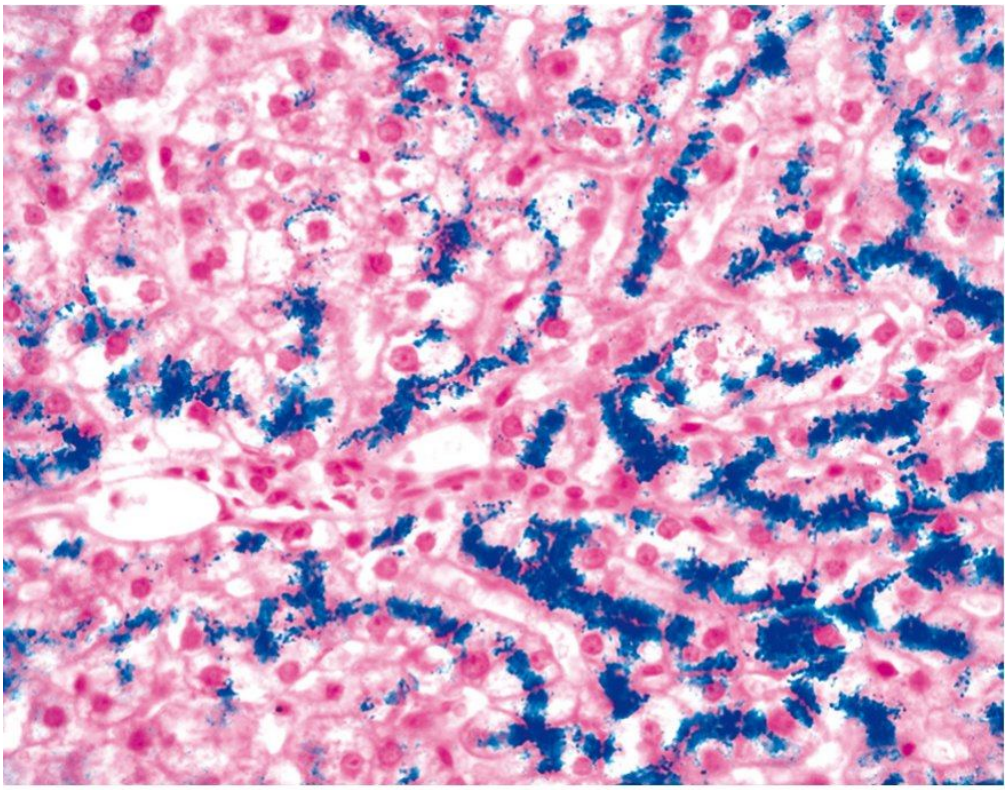


Fig. 18.25 Hereditary hemochromatosis. In this Prussian blue–stained — Robbins 11e, p. 773

Hereditary hemochromatosis (iron-pigment disorder)

- It is most commonly caused by loss-of-function mutations in **HFE**. The HFE product regulates intestinal iron uptake by increasing hepatic hepcidin synthesis.
- It is characterized by increased dietary iron absorption. Iron accumulates in the liver and pancreas, causing cirrhosis and diabetes.
- Morphology:
  - The liver is dark brown to nearly black from iron. In later stages it is small and shrunken with **micronodular cirrhosis**.

  - The pancreas is intensely pigmented, with atrophy and interstitial fibrosis.
  - The heart is enlarged and brown from hemosiderosis.
  - Skin shows **slate-gray** colour from combined iron and melanin pigments.
  - Joints may show synovitis. Calcium pyrophosphate deposition causes **pseudogout**.
  - The testes may be small and atrophic.
- Clinical: the classic **tetrad** is cirrhosis with hepatomegaly, skin pigmentation, diabetes mellitus and cardiac dysfunction. It may develop only late.
  - It is more often a disease of males, and rarely evident before 40 years.
  - The risk of **hepatocellular carcinoma** is 200-fold greater than in the general population.
- Diagnosis: serum iron measurements, confirmed by DNA sequencing for causative mutations.
  - Secondary causes of iron overload must be excluded.
  - Liver biopsy is not routine.
- Treatment: regular **phlebotomy** depletes iron stores, and life expectancy is then normal.
- Secondary (acquired) hemochromatosis:
  - It is commonly due to ineffective erythropoiesis, as in thalassemia and myelodysplastic syndrome.
  - In these, the excess iron comes from transfusions and increased absorption.
  - Repeated transfusions alone can also cause hemosiderosis.
  - Other forms of cirrhosis lower hepcidin and increase iron uptake.

Bilirubin (bile pigment)

- Bilirubin is a toxic end product of heme degradation. It is processed by the liver and excreted in bile.
- Origin and handling:
  - 0.2–0.3 g/day is produced. About **85%** comes from breakdown of effete red cells by macrophages.
  - The rest comes from turnover of hepatic heme proteins (e.g., P-450 cytochromes).
  - Heme is converted to bilirubin by phagocyte enzymes. In blood it binds **albumin**.
  - Hepatocytes take up bilirubin at the sinusoidal membrane and conjugate it with glucuronic acid in the endoplasmic reticulum.
  - The water-soluble bilirubin glucuronides are excreted into bile.
  - Gut bacterial β-glucuronidases deconjugate them to colorless **urobilinogens**.
  - About 20% of urobilinogens are reabsorbed and re-excreted in bile, and a little appears in urine.

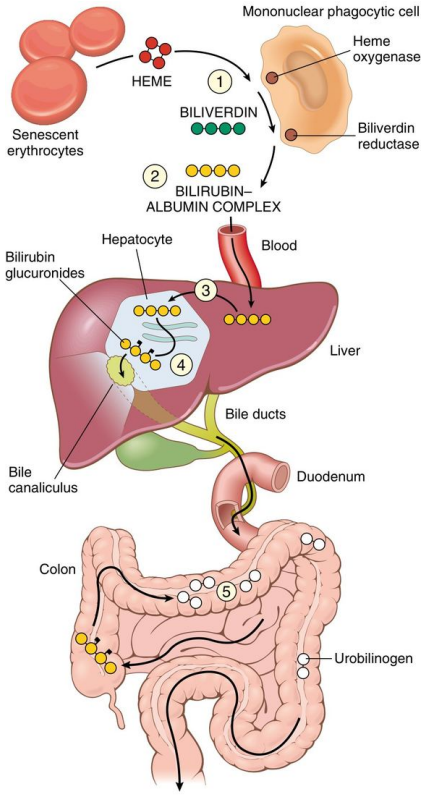


Fig. 18.28 Bilirubin metabolism and elimination. (1) Normal bilirubin pro- — Robbins 11e, p. 776

- Normal adult serum bilirubin is **0.3–1.2 mg/dL**. **Jaundice** becomes evident above **2 to 2.5 mg/dL**.
- Unconjugated hyperbilirubinemia:
  - It results from excess production or defective conjugation.
  - It is largely insoluble and is not excreted in urine.
  - At excessive levels the unbound fraction diffuses into brain in infants, causing **kernicterus**.
- Conjugated hyperbilirubinemia:
  - It results from hepatocellular disease, bile duct injury and biliary obstruction.
  - It is water soluble and loosely albumin-bound, so it appears in **urine**.

Table 1: Causes of jaundice

Type	Mechanism	Examples
Unconjugated	Excess production	Hemolytic anemias; internal hemorrhage resorption; ineffective erythropoiesis (pernicious anemia, thalassemia)
Unconjugated	Reduced hepatic uptake	Drug interference with carrier systems; some Gilbert syndrome
Unconjugated	Impaired conjugation	Physiologic newborn jaundice; breast milk jaundice; Crigler-Najjar I and II; Gilbert
Conjugated	Canalicular transporter deficiency	Dubin-Johnson; Rotor
Conjugated	Hepatocellular disease	Viral or drug hepatitis; cirrhosis
Conjugated	Impaired bile flow	Duct obstruction; autoimmune cholangiopathies

- **Physiologic jaundice of the newborn**:
  - UGT1A1 is low at birth and reaches adult levels only at 3–4 months.
  - Mild unconjugated hyperbilirubinemia is nearly universal in the first week.
  - Breastfeeding may worsen it.
  - Blue-light phototherapy converts bilirubin to a soluble isomer excreted in urine.
- **Hereditary hyperbilirubinemias**:
  - Crigler-Najjar type 1 is severe UGT1A1 deficiency and is fatal around birth.
  - Crigler-Najjar type 2 and Gilbert syndrome have residual UGT1A1 activity and a milder phenotype.

  - Dubin-Johnson and Rotor syndromes cause conjugated hyperbilirubinemia. Both are autosomal recessive and clinically innocuous.
  - Dubin-Johnson is due to **MRP2** mutation. It shows brown-black melanin-like pigment in hepatocytes, which can blacken the liver.
- **Cholestasis** morphology:
  - The hallmark is **green-brown bile plugs** in hepatocytes and dilated canaliculi.
  - Canalicular rupture extravasates bile, which Kupffer cells phagocytose.
  - Bile salt accumulation gives swollen, foamy cytoplasm ("**feathery degeneration**").
  - Hepatocytes are enlarged with dilated canaliculi, and apoptotic cells may be seen. Kupffer cells contain regurgitated bile pigment.
- Clinical features of cholestasis:
  - Jaundice and icterus, pruritus and xanthomas.
  - Fat-soluble vitamin (A, D, K) deficiency from malabsorption.
  - Raised **alkaline phosphatase and GGT**.
- Large duct obstruction:
  - Causes in adults: stones, malignancy of the biliary tree or pancreatic head, strictures.
  - Causes in children: biliary atresia, cystic fibrosis, choledochal cysts.
  - Persistent obstruction leads to fibrosis and **biliary cirrhosis**.
  - Biopsy shows portal edema, ductular reaction and neutrophils ("pericholangitis"), with Mallory hyaline in advanced disease.

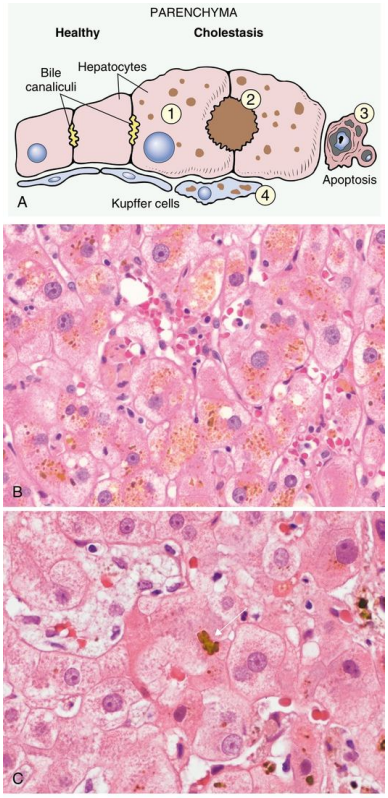


Fig. 18.29 *Cholestasis. (A) Morphologic features of cholestasis (right) — Robbins 11e, p. 777*

Pigment-like intracellular accumulations (differentiation from true pigments)

- **Glycogen** appears as clear vacuoles. It is stained rose-to-violet by Best carmine or PAS. It is proven by loss of staining after **diastase**.
- **PAS-positive, diastase-resistant** globules in hepatocytes characterize α1-antitrypsin deficiency.

Table 2: Comparison of endogenous pigments

Pigment	Colour	Origin	Special feature
Lipofuscin	Yellow-brown, perinuclear	Lipid peroxidation	Harmless; aging and atrophy
Melanin	Brown-black	Tyrosine via tyrosinase	Only endogenous brown-black pigment
Hemosiderin	Golden yellow-brown	Hemoglobin iron	Prussian blue positive
Homogentisic acid	Black	Alkaptonuria	Ochronosis
Bilirubin	Green-brown	Heme breakdown	Cholestasis, jaundice

DRAW IN THE EXAM

Flow diagram of bilirubin metabolism (heme → biliverdin → bilirubin-albumin → hepatocyte conjugation → bile → urobilinogen).

PEARLS

- Lipofuscin is the "wear-and-tear" pigment, a marker of lipid peroxidation, and is harmless.
- Melanin is the only endogenous brown-black pigment. Homogentisic acid (ochronosis) is the rare exception.
- Hemosiderin is ferritin aggregates, shown by Prussian blue. The bruise sequence is biliverdin → bilirubin → hemosiderin.
- Hemosiderosis causes: hemochromatosis, hemolytic anemia, repeated transfusions.
- Hemochromatosis: HFE mutation, slate-gray skin, tetrad, 200-fold HCC risk, phlebotomy treatment.
- Jaundice appears at a serum bilirubin above 2–2.5 mg/dL. Conjugated bilirubin appears in urine, unconjugated does not.
- Dubin-Johnson (MRP2) gives a black liver with conjugated hyperbilirubinemia.

17. Define apoptosis with examples. Discuss intrinsic pathway of apoptosis

ASKED 1× · 10-mark answer

Asked in: Paper I · Jun 2021 · Q10

Q: Define apoptosis with examples. Discuss intrinsic pathway of apoptosis. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Morphologic and Biochemical Changes in Apoptosis; §Mechanisms of Apoptosis; §Necroptosis — pp. 41–44

Definition of apoptosis

- **Apoptosis** is a regulated mechanism of cell death. It eliminates unwanted and irreparably damaged cells with the least possible host reaction.
- It is characterised by enzymatic degradation of proteins and DNA, initiated by **caspases**.
- Dead cells are recognised and removed by phagocytes.
- Caspases are proteases with a cysteine in the active site. They cleave proteins after aspartic residues.
- Caspases exist as inactive proenzymes and need enzymatic cleavage to become active. Active caspases are therefore a marker of apoptosis.
- Apoptosis is regulated by a balance between pro-apoptotic and anti-apoptotic proteins.
- Morphological hallmarks:
  - **Cell shrinkage** with dense eosinophilic cytoplasm. This contrasts with the early cell swelling of necrosis.
  - **Chromatin condensation**, the most characteristic feature. Chromatin aggregates peripherally under the nuclear membrane, and the nucleus may fragment.
  - **Membrane blebbing** followed by **apoptotic bodies**.

  - **Phagocytosis**, usually by macrophages.
- On H&E, the cell is a round or oval mass of intensely eosinophilic cytoplasm with dense nuclear chromatin fragments.
- There is no inflammatory response, so apoptosis can be hard to detect by light microscopy.

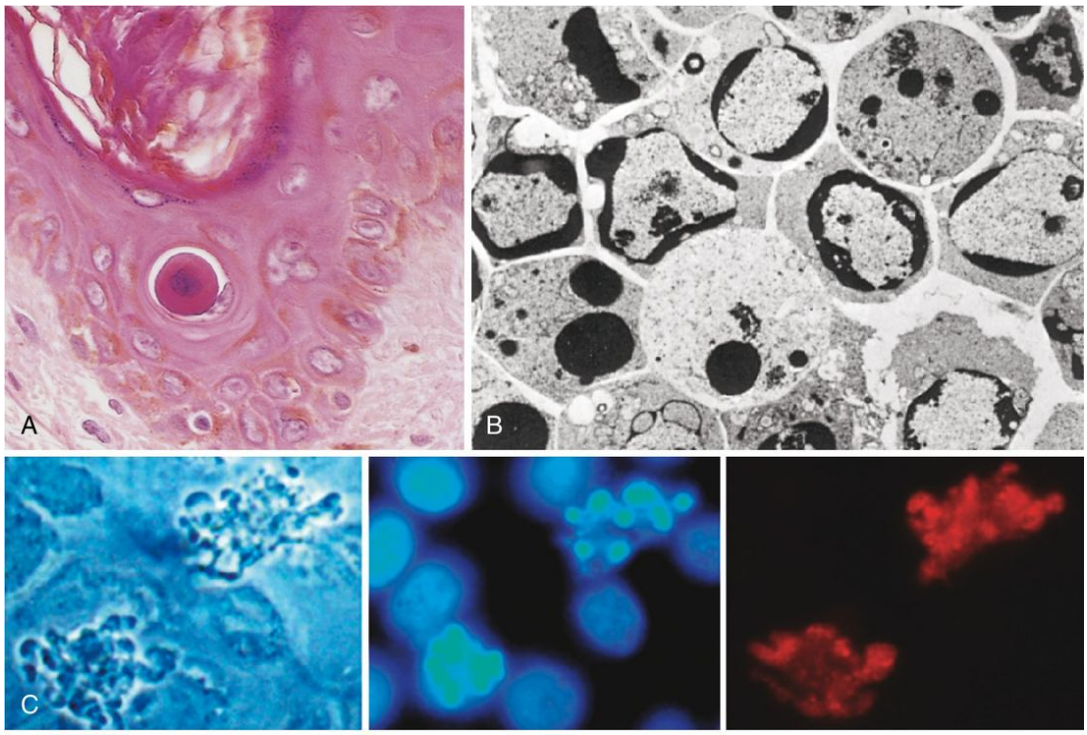


Fig. 2.15 Morphologic features of apoptosis. (A) Apoptosis of an epidermal cell. The cell is reduced in size and — Robbins 11e, p. 41

Examples of apoptosis

- **DNA damage.** If repair fails, the cell triggers intrinsic mechanisms that induce apoptosis. This protects against survival of cells with DNA mutations that could lead to malignant transformation.
- **Misfolded proteins.** Improperly folded intracellular proteins cause ER stress, which can end in cell death.
- **Viral infections:** apoptosis may be caused by the virus itself, as in adenovirus and HIV infection.
- **Host immune response to viruses:** apoptosis is caused by cytotoxic T lymphocytes (CTLs), as in viral hepatitis.
- **CTL-mediated apoptosis** also underlies:
  - killing of tumour cells
  - cellular rejection of transplants
  - tissue damage in graft-versus-host disease
- **Pathologic atrophy** of parenchymal organs after duct obstruction, for example pancreas, parotid and kidney.
- **Elimination of self-reactive lymphocytes** by Fas–FasL interaction.

Table 1: Apoptosis versus necrosis (as given in the text)

Feature	Apoptosis	Necrosis
Cell size	Shrinkage	Early swelling
Cytoplasm	Dense, eosinophilic	Swollen
Nucleus	Peripheral chromatin condensation, fragmentation	–
Inflammation	Absent or minimal	Present
Clearance	Apoptotic bodies phagocytosed	–

Intrinsic (mitochondrial) pathway of apoptosis

Overview

- This pathway is responsible for apoptosis in **most physiologic and pathologic situations**.

- It results from increased permeability of the **outer mitochondrial membrane**. Death-inducing (pro-apoptotic) molecules are released from the intermembrane space into the cytoplasm.
- **Cytochrome c** is essential for ATP production. When released into the cytoplasm, it initiates the suicide programme.
- Release is determined by the integrity of the outer mitochondrial membrane, which is controlled by the **BCL2 family**.
- BCL2 is named after a gene frequently overexpressed in certain B-cell lymphomas through chromosomal translocations.

Triggers

- Loss of survival signals or growth-factor withdrawal.
- DNA damage.
- ER stress from accumulated misfolded proteins.

BCL2 family (more than 20 members, three functional groups)

Table 2: BCL2 family groups in the intrinsic pathway

Group	Members	BH domains	Function
Anti-apoptotic	BCL2, BCL-XL, MCL1	BH1–4	Keep the outer membrane impermeable and prevent cytochrome c leak
Pro-apoptotic effectors	BAX, BAK	BH1–3	Oligomerise in the outer membrane and increase permeability
BH3-only initiators (sensors)	BAD, BIM, BID, Puma, Noxa	BH3 only	Activated by stress sensors and initiate apoptosis

- Anti-apoptotic proteins sit in the outer mitochondrial membrane, the cytosol and ER membranes.
- The precise mechanism by which BAX–BAK oligomers permeabilise membranes is not settled. One model proposes a channel for cytochrome c leakage.

Sequence of events

- **Survival signals.** Growth factors and other survival signals stimulate production of anti-apoptotic proteins such as BCL2, which protects against apoptosis.
- **Upregulation of BH3-only proteins.** With survival-signal deprivation, DNA damage or ER stress, these proteins rise through increased transcription or post-translational modification such as phosphorylation.
- **Activation of BAX and BAK.** BH3-only proteins directly activate them. BAX and BAK form oligomers that insert into the mitochondrial membrane.
- **Blocking of anti-apoptotic proteins.** BH3-only proteins may also bind and block BCL2 and BCL-XL. Synthesis of BCL2 and BCL-XL may fall because their transcription relies on survival signals.
- **Mitochondrial leakage.** The net result is leakage of cytochrome c and other mitochondrial proteins into the cytoplasm.
- **Apoptosome formation.** Cytosolic cytochrome c binds **APAF-1** (apoptosis-activating factor-1) to form the apoptosome.
- **Caspase-9 activation.** The apoptosome binds caspase-9, the critical initiator caspase of this pathway. This promotes its autocatalytic cleavage into active enzyme.
- **Caspase cascade.** Active caspase-9 cleaves and activates other pro-caspases such as caspase-3, which mediate the execution phase.
- **Removal of the IAP brake.** Smac/DIABLO enter the cytoplasm and neutralise the **inhibitors of apoptosis (IAPs)**. IAPs normally block inappropriate caspase activation, including executioners like caspase-3. Inhibiting IAPs therefore permits the cascade to start.

Flowchart 1: Intrinsic (mitochondrial) pathway of apoptosis

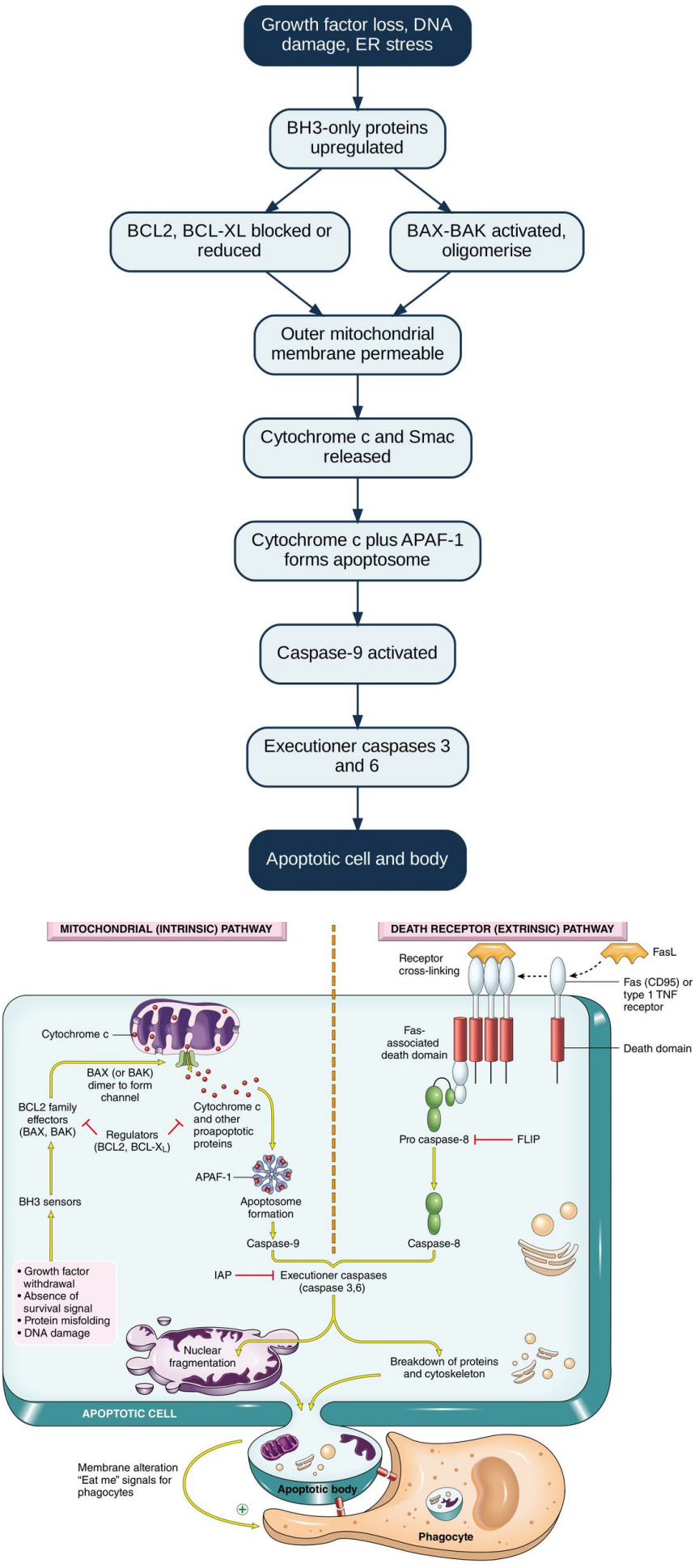


Fig. 2.16 Mechanisms of apoptosis. While the mitochondrial and death receptor pathways of apoptosis differ in — Robbins 11e, p. 42

Execution phase and clearance

- The intrinsic pathway activates initiator caspase-9. The extrinsic pathway activates caspase-8 and caspase-10. Both then activate the executioner caspases, caspase-3 and caspase-6.
- Executioner caspases cleave an inhibitor of a DNase. The freed DNase becomes active and DNA degradation begins.
- Caspases proteolyse nuclear matrix components, which promotes nuclear fragmentation.
- How membrane blebs and apoptotic bodies form is not known.
- **Phosphatidylserine** flips from the inner to the outer leaflet of the membrane. It is recognised by macrophage receptors as an "eat me" signal.
- Other "eat me" signals are natural antibodies and complement proteins, notably C1q, coating apoptotic bodies.
- Phagocytosis of apoptotic cells is called **efferocytosis**. It is so efficient that dead cells often vanish within minutes.
- Macrophages that have ingested apoptotic cells make fewer proinflammatory cytokines. This limits inflammation even when apoptosis is extensive.

Cross-talk with the extrinsic pathway

- In hepatocytes and pancreatic β cells, caspase-8 from Fas signalling cleaves the BH3-only protein **BID**. BID then feeds into the mitochondrial pathway.

Table 3: Intrinsic versus extrinsic pathway

Feature	Intrinsic (mitochondrial)	Extrinsic (death receptor)
Trigger	Survival-signal loss, DNA damage, misfolded proteins	Ligand binding to TNF-family death receptors such as Fas, TNFR1
Regulators	BCL2 family	FLIP
Adaptor	APAF-1 (apoptosome)	FADD
Initiator caspase	Caspase-9	Caspase-8 (or 10)
Executioners	Caspase-3 and caspase-6	Caspase-3 and caspase-6

DRAW IN THE EXAM

Flow diagram of the mitochondrial pathway: BH3-only proteins, BAX/BAK versus BCL2/BCL-XL, cytochrome c release, APAF-1 apoptosome, caspase-9, caspase-3 and 6.

PEARLS

- Apoptosis is caspase-dependent, regulated cell death with shrinkage, peripheral chromatin condensation and apoptotic bodies, and no inflammation.
- The **mitochondrial pathway** is the commonest route. It is controlled by the BCL2 family, and the sentinel event is cytochrome c leakage.
- **BAX and BAK** are pro-apoptotic. **BCL2, BCL-XL and MCL1** are anti-apoptotic. **BH3-only** proteins are the sensors.
- The apoptosome is cytochrome c plus APAF-1 plus caspase-9. Caspase-9 is the initiator caspase of the intrinsic pathway, and caspase-3 and 6 are the executioners.
- Smac/DIABLO neutralise IAPs. BCL2 overexpression from translocation in B-cell lymphoma blocks the intrinsic pathway.
- Caspase-8 cleaves BID to link the extrinsic and intrinsic pathways.
- Exam trap: necroptosis is caspase-independent (RIPK1, RIPK3, MLKL), unlike apoptosis.

18. Define Anoikis. Discuss the mechanism of Anoikis and its role in cancer progression

ASKED 1× · 10-mark answer

Asked in: Paper I · Apr 2023 · Q3

Q: Define Anoikis. Discuss the mechanism of Anoikis and its role in cancer progression. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Causes of Apoptosis; Morphologic and Biochemical Changes in Apoptosis; Mechanisms of Apoptosis; Necroptosis — pp. 39–44
- W1 · Cancers (MDPI) review via PMC — Anoikis-Associated Lung Cancer Metastasis: Mechanisms and Therapies — 2022
- W2 · Review via PMC — Mechanisms for Modulating Anoikis Resistance in Cancer and the Relevance of Metabolic Reprogramming — 2021
- W3 · J Cancer Res Clin Oncol — Uncovering a key to the process of metastasis in human cancers: a review of critical regulators of anoikis — 2013
- W4 · Cell Biology International — Understanding and targeting anoikis in metastasis for cancer therapies — 2023
- W5 · Apoptosis — Anoikis in cancer: molecular mechanisms, resistance, and therapeutic strategies — 2026
- W6 · Cancer Letters — Anoikis resistance in Cancer: Mechanisms, therapeutic strategies, potential targets, and models for enhanced understanding — 2025
- W7 · Biochim Biophys Acta Rev Cancer — The mechanics of anoikis resistance in cancer — 2025
- W8 · Same source as (introduction)

NOT IN THE REFERENCE BOOKS

Searched: the supplied Robbins 11e pages (ch. 2, Cell Injury, Cell Death, and Adaptations, pr.36–47) for "anoikis", detachment-induced apoptosis and anchorage-dependent survival. These pages cover apoptosis in general but do not name or describe anoikis. The definition, anoikis-specific mechanism and cancer role below come from peer-reviewed reviews. The general apoptotic machinery is from Robbins.

Definition of anoikis

- **Anoikis** (Greek, "homelessness") is apoptosis triggered when a cell loses adhesion to its extracellular matrix (ECM) or adheres inappropriately.
- It is a programmed cell death process that follows loss of cell–ECM interaction.
- It is a special type of apoptosis, induced by loss of anchorage from the ECM.
- It eliminates misplaced or detached cells under physiological and pathological conditions, which maintains tissue homeostasis.
- It stops cells from migrating arbitrarily and settling in the wrong tissue, so it is a barrier against metastasis.
- The parent process is apoptosis: a regulated, caspase-mediated suicide programme. The dead cell breaks into membrane-bound apoptotic bodies and is cleared by phagocytes without inflammation.
- Anoikis is therefore **anchorage-dependent survival failing**. Normal epithelial, endothelial and similar cells need matrix signals to stay alive.
- The detached cell is deprived of survival signals. Robbins lists loss of growth factor and matrix interaction as a cause of apoptosis.

Mechanism of anoikis

Initiation: sensing detachment

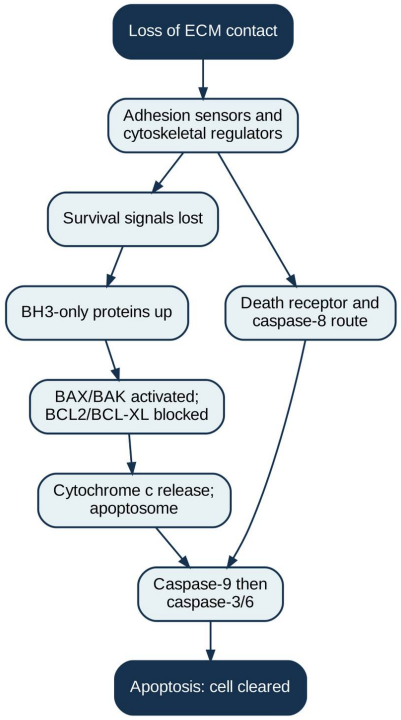
- Anoikis starts with a change in the external environment when the cell leaves its primary site.
- ECM components and cell-adhesion membrane proteins act as sensors that pass signals into the cell.

- Regulators of adhesion and the cytoskeleton, such as **p66Shc and RhoA**, activate the cell to start apoptosis.
- Activation of apoptotic signalling is the critical step of anoikis.
- Growth-promoting signals work the other way and counteract anoikis.
- The sensors, cytoskeletal regulators, EMT and ECM composition all feed into anoikis in lung cancer.

Execution: intrinsic and extrinsic pathways

- Anoikis regulators act through the intrinsic (mitochondrial) pathway, the extrinsic (death-receptor) pathway or caspase-independent pathways.
- This is the general apoptotic machinery, as follows.
- Intrinsic pathway
  - **Survival signals** from growth factors keep anti-apoptotic BCL2 proteins (BCL2, BCL-XL, MCL1) active. These keep the outer mitochondrial membrane impermeable, so cytochrome c is retained.
  - When survival signals are lost, **BH3-only proteins** (BAD, BIM, BID, Puma, Noxa) are upregulated or activated.
  - They activate **BAX/BAK**, which oligomerise in the outer mitochondrial membrane, and they also block BCL2/BCL-XL.
  - **Cytochrome c** leaks into the cytosol and binds APAF-1 to form the apoptosome. This activates the initiator **caspase-9**, which in turn activates caspase-3.
  - Smac/DIABLO released from mitochondria neutralise inhibitors of apoptosis (IAPs), allowing the caspase cascade to proceed.
- Extrinsic pathway
  - Death receptors of the TNF receptor family (Fas/CD95, TNFR1) bind their ligands. The adaptor **FADD** then recruits pro-caspase-8.
  - Active **caspase-8** activates the executioner caspases-3 and -6.
  - **FLIP** blocks FADD binding to pro-caspase-8 and so inhibits this pathway.
  - Caspase-8 can cleave BID, linking the extrinsic pathway to the mitochondrial pathway.
- Execution phase
  - Executioner caspases-3 and -6 activate a DNase and fragment the nucleus.
  - Phosphatidylserine flips to the outer membrane leaflet as an "eat me" signal, and phagocytes clear the cell (efferocytosis).
- Specific anoikis regulators
  - **Mcl-1, Cav-1, Bcl-xL, cFLIP and 14-3-3ζ** suppress anoikis, and their upregulation gives resistance.
  - **Bit1** promotes anoikis, and its downregulation gives resistance in metastatic cancer.

Flowchart 1: Sequence of events in anoikis



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Flow diagram of anoikis: loss of ECM/integrin contact → intrinsic (BH3-only, BAX/BAK, cytochrome c, caspase-9) and extrinsic (Fas/FADD, caspase-8) arms → caspase-3/6 → apoptosis, with the sites of anoikis resistance marked.

Role of anoikis in cancer progression

Anoikis as a barrier to metastasis

- Anoikis kills tumour cells that escape the native ECM and so limits their spread to other organs.
- ECM detachment in a localised tumour comes before abnormal dissemination and metastasis. Programmed cell death normally clears these detached cells through anoikis.
- A tumour cell must therefore overcome anoikis to acquire metastatic potential.

Anoikis resistance (AR)

- Neoplastic cells evolve mechanisms to evade anoikis. This is **anoikis resistance**, and it promotes survival, invasiveness, therapy resistance and relapse.
- Resisting death after detachment from the ECM creates the anoikis-resistant phenotype.
- Anoikis resistance is a prerequisite for distant metastasis and a major cause of treatment failure.
- It is an active, finely regulated adaptation, not a passive loss. It lets malignant cells survive and disseminate.

Table 1: Mechanisms of anoikis resistance in cancer

Mechanism	Examples	Effect
Upregulation of anti-apoptotic proteins	Mcl-1, Bcl-xL, cFLIP, Cav-1, 14-3-3ζ	Block intrinsic and extrinsic death signalling
Loss of pro-anoikis proteins	Bit1 downregulation	Removes the death signal
Altered apoptotic signalling, metabolic reprogramming, autophagy, epigenetic change	Reviewed in	Survival in the detached state
Growth-factor, pH, oxidative-stress and transcriptional signalling; cancer stem cells; lncRNA	Drivers of AR	Enhance proliferation and metastasis
Metabolic pathways	Amino acid and nucleotide metabolism	Bypass anoikis
EMT, ECM composition, adhesion proteins	Lung cancer	Alter sensing of detachment
Mechanotransduction and mechanical mimetics	Soluble mediators such as irisin activate matrix-like survival pathways	Cells survive detached from the ECM

Link to the metastatic cascade

- Anoikis resistance lets cells survive detachment from the primary site and survive transit in the circulation. These are the first steps of metastasis.
- Cell types that evade anoikis can then colonise other organs.
- Resistance also carries therapy resistance and relapse.

Therapeutic relevance

- Restoring anoikis sensitivity by targeting key regulators is a strategy against metastatic cancer.
- Several natural and synthetic compounds have pro-anoikis activity in lung cancer cells and in vivo models.
- Examples are artonin E, imperatorin, oroxylin A, sulforaphane and carbenoxolone.

PEARLS

- **Anoikis** is apoptosis caused by loss of cell–ECM adhesion. The Greek meaning is "homelessness".
- It uses the standard apoptotic machinery: the BCL2-family/cytochrome c/caspase-9 intrinsic pathway and the Fas/FADD/caspase-8 extrinsic pathway.
- Suppressors of anoikis: **Mcl-1, Bcl-xL, cFLIP, Cav-1, 14-3-3ζ**. Promoter: **Bit1**.
- Normal role: it eliminates misplaced cells and maintains tissue homeostasis. In cancer it is a barrier to metastasis.
- **Anoikis resistance** is a prerequisite for metastasis. Classic trap: resistance means detached cells survive, whereas anoikis itself kills them.
- Anoikis is non-inflammatory, like all apoptosis. Cell shrinkage, chromatin condensation and apoptotic bodies are the morphology.

19. Autophagy — mechanism, molecular biology, role in health & disease

ASKED 2× · 10-mark answer

Asked in: Paper I · Jun 2021 · Q3(a) · Paper I · Jun 2022 · Q9

Q: Autophagy. [5]

Q: Describe the mechanism, molecular biology and role of autophagy in health and disease. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Autophagy; §Reversible Cell Injury; §Dysregulated Nutrient Sensing — pp. 34–35, 61
- Robbins — ch. 5 Genes and Human Diseases — §Lysosomal Storage Diseases — pp. 134–135

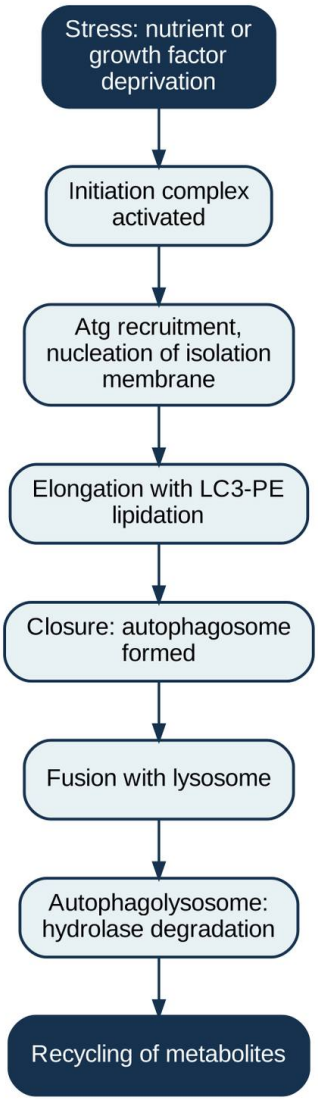
Definition

- **Autophagy** means "self-eating" (Greek: auto, self; phagy, eating).
- The cell delivers its own cytoplasmic materials to the lysosome for degradation.
- It is an evolutionarily conserved survival mechanism during nutrient deprivation. Starved cells live by cannibalizing themselves and recycling the digested contents.
- It is also a fundamental stress response in physiologic states (e.g., aging, exercise).
- It is a stress response in pathologic processes too (e.g., hypoxia, oxidative stress, organelle and membrane damage).

Mechanism

- Triggers: environmental cues such as nutrient deprivation or depletion of growth factors activate an **initiation complex**.
- The initiation complex promotes hierarchical recruitment of Atg proteins to nucleate the initiation membrane.
- The process runs in three steps.
  - **Nucleation** and formation of an isolation membrane, also called a **phagophore**.
  - Formation of the **autophagosome** from the isolation membrane. Intracellular organelles and cytosolic structures are sequestered inside it.
  - **Maturation** of the autophagosome by fusion with lysosomes. This delivers digestive enzymes that degrade the contents.
- Source of the isolation membrane: it is believed to be derived from the ER. The plasma membrane and mitochondria may also contribute.
- The initiation membrane elongates, surrounds and captures its cytosolic cargo, then closes to form the autophagosome.
- The newly formed autophagosome fuses with lysosomes to form an **autophagolysosome**.
- In the terminal step, lysosomal enzymes degrade the inner membrane and the enclosed cargo.
- The digested material is released for **recycling of metabolites**.
- The autophagosome is a double-membrane-bound vacuole.

Flowchart 1: Sequence of autophagy



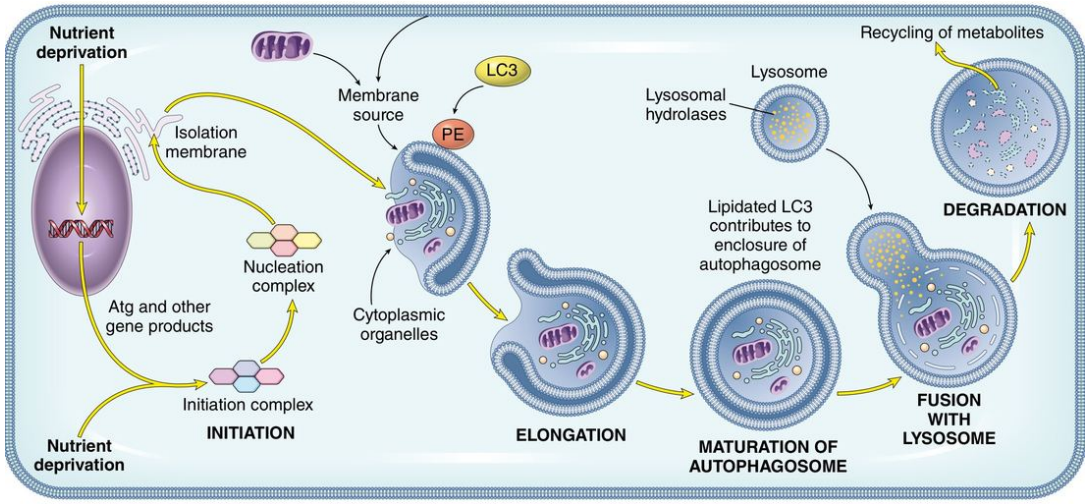


Fig. 2.5 Autophagy. Cellular stresses such as nutrient deprivation activate the autophagy pathway via nuclear — Robbins 11e, p. 34

Molecular biology

- **Atg genes**: over 30 "autophagy-related genes" (Atgs) have been identified. Their products are required to create the autophagosome.
- Atgs and other gene products act together with the nucleation complex and membrane source at the early steps.
- Two **ubiquitin-like conjugation systems** act in a coordinated way.
  - They are needed for elongation and closure of the initiation membrane.

  - They covalently link the lipid **phosphatidylethanolamine (PE)** to **microtubule-associated protein light chain 3 (LC3)**.
- **Lipidated LC3 (LC3-PE)** contributes to enclosure of the autophagosome.
- LC3-PE is increased during autophagy. It is therefore a useful **marker** for identifying cells in which autophagy is occurring.
- **Selectivity**: autophagy is not a random process that engulfs cytosolic contents indiscriminately. Cargo loading into the autophagosome is selective.
- One function of lipidated LC3 is to target **protein aggregates** and **effete organelles**.
- Lysosomal hydrolases complete the process. Lysosomal enzymes are synthesized in the ER and tagged with mannose-6-phosphate in the Golgi for targeting to lysosomes.
- **mTOR**: rapamycin inhibits the mTOR pathway and promotes autophagy, among its multiple effects.
- **Linked quality-control systems**: protein homeostasis also depends on chaperones and on the ubiquitin-proteasome system.
- Like the unfolded protein response, autophagy can trigger cell death if it cannot cope with the stressor.

Role in health

- **Survival under stress**: autophagy frequently acts as a survival mechanism. It maintains cell integrity by recycling essential metabolites and clearing intracellular debris.
- **Atrophy**: it is prominent in atrophic cells exposed to severe nutrient deprivation.
- **Organelle turnover**: autophagy turns over the ER, mitochondria and lysosomes.
  - Selective removal of mitochondria is called **mitophagy**. It is a quality-control system that degrades dysfunctional mitochondria.
- **Aggregate clearance**: it clears intracellular aggregates that accumulate during aging, stress and various disease states.
- **Host defence**: many pathogens are degraded by autophagy. This is one way microbial proteins are digested and delivered to antigen presentation pathways.
- **Aging and longevity**: impaired protein homeostasis (reduced autophagy-lysosome and proteasome degradation) is a mechanism of cellular aging.
  - Rapamycin, which promotes autophagy, increases the life span of middle-aged mice.
  - Caloric restriction increases life span in all eukaryotic species tested. It is proposed to act by reducing insulin/IGF-1 signaling and increasing sirtuins.
- **Lysosomal link**: lysosomes play critical roles in autophagy (fusion with the autophagosome), in immunity (fusion with phagosomes) and in membrane repair.

Role in disease

Cell death

- Multiple death pathways can be activated with excessive autophagy: apoptosis, necroptosis and ferroptosis.
- It is not clear whether cell death is caused by autophagy itself or by the stress that triggered autophagy.

Disease associations

Table 1: Autophagy in human disease

Disease group	Autophagy abnormality	Example
Cancer	Can promote cancer growth and also defend against cancer	Dual role
Neurodegenerative disorders	Dysregulated autophagy worsens defects in proteostasis and organelle function	Alzheimer disease: impaired autophagosome maturation
Neurodegenerative disorders	Mutant protein impairs autophagy	Huntington disease: mutant huntingtin
Infectious diseases	Pathogens degraded by autophagy	Mycobacteria, Shigella spp., HSV-1
Infectious diseases	Loss of host defence	Macrophage-specific Atg5 deletion raises susceptibility to tuberculosis
Inflammatory bowel disease	SNPs in the autophagy-related gene ATG16L1	Crohn disease and ulcerative colitis
Lysosomal storage diseases	Defective fusion and degradation of autophagic cargo	Gaucher disease

- **Cancer**: the dual role is an area of active investigation.
- **Neurodegeneration**: in mouse models of Alzheimer disease, genetic defects in autophagy accelerate neurodegeneration.
- **Infection**: autophagy removes mycobacteria, Shigella spp. and HSV-1.
- **IBD**: how the ATG16L1 polymorphisms promote intestinal inflammation is not known.

Lysosomal storage diseases

- Lysosomal enzyme defects cause accumulation of undigested macromolecules in lysosomes. These interfere with processing of organelles delivered by autophagic vacuoles, including mitochondria.
- Damaged mitochondria persist. They have poor calcium-buffering capacity and altered membrane potentials, and they generate free radicals.
- These mitochondria release molecules that trigger the **intrinsic pathway of apoptosis**.
- Impaired autophagy gives **secondary accumulation** of autophagic substrates. Examples are ubiquitinated and aggregation-prone polypeptides such as α-synuclein and Huntingtin protein.
- This is a molecular link between neurodegenerative disorders and lysosomal storage diseases such as Gaucher disease.
- Virtually all patients with Gaucher disease develop Parkinson disease, so the carrier state is an important genetic risk factor for Parkinson disease.
- Niemann-Pick type C increases the risk of Alzheimer disease.
- Combined frequency of lysosomal storage disorders is about 1 in 5000 live births.

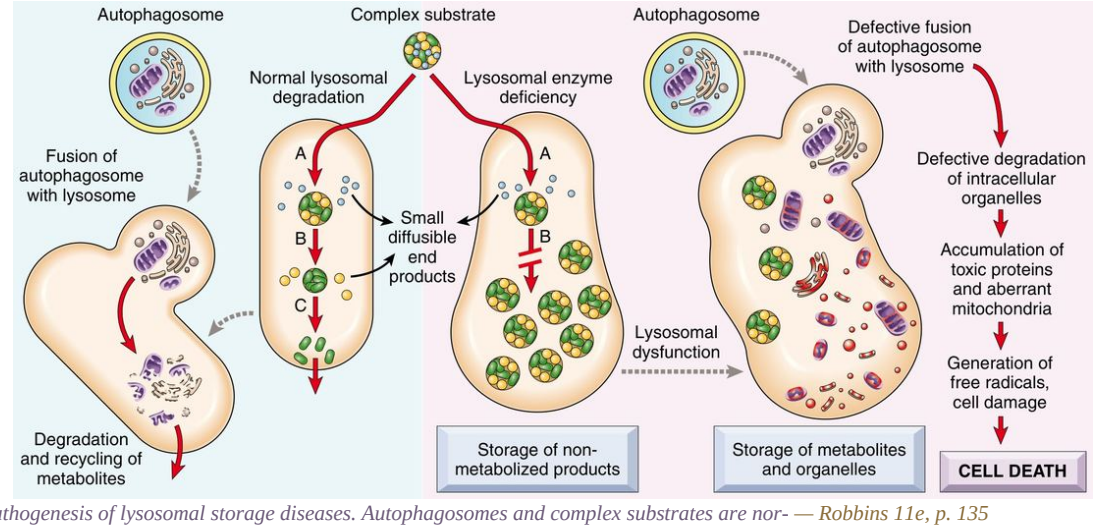


Fig. 5.11 Pathogenesis of lysosomal storage diseases. Autophagosomes and complex substrates are nor- — Robbins 11e, p. 135

Misfolded-protein diseases

- Abnormal folding with aggregation and apoptosis occurs in Alzheimer disease (Aβ peptide) and in Creutzfeldt-Jakob disease (PrPsc).
- In α1-antitrypsin deficiency, nonfunctional protein is stored in hepatocytes and causes apoptosis.

DRAW IN THE EXAM

Fig. 2.5 style flow diagram: stress → initiation complex → isolation membrane (phagophore) with LC3-PE → autophagosome → fusion with lysosome → autophagolysosome → recycling of metabolites.

PEARLS

- Autophagy is a lysosome-mediated self-digestion of cytoplasmic contents. It is a survival response to nutrient deprivation and stress.
- Steps: nucleation (phagophore, probably ER-derived) → autophagosome → fusion with lysosome → degradation and recycling.
- Over 30 Atg genes are involved. Two ubiquitin-like conjugation systems link PE to LC3.
- Lipidated LC3 is the marker of autophagy and targets protein aggregates and effete organelles.
- Autophagy has a dual role in cancer. Excess or failed autophagy can trigger apoptosis, necroptosis or ferroptosis.
- Disease links to remember: Alzheimer disease (impaired autophagosome maturation), Huntington disease, ATG16L1 in Crohn disease, Atg5 in tuberculosis, Gaucher disease and Parkinson disease.
- Rapamycin inhibits mTOR and promotes autophagy.

20. Fibrinoid necrosis

ASKED 1× · 5-mark answer
Asked in: Paper I · Jun 2020 · Q9(a)
Q: Fibrinoid necrosis. [5]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Causes of Apoptosis — p. 40
- Robbins — ch. 11 Blood Vessels — §Medium Vessel Vasculitis; Small Vessel Vasculitis; Raynaud Phenomenon — pp. 467–470

Definition

- **Fibrinoid necrosis** is a pattern of necrosis seen in the **walls of arteries** in vasculitis. It appears as a circumferential, **bright pink** zone of necrosis with inflammation.
- It is listed with coagulative, liquefactive, caseous and fat necrosis among the morphological patterns of necrosis.
- In vasculitis it is a marker of **immune-mediated vessel wall injury**. Vasculitis is inflammation of vessel walls, and it most commonly has an immunologic basis.

Pathogenesis (immune-mediated vessel injury)

- Immune complex vasculitis shows moderate to extensive **immunoglobulin and/or complement deposits** in the vessel wall.
- Damage follows **complement activation** and/or recruitment and activation of **Fc-receptor-bearing cells** (neutrophils, monocytes/macrophages).
- Examples of immune complex deposition:
  - **PAN**: HBsAg–anti-HBsAg complexes, seen in about one-third of patients. Another passage gives up to 30%.
  - **Drug hypersensitivity** (e.g. penicillin acting as a hapten).
  - **SLE**: DNA/anti-DNA complexes.
- ANCA-associated vasculitis is **pauci-immune**. ANCAs directly activate neutrophils, which release reactive oxygen species and proteolytic enzymes, and this causes destructive neutrophil–endothelial interactions.

- **Kawasaki disease**: a delayed-type hypersensitivity response with autoantibodies to endothelial cells and smooth muscle cells.

Morphology

Light microscopy

- The arterial wall shows a **circumferential bright pink area of necrosis**. There is an inflammatory infiltrate with neutrophils that have dark nuclei.

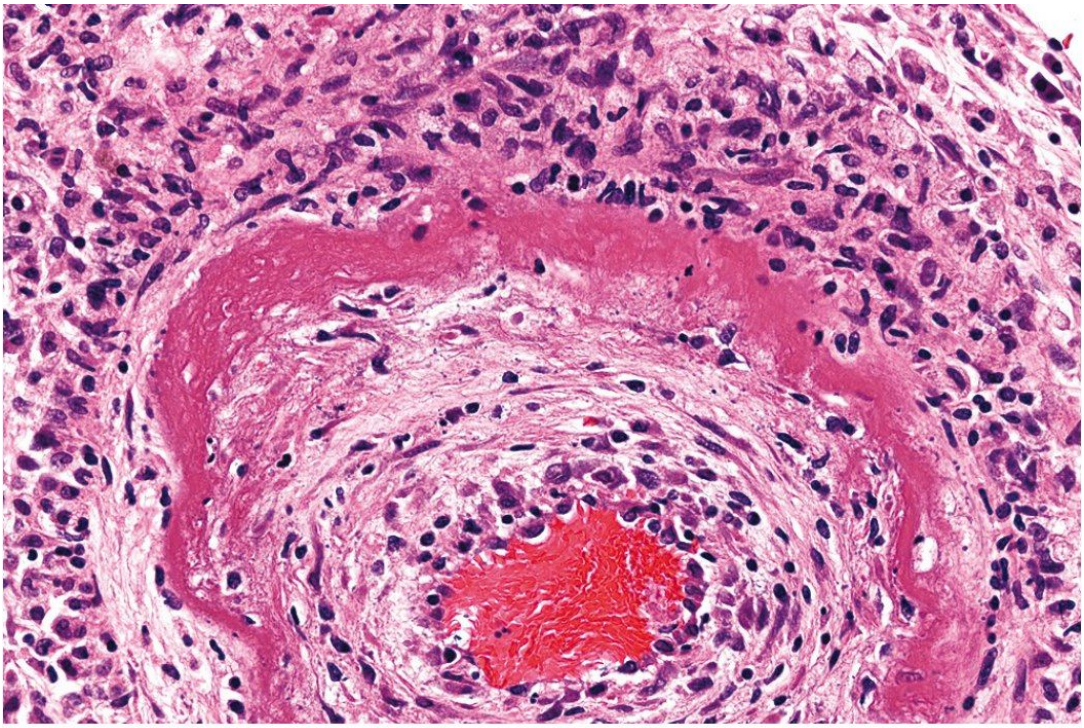


Fig. 2.14 *Fibrinoid necrosis in an artery. The wall of the artery shows a — Robbins 11e, p. 40*

- In acute PAN there is **transmural inflammation** with a mixed infiltrate of neutrophils, eosinophils and mononuclear cells. It is frequently accompanied by fibrinoid necrosis and luminal thrombosis.
- PAN lesions are **segmental**, so part of the vessel wall may be uninvolved.

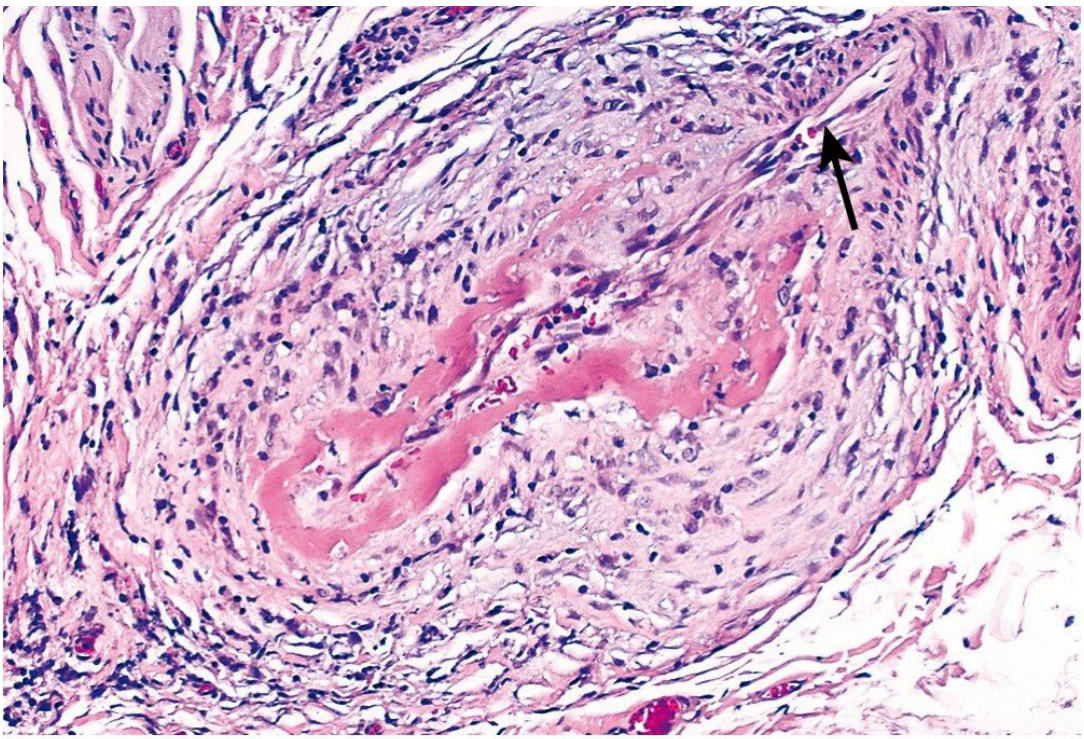


Fig. 11.26 *Polyarteritis nodosa. There is segmental fibrinoid necrosis — Robbins 11e, p. 467*

- Older PAN lesions show fibrous thickening of the wall extending into the adventitia.

Distribution in different vasculitides

Table 1: Fibrinoid necrosis in vasculitis (ROB 11e)

Disease	Vessels	Fibrinoid necrosis / key feature
PAN	Small and medium muscular arteries	Segmental transmural necrotizing inflammation; **all stages coexist**
Microscopic polyangiitis	Capillaries, arterioles, venules	Segmental fibrinoid necrosis of the media; **all lesions of the same age**; no granulomas
Kawasaki disease	Large and medium arteries (coronary)	Resembles PAN, but fibrinoid necrosis is **less prominent**
GPA	Small to medium vessels	Necrotizing or granulomatous vasculitis, with granulomas
EGPA	Small vessels	Granulomas and eosinophils

- Fibrinoid necrosis is the same lesion across these diseases. The distinguishing features are vessel size, lesion age, granulomas and eosinophils.

Diagnosis and correlation

- Biopsy of the affected vessel shows fibrinoid necrosis with an inflammatory infiltrate.
- Immunoglobulin and complement deposition is seen in immune complex vasculitis. Most MPA lesions are pauci-immune, although early skin lesions may show deposits.
- ANCA type points to the disease:
  - **PR3-ANCA** is associated with GPA.
  - **MPO-ANCA** is associated with MPA and EGPA.
- Consequences of PAN lesions:
  - Impaired perfusion causes ulcers, infarcts, ischemic atrophy or hemorrhage.
  - Clinically this gives hypertension (renal artery involvement), abdominal pain and bloody stools, myalgias and peripheral neuritis.
- **Leukocytoclastic vasculitis** is a related pattern. Fragmenting neutrophils are seen, typically in postcapillary venules. It is not ANCA-specific.

DRAW IN THE EXAM

Cross-section of a muscular artery with a segmental pink fibrinoid necrotic wall, neutrophil/eosinophil infiltrate and luminal thrombus, labelled against the uninvolved wall segment.

PEARLS

- Fibrinoid necrosis = bright pink, circumferential necrosis of the arterial wall with inflammation.
- PAN: segmental, transmural, with **all stages coexisting**. MPA: all lesions of the **same age**.
- Kawasaki disease: fibrinoid necrosis is **less prominent** than in PAN.
- ANCA vasculitis is **pauci-immune**. Immune complex vasculitis shows Ig and complement deposits.
- PR3-ANCA goes with GPA. MPO-ANCA goes with MPA and EGPA.
- Trap: neutrophil fragmentation (leukocytoclasia) is not specific for ANCA vasculitis.

21. What are the cellular responses to stress and injury. Describe briefly pathologic calcification

ASKED 1× · 10-mark answer

Asked in: Paper I · Jun 2022 · Q1

Q: What are the cellular responses to stress and injury. Describe briefly pathologic calcification. [10]

SOURCE

- Robbins — ch. 2 Cell Injury, Cell Death, and Adaptations — §Introduction to Pathology; Overview of Cellular Responses; Physical Agents; Hyperplasia; Dystrophic Calcification; Metastatic Calcification — pp. 29–31, 53, 59–60

Cellular responses to stress and injury

Basis of the response

- The normal cell lives in a narrow range of function and structure. This range is set by its metabolism, differentiation and specialization, by constraints from neighbouring cells, and by the availability of substrates.
- It handles physiologic demands and maintains a steady state called **homeostasis**.
- Adaptation, reversible injury and cell death are **stages of progressive impairment** after different insults.
- The stages do not always occur in sequence. Sudden complete occlusion of a major coronary artery can cause irreversible injury without preceding adaptation.

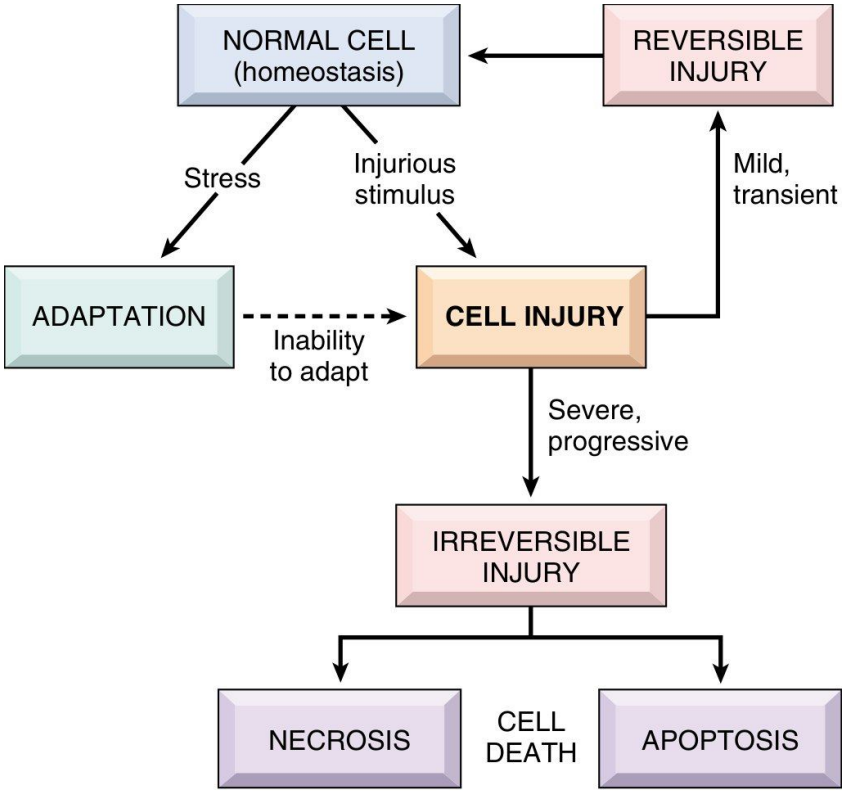
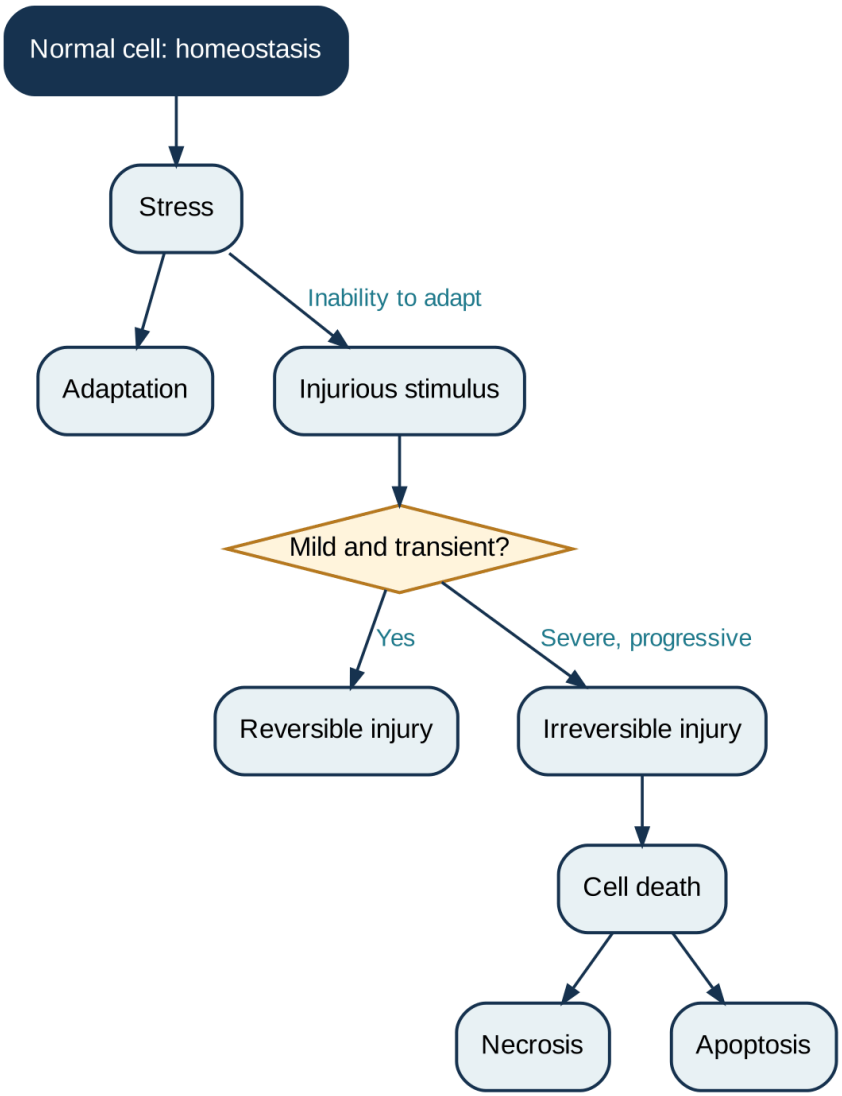


Fig. 2.1 Stages of the cellular response to stress and injurious stimuli. — Robbins 11e, p. 30

Flowchart 1: Stages of cellular response to stress and injury



Causes of cell injury

- **Oxygen deprivation (hypoxia)** is an extremely common cause. It reduces aerobic oxidative respiration.
  - Reduced blood flow (ischemia).
  - Inadequate oxygenation of blood from cardiorespiratory failure.
  - Reduced oxygen-carrying capacity, as in anemia, carbon monoxide poisoning and severe blood loss.
- **Physical agents** include mechanical trauma, extremes of temperature, sudden pressure change, radiation and electric shock.
- **Chemical agents and drugs** include hypertonic glucose or salt and high-concentration oxygen. Trace amounts of arsenic, cyanide or mercury can kill enough cells within minutes to hours.
- Other categories are infectious agents, immunologic reactions, genetic abnormalities and nutritional imbalances.
- Etiology falls into two broad classes: genetic and environmental. Most common diseases are multifactorial.

Adaptation

- Adaptations are **reversible** functional and structural responses to changes in physiologic state (e.g., pregnancy) and to some pathologic stimuli.
- The cell reaches a new but altered steady state in which it survives and continues to function.
- If the stress is removed, the cell returns to its original state without harm.
- The four adaptive patterns are listed below.
  - **Hypertrophy**: increase in cell size and functional activity.
  - **Hyperplasia**: increase in cell number.

- **Atrophy**: decrease in cell size and metabolic activity.
- **Metaplasia**: change in cell phenotype.

Hypertrophy

- Cardiac hypertrophy is the illustrated example. It follows an increased hemodynamic load and thickens the left ventricular wall.
- Mechanism of myocardial hypertrophy:
  - Mechanical sensors detect the increased load.
  - They activate signalling pathways. The **PI3K/AKT** pathway is thought to be most important in physiologic (exercise-induced) hypertrophy. **G-protein–coupled receptor** pathways, activated by growth factors and vasoactive agents, are thought to be more important in pathologic hypertrophy.
  - These pathways increase production of growth factors (TGF-β, IGF-1, FGF) and vasoactive agents (α-adrenergic agonists, endothelin-1, angiotensin II).
  - Transcription factors **GATA4, NFAT and MEF2** increase expression of contractile protein genes.
- There is a switch to **fetal isoforms**. The α isoform of myosin heavy chain is replaced by the β isoform, which gives slower, more economical contractions.
- **Atrial natriuretic factor** gene expression increases. It causes renal salt excretion and lowers blood volume and pressure, so it reduces hemodynamic load.
- Hypertrophy reaches a limit. Beyond it, myofibrillar contractile elements are degraded and lost, myocytes may die, and cardiac failure follows.
- A hypertrophied heart has increased metabolic demands and is more susceptible to injury.

Hyperplasia

- Hyperplasia is an increase in cell number in response to a stimulus.
- It occurs only if the tissue contains cells **capable of dividing**.
- It often coexists with hypertrophy and may share the same triggers.
- **Physiologic hyperplasia**
  - Hormonal: proliferation of breast glandular epithelium at puberty and in pregnancy.
  - Compensatory: liver regeneration after donor lobe resection.
  - Bone marrow: in acute bleeding or hemolysis, erythropoietin can raise red cell production up to **eightfold**.
- **Pathologic hyperplasia**
  - It is caused by excessive or inappropriate hormone or growth-factor action.
  - Endometrial hyperplasia results from an absolute or relative excess of estrogen over progesterone. It is a common cause of abnormal uterine bleeding.
  - Benign prostatic hyperplasia is driven by androgens.
  - These remain controlled and regress or stabilize if the stimulus is removed.
  - Increased cell division raises the risk of genetic aberrations that may lead to cancer.

Atrophy and metaplasia

- Atrophy example: if an artery is narrowed, the supplied tissue may initially shrink. More severe or sudden hypoxia causes injury and death.
- Metaplasia is a change in the phenotype of cells.

Cell injury and cell death

- Reversible injury is seen in myocardium as functional alterations, usually without gross change. Microscopic changes such as **cellular swelling and fat accumulation** may occur.
- Injury is reversible only up to a point. If the stimulus is persistent or severe, the cell suffers **irreversible injury** and dies.
- Myocardial example: reversible injury occurs if blood supply is inadequate. Unless supply is restored quickly, cells die.
- Triphenyltetrazolium chloride stains viable myocardium brick-red. Infarcted (necrotic) myocardium stays unstained.

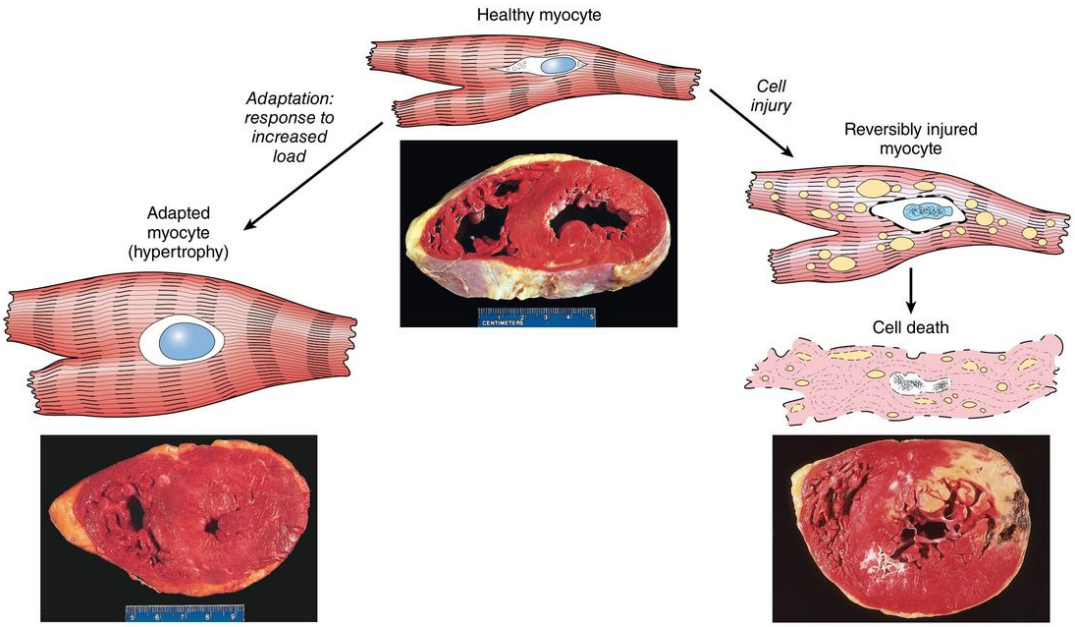


Fig. 2.2 The relationship among healthy, adapted, reversibly injured, and dead myocardial cells. All three — Robbins 11e, p. 31

- Cell death is normal in embryogenesis, organ development and adult homeostasis. Excessive death from ischemia, infection or toxins is crucial in the evolution of disease.
- The two principal pathways are **necrosis and apoptosis**. Recent evidence adds necroptosis, pyroptosis and ferroptosis.

Other responses

- Metabolic derangements and chronic injury cause **intracellular accumulations** of proteins, lipids and carbohydrates.
- Calcium may deposit at sites of cell death (pathologic calcification).
- Cellular **aging** is a progressive decline in function and viability. It results from genetic abnormalities and accumulated molecular damage.

Pathologic calcification

Definition and types

- Pathologic calcification is abnormal tissue deposition of **calcium salts**, with smaller amounts of iron, magnesium and other mineral salts.
- There are two forms, **dystrophic** and **metastatic** calcification.

Table 1: Dystrophic vs metastatic calcification

Feature	Dystrophic	Metastatic
Tissue	Dying or damaged tissue	Otherwise normal tissue
Serum calcium	Normal	Hypercalcemia
Calcium metabolism	No derangement	Disturbed
Cause	Necrosis, atheroma, damaged valves	PTH excess, bone resorption, vitamin D disorders, renal failure
Dysfunction	Often causes organ dysfunction	Usually none

Dystrophic calcification

- It occurs in areas of coagulative, caseous or liquefactive necrosis, and in foci of enzymatic fat necrosis.
- It is almost always present in atheromas of advanced atherosclerosis.
- It commonly develops in aging or damaged heart valves, further hampering function (calcific aortic stenosis).
- **Gross**: fine white granules or clumps, often gritty. A tuberculous lymph node may be converted to stone.
- **Microscopy**:
  - On H&E, calcium salts are **basophilic**, amorphous and granular, sometimes clumped.
  - They may be intracellular, extracellular or both.

- Heterotopic bone may form in time.
- Single necrotic cells can act as seed crystals that become encrusted with mineral.
- Progressive outer layering gives lamellated **psammoma bodies**, seen in some papillary cancers (e.g., thyroid).
- In asbestosis, calcium and iron salts coat asbestos spicules to form beaded, dumbbell-shaped **asbestos bodies**.

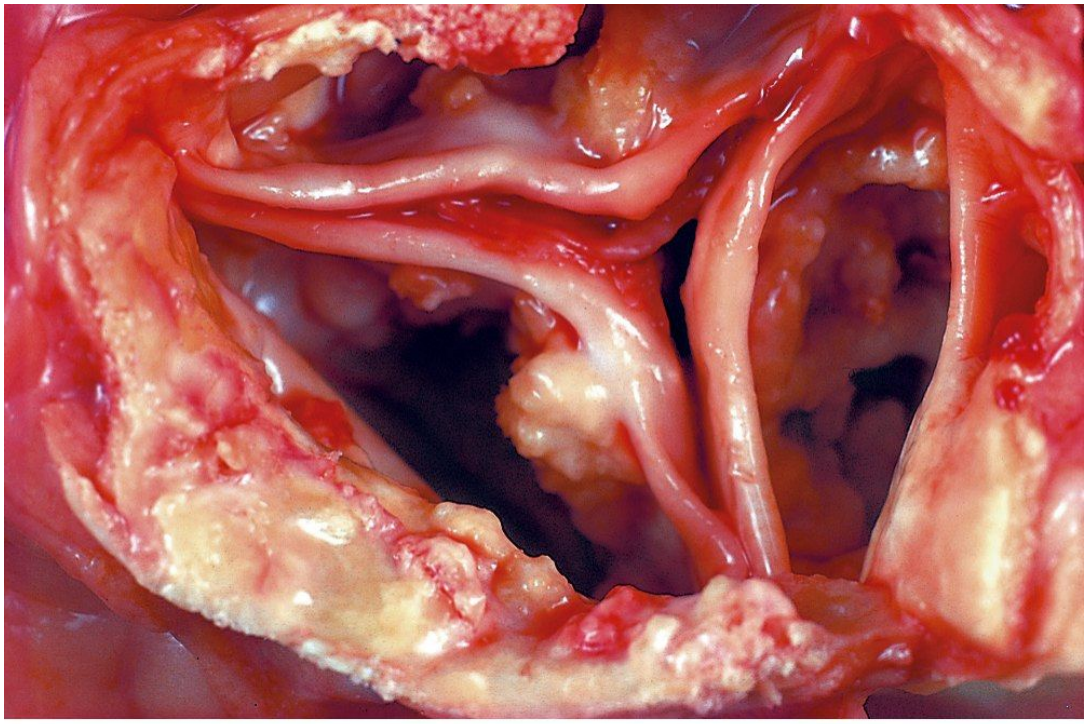


Fig. 2.34 *Dystrophic calcification of the aortic valve. View looking down — Robbins 11e, p. 59*

- Dystrophic calcification may be only a sign of earlier injury, but it is often a cause of organ dysfunction, as in calcific valve disease and atherosclerosis. Serum calcium is normal.

Metastatic calcification

- It occurs in normal tissues whenever there is **hypercalcemia**. Hypercalcemia also accentuates dystrophic calcification.
- The four principal causes of hypercalcemia are listed below.
  - Increased **PTH** secretion, as in parathyroid tumors, or ectopic PTH-related protein from malignant tumors.
  - Bone resorption from multiple myeloma, diffuse skeletal metastasis (e.g., breast cancer), Paget disease or immobilization.
  - **Vitamin D** disorders: intoxication, sarcoidosis, and Williams syndrome (idiopathic hypercalcemia of infancy).
  - **Renal failure**, where phosphate retention causes secondary hyperparathyroidism.
- Less common causes are aluminum intoxication in chronic dialysis and milk-alkali syndrome.
- **Sites**: interstitial tissues of gastric mucosa, kidneys, lungs, systemic arteries and pulmonary veins.
  - All of these excrete acid and have an internal **alkaline compartment**, which predisposes them to calcification.
- Morphology resembles dystrophic calcification: amorphous noncrystalline deposits or hydroxyapatite crystals.
- Clinical effects are usually absent. Massive lung involvement can cause respiratory compromise, and massive renal deposits (nephrocalcinosis) can cause renal damage.

PEARLS

- Adaptations are reversible and include hypertrophy, hyperplasia, atrophy and metaplasia. Injury is reversible only up to a point, then becomes irreversible and ends in necrosis or apoptosis.
- Hyperplasia needs cells capable of dividing. Hypertrophy and hyperplasia often coexist, and hyperplasia raises cancer risk through more cell divisions.
- Cardiac hypertrophy: PI3K/AKT drives physiologic hypertrophy, and GPCR pathways drive pathologic hypertrophy. Fetal isoforms (β-myosin heavy chain, ANF) are re-expressed.
- Dystrophic calcification occurs in dead tissue with **normal serum calcium**. Metastatic calcification occurs in normal tissue with **hypercalcemia**.
- Psammoma bodies are lamellated calcifications in papillary tumors (e.g., thyroid). Asbestos bodies are beaded dumbbell forms.
- Metastatic calcification favours the stomach, kidneys, lungs, systemic arteries and pulmonary veins, because of their alkaline compartment.

PART 2

Inflammation, Repair and Haemodynamic Disorders

22. Latest marker for early diagnosis of sepsis

ASKED 3× · 10-mark answer

Asked in: Paper I · Apr 2023 · Q6(a) · Paper I · Dec 2022 · Q9 · Paper I · Jun 2020 · Q1

Q: Discuss mechanism of septic shock and MODS. Enumerate and elaborate the role of various serum prognostic markers of sepsis. [10]
Q: Discuss shock with special reference to Systemic Inflammatory Response Syndrome. [10]
Q: Latest marker for early diagnosis of sepsis. [5]

SOURCE

- Robbins — ch. 4 Hemodynamic Disorders, Thromboembolic Disease, and Shock — §Factors That Influence Development of an Infarct; §Stages of Shock — pp. 117–121
- W1 · SCCM/ESICM Task Force — Sepsis-3 (Third International Consensus Definitions), JAMA 2016;315(8):801–810 — 2016
- W2 · SCCM/ESICM — Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2026 — 2026
- W3 · ISTH SSC Subcommittee on DIC — Updated definition and scoring of DIC — 2025
- W4 · ISTH SSC on DIC — SIC criteria (Iba et al., J Thromb Haemost) — 2019
- W5 · PMC — SIC criteria review

Shock: definition and types

- **Shock** is a state of circulatory failure that impairs tissue perfusion and causes cellular hypoxia.
  - Initial cellular injury is reversible. Prolonged shock causes irreversible injury and can be fatal.
- Causes include severe haemorrhage, extensive trauma or burns, myocardial infarction, pulmonary embolism and microbial sepsis.
- Less common forms are **neurogenic** (spinal cord injury) and **anaphylactic** (IgE-mediated) shock. Both cause acute vasodilation, hypotension and hypoperfusion.

TABLE 4.3 Three Major Types of Shock — Robbins 11e, p. 118

Type of Shock	Clinical Example	Principle Mechanisms
Cardiogenic	Myocardial infarction Ventricular rupture Arrhythmia Cardiac tamponade Pulmonary embolism	Failure of myocardial pump resulting from intrinsic myocardial damage, extrinsic compression, or obstruction to outflow
Hypovolemic	Fluid loss (e.g., hemorrhage, vomiting, diarrhea, burns, or trauma)	Inadequate blood or plasma volume
Septic	Overwhelming microbial infections (bacterial and fungal)	Activation of cytokine cascades; peripheral vasodilation and pooling of blood; endothelial activation/injury; leukocyte-induced damage, disseminated intravascular coagulation

Table 1: Major types of shock

Type	Example	Principal mechanism
Cardiogenic	MI, arrhythmia, tamponade, pulmonary embolism	Pump failure, extrinsic compression or outflow obstruction
Hypovolaemic	Haemorrhage, vomiting, diarrhoea, burns	Inadequate blood or plasma volume
Septic	Overwhelming bacterial or fungal infection	Cytokine cascades, vasodilation and pooling, endothelial injury, DIC

SIRS, sepsis and septic shock: definitions

- **SIRS** is a sepsis-like condition with systemic inflammation. It is triggered by non-microbial insults such as burns, trauma or pancreatitis.
- **Sepsis** is life-threatening organ dysfunction caused by a dysregulated host response to infection.
- **Septic shock** is a subset of sepsis with profound circulatory, cellular and metabolic abnormalities and a greater mortality risk than sepsis alone.

Table 2: Older scheme versus current definitions

ROB (11e) / older scheme	Current (Sepsis-3, SCCM/ESICM, 2016)
Sepsis-1/2 used SIRS criteria	Organ dysfunction defined as an increase in SOFA score of ≥2 points, with in-hospital mortality >10%
Earlier SSC definitions used lactate 4 mmol/L	Septic shock: vasopressors needed for MAP ≥65 mm Hg plus lactate >2 mmol/L despite adequate volume; mortality >40%

- Baseline SOFA is taken as zero unless organ dysfunction was present before the infection.
- **qSOFA** (any 2 of 3): respiratory rate ≥22/min, altered mentation, systolic BP ≤100 mm Hg. It is a bedside prompt for adults with suspected infection outside the ICU.

Mechanism of septic shock

- Sepsis occurs when the response to infection becomes generalised and injures normal tissues remote from the infection site. It represents failure of the normal mechanisms that contain infection.
- Triggers are diverse. Gram-positive bacteria are most frequent, followed by gram-negative bacteria and fungi, and viruses such as SARS-CoV-2 can also cause sepsis.
  - Microbial constituents include endotoxin, staphylococcal enterotoxin B and streptococcal M protein.

Flowchart 1: Pathogenesis of septic shock and MODS

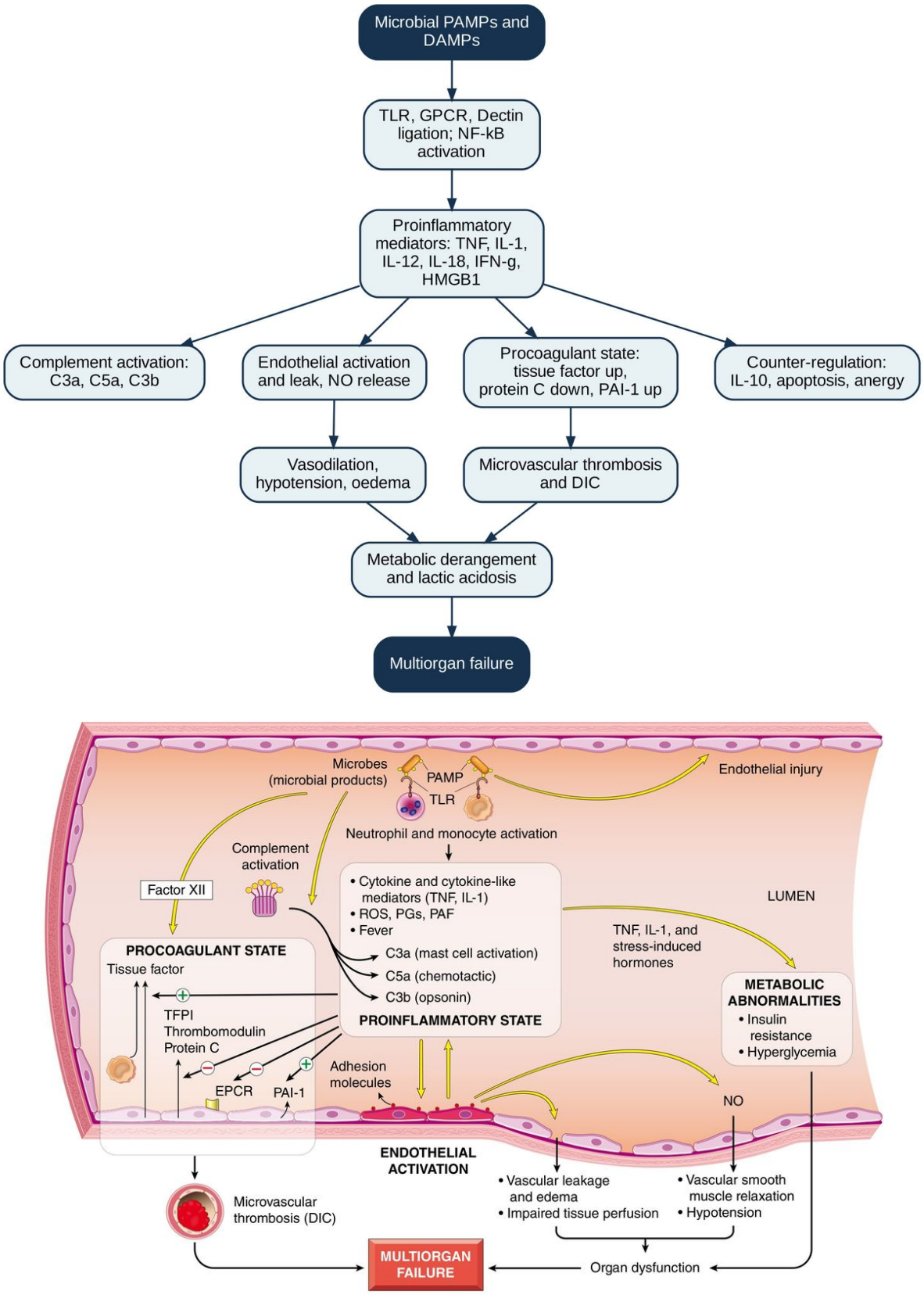


Fig. 4.20 Major pathogenic pathways in septic shock. Microbial products (PAMPs, or pathogen-associated — Robbins 11e, p. 119

Inflammatory and counter-inflammatory responses

- Microbial products engage **TLRs** (recognising PAMPs and DAMPs), G protein–coupled receptors and C-type lectin receptors (Dectins, for fungal antigens).
- Receptor ligation activates **NF-kB**. This upregulates TNF, IL-1, IL-12, IL-18, IFN-γ and HMGB1; CRP and procalcitonin also rise.
- Reactive oxygen species, prostaglandins and PAF are also produced.

- Complement activation yields anaphylatoxins (C3a, C5a), a chemotactic fragment (C5a) and an opsonin (C3b).
- Microbial components activate coagulation directly through factor XII. Thrombin then augments inflammation through protease-activated receptors.
- Counter-regulation causes immunosuppression, so patients may oscillate between hyperinflammatory and immunosuppressed states.
  - Proposed mechanisms are a Th1 to anti-inflammatory cytokine shift, soluble TNF receptor, IL-1 receptor antagonist, IL-10, lymphocyte apoptosis and anergy.

Endothelial activation and injury

- Cytokines loosen endothelial tight junctions. The result is vascular leak and protein-rich oedema, which impede perfusion and may be worsened by IV fluids.
- Activated endothelium produces NO, C3a, C5a and PAF, causing smooth-muscle relaxation and hypotension.
- Microvascular dysfunction (intermittent capillary flow, lost autoregulation) causes an oxygen supply–demand mismatch.

Procoagulant state

- **DIC** develops in up to one-half of septic patients.
- Cytokines increase tissue factor on monocytes. They decrease TFPI, thrombomodulin and protein C, and raise PAI-1 expression to dampen fibrinolysis.
- Neutrophil extracellular traps may promote coagulation.
- Systemic thrombin activation deposits fibrin thrombi in small vessels. In full-blown DIC, consumption of factors and platelets leads to bleeding.

Metabolic abnormalities

- Insulin resistance and hyperglycaemia occur, with raised glucose, triglycerides and lactate.
  - TNF, IL-1, glucagon, growth hormone, glucocorticoids and catecholamines drive gluconeogenesis, while cytokines impair GLUT-4 expression.
- Hyperglycaemia impairs neutrophil bactericidal function.
- An initial glucocorticoid surge may be followed by adrenal insufficiency. DIC-related adrenal necrosis is Waterhouse-Friderichsen syndrome.
- Cellular hypoxia and reduced oxidative phosphorylation cause lactic acidosis.

Multiple organ dysfunction syndrome (MODS)

- Hypotension, interstitial oedema, microvascular dysfunction and small-vessel thrombosis reduce oxygen and nutrient delivery.
- Mitochondrial damage from oxidative stress impairs oxygen use.
- Cytokines reduce myocardial contractility and cardiac output. Increased permeability and endothelial injury can cause **ARDS**.
- Failure involves chiefly the kidneys, liver, lungs and heart, culminating in death.
- Outcome depends on infection extent and virulence, host immune status, comorbidity and the pattern and level of mediator production.
- Specific mediator antagonists have mostly failed, partly because anti-inflammatory mediators are activated concurrently.
- **Superantigens** (e.g. toxic shock syndrome) are polyclonal T-cell activators that cause a similar syndrome.

Stages of shock

- **Non-progressive:** compensatory reflexes maintain vital organ perfusion.
  - Mechanisms are baroreceptor reflexes, catecholamines, ADH and the renin-angiotensin-aldosterone axis.
  - The net effect is tachycardia, peripheral vasoconstriction and renal fluid conservation.
  - Skin is cool and pale, but early septic shock may show warm, flushed skin.

- **Progressive:** tissue hypoperfusion with anaerobic glycolysis and lactic acidosis.
  - Acidosis blunts vasomotor response, arterioles dilate and blood pools. This risks endothelial anoxic injury and DIC.
- **Irreversible:** lysosomal enzyme leakage and worsening myocardial function, partly from increased NO. Gut flora may enter the circulation, and renal failure follows.

Morphology and clinical features of shock

- Changes are those of hypoxic injury, from hypoperfusion plus microvascular thrombosis. The brain, heart, kidneys, adrenals and GIT are most involved.
- Fibrin thrombi are best seen in glomeruli. Adrenal cortical lipid depletion occurs.
- Sepsis or trauma can cause diffuse alveolar damage ("shock lung").
- Hypovolaemic and cardiogenic shock show hypotension, weak rapid pulse, tachypnoea and cool, clammy, cyanotic skin.
- Survival exceeds 90% in young healthy patients with hypovolaemic shock, but septic and cardiogenic shock have poorer outcomes.

Serum prognostic and diagnostic markers of sepsis

Table 3: Serum markers in sepsis

Marker	Behaviour / role	Significance
CRP	Acute-phase marker, raised in sepsis	Marker of acute inflammation
Procalcitonin	Raised in sepsis	Marker of acute inflammation
TNF, IL-1, IL-12, IL-18, IFN-γ	Upregulated via NF-kB	Proinflammatory cytokine burden
HMGB1	Upregulated inflammatory mediator	Late mediator
IL-10, soluble TNF receptor, IL-1Ra	Anti-inflammatory mediators	Immunosuppressive phase
Lactate	Rises with hypoxia and impaired oxidative phosphorylation	Septic shock if >2 mmol/L despite volume, with vasopressor need; mortality >40%
Glucose	Hyperglycaemia from insulin resistance	Reflects metabolic stress
Platelets, PT/INR, D-dimer, fibrinogen	Coagulopathy markers	See Table 4
SOFA score	Organ dysfunction score	Increase ≥2 points, with mortality >10%

- Serial lactate measurement is suggested to guide resuscitation in sepsis with raised lactate or septic shock (SSC 2026, conditional recommendation, low certainty).

Early diagnosis: sepsis-induced coagulopathy (SIC)

- SIC detects coagulopathy earlier than overt DIC. It excludes fibrinogen and D-dimer and uses SOFA to reflect concurrent organ dysfunction.
- Stepwise use: septic patients with platelets <150 × 10^9/L are screened with SIC criteria (step 1), then overt DIC criteria (step 2).

Table 4: SIC score (Iba 2019) and overt DIC score (ISTH 2025)

Parameter	SIC score	Overt DIC 2025
Platelets (×10^9/L)	100–150 = 1; <100 = 2	≥50 to <100 = 1; <50 = 2
PT / INR	INR 1.2–1.4 = 1; >1.4 = 2	PT prolongation ≥3 to <6 s = 1; ≥6 s = 2
SOFA	1 = 1; ≥2 = 2 (capped at 2)	Not used
D-dimer	Not used	>3× ULN = 2; >7× ULN = 3
Fibrinogen	Not used	<100 mg/dL = 1
Diagnostic total	≥4, with PT/INR + platelet score >2	≥5

- The 2001 overt DIC score used qualitative D-dimer categories. Moderate (1–10× ULN) scored 2 and strong (>10× ULN) scored 3.

- SSC 2026 recommends antimicrobials immediately, ideally within 1 hr, for possible, probable or definite septic shock and for probable or definite sepsis without shock.
- SSC 2026 suggests at least 30 mL/kg IV crystalloid in the first 3 hr for sepsis-induced hypoperfusion or septic shock.

DRAW IN THE EXAM

Flowchart of septic shock pathogenesis: PAMP–TLR–NF-kB, cytokines, endothelial leak and NO, procoagulant state with DIC, lactic acidosis, multiorgan failure (as in ROB Fig. 4.20).

PEARLS

- Sepsis = life-threatening organ dysfunction from a dysregulated host response (SOFA rise ≥2). Septic shock = vasopressors for MAP ≥65 plus lactate >2 mmol/L.
- SIRS is non-infectious systemic inflammation (burns, trauma, pancreatitis). It is no longer the basis of sepsis definition.
- DIC occurs in up to one-half of septic patients. Fibrin thrombi are best seen in glomeruli.
- Waterhouse-Friderichsen syndrome = adrenal necrosis from DIC.
- Early septic shock may show warm, flushed skin from vasodilation.
- CRP and procalcitonin are inflammation markers. Lactate and the SIC score (≥4) are the key prognostic and early-coagulopathy markers.
- Overt DIC ≥5 (ISTH 2025); the 2025 update uses D-dimer >3× and >7× ULN.

23. Describe the pathophysiology of hypovolemic shock. Discuss the expected autopsy findings in a case of death from hypovolemic shock due to upper GI bleed

ASKED 1× · 10-mark answer

Asked in: Paper I · Dec 2020 · Q1

Q: Describe the pathophysiology of hypovolemic shock. Discuss the expected autopsy findings in a case of death from hypovolemic shock due to upper GI bleed. [10]

SOURCE

- Robbins — ch. 4 Hemodynamic Disorders, Thromboembolic Disease, and Shock — §Factors That Influence Development of an Infarct; §Stages of Shock; §Pulmonary Embolism — pp. 114, 117–121

Definition and causes of hypovolemic shock

- **Shock** is a state of circulatory failure that impairs tissue perfusion and leads to cellular hypoxia.
- Early cellular injury is reversible. Prolonged shock causes irreversible tissue injury and can be fatal.
- **Hypovolemic shock** is low cardiac output due to low blood volume, as in massive hemorrhage or fluid loss from severe burns.
- Clinical examples are hemorrhage, vomiting, diarrhea, burns and trauma.
- The principal mechanism is inadequate blood or plasma volume.
- Shock is defined as systemic tissue hypoperfusion from reduced cardiac output and/or reduced effective circulating blood volume.

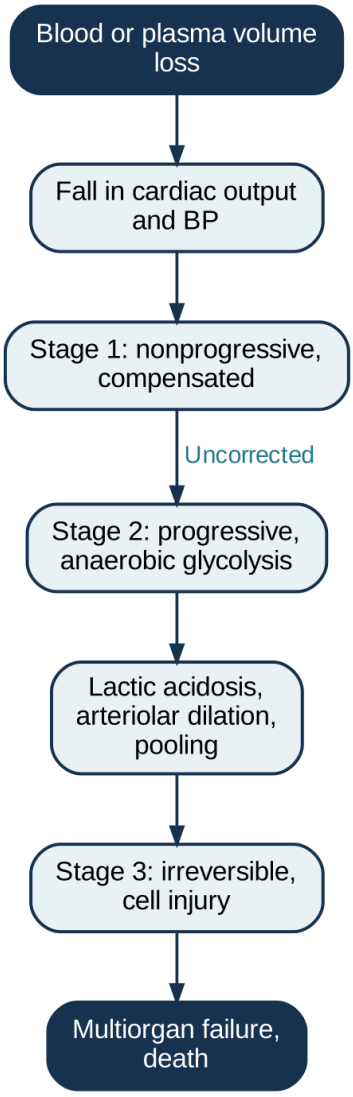
TABLE 4.3 Three Major Types of Shock — Robbins 11e, p. 118

Type of Shock	Clinical Example	Principle Mechanisms
Cardiogenic	Myocardial infarction Ventricular rupture Arrhythmia Cardiac tamponade Pulmonary embolism	Failure of myocardial pump resulting from intrinsic myocardial damage, extrinsic compression, or obstruction to outflow
Hypovolemic	Fluid loss (e.g., hemorrhage, vomiting, diarrhea, burns, or trauma)	Inadequate blood or plasma volume
Septic	Overwhelming microbial infections (bacterial and fungal)	Activation of cytokine cascades; peripheral vasodilation and pooling of blood; endothelial activation/injury; leukocyte-induced damage, disseminated intravascular coagulation

Pathophysiology of hypovolemic shock

- Shock is a progressive disorder that leads to death if the underlying problem is not corrected.
- Unless the insult is massive and rapidly lethal, it passes through three somewhat artificial stages. These are best documented in hypovolemic shock.

Flowchart 1: Stages of hypovolemic shock



Stage 1: initial nonprogressive (compensated) stage

- Reflex compensatory mechanisms are activated and vital organ perfusion is maintained.
- Neurohumoral mechanisms maintain cardiac output and blood pressure. These are baroreceptor reflexes, catecholamine and ADH release, activation of the renin-angiotensin-aldosterone axis, and generalized sympathetic stimulation.
- The net effect is **tachycardia, peripheral vasoconstriction and renal fluid conservation**.

- Cutaneous vasoconstriction causes the "shocky" skin coolness and pallor.
- Coronary and cerebral vessels are less sensitive to sympathetic signals. They keep a relatively normal caliber, blood flow and oxygen delivery.
- Blood is therefore shunted from the skin to the heart and brain.

Stage 2: progressive stage

- If the cause is not corrected, shock passes imperceptibly into this stage. It is characterized by widespread tissue hypoxia.
- Persistent oxygen deficit replaces intracellular aerobic respiration with **anaerobic glycolysis** and excess lactic acid.
- The resulting metabolic **lactic acidosis** lowers tissue pH and blunts the vasomotor response. Arterioles dilate and blood pools in the microcirculation.
- Peripheral pooling worsens cardiac output. It also puts endothelial cells at risk of anoxic injury with subsequent **DIC**.
- With widespread hypoxia, vital organs begin to fail.

Stage 3: irreversible stage

- Cell and tissue injury is so severe that survival is not possible even if the hemodynamic defects are corrected.
- Widespread cell injury shows as lysosomal enzyme leakage, which aggravates shock.
- Myocardial contractility worsens, in part from increased NO synthesis.
- The ischemic bowel may let intestinal flora enter the circulation, so bacteremic shock may be superimposed.
- Ischemic renal injury commonly leads to renal failure, and the downward spiral ends in death.

Clinical features and prognosis

- Patients have hypotension, a weak rapid pulse, tachypnea, and cool, clammy, cyanotic skin.
- Worsening renal function can produce a phase of progressive oliguria, acidosis and electrolyte imbalance.
- More than 90% of young, otherwise healthy patients with hypovolemic shock survive with appropriate management. Septic or cardiogenic shock has a substantially poorer outcome.

Expected autopsy findings in death from hypovolemic shock due to upper GI bleed

General principle

- The cellular and tissue effects of shock are essentially those of **hypoxic injury**. They result from hypoperfusion plus microvascular thrombosis.
- The organs most involved are the **brain, heart, kidneys, adrenals and gastrointestinal tract**.
- Findings reflect the speed of death. Many changes are microscopic and may be absent if death was rapid.
  - If occlusion or ischemia occurred minutes to hours before death, histologic changes may be absent.
  - It takes 4–12 hours for dead tissue to show microscopic evidence of necrosis.

Table 1: Organ-wise autopsy findings in hypovolemic (hemorrhagic) shock

Organ	Finding	Basis
Kidney	Fibrin thrombi in glomeruli, most readily visualized here	Microvascular thrombosis (DIC)
Kidney	Ischemic injury leading to renal failure	Hypoperfusion
Adrenal	Cortical cell lipid depletion	Stress; stored lipids used for steroid synthesis
Lung	Usually resistant to hypoxic injury in hemorrhagic hypovolemic shock	Diffuse alveolar damage ("shock lung") is typical of sepsis or trauma
Heart	Hypoxic injury with possible cardiomyocyte loss	Myocardial cells die after 20–30 min of ischemia
Brain	Hypoxic injury with neuronal loss	Neurons are damaged irreversibly after 3–4 min
GI tract	Ischemic bowel	Allows entry of intestinal flora

Points to describe

- **Brain:** neuronal loss is an exception to complete recovery. Neurons are irreversibly damaged after only 3–4 minutes without blood supply.

- **Heart:** cardiomyocyte loss is likewise not recoverable. Myocardium is hardier than neurons but dies after only 20–30 minutes of ischemia. Changes in the appearance of the dead cells take 4–12 hours to develop.
- **Kidneys:** fibrin thrombi are best seen in the glomeruli. These reflect microvascular thrombosis, with hypoperfusion as the other mechanism.
- **Adrenals:** lipid depletion of the cortical cells, as in all forms of stress.
  - Adrenal necrosis from DIC (Waterhouse-Friderichsen syndrome) is described in septic shock.
- **Lungs:** these are resistant to hypoxic injury in hypovolemic shock after hemorrhage. Diffuse alveolar damage is not expected unless trauma or sepsis supervenes.
- **GI tract:** ischemic bowel may allow flora to enter the circulation.
- **DIC:** this is a possible complication of shock.
  - Anoxic endothelial injury can lead to DIC.
  - DIC is widespread microcirculatory thrombosis. It consumes platelets and coagulation factors, so bleeding can follow.
- **Reversibility:** except for neuronal and cardiomyocyte loss, affected tissues can recover completely if the patient survives.
  - In a fatal case dying early, therefore, many organs show only subtle or no microscopic change.

Histologic timing relevant at autopsy

- If the patient survived for hours to days, coagulative necrosis may be seen. It is the dominant histologic feature of infarction.
- Acute inflammation appears at infarct margins within a few hours. It is usually well defined within 1–2 days.
- Infarcts are then replaced by scar.
- Brain infarction undergoes liquefactive necrosis, unlike other organs.
- Ischemic injury is more likely where collateral supply is poor. Renal and splenic circulations are end-arterial, so obstruction generally causes tissue death.

DRAW IN THE EXAM

Flowchart of the stages of hypovolemic shock (compensated → progressive → irreversible) with neurohumoral responses, lactic acidosis and DIC.

PEARLS

- **Hypovolemic shock** is low cardiac output from inadequate blood or plasma volume (hemorrhage, burns, vomiting, diarrhea).
- Three stages: **nonprogressive → progressive → irreversible**.
- In the early stage, skin vasoconstriction shunts blood to the coronary and cerebral circulations.
- In the progressive stage, **anaerobic glycolysis → lactic acidosis → arteriolar dilation and pooling → endothelial anoxia → DIC**.
- At autopsy, the organs most affected are the brain, heart, kidneys, adrenals and GI tract.
- Key microscopic clues are **glomerular fibrin thrombi** and **adrenal cortical lipid depletion**.
- Lungs are usually spared in hemorrhagic shock, unlike the "shock lung" of sepsis or trauma.
- Neuronal and cardiomyocyte loss are the only irreversible tissue changes. Histologic necrosis needs 4–12 hours to appear.

24. Pathophysiology of bone healing

ASKED 1× · 10-mark answer

Asked in: Paper I · Jun 2020 · Q10

Q: Pathophysiology of bone healing. [10]

SOURCE

- Robbins — ch. 26 Bones, Joints, and Soft Tissue Tumors — §Fractures; §Healing of Fractures; §Pyogenic Osteomyelitis — pp. 1074–1076

Definition and types of fracture

- **Fracture** is a loss of bone integrity resulting from mechanical injury and/or diminished bone strength.
- Fractures are the most common pathologic conditions affecting bone.
- Bone has a remarkable capacity for repair.
- Fracture types affect treatment and healing:
  - **Simple**: the overlying skin is intact.
  - **Compound**: the bone communicates with the skin surface.
  - **Comminuted**: the bone is fragmented.
  - **Displaced**: the ends of the bone at the fracture site are not aligned.
  - **Stress**: a slowly developing fracture after a period of increased physical activity, with repetitive loading of the bone.
  - **Greenstick**: extends only partially through the bone. It is common in infants and young children, whose bones are soft.
  - **Pathologic**: involves bone weakened by an underlying disease process, such as a tumour.

Sequence of events in fracture healing

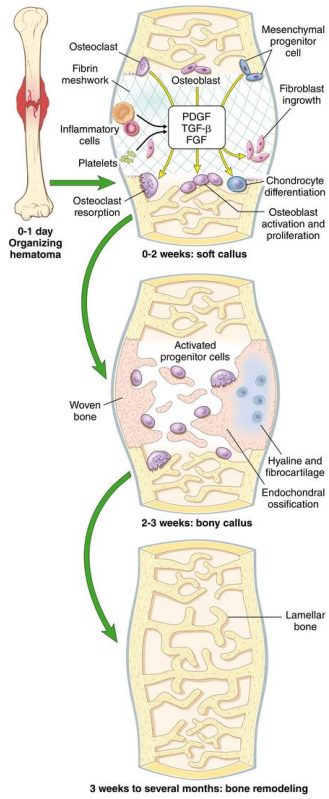
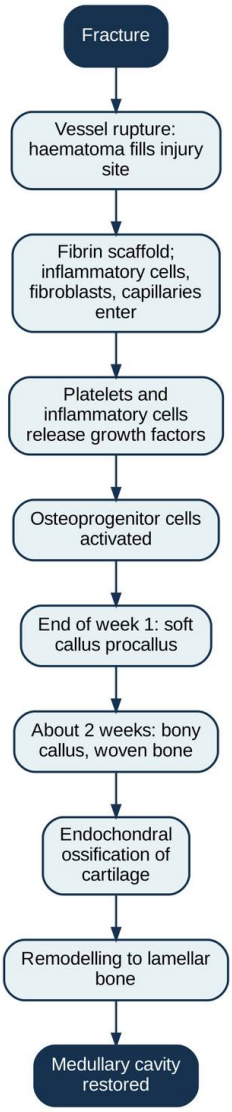


Fig. 26.14 Healing of a fracture. Healing begins with an organizing he- — Robbins 11e, p. 1075

Flowchart 1: Stages of healing of a simple, well-immobilised fracture



Stage 1: Haematoma and inflammation

- At the time of fracture, rupture of blood vessels produces a **haematoma** that fills and surrounds the area of injury.
- The clotted blood seals off the fracture site.
- The clot provides a **fibrin scaffold**. This guides two things:
  - the influx of inflammatory cells
  - the ingrowth of fibroblasts and new capillaries
- Healing therefore begins with an **organising haematoma**.

Stage 2: Growth factor signalling

- Degranulated platelets and migrating inflammatory cells release **PDGF, TGF-β, FGF** and other growth factors.
- These factors activate **osteoprogenitor cells** in three sites:
  - the periosteum
  - the medullary cavity
  - the surrounding soft tissues
- They also stimulate both **osteoclastic and osteoblastic activity**.

Stage 3: Soft callus (procallus)

- By the end of the **first week**, a mass of predominantly uncalcified tissue bridges the fractured ends. This is the **soft callus or procallus**.
- It gives some anchorage but **not structural rigidity** for weight bearing.

- Within 2 weeks, the bone ends are bridged by a fibrin meshwork. Osteoclasts, osteoblasts and chondrocytes differentiate from precursors within it.

Stage 4: Bony callus

- By about **2 weeks** after injury, the procallus is converted into a **bony callus** by deposition of woven bone.
- Activated osteoprogenitor cells deposit subperiosteal trabeculae of woven bone. These are oriented **perpendicular to the cortical axis** within the medullary cavity.
- In some cases, activated soft tissue mesenchymal cells differentiate into **chondrocytes**. These produce fibrocartilage and hyaline cartilage.
- The cartilage undergoes **endochondral ossification** and forms new bone.
- The fractured ends are thus bridged. With progressive mineralisation, the stiffness and strength of the callus increase enough to allow weight bearing.
- Two routes therefore contribute to the callus: direct woven bone deposition (intramembranous-type) and cartilage with endochondral ossification.

Stage 5: Remodelling

- In early callus formation, **excess fibrous tissue, cartilage and woven bone** are produced.
- As the callus matures and bears weight, portions that are not physically stressed are **resorbed**.
- This remodelling reduces the size of the callus. The shape and outline of the bone are re-established as **lamellar bone**.
- Healing is completed by **restoration of the medullary cavity**.
- In simple fractures with adequate immobilisation and no comorbidities, **near-perfect union** is the norm.

Cells and mediators

Table 1: Cells and mediators in bone healing

Component	Source / location	Role
Fibrin clot	Haematoma	Seals fracture site; scaffold for cell and capillary ingrowth
Platelets	Degranulating in haematoma	Release PDGF, TGF-β, FGF
Inflammatory cells	Migrate into the clot	Release growth factors
Osteoprogenitor cells	Periosteum, medullary cavity, soft tissues	Activated to form osteoblasts
Osteoblasts	Differentiate from precursors	Woven bone, then new bone matrix
Osteoclasts	Differentiate from precursors	Resorb unstressed callus during remodelling
Chondrocytes	From mesenchymal cells	Fibrocartilage and hyaline cartilage, then endochondral ossification
Fibroblasts and capillaries	Ingrowth along fibrin	Soft callus

Factors impeding healing

Table 2: Factors impairing fracture healing and their consequences

Factor	Effect
Displaced and comminuted fractures	Frequently result in some deformity
Primary or metastatic bone neoplasm (pathologic fracture)	Often inhibits normal healing
Inadequate immobilisation	Callus moves and maturation is disturbed; delayed union or nonunion
Infection, especially in open fractures	A serious obstacle to healing
Malnutrition, diabetes, skeletal dysplasia (e.g., osteoporosis)	Hinder healing

Immobilisation and nonunion

- Inadequate immobilisation permits movement of the callus and interferes with normal maturation. This may result in **delayed union or nonunion**.
- If nonunion persists, the malformed callus undergoes **cystic degeneration**.

- The luminal surface can become lined by **synovial-like cells**. This creates a **false joint (pseudoarthrosis)**.

Infection

- Compound fractures and orthopaedic procedures provide a route for **direct implantation** of bacteria.
- In adults, osteomyelitis is most often a complication of open fractures, surgical procedures or diabetes.
- **Staphylococcus aureus** causes 80% to 90% of culture-positive pyogenic osteomyelitis.
- Mixed bacterial infections are common with open fractures, surgery or direct spread.
- In acute osteomyelitis, necrosis of bone cells and marrow ensues within **48 hours**.
- Chronic infection leaves **sequestrum** (dead bone) surrounded by **involucrum** (a shell of reactive new bone).

Vascular compromise

- Ischaemic necrosis (osteonecrosis) of bone and marrow can follow mechanical vessel disruption, such as fracture.
- Other mechanisms are thrombotic occlusion and extravascular compression.
- Dead bone is recognised by **empty lacunae** lacking osteocytes.
- Remaining trabeculae act as scaffolding for new bone, while osteoclasts resorb the necrotic trabeculae.
- Repair in subchondral infarcts is slow. This leads to collapse and fracturing of the necrotic bone.

Pathologic fracture and weakened bone

- In Paget disease, weakened pagetic bone is vulnerable to fractures. **Chalk stick–type fractures** are common and usually involve the long bones of the lower limbs.
- Compression fractures of the spine in Paget disease can cause kyphosis and spinal cord injury.
- Osteoporosis, a skeletal dysplasia, hinders healing.

Outcome of healing

- **Normal**: union and remodelling to lamellar bone, with restoration of the medullary cavity.
- **Abnormal**:
  - deformity after displaced or comminuted fractures
  - delayed union or nonunion
  - pseudoarthrosis
  - infection

DRAW IN THE EXAM

Flow diagram of fracture healing: haematoma → soft callus (week 1) → bony callus (about 2 weeks) → remodelling to lamellar bone, with PDGF, TGF-β and FGF marked.

PEARLS

- **Sequence**: haematoma with fibrin scaffold → growth factors (PDGF, TGF-β, FGF) → soft callus (end of week 1) → bony callus (about 2 weeks) → remodelling to lamellar bone.
- **Soft callus** gives anchorage but not rigidity for weight bearing.
- Bony callus forms by woven bone deposition and by **endochondral ossification** of cartilage.
- Remodelling resorbs the unstressed parts of the callus and restores the medullary cavity.
- **Nonunion** with cystic degeneration and synovial-like lining gives **pseudoarthrosis**.
- Impeding factors: displacement or comminution, tumour, poor immobilisation, infection, malnutrition, diabetes, osteoporosis.
- Exam trap: the pathologic fracture (tumour-weakened bone) heals poorly, unlike a simple fracture with adequate immobilisation.

25. Role of growth factors in tissue repair

ASKED 2× · 5-mark answer

Asked in: Paper I · Dec 2020 · Q7(a) · Paper I · Dec 2021 · Q3(b)

Q: Role of growth factors in tissue repair. [5]

Q: Role of extracellular matrix components in wound healing. [5]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Steps in Scar Formation; Angiogenesis; Remodeling of Connective Tissue; Healing of Skin Wounds — pp. 94–97

Role of growth factors in tissue repair

- Cell proliferation in repair is stimulated by **growth factors** and by cell interactions with the ECM.
- **Macrophages**, mostly alternatively activated (M2), are the main source. They clear offending agents and dead tissue, supply growth factors and secrete cytokines that stimulate fibroblasts.
- M2 macrophages, mast cells and some lymphocytes at the injury site all secrete cytokines and growth factors that drive fibroblast proliferation and activation.
- **VEGF (mainly VEGF-A)**:
  - Starts capillary sprouting (the "angiogenic switch") by stimulating endothelial migration and proliferation.
  - Causes vasodilation through nitric oxide and helps form the vascular lumen.
  - Increases vascular permeability.
  - Induces Notch ligands, which regulate vessel branching and spacing.
- **FGF (mainly FGF-2)**:
  - Stimulates endothelial proliferation.
  - Promotes migration of macrophages and fibroblasts to the damaged area.
  - Stimulates epithelial cell migration to cover epidermal wounds.
- **PDGF**:
  - Recruits smooth muscle cells and pericytes to stabilise new vessels.
  - Acts with FGF-2 and TGF-β in fibroblast migration and proliferation.
- **Angiopoietins 1 and 2** are involved in angiogenesis and in the structural maturation of new vessels.
- **TGF-β** is the most important cytokine for synthesis and deposition of connective tissue proteins.
  - It is made by most cells in granulation tissue, including M2 macrophages.
  - It stimulates fibroblast migration and proliferation.
  - It increases synthesis of collagen and fibronectin.
  - It decreases ECM degradation by inhibiting metalloproteinases.
  - It suppresses endothelial proliferation and migration and enhances ECM protein production, which stabilises vessels.
  - It is also anti-inflammatory: it inhibits lymphocyte proliferation and the activity of other leukocytes, which limits and terminates inflammation.
  - Excess TGF-β contributes to fibrosis in lung, liver and kidney after chronic inflammation.
  - Its level is controlled mainly by activation of latent TGF-β, not by gene transcription.
- Glucocorticoids inhibit TGF-β production, giving weak scars with diminished fibrosis.

Table 1: Growth factors in repair

Factor	Main action	Page
VEGF-A	Endothelial sprouting, permeability, NO-mediated vasodilation	ROB pr.95–96
FGF-2	Endothelial proliferation; macrophage, fibroblast and epithelial migration	ROB pr.96
PDGF	Recruits smooth muscle cells and pericytes; fibroblast proliferation	ROB pr.96
TGF-β	Collagen and fibronectin synthesis; inhibits MMPs; anti-inflammatory	ROB pr.96
Angiopoietins 1 and 2	Vessel maturation	ROB pr.96

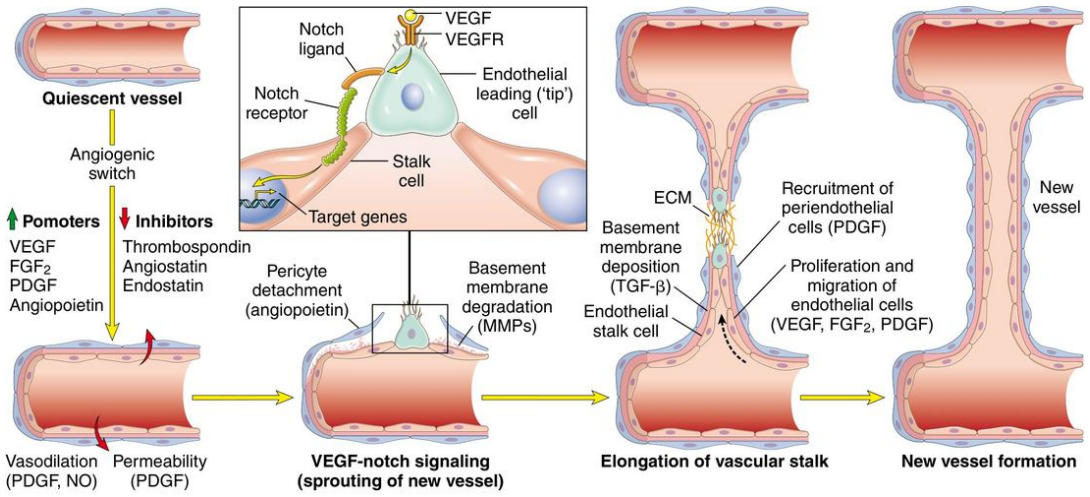


Fig. 3.26 *Angiogenesis. In tissue repair, angiogenesis occurs mainly by sprouting of new vessels. The steps — Robbins 11e, p. 95*

Role of extracellular matrix components in wound healing

- **ECM proteins** support vessel sprouting in angiogenesis. They act through integrin receptors on endothelial cells and provide the scaffold for vessel growth.
- Cell proliferation depends on growth factors together with cell–ECM interactions.
- Damage to the connective tissue framework, which normally scaffolds regenerating cells, leads to scarring instead of regeneration.
- **Granulation tissue** is fibroblasts and thin-walled capillaries in a loose ECM, with macrophages. It is later replaced by collagen to form a stable scar.
- **Deposition of connective tissue** occurs in two steps: fibroblast migration and proliferation into the injury site, then deposition of ECM proteins made by these cells.
  - **Collagen** is critical for developing wound strength.
  - **Fibronectin** synthesis is increased by TGF-β.
  - As healing progresses, fibroblasts become more synthetic, so ECM deposition increases. Vascular regression then turns granulation tissue into a pale, avascular scar.
  - **Myofibroblasts** have actin filaments and contribute to scar contraction.
- **ECM remodelling** depends on the balance between ECM synthesis and degradation.
  - **MMPs** are zinc-dependent enzymes that degrade collagen and other ECM components.
  - Interstitial collagenases (MMP-1, -2, -3) cleave fibrillar collagen.
  - Gelatinases degrade amorphous collagen and fibronectin.
  - Stromelysins degrade proteoglycans, laminin, fibronectin and amorphous collagen.
  - MMPs are made as inactive zymogens, activated by proteases such as plasmin at the injury site, and then inhibited by **TIMPs**.
  - MMPs also degrade ECM during angiogenesis to allow extension of the vascular tube.
- Basement membrane degradation by MMPs allows the vessel sprout to form, and TGF-β later drives basement membrane deposition.
- ECM deposition therefore depends on the balance of fibrogenic agents (TGF-β), MMPs and TIMPs.

Table 2: Kinetics of ECM events in skin wound healing

Time	Event
Day 3	Early granulation tissue; angiogenesis and ECM deposition begin
Day 5	Maximum neovascularisation and granulation tissue; collagen and ECM deposition
Day 14	Continued collagen deposition and fibroblast proliferation
Day 30	Complete scar formation

(Source: ROB pr.97)

TABLE 3.11 Kinetics of Healing of Skin Wounds — Robbins 11e, p. 97

Time	Sequence of Events in Wound Healing
Immediate	Formation of blood clot
24 hours	Neutrophils at incision margins
24–48 hours	Epithelial cells from both edges proliferate to form a thin continuous layer
Day 3	Neutrophils replaced by macrophages; early granulation tissue appears; initiation of angiogenesis and ECM deposition
Day 5	Maximum neovascularization and granulation tissue; collagen and ECM deposition
Day 14	Continued collagen deposition and fibroblast proliferation
Day 30	Complete scar formation

ECM, Extracellular membrane.

PEARLS

- **TGF-β** is the most important fibrogenic cytokine. It also acts as an anti-inflammatory brake.
- **VEGF-A** is the angiogenic switch; FGF-2 drives endothelial proliferation; PDGF recruits pericytes and smooth muscle cells.
- Macrophages (M2) are the main source of repair growth factors.
- The ECM scaffold, integrins and the **MMP/TIMP** balance determine the quality of the scar.
- Latent TGF-β activation is regulated by the ECM (integrins, microfibrils).
- Granulation tissue is not a granuloma.

26. Types of collagen

ASKED 1× · 5-mark answer
Asked in: Paper I · Dec 2022 · Q2(b)
Q: Types of collagen. [5]

SOURCE

- Robbins — ch. 5 Genes and Human Diseases — §Enzyme Defects and Their Consequences; §Ehlers-Danlos Syndrome; §Familial Hypercholesterolemia — pp. 127, 130–131
- W1 · PMC (Extracellular Matrix and Liver Disease) — 2014
- W2 · PMC (A Review of the Effects of Collagen Treatment in Clinical Studies) — 2021
- W3 · PMC (The role of collagen and collagen I/III ratio in pathological conditions) — 2025
- W4 · PMC (Collagen: quantification, biomechanics, and role of minor subtypes in cartilage) — 2020
- W5 · PMC (Main and Minor Types of Collagens in the Articular Cartilage) — 2021
- W6 · PMC (Advances in Molecular Function and Recombinant Expression of Human Collagen) — 2025

NOT IN THE REFERENCE BOOKS

Searched the supplied Robbins 11e pages (ch. 5, Table 5.4, EDS, Table 5.5) for a classification of collagen types. They list collagen genes in disease only, so the type-wise description comes from web sources: PubMed Central reviews found with the search "collagen types I II III IV distribution function review PMC".

Definition and classification of collagens

- Collagen is the main structural protein of the extracellular matrix. It is built from three α-chains wound into a triple helix.
- Types differ by α-chain composition; about 28 types are known, and type I is dominant.
- More than 90% of body collagen is type I.
- Collagens are grouped by structure and function:
  - **Fibrillar (fibril-forming)**: types I, II, III, XI (and XXIV, XXVII); the most abundant; give tensile strength.
  - **Network-forming**: types IV, VIII, X. Their triple helix is interrupted, which gives flexibility and allows network formation.
  - **FACIT** (fibril-associated collagens with interrupted triple helix) form a third group.

Table 1: Major collagen types, distribution and associated disorders

Type	Group · composition	Distribution / function	Disorder (gene)
I	Fibrillar · [α1(I)]2α2(I)	Skin, bone, teeth, tendon, ligament; stiffness and tensile strength	Osteogenesis imperfecta; arthrochalasia EDS (COL1A1, COL1A2)
II	Fibrillar · [α1(II)]3	Cartilage, vitreous, intervertebral disc	Cartilage repair quality (hyaline vs fibrocartilage)
III	Fibrillar · [α1(III)]3	Reticular fibres, blood vessels, intestine, skin	Vascular EDS (COL3A1)
IV	Network-forming · [α1(IV)]2α2(IV) and variants	Basement membrane, basal lamina	Alport syndrome
V	Fibrillar	Hair, cornea, placenta, skin, bone, cell surfaces	Classic EDS (COL5A1)

Type-wise features

Type I

- It is the commonest collagen, in skin, bone, tendon and ligament.
- It forms large fibrils in bone, skin, tendons and ligaments.
- Mutations in the type I genes cause osteogenesis imperfecta.
- COL1A1/COL1A2 mutations also cause arthrochalasia EDS.
  - In arthrochalasia EDS, abnormal pro-α chains resist N-terminal cleavage.
  - A single mutant allele makes 50% of type I chains abnormal. These interfere with normal helix formation, so heterozygotes are affected.

Type II

- It is a fibrillar collagen restricted to cartilage, vitreous and intervertebral disc.
- It makes up more than 90–95% of mature articular cartilage collagen.
- It is the main fibrillar type of hyaline cartilage.

Type III

- It is a homotrimer of three identical α1(III) chains.
- It usually forms hetero-fibrils with type I. It provides distension and flexibility to skin, vessels and intestine.
- The type I/III ratio regulates tissue elasticity and tensile strength.
- It is the second most abundant collagen.
- Type III collagen defects cause **vascular EDS** (COL3A1).

- Blood vessels and intestines are rich in type III, so there is a risk of spontaneous rupture.
- The skin is usually not hyperextensible.
- Some mutant alleles act as dominant negatives, so heterozygotes have a severe phenotype.

Type IV

- It is the key structural component of the basement membrane.
- It is a flexible mesh.
- α1 and α2 chains occur in all basement membranes. α3–α6 chains are tissue-specific.
- Its role in the glomerulus is filtration; Alport syndrome is a collagen-synthesis mutation disorder.

Other types

- Type V mutations (COL5A1, COL5A2) cause classic EDS.
- Type XII (COL12A1) mutations cause myopathic EDS.

Collagen defects (Ehlers-Danlos syndrome)

- EDS arises from mutations in collagen genes, collagen-modifying enzymes, or other ECM proteins.
- The 2017 International Classification lists 13 subtypes.
- Examples of enzyme defects:
  - Kyphoscoliotic EDS: PLOD1 (lysyl hydroxylase) deficiency; collagen lacks stable cross-links.
  - Dermatosparaxis EDS: ADAMTS2 (procollagen N-peptidase) deficiency; autosomal recessive.
- Common clinical features:
  - Hyperextensible, fragile skin.
  - Hypermobile joints.
  - Rupture of colon, cornea or large arteries.
  - Poor wound healing.

TABLE 5.5 *Classification of Ehlers-Danlos Syndrome — Robbins 11e, p. 130*

EDS Type	Inheritance	Gene Defects
Classical	Autosomal dominant	*COL5A1* *COL1A1* (rare)
Vascular	Autosomal dominant	*COL3A1* *COL1A1* (rare)
Hypermobile	Autosomal dominant	Unknown
Arthrochalasia	Autosomal dominant	*COL1A1* *COL1A2*
Periodontal	Autosomal dominant	C1r C1s
Myopathic	Autosomal dominant or autosomal recessive	*COL12A1*
Classical-like	Autosomal recessive	*TNXB*
Cardiac-valvular	Autosomal recessive	*COL1A2*
Dermatosparaxis	Autosomal recessive	*ADAMTS2*
Kyphoscoliotic	Autosomal recessive	*PLOD1* *FKBP14*
Brittle cornea syndrome	Autosomal recessive	*ZNF469* *PRDM5*
Spondylodysplastic	Autosomal recessive	*B4GALT7* *B3GALT6* *SLC39A13*
Musculocontractural	Autosomal recessive	*CHST14* *DSE*

PEARLS

- Type I is the most abundant. Type II is cartilage. Type III is reticulin, in vessels and gut. Type IV is basement membrane. Type V is classic EDS.
- Fibrillar types are I, II, III and V (plus XI); type IV is network-forming.
- Remember the gene–disease pairs: COL3A1 and vascular EDS, COL5A1 and classic EDS, COL1A1/COL1A2 and osteogenesis imperfecta or arthrochalasia EDS.
- The type I/III ratio governs tissue elasticity and strength.
- Exam trap: vascular EDS has non-hyperextensible skin.

27. Chemokines in inflammation

ASKED 2× · 10-mark answer

Asked in: Paper I · Dec 2021 · Q1 · Paper I · Jun 2025 · Q9(b)

Q: Enumerate the mediators of inflammation and describe their roles in acute inflammation. [10]

Q: Chemokines in inflammation. [5]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Overview of Inflammation; Other Functional Responses of Activated Leukocytes; Vasoactive Amines: Histamine and Serotonin; Complement System; Other Mediators of Inflammation; Serous Inflammation — pp. 76–78, 81–83

Enumeration of mediators of inflammation

- **Definition:** inflammatory mediators are substances that initiate and regulate inflammatory reactions.
- **Principal mediators of acute inflammation:** vasoactive amines, lipid products (prostaglandins and leukotrienes), cytokines including chemokines, and complement products.
- **Cell-derived mediators:**
  - Histamine and serotonin are preformed in granules and released by exocytosis.
  - Prostaglandins, leukotrienes and cytokines are synthesised de novo in response to a stimulus.
  - Sentinel cells make most of them: macrophages, dendritic cells and mast cells. Platelets, neutrophils, endothelium and epithelia can also make some.
- **Plasma-derived mediators:**
  - Complement and kinins are produced mainly in the liver.
  - They circulate as inactive precursors and are activated by proteolytic cleavage.
- **Other mediators:** platelet-activating factor (PAF), products of coagulation, kinins and neuropeptides.

General properties of mediators

- Active mediators are produced only in response to microbial products or substances from injured or necrotic cells.
- Most are short-lived. They decay, are inactivated by enzymes, or are scavenged.
- One mediator can release others, which amplifies or counteracts the first action.
  - Complement products stimulate histamine release.
  - TNF acts on endothelium to produce IL-1 and many chemokines.
- Several mediators have overlapping actions (redundancy), so the response is robust.

Table 1: Principal mediators, sources and actions in acute inflammation

Mediator	Principal sources	Actions
Histamine	Mast cells, basophils, platelets	Vasodilation, increased permeability, endothelial activation
Prostaglandins	Mast cells, leukocytes	Vasodilation, pain, fever
Leukotrienes	Mast cells, leukocytes	Increased permeability, chemotaxis, leukocyte adhesion and activation
TNF, IL-1, IL-6	Macrophages, endothelium, mast cells	Local: endothelial activation. Systemic: fever, metabolic change, hypotension
Chemokines	Leukocytes, activated macrophages	Chemotaxis, leukocyte activation
PAF	Leukocytes, mast cells	Vasodilation, permeability, adhesion, chemotaxis, degranulation, oxidative burst
Complement	Plasma (liver)	Chemotaxis, activation, MAC killing, vasodilation
Kinins	Plasma (liver)	Permeability, smooth muscle contraction, vasodilation, pain

Roles of mediators in acute inflammation

Vasoactive amines

- Histamine and serotonin are the two major vasoactive amines. Both are preformed and among the first mediators released.
- **Histamine sources:** the richest source is mast cells beside vessels. It is also found in basophils and platelets.
- **Triggers for release:**
  - Physical injury, cold and heat.
  - Antigen binding to IgE on mast cells (immediate hypersensitivity).
  - Anaphylatoxins C3a and C5a.
  - Neuropeptides (substance P) and cytokines (IL-1, IL-8).
- **Actions of histamine:**
  - Dilates arterioles and increases venular permeability.
  - It is the principal mediator of the immediate transient phase of increased permeability. It produces interendothelial gaps in postcapillary venules.
  - Effects act mainly via H1 receptors on microvascular endothelium.
- **Serotonin:** present in platelets and neuroendocrine cells. It is a vasoconstrictor, but its role in inflammation is unclear.

Arachidonic acid (AA) metabolites

- AA is a 20-carbon polyunsaturated fatty acid from membrane phospholipids. Phospholipase A2 releases it.
- Cyclooxygenases make prostaglandins. Lipoxygenases make leukotrienes and lipoxins.
- Eicosanoids bind G protein–coupled receptors and mediate virtually every step of inflammation.

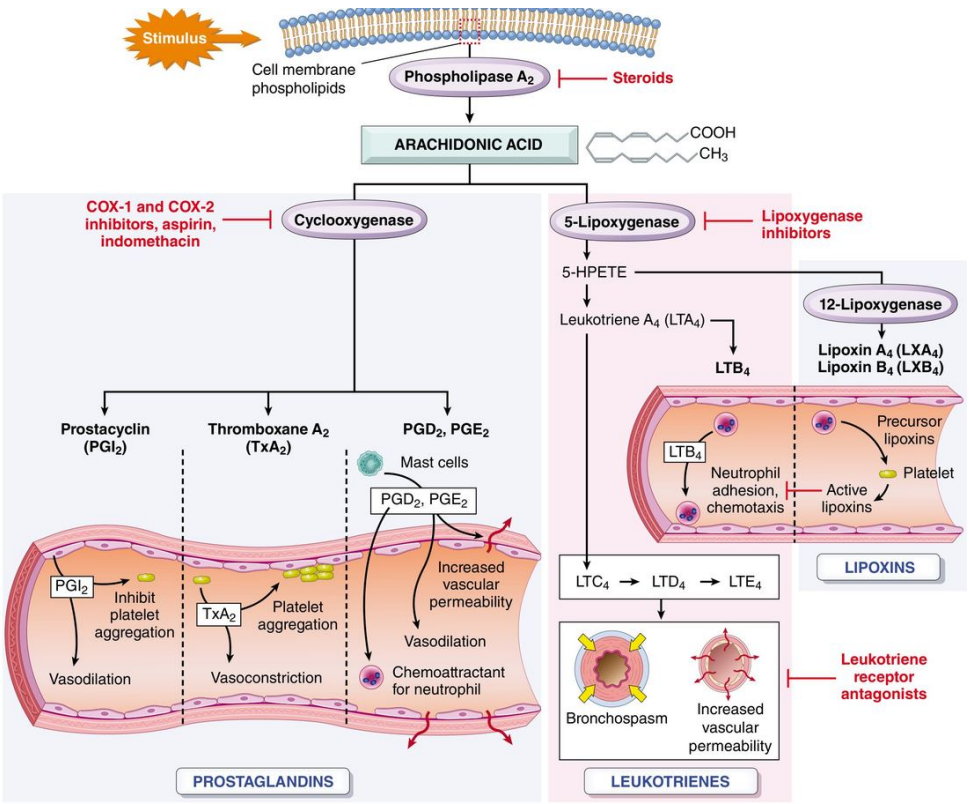


Fig. 3.9 Production of arachidonic acid metabolites and their roles in inflammation. Note the enzymatic ac- — Robbins 11e, p. 78

Table 2: Actions of arachidonic acid metabolites

Action	Eicosanoid
Vasodilation	PGI2, PGE1, PGE2, PGD2
Vasoconstriction	Thromboxane A2, LTC4, LTD4, LTE4
Increased permeability	LTC4, LTD4, LTE4
Chemotaxis, leukocyte adhesion	LTB4, HETE

- **COX-1 and COX-2:**
  - COX-1 is constitutive and homeostatic. Examples are renal fluid balance and gastric cytoprotection.
  - COX-2 is mainly confined to cells in inflammatory reactions.
- **PGD2 and PGE2:** PGD2 is the major prostaglandin of mast cells. With PGE2 it causes vasodilation and increases venular permeability. PGD2 also attracts neutrophils.
- **Thromboxane A2:** made by platelets. It is a potent platelet aggregator and vasoconstrictor.
- **Prostacyclin (PGI2):** made by endothelium. It is a vasodilator and inhibits platelet aggregation. It potentiates the permeability and chemotactic effects of other mediators.
- **Thromboxane–prostacyclin imbalance** is implicated in early thrombus formation.
- **Pain and fever:** PGE2 is hyperalgesic. Prostaglandins are involved in pain and fever.
- **Lipoxins** are antiinflammatory. The switch from leukotrienes to lipoxins helps terminate inflammation.
- **Drug targets:** steroids (phospholipase A2), COX inhibitors (aspirin, indomethacin), lipoxygenase inhibitors and leukotriene receptor antagonists.

Cytokines

- **Key cytokines:** TNF, IL-1 and chemokines are the principal cytokines in acute inflammation.
- **Local effect:** endothelial activation, with expression of adhesion molecules.
- **Systemic effects:** fever, metabolic abnormalities and hypotension (shock).
- **IL-6:** made by macrophages and others. It acts in local and systemic reactions. Anti-IL-6 receptor therapy is used in juvenile arthritis.
- **IL-17:**

- Made mainly by T cells (Th17). It promotes neutrophil recruitment.
- It induces chemokines that recruit other leukocytes.
- Without Th17 responses, patients get "cold abscesses" that lack warmth and redness. They are also susceptible to fungal and bacterial infections.
- Anti-IL-17 therapy is used in psoriasis.

- **Chemokines** are dealt with in their own section below.

Complement system

- Complement is more than 20 plasma proteins, including C1–C9. It acts in innate and adaptive immunity.
- C3 cleavage is the critical step. It can occur by three pathways:
  - **Classical:** C1 bound to antibody (IgM or IgG) with antigen.
  - **Alternative:** microbial surface molecules such as LPS, without antibody.
  - **Lectin:** mannose-binding lectin binds microbial carbohydrates.
- All three form C3 convertase, which splits C3 into C3a and C3b. C5 convertase then cleaves C5 to C5a and C5b. C5b with C6–C9 forms the membrane attack complex (MAC).

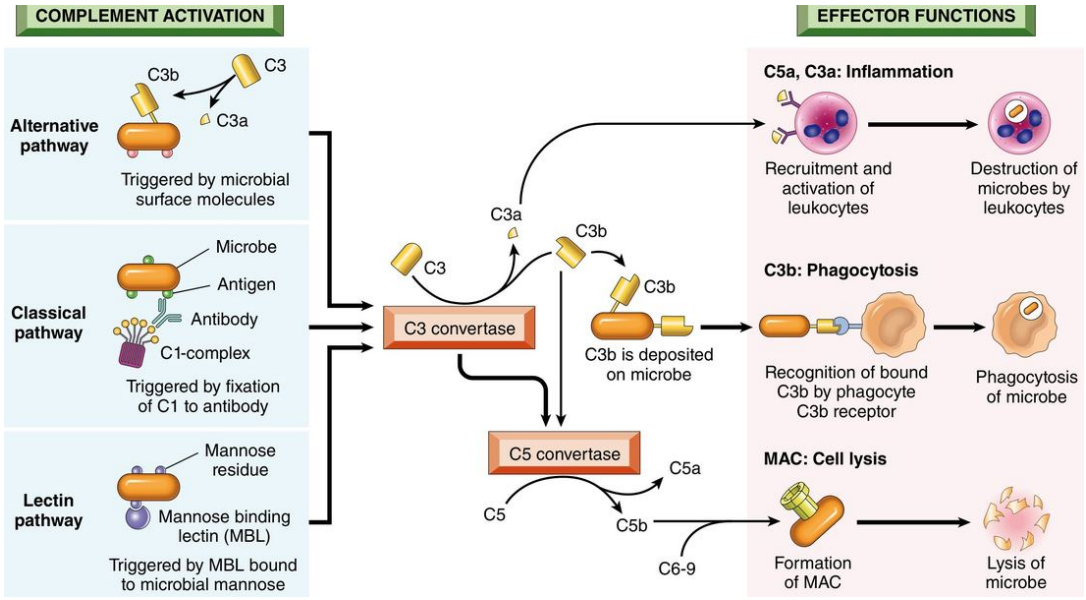


Fig. 3.11 Activation and functions of the complement system. Activation of complement by different path- — Robbins 11e, p. 82

- **Three functions:**
  - **Inflammation:**
  - C5a, C3a and (less) C4a are anaphylatoxins. They release histamine, increasing permeability and causing vasodilation.
  - C5a is chemotactic for neutrophils, monocytes, eosinophils and basophils.
  - C5a also activates the lipoxygenase pathway.
  - **Opsonization:** C3b and iC3b promote phagocytosis.
  - **Cell lysis:** the MAC causes osmotic lysis. This is important for Neisseria; terminal component deficiency predisposes to Neisseria infection.
- **Regulators:**
  - C1 inhibitor: its deficiency causes hereditary angioedema.
  - DAF (CD55) and CD59: GPI-anchored. Their deficiency causes paroxysmal nocturnal hemoglobinuria (PNH).
  - Factor H: defects cause atypical hemolytic uremic syndrome.

Other mediators

- **PAF:**
  - It is phospholipid-derived. Platelets, basophils, mast cells, neutrophils, macrophages and endothelium make it.
  - It causes platelet aggregation, vasoconstriction and bronchoconstriction. At low concentration it causes vasodilation and increased venular permeability.

- **Coagulation products:** thrombin acts on protease-activated receptors on leukocytes. Fibrinopeptides may stimulate inflammation.
- **Kinins:**
  - Kallikrein cleaves high-molecular-weight kininogen to produce bradykinin.
  - Bradykinin increases permeability, contracts smooth muscle, dilates vessels and causes pain. Kininase quickly inactivates it.
- **Neuropeptides:** substance P transmits pain and increases permeability. Vagal activation inhibits TNF production.

Table 3: Mediators responsible for each reaction of acute inflammation

Reaction	Principal mediators
Vasodilation	Histamine
Increased permeability	Histamine, serotonin, C3a, C5a, LTC4, LTD4, LTE4
Chemotaxis and leukocyte recruitment	TNF, IL-1, chemokines, C3a, C5a, LTB4
Fever	IL-1, TNF, prostaglandins
Pain	Prostaglandins, bradykinin, substance P
Tissue damage	Lysosomal enzymes, ROS

Chemokines in inflammation

- **Definition:** chemokines are cytokines that mediate leukocyte chemotaxis and activation.
- **Receptors:**
  - They bind seven-transmembrane G protein–coupled receptors. Most leukocytes express more than one type.
  - CXCR4 and CCR5 are coreceptors for HIV entry.
- **Display:** they are displayed at high concentration on endothelial proteoglycans and in the extracellular matrix.

Table 4: Chemokine classes

Class	Structural feature	Example	Target cells
Neutrophil-act ing class	Chemotactic for neutrophils; limited effect on monocytes and eosinophils	Induced by microbial products, IL-1, TNF	Neutrophils
C-C	First two cysteines adjacent	MCP-1 (CCL2), eotaxin (CCL11), MIP-1α (CCL3)	Monocytes, eosinophils, basophils, lymphocytes; weak for neutrophils. Eotaxin is selective for eosinophils
C	Lack the first and third cysteines	Lymphotactin (XCL1)	Lymphocytes
CX3C	Three amino acids between the first two cysteines	Fractalkine (CX3CL1)	Monocytes and T cells

- **Fractalkine:** the cell-surface form is induced on endothelium by inflammatory cytokines and promotes strong adhesion. The soluble form is a potent chemoattractant for the same cells.
- **Functions:**
  - **Inflammatory chemokines** are induced by microbes and other stimuli. They increase integrin affinity on leukocytes, which promotes attachment to endothelium. They also drive chemotaxis to the site of infection or damage.
  - **Homeostatic chemokines** are produced constitutively. They organise tissue architecture, for example T and B cells in separate areas of spleen and lymph node.
- **Therapy:** it has been difficult to develop effective chemokine antagonists.

DRAW IN THE EXAM

Flow diagram of the arachidonic acid pathway (phospholipase A2 → COX and lipoxygenase products) with their actions and drug inhibition sites.

PEARLS

- Histamine is the principal mediator of the immediate transient increase in permeability. It acts mainly via H1 receptors.
- COX-1 is constitutive. COX-2 is induced at inflammatory sites.
- Thromboxane A2 (platelets) causes vasoconstriction and aggregation. Prostacyclin (endothelium) does the opposite.
- C3 cleavage is the critical step of complement. C5a is the chemotactic anaphylatoxin.
- C-C chemokines attract monocytes, eosinophils, basophils and lymphocytes. Eotaxin is eosinophil-selective.
- CXCR4 and CCR5 are HIV coreceptors.
- Deficiencies of C1 inhibitor, DAF/CD59 and terminal complement components cause hereditary angioedema, PNH and Neisseria infection respectively.
- TNF and IL-1 are the main cytokines of acute inflammation. They cause fever and endothelial activation.

28. Clinical use of inhibitors of prostaglandins & leukotrienes

ASKED 1× · 5-mark answer

Asked in: Paper I · May 2024 · Q3(a)

Q: Clinical use of inhibitors of prostaglandins & leukotrienes. [5]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Vasoactive Amines: Histamine and Serotonin; §Cytokines and Chemokines — pp. 77–79

Sites of inhibition in the arachidonic acid pathway

- Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid. Phospholipases, mainly **phospholipase A2**, release it from membrane phospholipids.
- **Cyclooxygenases** (COX-1, COX-2) generate prostaglandins. **Lipoxygenases** generate leukotrienes and lipoxins.
- Each drug class blocks a different step of this pathway, as Fig. 3.9 shows.

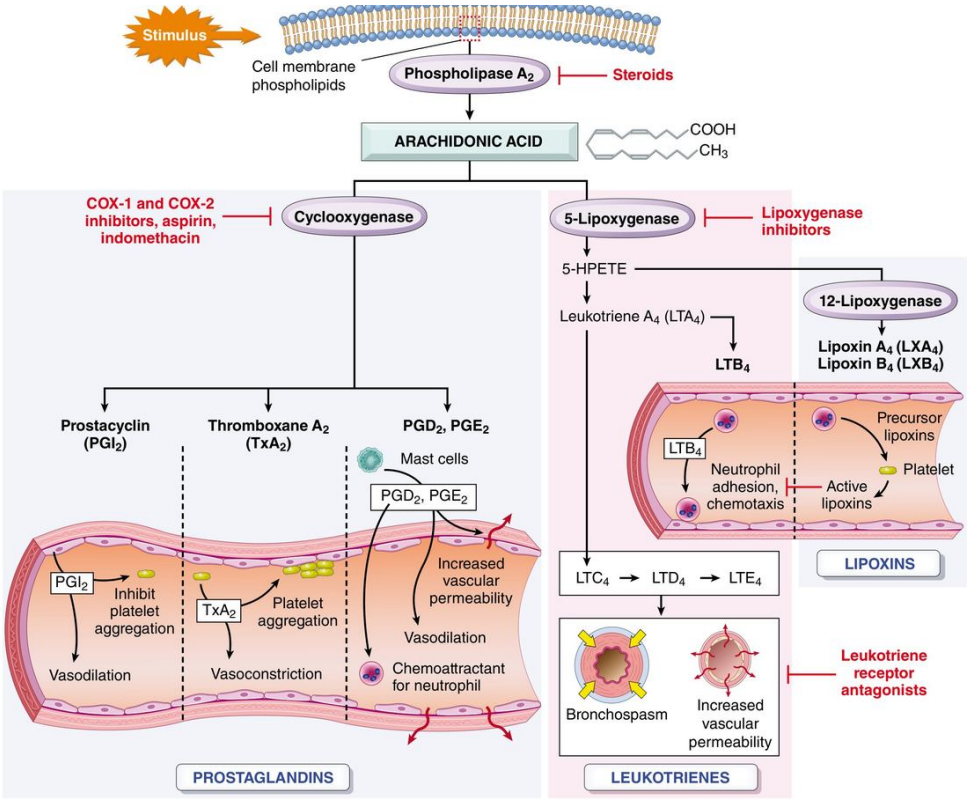


Fig. 3.9 Production of arachidonic acid metabolites and their roles in inflammation. Note the enzymatic ac- — Robbins 11e, p. 78

- Prostaglandin actions that are targeted:

  - PGE2 and PGD2 cause vasodilation and raise venular permeability.
  - PGE2 is hyperalgesic and mediates cytokine-induced fever.
- Leukotriene actions that are targeted:
  - LTB4 is chemotactic and causes neutrophil adhesion.
  - LTC4, LTD4 and LTE4 cause **bronchospasm** and increased venular permeability.

Inhibitors and their clinical use

Table 1: Inhibitors of eicosanoid synthesis or action and their clinical use

Drug class	Target	Clinical use
Aspirin and other NSAIDs	COX-1 and COX-2	Pain and fever
Selective COX-2 inhibitors	COX-2 (200–300-fold more potent than on COX-1)	Anti-inflammatory use with less gastric toxicity
Lipoxygenase inhibitors	5-Lipoxygenase	Asthma
Leukotriene receptor antagonists	Leukotriene receptors	Asthma
Corticosteroids	Gene transcription	Broad-spectrum anti-inflammatory
Fish oil (dietary)	Altered substrate	Modifies lipid mediator profile

Cyclooxygenase inhibitors (NSAIDs)

- NSAIDs inactivate both COX-1 and COX-2, so they inhibit prostaglandin synthesis. This explains their efficacy in **pain and fever**.
- **Aspirin** acts by irreversibly acetylating and inactivating cyclooxygenases.
- Rationale for COX-2 selectivity:
  - COX-1 is constitutive. It serves homeostatic functions such as renal fluid and electrolyte balance and gastric cytoprotection.
  - COX-2 expression is mainly confined to cells in inflammatory reactions.
  - Selective COX-2 inhibitors were therefore expected to be anti-inflammatory without causing gastric ulceration.
- Limitations of selective COX-2 inhibitors:
  - The COX-1/COX-2 distinction is not absolute, because COX-2 also plays a role in cytoprotection.
  - They may increase **cardiovascular and cerebrovascular events**.
  - Mechanism: they impair endothelial PGI2 (prostacyclin), a vasodilator and inhibitor of platelet aggregation. Platelet COX-1-derived thromboxane A2, which activates platelets and constricts vessels, is left intact.
  - The balance tilts towards thromboxane and vascular thrombosis, especially in patients with other thrombotic risk factors.
  - They are still used in patients without cardiovascular risk factors when benefits outweigh risks.
- Thromboxane A2 is the major eicosanoid in platelets. It is a potent platelet aggregator and vasoconstrictor.
- A thromboxane–prostacyclin imbalance is implicated early in coronary and cerebral thrombus formation.

Lipoxygenase inhibitors and leukotriene receptor antagonists

- 5-Lipoxygenase is **not affected by NSAIDs**. Separate inhibitors have been developed for it.
- Agents that inhibit leukotriene production are useful in **asthma**.
- Leukotriene receptor antagonists block leukotriene receptors and are also used in asthma.
- Rationale: the cysteinyl leukotrienes LTC4, LTD4 and LTE4 cause bronchospasm, which is important in asthma.

Corticosteroids

- They are broad-spectrum anti-inflammatory agents.
- They reduce transcription of genes for **COX-2, phospholipase A2**, pro-inflammatory cytokines (IL-1, TNF) and iNOS.
- Phospholipase A2 inhibition blocks release of AA, so both the prostaglandin and the leukotriene pathways are suppressed (Fig. 3.9).

Dietary modification

- Increasing fish oil intake modifies the lipid mediators. Its polyunsaturated fatty acids are poor substrates for the cyclooxygenase and lipoxygenase pathways.
- They are better substrates for anti-inflammatory products such as **resolvins**.

DRAW IN THE EXAM

Flow diagram of the arachidonic acid pathway (phospholipase A2, COX, 5-lipoxygenase) with the sites of action of steroids, NSAIDs, lipoxygenase inhibitors and leukotriene receptor antagonists.

PEARLS

- NSAIDs block COX-1 and COX-2, so they give anti-inflammatory, analgesic and antipyretic effects. They spare 5-lipoxygenase.
- Aspirin acetylates COX **irreversibly**.
- Selective COX-2 inhibitors spare gastric COX-1 but carry a cardiovascular and thrombotic risk. The cause is reduced PGI2 with preserved platelet TxA2.
- Leukotriene inhibitors (lipoxygenase inhibitors and receptor antagonists) are used in **asthma**.
- Corticosteroids act at the gene transcription level (COX-2, PLA2, IL-1, TNF, iNOS).
- Lipoxins and resolvins are anti-inflammatory AA derivatives. Fish oil favours resolvin production.

29. Neutrophil extracellular traps (NETs / NETosis)

ASKED 3× · 10-mark answer

Asked in: Paper I · Dec 2022 · Q3(a) · Paper I · Jun 2021 · Q3(b) · Paper I · Oct 2024 · Q5

Q: Neutrophil Extracellular Traps. [5]
Q: Neutrophil Extracellular traps (NETs) and its implications in inflammation. [5]
Q: NETosis (Neutrophil Extracellular traps): Role in Pathology, with emphasis on pathophysiology. [10]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Neutrophil Extracellular Traps; §Other Functional Responses of Activated Leukocytes — pp. 75–76
- W1 · SCCM/ESICM Task Force — Sepsis-3 (Third International Consensus Definitions), JAMA 2016;315(8):801–810 — 2016

Definition

- **Neutrophil extracellular traps (NETs)** are extracellular fibrillar networks. They concentrate antimicrobial substances at sites of infection and trap microbes, helping to prevent their spread.
- **NETosis** is the regulated neutrophil cell death that produces NETs. The nucleus is lost and the cell dies.
- NETs consist of a viscous meshwork of **nuclear chromatin** (histones and DNA). The chromatin binds and concentrates granule proteins such as antimicrobial peptides and enzymes.
- NET release is one of the ways leukocytes eliminate microbes, alongside phagocytosis and destruction in phagolysosomes.
- Sepsis-3 defines sepsis as "life-threatening organ dysfunction caused by a dysregulated host response to infection." This definition frames why NETs in blood matter.

Pathophysiology of NET formation

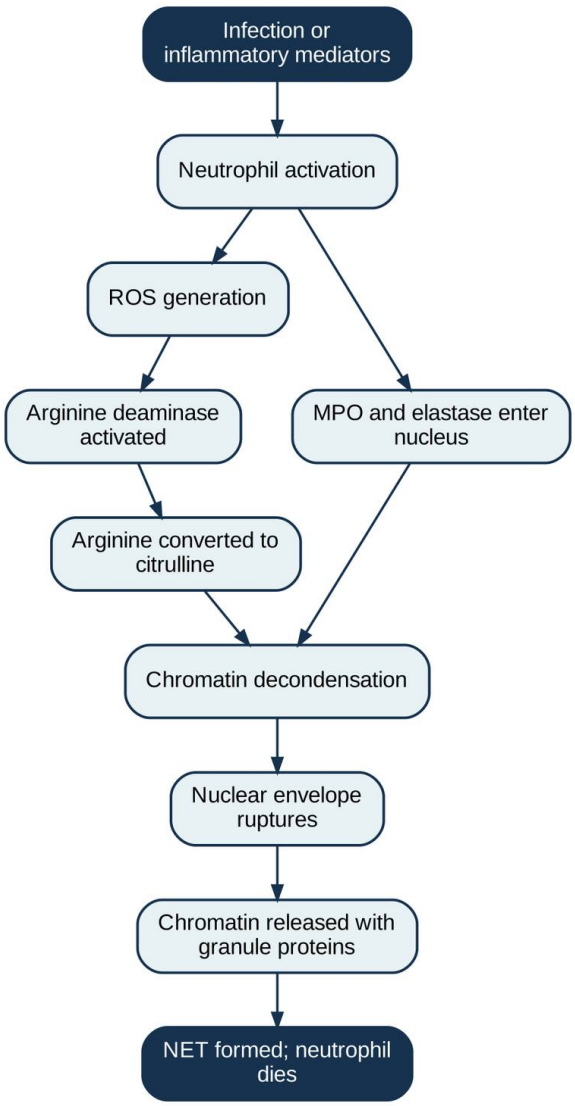
Triggers

- Neutrophils make NETs in response to **infectious pathogens**, mainly bacteria and fungi.
- They also respond to **inflammatory mediators**: chemokines, cytokines (mainly interferons), complement proteins and ROS.

Stepwise mechanism

- **Step 1:** ROS-dependent activation of an **arginine deaminase** converts arginines to **citrulline**. This causes **chromatin decondensation**.
- **Step 2:** Other enzymes of activated neutrophils, **MPO and elastase**, enter the nucleus. They cause further chromatin decondensation.
- **Step 3:** The nuclear envelope ruptures and chromatin is released.
- **Step 4:** The neutrophil loses its nucleus and dies. This is "beneficial suicide", as the Fig. 3.8 source title puts it.
- **Step 5:** Released chromatin carries granule proteins, which are concentrated on the mesh.

Flowchart 1: Pathophysiology of NETosis



Granule contents that load the NET

- **Azurophil (primary) granules** contain MPO, bactericidal proteins (lysozyme, defensins), acid hydrolases, and neutral proteases (elastase, cathepsin G, nonspecific collagenases, proteinase 3).
- **Specific (secondary) granules** contain lysozyme, collagenase, gelatinase, lactoferrin, plasminogen activator, histaminase and alkaline phosphatase.
- **Defensins** are cationic arginine-rich peptides toxic to microbes. **Cathelicidins** are antimicrobial proteins.
- **Lysozyme** hydrolyses the muramic acid–N-acetylglucosamine bond in the bacterial glycopeptide coat. **Lactoferrin** is an iron-binding protein.
- **Neutral proteases** degrade collagen, basement membrane, fibrin, elastin and cartilage. They also cleave C3 and C5 and release a kinin-like peptide from kininogen.

- These properties explain why NET-bound enzymes can both kill microbes and injure tissue.

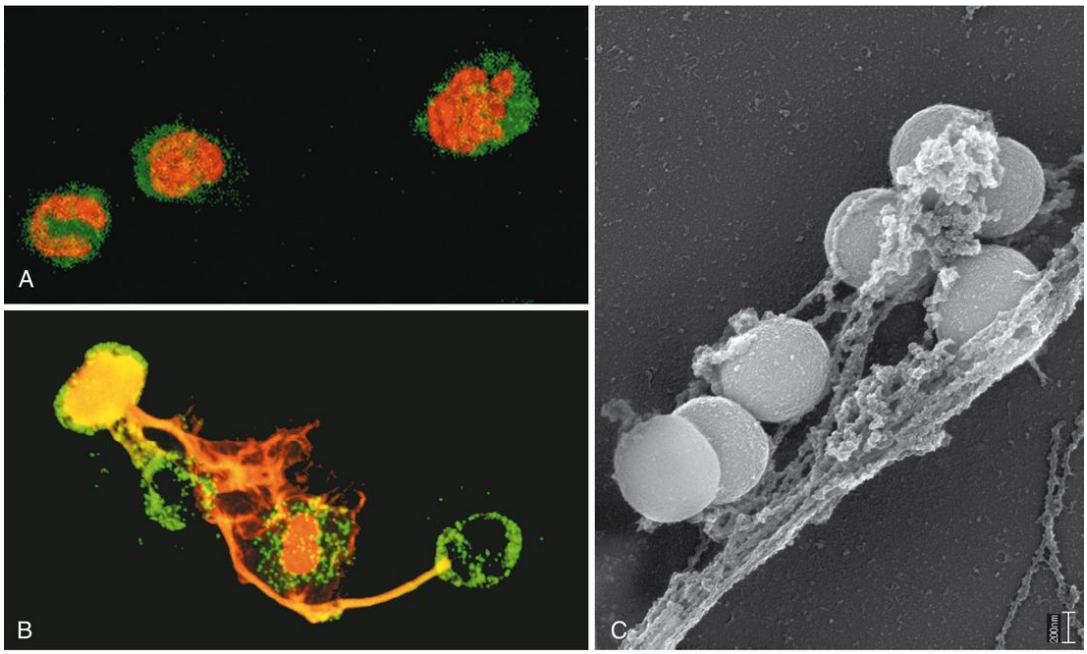


Fig. 3.8 *Neutrophil extracellular traps (NETs). (A) Healthy neutrophils with nuclei stained red and cytoplasm — Robbins 11e, p. 75*

DRAW IN THE EXAM

Neutrophil releasing decondensed chromatin and granule proteins to form an extracellular mesh trapping bacteria, labelled with the steps (ROS, arginine deaminase, citrulline, MPO/elastase, nuclear rupture).

Role in host defence (physiological implications)

- NETs give **high local concentrations** of antimicrobial substances.
- They **trap large microbes**, such as fungi, which may be too large to be phagocytosed.
- They **prevent systemic dissemination** of microbes.
- Appearance on EM (Fig. 3.8C): bacteria (staphylococci) are seen trapped in NET fibres.
- Appearance on staining (Fig. 3.8A–B): healthy neutrophils show a discrete nucleus. After NET release, nuclear material is extruded and some cells have lost their nuclei.

Role in pathology (implications in inflammation and disease)

Autoimmunity

- NET chromatin (histones and associated DNA) is postulated to be a **source of nuclear antigens** in systemic autoimmune disease.
- The classic example is **lupus**, in which individuals react against their own DNA and nucleoproteins.

Sepsis

- **NETs have been detected in the blood during sepsis**.
- Sepsis-3 operational criterion: an increase in SOFA score of **2 points or more**, associated with in-hospital mortality greater than 10%.
- Septic shock: persisting hypotension needing vasopressors to maintain MAP ≥65 mm Hg and serum lactate >2 mmol/L despite adequate volume resuscitation.
- qSOFA (any 2 of 3): respiratory rate ≥22/min, altered mentation, systolic BP ≤100 mm Hg.
- Older scheme: Sepsis-1/2 used SIRS criteria. Sepsis-3 replaced these with SOFA-based criteria.

Leukocyte-mediated tissue injury

- Leukocytes injure normal tissue in three settings:

  - Collateral damage during a normal defence reaction. In difficult infections such as tuberculosis, the prolonged host response contributes more to the pathology than the microbe does.
  - Inappropriate response against host tissues, as in autoimmune disease.
  - Excessive reaction to harmless environmental substances, as in allergy and asthma.
- Effector mechanisms **do not distinguish offender from host**. ROS, NO and lysosomal enzymes can be released into the extracellular space and amplify the original injury.
- NETs are the extreme form of this extracellular release. Granule enzymes and chromatin leave the cell.
- **Frustrated phagocytosis** also releases lysosomal enzymes extracellularly. It occurs when immune complexes sit on large surfaces such as the glomerular basement membrane.
- **Urate crystals** can damage the phagolysosome membrane and release granule contents.
- Uncontrolled elastase activity is damaging. In **α1-antitrypsin deficiency** there is sustained leukocyte protease action, causing emphysema through destruction of elastic fibres.
- The major inhibitor of neutrophil elastase is **α1-antitrypsin**. **α2-macroglobulin** is another serum antiprotease.
- Elastase is a component of NET formation and NET-associated damage. Antiproteases therefore limit the harm of NET-bound enzymes.

Contrast with other neutrophil responses

Table 1: NETosis compared with phagocytic killing and frustrated phagocytosis

Feature	Phagocytosis	NETosis	Frustrated phagocytosis
Site of killing	Phagolysosome	Extracellular mesh	Extracellular release
Trigger	Ingestible microbe	Pathogen or mediator (e.g. interferons, complement, ROS)	Material too large to ingest, e.g. immune complexes on GBM
Fate of neutrophil	Usually survives briefly	Nucleus lost, cell dies	Strong activation
Key content	ROS, NO, lysosomal enzymes	Chromatin, MPO, elastase, peptides	Lysosomal enzymes
Pathological role	Microbial killing	Autoantigen source, sepsis	Tissue destruction

Control and termination

- Mediators are produced only while the stimulus persists. They have short half-lives and are degraded after release.
- Neutrophils have a short tissue half-life. They die by **apoptosis** within hours to a few days of leaving the blood.
- Active stop signals include a switch from proinflammatory leukotrienes to antiinflammatory **lipoxins**. **TGF-β and IL-10** are also released.
- Cholinergic discharge inhibits macrophage TNF production.
- Th17 cells produce IL-17, which induces chemokines that recruit leukocytes. When they are deficient, patients are prone to fungal and bacterial infection and "cold abscesses".

PEARLS

- NETs are extracellular meshes of decondensed nuclear chromatin carrying granule proteins (MPO, elastase, antimicrobial peptides). The neutrophil dies in the process.
- Mechanism in order: ROS → arginine deaminase → citrullination → chromatin decondensation → MPO/elastase enter the nucleus → nuclear envelope ruptures.
- Triggers: bacteria, fungi, interferons, complement, chemokines, ROS.
- Useful: they trap fungi too large to phagocytose and prevent systemic spread.
- Harmful: they are a postulated source of nuclear autoantigens in lupus, and they are found in blood in sepsis.
- Classic trap: NETs are not formed by phagocytosis. They represent cell death with chromatin extrusion. α1-antitrypsin is the elastase inhibitor.
- Sepsis-3: SOFA rise of ≥2, with mortality >10%.

30. Regulators of angiogenesis

ASKED 2× · 5-mark answer

Asked in: Paper I · Dec 2020 · Q4(a) · Paper I · Jun 2020 · Q5(a)

Q: Regulators of angiogenesis. [5]

Q: Regulators of angiogenesis. [5]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Angiogenesis, §Remodeling of Connective Tissue — pp. 95–96
- Robbins — ch. 7 Neoplasia — §Angiogenesis — p. 272

Definition and importance

- **Angiogenesis** is the development of new blood vessels from existing vessels.
- It is critical in healing at injury sites, in collateral circulation at ischaemic sites, and in allowing tumours to grow beyond their original blood supply.
- It can be augmented (e.g. in coronary atherosclerosis) or inhibited (tumour growth, diabetic retinopathy) therapeutically.
- A solid tumour cannot enlarge beyond **1 to 2 mm** in diameter unless it induces angiogenesis. This is presumably the maximal distance across which oxygen and nutrients can diffuse.

Steps of angiogenesis (sprouting)

- Vasodilation in response to **NO**, and increased permeability induced by **VEGF**.
- Pericytes separate from the abluminal surface, and the basement membrane breaks down to allow a vessel sprout.
- Endothelial cells migrate toward the area of injury.
- Endothelial cells proliferate just behind the leading "tip" cell.
- The sprout remodels into capillary tubes.
- Periendothelial cells are recruited: pericytes for small capillaries and smooth muscle cells for larger vessels.
- Endothelial proliferation and migration are suppressed, and the basement membrane is deposited.

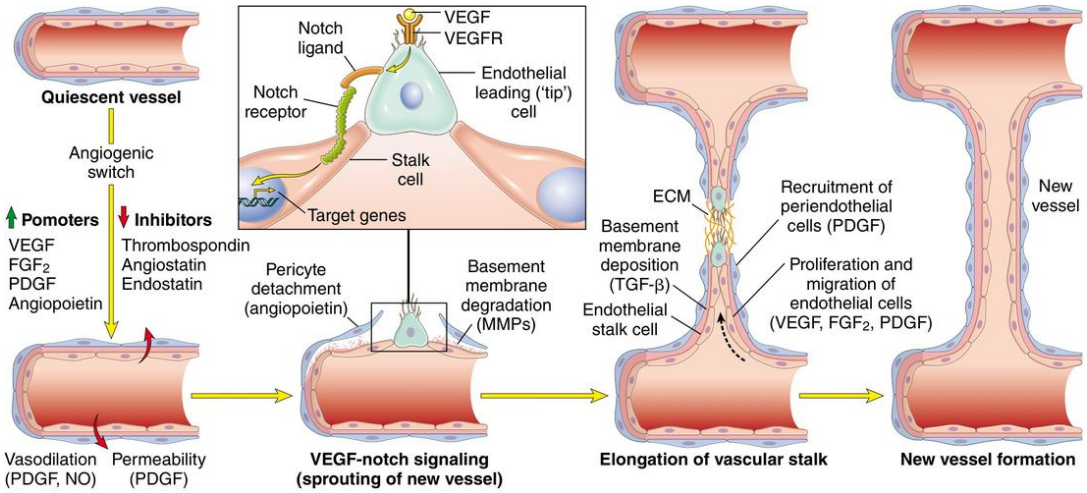


Fig. 3.26 Angiogenesis. In tissue repair, angiogenesis occurs mainly by sprouting of new vessels. The steps — Robbins 11e, p. 95

Regulators (promoters and inhibitors)

Positive regulators

- **VEGF (mainly VEGF-A)** stimulates both migration and proliferation of endothelial cells and starts capillary sprouting, the "angiogenic switch".
  - It promotes vasodilation by stimulating NO production and contributes to lumen formation.
  - It increases permeability.
- **FGFs (mainly FGF-2)** stimulate endothelial proliferation.

  - They promote migration of macrophages and fibroblasts to the damaged area.
  - They stimulate epithelial migration to cover epidermal wounds.
- **Angiopoietins 1 and 2** play a role in angiogenesis and in structural maturation of new vessels.
  - Angiopoietin is shown in the figure as the signal for pericyte detachment.
- **Notch signalling** regulates sprouting and branching, which ensures proper spacing of new vessels.
  - VEGF stimulates expression of Notch ligands, which bind the Notch receptor on endothelial cells and set the branching pattern.
- **MMPs** degrade the ECM and basement membrane to permit remodelling and extension of the vascular tube.
- **ECM proteins and integrins** give the scaffold for vessel growth and signal through integrin receptors on endothelial cells.

Stabilising regulators

- **PDGF** recruits smooth muscle cells (periendothelial cells) to stabilise new vessels.
- **TGF-β** suppresses endothelial proliferation and migration and enhances ECM protein production.
  - It also drives basement membrane deposition.
- Pericytes, smooth muscle cells and connective tissue deposition are needed to stabilise newly formed vessels.

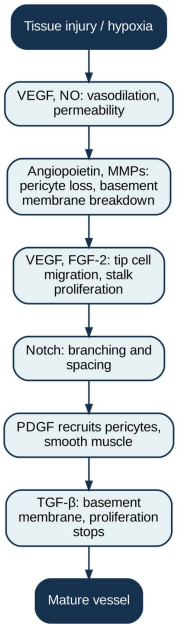
Inhibitors

- **Thrombospondin, angiostatin and endostatin** are the inhibitors listed in the figure.
- TGF-β acts as a brake on endothelial proliferation, as above.

Table 1: Regulators of angiogenesis and their actions

Regulator	Class	Main action
VEGF-A	Promoter	Endothelial migration and proliferation; vasodilation via NO; permeability
FGF-2	Promoter	Endothelial proliferation; macrophage and fibroblast migration
Angiopoietin 1/2	Promoter	Pericyte detachment; vessel maturation
Notch ligand/receptor	Regulator	Sprouting and branching pattern; vessel spacing
MMPs	Promoter	Degrade basement membrane and ECM
PDGF	Stabiliser	Recruits smooth muscle cells
TGF-β	Stabiliser/inhibitor	Suppresses endothelial proliferation; basement membrane deposition
Thrombospondin, angiostatin, endostatin	Inhibitors	Block new vessel formation

Flowchart 1: Sequence and regulators in angiogenesis



DRAW IN THE EXAM

Labelled diagram of sprouting angiogenesis showing the tip and stalk cells, with promoters (VEGF, FGF-2, PDGF, angiopoietin) and inhibitors (thrombospondin, angiostatin, endostatin).

PEARLS

- Angiogenesis is new vessel growth by sprouting from existing vessels. VEGF-A is the key initiator, the "angiogenic switch".
- FGF-2 is the second major endothelial mitogen. PDGF recruits smooth muscle cells and TGF-β suppresses endothelial proliferation, so both stabilise the vessel.
- Notch (driven by VEGF) controls branching and spacing. MMPs clear the basement membrane.
- Endogenous inhibitors are thrombospondin, angiostatin and endostatin.
- A tumour larger than 1–2 mm needs angiogenesis. Anti-angiogenic therapy is also used in diabetic retinopathy.

31. Role of angiogenesis in tissue engineering

ASKED 1× · 5-mark answer

Asked in: Paper II · Jun 2021 · Q7(b)

Q: Role of angiogenesis in tissue engineering. [5]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Liver Regeneration; §Steps in Scar Formation; §Angiogenesis; §Remodeling of Connective Tissue — pp. 93–96
- W1 · PMC (Vascularization of Engineered Tissues: Approaches to Promote Angiogenesis in Biomaterials) — review
- W2 · PMC (Angiogenesis in Tissue Engineering: As Nature Intended?) — review
- W3 · PMC (Neovascularization in Tissue Engineering) — review
- W4 · PMC (Vascular tissue engineering: biodegradable scaffold platforms to promote angiogenesis) — review
- W5 · PMC (Current Strategies for Engineered Vascular Grafts and Vascularized Tissue Engineering) — review

NOT IN THE REFERENCE BOOKS

Searched: Robbins 11e ch.3 (Inflammation and Repair) pages on regeneration, scar formation and angiogenesis. Searched the web for "angiogenesis vascularization tissue engineering review PMC". Robbins gives the biology of angiogenesis. The tissue-engineering applications below come from PMC reviews.

Angiogenesis: the biological basis

- **Angiogenesis** is the development of new blood vessels from existing vessels. It is critical for healing, for collateral circulation in ischaemia, and for tumour growth.
- Tissue engineering borrows this process of repair. Repair by regeneration needs proliferating cells, growth factors and an intact ECM scaffold.
- Granulation tissue, with new thin-walled capillaries in a loose ECM, supplies nutrients for repair.

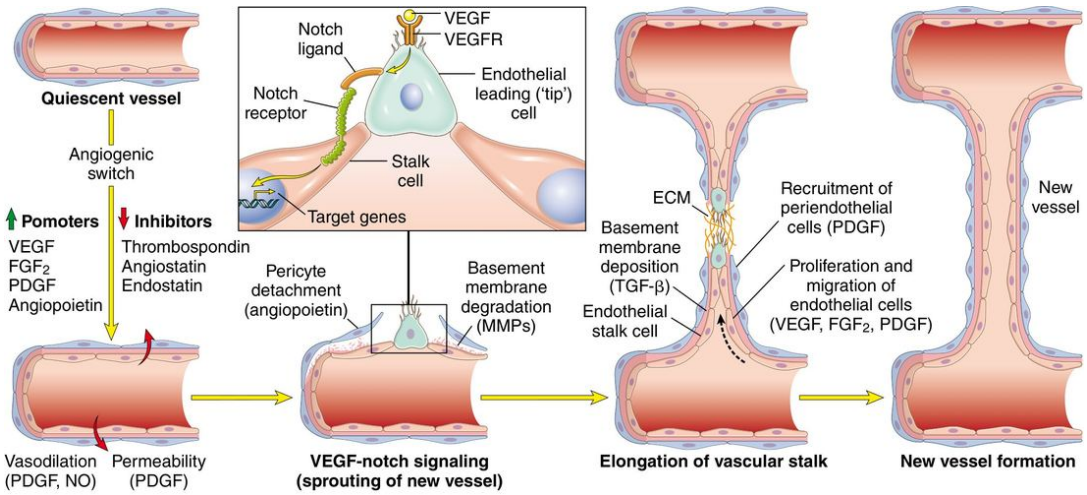


Fig. 3.26 Angiogenesis. In tissue repair, angiogenesis occurs mainly by sprouting of new vessels. The steps — Robbins 11e, p. 95

Why angiogenesis is needed in tissue engineering

- **Vascularization** is the major hurdle in building complex tissues. Engineered constructs need vascular networks to deliver oxygen and nutrients throughout.
- A prerequisite for success is adequate vascularization, which lets the construct survive and grow.
- Lack of mature, functional vasculature limits the size and complexity of engineered constructs.
- The goal is analogous to **therapeutic angiogenesis**, which revascularizes ischaemic disease such as myocardial infarction.
- Controlled angiogenesis helps integrate the transplanted tissue with host tissue.

Steps of angiogenesis the construct must support

- **Vasodilation** by nitric oxide and **increased permeability** by VEGF.
- **Pericyte detachment** and basement-membrane breakdown by MMPs, allowing a sprout to form.
- **Endothelial migration** toward the injury, with proliferation behind the leading "tip" cell.
- **Remodelling** into capillary tubes.
- **Recruitment of pericytes** (small vessels) or smooth muscle cells (larger vessels), and deposition of basement membrane to stabilise the vessel.
- Engineered scaffolds aim to promote endothelial adhesion and migration, sprouting, lumen formation, capillary network development, and finally vessel stabilisation and maturation.

Key mediators and how they are used

- **VEGF-A** starts the angiogenic switch, stimulating endothelial migration and proliferation. It also promotes vasodilation through NO and helps form the lumen.
- **FGF-2** stimulates endothelial proliferation and fibroblast and macrophage migration.
- **Angiopoietins 1 and 2** help maturation of new vessels.
- **PDGF** recruits smooth muscle cells. **TGF-β** suppresses endothelial proliferation and increases ECM production.
- The **Notch pathway** regulates sprouting and branching, so vessels are properly spaced to supply the tissue.
- **Integrins and the ECM** give the scaffold for vessel growth.
- VEGF can be bound to scaffold surfaces to present pro-angiogenic signals to surrounding tissue.

Engineering strategies that exploit angiogenesis

Table 1: Strategies to promote angiogenesis in engineered constructs

Strategy	Principle	Source
Angiogenic growth factors	Alone or in combination, with gene therapy for sustained release	
Cell therapy	Endothelial cells and endothelial progenitor cells	
Co-culture	Endothelial cells with supporting cell types	
Scaffold modification	ECM proteins and peptides in micropatterns to guide angiogenesis	
Scaffold design	Composition, mechanics, degradation and dimensionality control vascular network development	
Injectable scaffolds	Less invasive; mould to fill cavities and necrotic areas	
Fabrication	3D bioprinting and electrospinning	

- Sufficient neovascularization is achieved by combining angiogenic factors with suitable cell types in the biomaterial.
- **Spatial and temporal control**: angiogenesis in vivo is regulated by spatial cues, so patterning of bioactive molecules in scaffolds is used.
- **Bone**: VEGF couples angiogenesis with osteogenesis. Prevascularised scaffolds for large defects remain difficult.

Limitations

- Constructs lack mature, hierarchically organised vasculature.
- Random growth-factor distribution is a limitation, so biomolecules are encapsulated before patterning.
- Scaffolds must degrade in time and avoid toxicity.

DRAW IN THE EXAM

Sprouting angiogenesis (tip cell, stalk cell, VEGF, Notch, pericyte recruitment) inside a scaffold pore.

PEARLS

- Angiogenesis is the sprouting of new vessels from existing ones, driven mainly by VEGF-A.
- Vascularization is the rate-limiting step for engineered tissue size and survival.
- VEGF starts the angiogenic switch, FGF-2 drives proliferation, PDGF and TGF-β stabilise the vessel.
- MMPs clear the path for sprouts, and TIMPs shut down remodelling.
- Strategies are growth factors, endothelial or progenitor cells, co-culture and scaffold design.

32. Atherosclerosis — cellular & immunological interactions in plaque, role of neutrophils

ASKED 3× · 5-mark answer

Asked in: Paper I · Dec 2020 · Q3(a) · Paper I · Jun 2020 · Q6(b) · Paper III · Dec 2020 · Q5(b)

Q: Cellular and immunological interactions in an atherosclerotic plaque. [5]
Q: Cellular and immunological interactions in atherosclerotic plaque. [5]
Q: Role of neutrophils in atherosclerosis. [5]

SOURCE

- Robbins — ch. 11 Blood Vessels — §Additional Risk Factors; §Acute Plaque Change — pp. 452–454, 458
- W1 · PMC — Emerging insights into inflammation-driven atherosclerosis: immune cell mechanisms
- W2 · PMC — Inflammasome Activation and Neutrophil Extracellular Traps in Atherosclerosis
- W3 · PMC — Neutrophil Extracellular Traps in Cardiovascular and Aortic Disease: A Narrative Review
- W4 · PMC — Glycolysis and neutrophil extracellular traps in atherosclerosis
- W5 · PMC — Worsening Thrombotic Complication of Atherosclerotic Plaques Due to Neutrophils Extracellular Traps: A Systematic Review

Cells involved and their interactions

- Atherosclerosis is a chronic inflammatory response in the vessel wall. Atheromas are dynamic lesions of dysfunctional endothelial cells (ECs), proliferating smooth muscle cells (SMCs), T lymphocytes and macrophages. All four cell types release mediators that influence atherogenesis.
- **Endothelium:** Dysfunction comes from hemodynamic disturbance, hypercholesterolemia and inflammation. It causes increased permeability, enhanced leukocyte adhesion and altered gene expression.
- **Monocyte/macrophage:**
  - Monocytes adhere to the injured endothelium and migrate into the intima.
  - Macrophages take up modified (oxidized or aggregated) LDL through scavenger receptors. They become **foam cells**.
  - Activated macrophages make reactive oxygen species, which enhance LDL oxidation. They also make growth factors that drive SMC proliferation.
- **SMC:**
  - SMCs migrate from the media into the intima, proliferate and synthesize ECM, notably collagen. This forms the fibrous cap.
  - SMCs can also become foam cells by ingesting modified lipids.
- **T lymphocyte:** Activated T cells in the intima release interferon-γ. This activates macrophages, ECs and SMCs.
- **Platelets:** They adhere at injured endothelium. They release PDGF, which stimulates SMC proliferation.

Table 1: Cell–mediator interactions in the plaque

Cell	Mediator / product	Effect on other cells
EC	Adhesion molecules, increased permeability	Monocyte and platelet adhesion
Macrophage	ROS, growth factors, cytokines	LDL oxidation; SMC proliferation
T cell	Interferon-γ	Activates macrophage, EC, SMC
Platelet, macrophage, EC, SMC	PDGF, FGF, TGF-α	SMC proliferation and ECM synthesis
SMC	Collagen	Stabilizes plaque
Macrophage, SMC	Metalloproteinases (MMPs)	Collagen degradation, so a fragile cap
EC, SMC, macrophage	TIMPs	Inhibit MMP activity

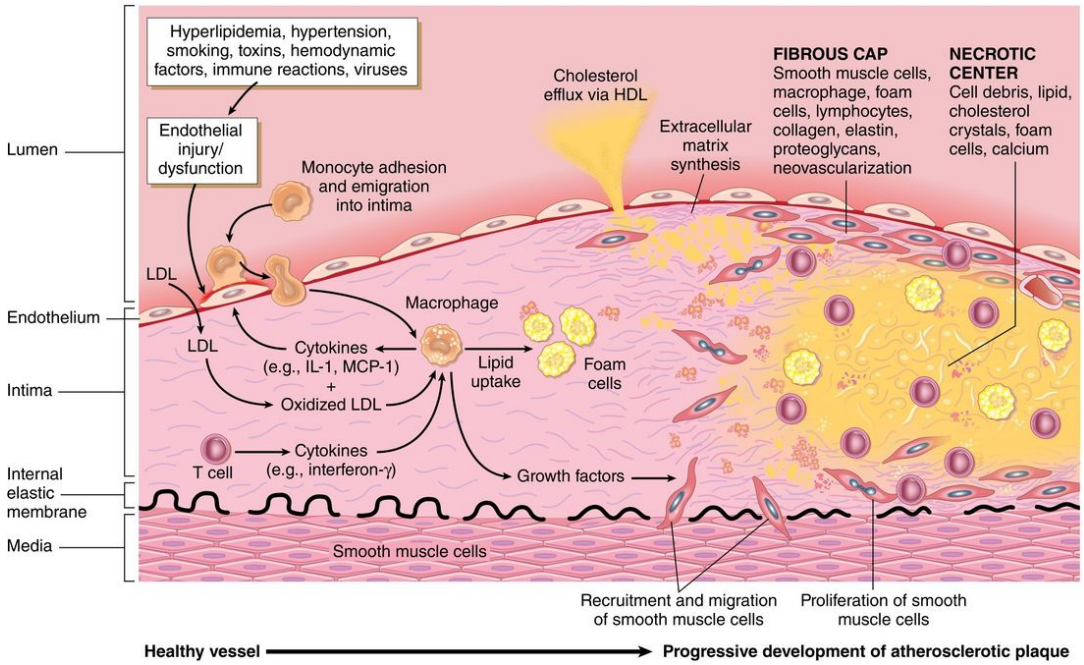


Fig. 11.10 *Cells and inflammatory molecules contributing to atheroma formation and progression. Hyperlip- — Robbins 11e, p. 454*

Immunological and inflammatory interactions

- **Inflammasome pathway:**

  - Cholesterol crystals and free fatty acids accumulate in macrophages and other cells. Cytosolic innate immune receptors of the inflammasome sense them.
  - Oxidized LDL acts through toll-like receptors (TLRs) to activate NF-κB. The sensor NLRP3 then assembles the inflammasome. It cleaves pro-IL-1β and pro-IL-18 into active cytokines.
  - IL-1 activates ECs and recruits and activates mononuclear cells, including macrophages and T cells. These produce more cytokines and chemokines, so inflammation becomes a self-perpetuating cycle.
  - Inflammasome activation can also trigger pyroptosis.
- **Vicious cycle:** Modified lipoproteins are toxic to ECs, SMCs and macrophages. Their uptake releases growth factors, cytokines and chemokines that recruit more monocytes.
- **Plaque stability:**
  - Inflammation causes a net increase in collagen degradation and reduced synthesis. This destabilizes the fibrous cap.
  - Plaques with thin caps and active inflammatory cells over a necrotic core are **vulnerable plaques**. They are prone to rupture.
- **Infection:** Herpesvirus, CMV and *Chlamydophila pneumoniae* are linked by circumstantial evidence only. There is no established causal role.

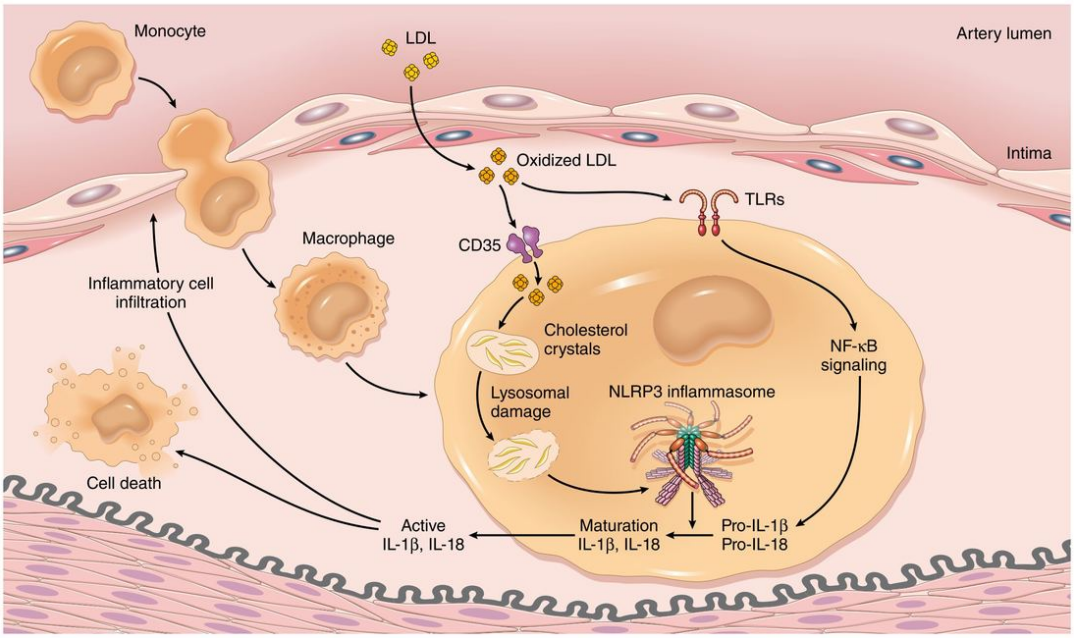


Fig. 11.9 The role of cholesterol crystals in inflammasome activation and cytokine production in atherosclе- — Robbins 11e, p. 453

Role of neutrophils in atherosclerosis

- Neutrophils are numerous in the vasculature of patients with unstable plaques. Their number correlates with histological features of plaque vulnerability.
- Human carotid plaque neutrophil count is strongly associated with rupture-prone histology. Neutropenia increases SMC content and cap thickness; neutrophilia has the opposite effect.
- **Recruitment:** Cholesterol crystals activate the NLRP3 inflammasome. This induces neutrophil infiltration into the plaque.
- **NETs (neutrophil extracellular traps):**
  - NETs are web-like structures of chromatin and granular proteins released by activated neutrophils.
  - Cholesterol crystals trigger NET release.
- **Effects of NETs:**
  - They activate the NLRP3 inflammasome, injure endothelium and recruit inflammatory cells. They also promote oxidative stress and thrombosis, favouring vulnerable plaques.
  - NET histone H4 lyses SMCs, which destabilizes the plaque.
  - NETs prime macrophages for IL-1β production. This leads to T-cell IL-17 release, which recruits more immune cells.
  - NET-derived dsDNA triggers AIM2 inflammasome activation, causing plaque destabilization.

- **Necrotic core:** Neutrophils release proteases and are a source of apoptotic and necrotic cells in the necrotic core.
- **Clinical correlation:** In human coronary plaques, NETs were present in complicated plaques (rupture, erosion, hemorrhage) and absent from intact ones.

DRAW IN THE EXAM

Schematic of the plaque: injured endothelium, monocyte → foam cell, SMC migration to the fibrous cap, T cell with interferon-γ, cholesterol crystal → NLRP3 → IL-1β, and neutrophil releasing NETs.

PEARLS

- Atherosclerosis is a chronic inflammatory response to endothelial injury and lipid accumulation.
- Foam cells come from macrophages and SMCs that take up modified LDL through scavenger receptors.
- SMCs make collagen, which stabilizes the plaque. MMPs from macrophages degrade collagen and destabilize it.
- Cholesterol crystals activate NLRP3. This gives active IL-1β and IL-18, with pyroptosis as a possible outcome.
- Vulnerable plaque = thin cap + large lipid core + dense inflammatory infiltrate.
- NETs link neutrophils to plaque destabilization and thrombosis.

33. Role of complement system in inflammation

ASKED 1× · 5-mark answer

Asked in: Paper I · Jun 2021 · Q7(a)

Q: Role of complement system in inflammation. [5]

SOURCE

- Robbins — ch. 3 Inflammation and Repair — §Complement System; §Other Mediators of Inflammation; §Serous Inflammation — pp. 81–83
- Robbins — ch. 20 The Kidney — §C3-Dominant Glomerulopathy — p. 834

Definition and components

- The **complement system** is a collection of plasma proteins that function mainly in host defence against microbes and in pathologic inflammatory reactions.
- It has more than 20 proteins, some numbered **C1 to C9**.
- It takes part in both innate and adaptive immunity.
- The proteins circulate in inactive form. Many become proteolytic enzymes that degrade other complement proteins, forming an enzymatic cascade capable of tremendous amplification.
- The critical step is proteolysis of **C3**, the third and most abundant component.

Pathways of activation

- **Classical pathway**
  - Triggered when C1 binds antibody (IgM or IgG) that has combined with antigen.
  - C2 and C4 then bind in sequence, forming a multiprotein enzyme that cleaves C3.
- **Alternative pathway**
  - Triggered by microbial surface molecules (endotoxin/LPS), complex polysaccharides, cobra venom and other substances, **without antibody**.
  - Spontaneous C3 cleavage is amplified and stabilised on the microbe.
- **Lectin pathway**
  - Plasma **mannose-binding lectin** binds carbohydrates on microbes and activates a protease homologous to C1. Later steps are as in the classical pathway.
- All three pathways form **C3 convertase**, which splits C3 into C3a and C3b.
  - C3a is released. C3b becomes covalently attached to the site of activation.

– More C3b binds to form **C5 convertase**, which cleaves C5 into C5a and C5b.
– C5b binds C6–C9 to form the **membrane attack complex (MAC)**, made of multiple C9 molecules.

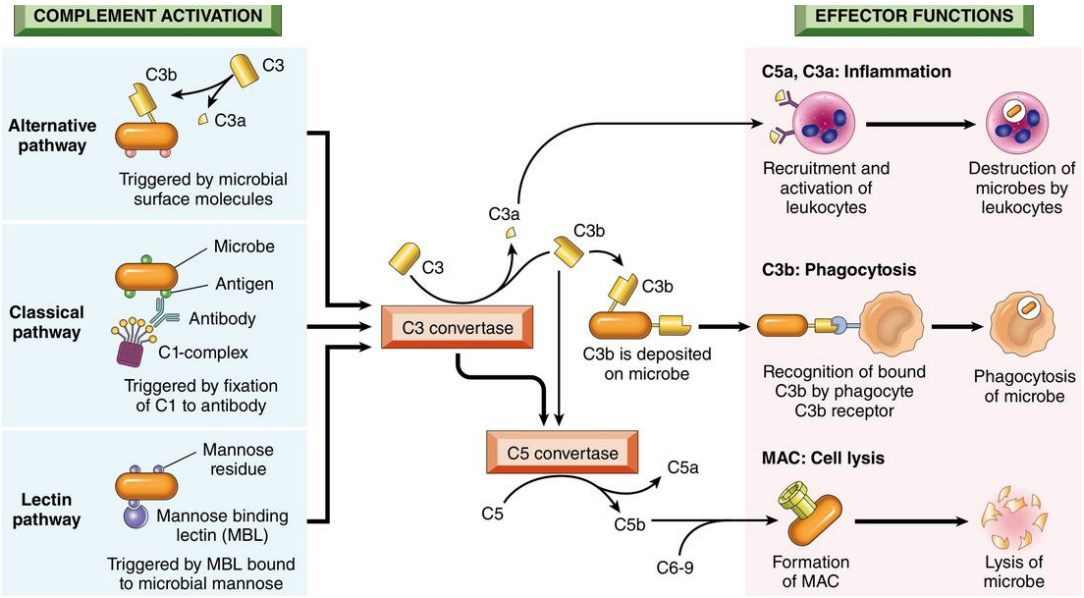
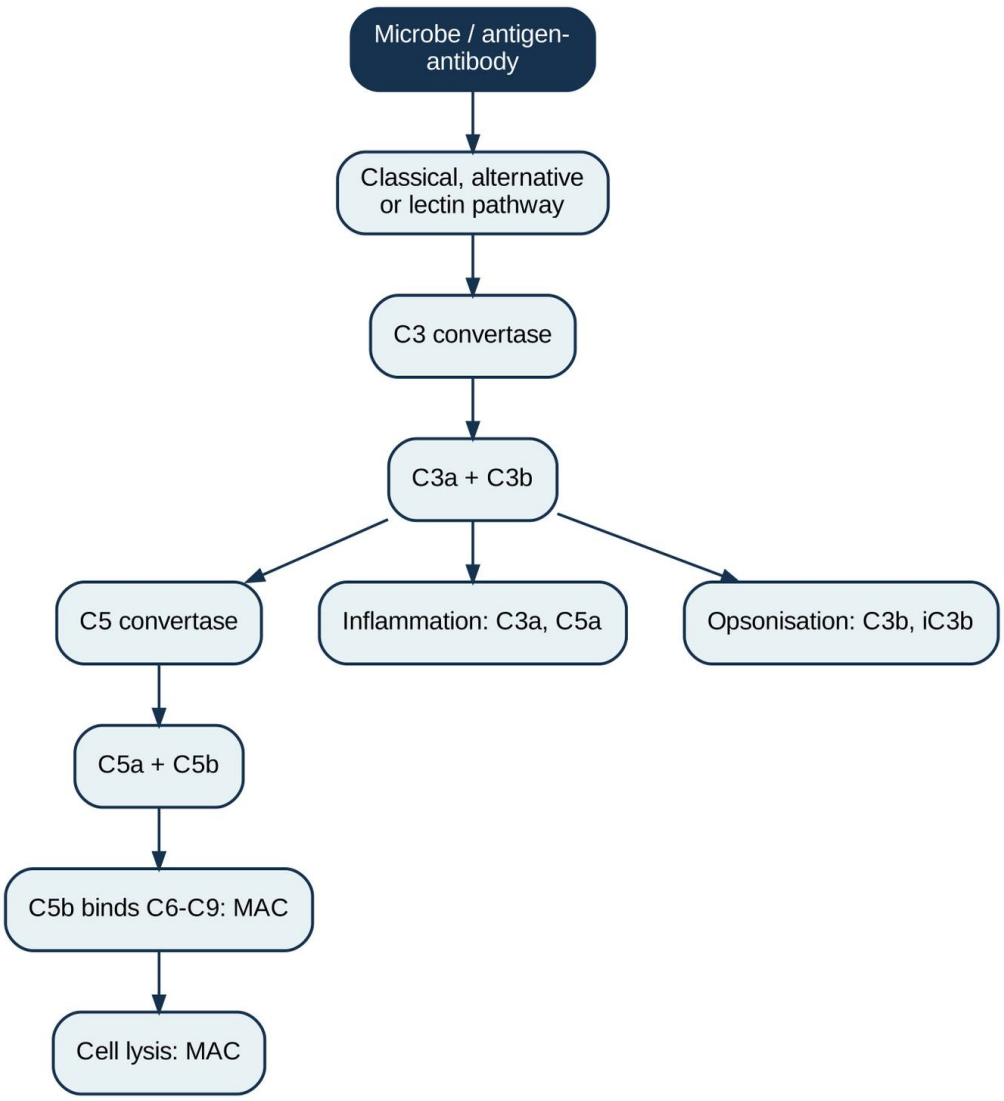


Fig. 3.11 Activation and functions of the complement system. Activation of complement by different path- — Robbins 11e, p. 82

Flowchart 1: Complement activation and effector functions



Role in inflammation: three main functions

Table 1: Functions of complement

Function	Mediators	Effect
Inflammation	C5a, C3a, C4a (lesser)	Mast cell histamine release, vasodilation, increased permeability
Opsonisation and phagocytosis	C3b, iC3b	Phagocytosis by neutrophils and macrophages via complement receptors
Cell lysis	MAC	Osmotic lysis of cells

- **Inflammation**
  - C5a, C3a and, to a lesser extent, C4a are **anaphylatoxins**. They stimulate histamine release from mast cells, so vascular permeability rises and vessels dilate.
  - Their effects resemble those of mast cell mediators in anaphylaxis.
  - **C5a** is also chemotactic for neutrophils, monocytes, eosinophils and basophils.
  - C5a activates the **lipoxygenase pathway** of arachidonic acid metabolism in neutrophils and monocytes, causing further release of inflammatory mediators.
  - In the summary of mediators, C3a and C5a are listed for increased permeability and for chemotaxis and leukocyte recruitment.
- **Opsonisation**
  - C3b and inactive C3b (iC3b) fixed to the microbial wall act as opsonins. Phagocytes bear surface receptors for these fragments.
- **Cell lysis**
  - MAC deposition makes cells permeable to water and ions, causing osmotic lysis.
  - This is important for microbes with thin cell walls, such as *Neisseria*. Deficiency of the terminal components predisposes to **Neisseria** infections.

Regulation

- Activation is tightly controlled by cell-associated and circulating regulatory proteins. These are expressed on normal host cells to protect healthy tissue.
- Regulators can be overwhelmed when large amounts of complement are deposited, as in autoimmune diseases with complement-fixing autoantibodies.
- **C1 inhibitor** blocks C1 activation. Its inherited deficiency causes **hereditary angioedema**.
- **DAF (CD55)** prevents C3 convertase formation. **CD59** inhibits MAC formation.
  - Both are GPI-anchored. Acquired deficiency of the GPI-anchor enzyme causes excessive complement lysis of red cells in **PNH**.
- **Factor H** inhibits the alternative pathway by promoting cleavage of C3b and turnover of C3 convertases.
  - Inherited Factor H defects cause **atypical haemolytic uraemic syndrome**.
  - Factor H polymorphisms are linked to age-related macular degeneration.

Complement in disease

- Antibody or immune-complex activation of complement on host tissue is an important mechanism of tissue injury.
- Inherited complement deficiencies increase susceptibility to infection.
- **C3-dominant glomerulopathy** (dense deposit disease and C3GN) results from excessive alternative pathway activation.
  - Serum C3 is low, with normal C1 and C4. Most patients with acquired C3GN have **C3 nephritic factor (C3NeF)**, which stabilises C3bBb.
- Inhibitors of C5 and C3 activation are approved for several of these disorders.

PEARLS

- Complement = more than 20 plasma proteins. The central step is cleavage of **C3** by C3 convertase.
- Three pathways: classical (antibody, C1), alternative (microbial surface, no antibody), lectin (MBL).
- Anaphylatoxins = **C3a, C4a, C5a**. C5a is the main chemotaxin.
- Opsonins = C3b and iC3b. MAC (C5b–C9) lyses cells.
- Terminal component deficiency leads to *Neisseria* infections. C1 INH deficiency leads to hereditary angioedema. CD55/CD59 loss leads to PNH.

34. Role of complement in Renal diseases

ASKED 1× · 10-mark answer

Asked in: Paper I · Oct 2024 · Q2

Q: Role of complement in Renal diseases. [10]

SOURCE

- Robbins — ch. 20 The Kidney — §Mediators of Glomerular Injury; §Mechanisms of Progression in Glomerular Diseases; §Acute Proliferative (Postinfectious and Infection-Associated) Glomerulonephritis; §Membranoproliferative Glomerulonephritis; §C3-Dominant Glomerulopathy; §IgA Nephropathy — pp. 821–824, 833–836

Complement in glomerular injury: general role

- Complement activation is one of the main routes by which antibody-mediated glomerular injury occurs. Antibodies may activate complement and engage Fc receptors on leukocytes.
- Antibody-mediated injury acts mainly through complement- and leukocyte-mediated pathways.
- **Chemotaxis:** complement activation generates chemotactic products, mainly **C5a**, which cause neutrophil and monocyte influx (complement-neutrophil–dependent injury).
- Neutrophils then release three groups of mediators:
  - proteases, which degrade the GBM;
  - oxygen-derived free radicals, which damage cells;
  - arachidonic acid metabolites, which lower GFR.
- **Membrane attack complex (C5b–C9):** it causes cell lysis. It may also stimulate mesangial cells to produce oxidants, proteases and other mediators.
- Even without neutrophils, C5b–C9 can cause proteinuria, as shown in experimental membranous glomerulopathy.
- **Defective regulation:** in the C3 glomerulopathies, complement activation results from defective regulation of the alternative pathway. It can be triggered or worsened by immune complexes or monoclonal immunoglobulins.
- **Site of deposition decides the response:**
  - Subendothelial immune complexes provoke leukocyte infiltration and crescent formation.
  - Mesangial deposits can elicit inflammation, because the mesangium is not separated from the capillary lumen by GBM.
  - Subepithelial deposits are shielded from the circulation by the GBM and do not induce inflammatory responses.
- Several factors affect where complexes localise: charge and size of the reactants, glomerular haemodynamics, mesangial function and the integrity of the charge-selective barrier.
- The coagulation system acts alongside complement. Fibrin in glomeruli and Bowman space indicates cascade activation, and thrombin may stimulate crescent formation.

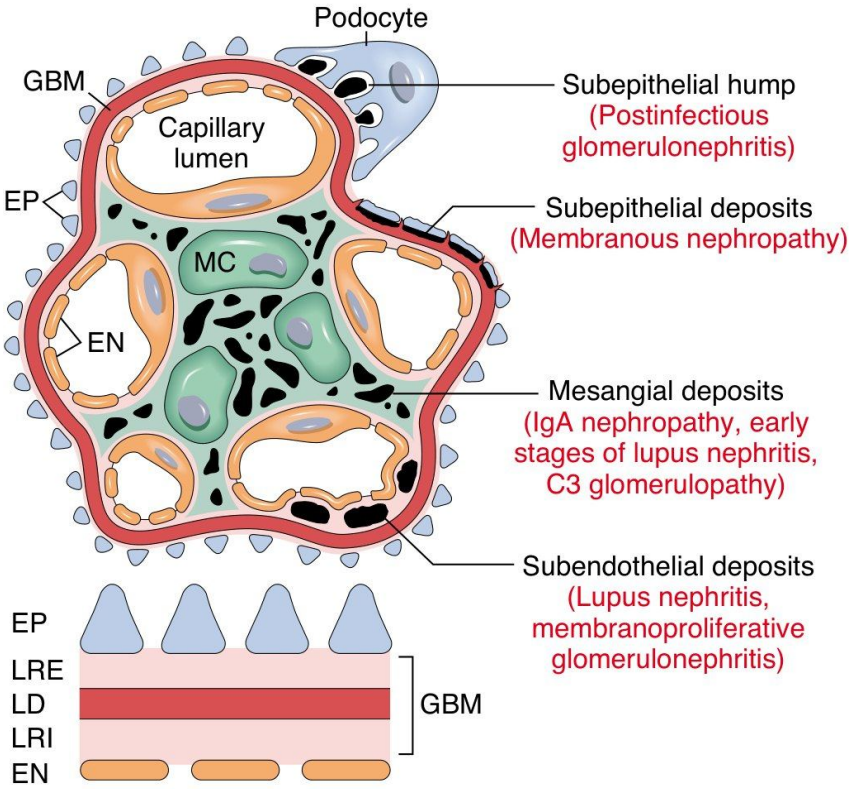


Fig. 20.6 Localization of immune complexes in the glomerulus: isolated — Robbins 11e, p. 821

Classical pathway: complement in immune complex–mediated glomerulonephritis

Postinfectious (poststreptococcal) GN

- It is caused by immune complexes containing streptococcal antigens and specific antibodies. More than 90% of cases trace to nephritogenic group A β-haemolytic types **1, 4 and 12**.
- Evidence for an immunological basis includes the following.
  - A latent period of 1–4 weeks between infection and nephritis.
  - Raised antistreptococcal antibody titres.
  - Low serum complement, from activation and consumption of components.
  - Granular immune deposits in glomeruli.
- **SpeB** (streptococcal pyogenic exotoxin B) is the principal antigen in most cases. It localises to the "humplike" deposits.
- Antigen is first planted subendothelially. The complexes then dissociate, migrate across the GBM and re-form as **subepithelial humps**.
- Immunofluorescence shows granular IgG and C3 in the GBM and mesangium. Electron microscopy shows mainly subepithelial humps.
- **Morphology:** glomeruli are enlarged and hypercellular, with diffuse global proliferation and leukocyte infiltration. Capillary lumens are obliterated, and red cell casts are present in tubules.
- Complement-attracted neutrophils and monocytes degrade the complexes. If antigen exposure is short-lived, the glomerulus recovers completely.

Immune complex–mediated MPGN (formerly type I)

- It is caused by deposition of immune complexes containing IgG and complement.
- Both **classical and alternative** complement pathways are frequently activated.
- Immunofluorescence shows granular IgG and C3, with **C1q and C4** often present. This indicates an immune complex–mediated mechanism.
- Electron microscopy shows discrete **subendothelial deposits**.

- Light microscopy shows lobular accentuation and a "tram-track" (double-contour) GBM, from new basement membrane synthesis in response to subendothelial deposits.
- Secondary MPGN is invariably immune complex–mediated. It arises in SLE, hepatitis B and C (with cryoglobulinaemia), endocarditis, infected shunts, chronic abscesses, HIV and schistosomiasis.
- It can also arise in malignancies producing autoantibodies or monoclonal immunoglobulins.

Membranous nephropathy

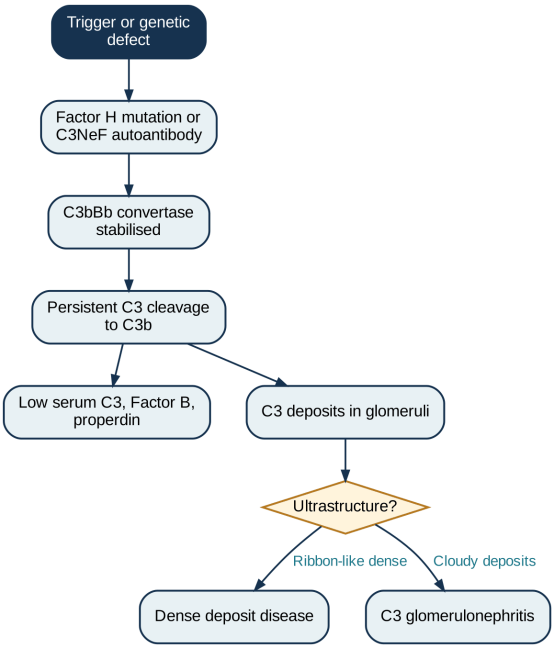
- It results from in situ immune complex formation, most often against PLA2R on podocytes.
- **C5b–C9** is the mediator of proteinuria, even without neutrophils.

Alternative pathway: C3 glomerulopathy (C3-dominant glomerulopathy)

Definition

- This entity includes **dense deposit disease** (formerly MPGN type II) and **C3 glomerulonephritis (C3GN)**.
- It results from genetic or acquired abnormalities of the alternative pathway, causing excessive activation and deposition.
- MPGN is a *pattern* of injury. Its present classification separates immune complex–mediated disease from complement overactivation without immune complexes.

Pathogenesis

- In the alternative pathway, **C3 is directly cleaved to C3b**. This needs activators such as bacterial polysaccharides, endotoxin and IgA aggregates.
- The pathway runs through **Factors B and D** and generates **C3bBb**, the alternative pathway C3 convertase.
- Normally C3bBb is labile. Factors H and I degrade C3b, and properdin stabilises the convertase.
- **C3 nephritic factor (C3NeF):** most patients with acquired C3GN have this autoantibody.
  - It binds C3bBb and protects it from inactivation.
  - This causes persistent C3 activation and hypocomplementaemia.
  - It mimics the action of properdin.
- Hepatic C3 synthesis is also decreased, which deepens the hypocomplementaemia.
- **Genetic causes:** mutations in alternative pathway components such as **Factor H** are associated with dense deposit disease and C3GN.
- **Paraproteins:** aberrant C3 activation can occur with circulating paraproteins. Masked monoclonal immunoglobulin deposits should be ruled out.
- Apolipoprotein E is enriched in dense deposits and is a biomarker of dense deposit disease.

Flowchart 1: Alternative pathway dysregulation in C3 glomerulopathy