



Generation of Free Radicals

Cell injury · oxygen and nitrogen redox chemistry

Dr Abu Suraih Sakhri

MBBS, MD (Pathology)

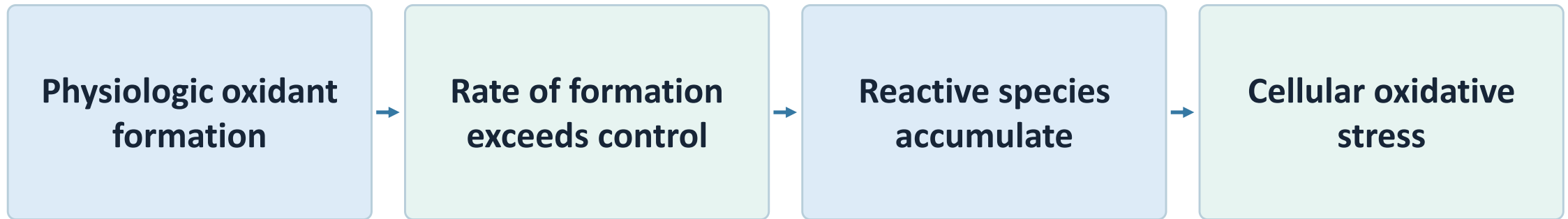
www.abusuraihsakhri.com

Learning objectives: Sources, species, reactions

- Classify oxygen- and nitrogen-derived reactive intermediates.
- Explain sequential reduction of molecular oxygen.
- Identify enzymatic, inflammatory, physical and chemical sources.
- Trace Fenton chemistry and the formation of peroxynitrite.
- Connect generation mechanisms to selected clinical scenarios.

Oxidative stress belongs to the core mechanisms of cell injury

The context



- Oxidative stress with chemical injury, radiation and reperfusion.
- The same chemistry participates in host antimicrobial defense.

What makes a molecule a free radical?

Unpaired electron

A free radical has at least one unpaired electron. That electron contributes to chemical reactivity.

Reaction propagation

Transfer of an electron or hydrogen atom can generate a new radical, sometimes sustaining a chain reaction.

Not every biologically important oxidant contains an unpaired electron.

Free radical is a chemical category—not a synonym for ROS

Superoxide	$\text{O}_2\bullet^-$	Oxygen-derived radical
Hydrogen peroxide	H_2O_2	Reactive oxygen species; Not a radical
Hydroxyl radical	$\bullet\text{OH}$	Very reactive oxygen-derived radical
Nitric oxide	$\text{NO}\bullet$	Nitrogen-centered free radical
Peroxynitrite	ONOO^-	Reactive nitrogen species; Not a radical

ROS and RNS are overlapping, but distinct, concepts

Reactive oxygen species (ROS)

- Include oxygen-centered radicals and nonradicals.
- Examples: $\text{O}_2^{\bullet-}$, H_2O_2 and $\bullet\text{OH}$.
- Chemical behavior differs markedly between species.

Reactive nitrogen species (RNS)

- Involve nitrogen-centered or nitrogen-containing chemistry.
- $\text{NO}\bullet$ is a free radical.
- ONOO^- is reactive but is not a free radical.

Six principal routes to reactive species production

Basal metabolism



Mitochondria · ER · cytosol · peroxisomes · lysosomes

Activated host defense



Phagocyte NADPH oxidase

Physical and chemical



Radiation · xenobiotic metabolism

Catalytic chemistry



Fe²⁺ / Cu⁺ cycling · NO + O₂•⁻

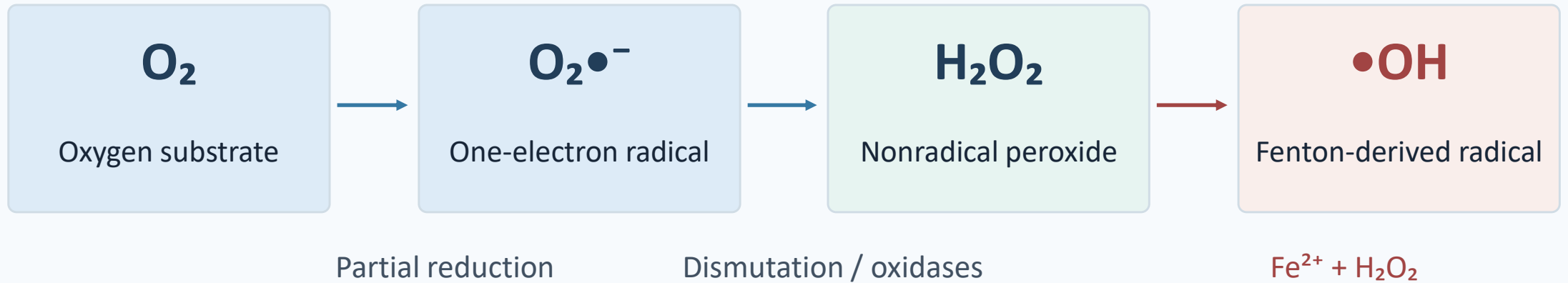
Normal oxygen reduction uses four electrons

Complete reduction



- This is the net chemistry of oxygen reduction to water.
- Biologic respiratory enzymes coordinate the electron-transfer process.
- Incomplete reduction gives rise to reactive intermediates.

Partial oxygen reduction generates reactive intermediates



Separate complete reduction: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$

These are possible generating routes—not obligatory stages of respiration.

One-electron reduction produces superoxide

First electron



- Superoxide is a radical anion.
- It forms during incomplete oxygen reduction and through oxidase activity.
- Its local abundance depends on production and dismutation.

Two-electron reduction produces hydrogen peroxide

Peroxide formation



- Hydrogen peroxide contains no unpaired electron.
- It can arise through superoxide dismutation or direct oxidase reactions.
- It can feed further chemistry, especially metal-catalyzed $\bullet\text{OH}$ generation.

Hydroxyl radical: High reactivity, short range

Formation

Ionizing radiation can generate $\bullet\text{OH}$ from water.
 Fe^{2+} can generate $\bullet\text{OH}$ from hydrogen peroxide via Fenton chemistry.

Consequence of reactivity

The hydroxyl radical reacts rapidly close to where it forms; The local generation site matters greatly.

$\bullet\text{OH}$ is the radical species; OH^- is a distinct hydroxide ion.

Do not conflate superoxide, peroxide and hydroxyl radical



Radical anion; One-electron product



Nonradical; Precursor for $\bullet\text{OH}$

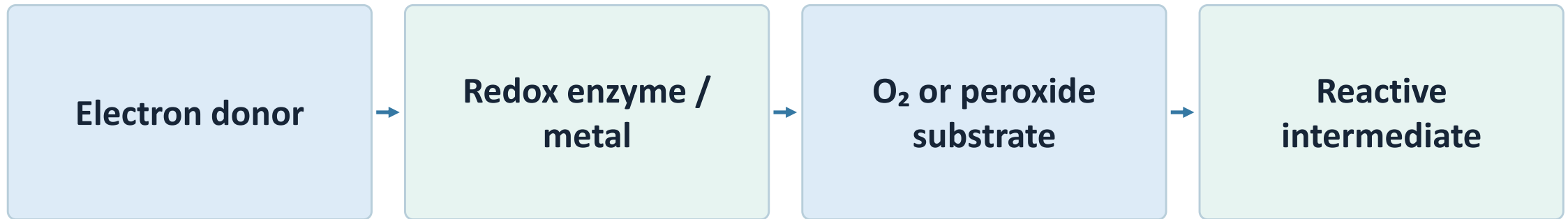


Highly reactive radical

- These species are connected by chemistry but are not interchangeable.
- Naming the particular oxidant improves mechanistic interpretation.

Radical generation depends on electron-transfer context

Sequential mechanism



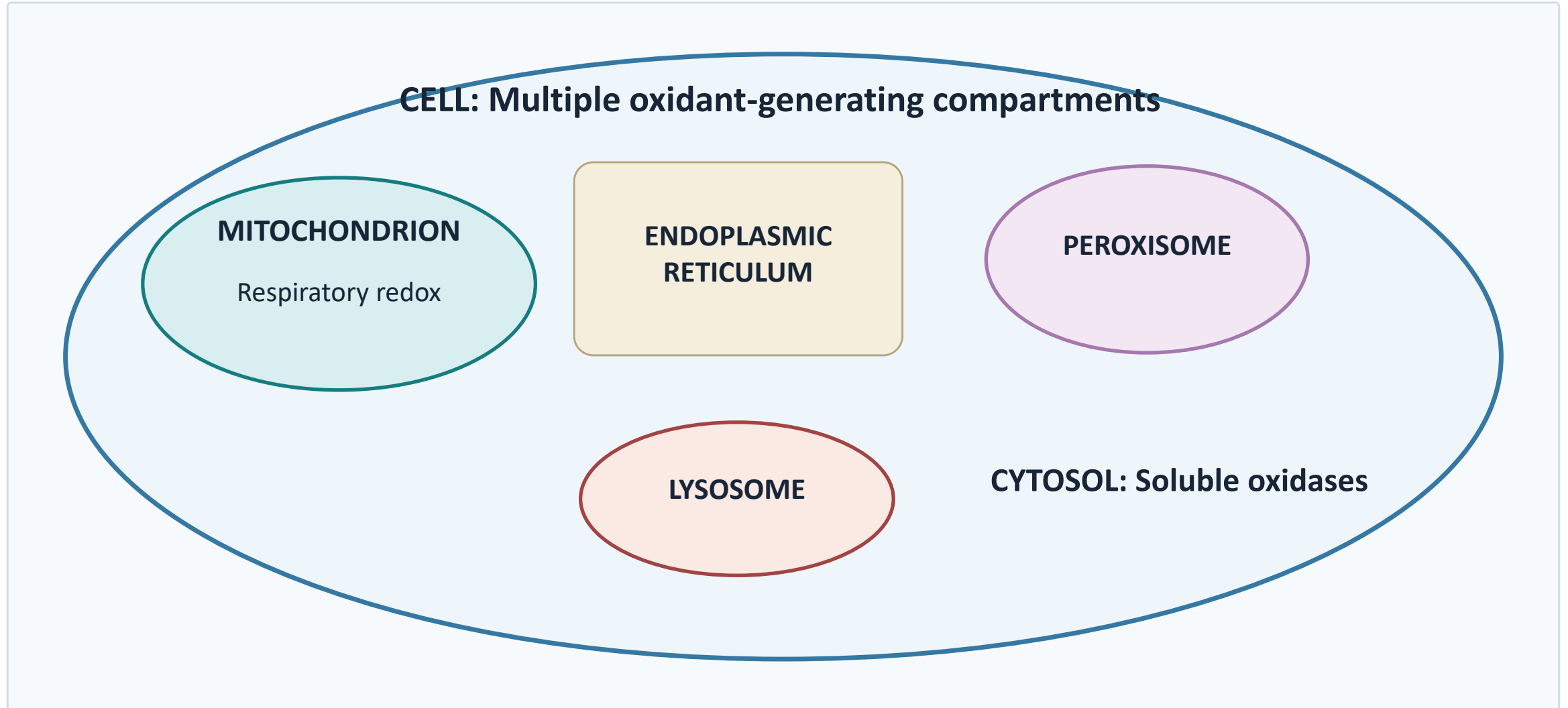
- Both the substrate and the catalytic setting determine the product.
- Compartmentalization directs which biomolecules can be exposed.

Reactive species formation also occurs in healthy cells

- Basal redox metabolism produces small quantities of reactive intermediates.
- Formation is not synonymous with tissue injury.
- Cells normally maintain a dynamic balance between production and removal.
- Oxidative stress denotes an imbalance in favor of oxidants.

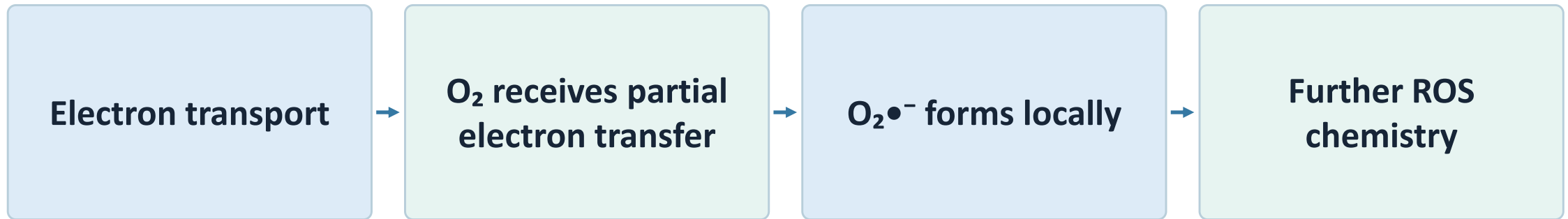
Normal generation is expected; Pathologic accumulation is context-dependent.

Oxygen-derived species arise in several compartments



Mitochondria link respiration to superoxide production

Unintended side products of respiratory electron transfer



- Mitochondrial respiration is a normal source of oxygen-derived intermediates.
- The central distinction is complete versus incomplete O₂ reduction.

Electron leakage is a route to mitochondrial ROS

Ordered electron transfer

Respiration channels electrons toward four-electron reduction of oxygen to water.

Side transfer to O₂

Premature, single-electron reduction can generate O₂•⁻ before complete water formation.

Mitochondrial generation is local but has broader consequences

- Superoxide is produced near the electron-transfer source.
- Related oxidants may be formed as superoxide is converted.
- H_2O_2 can influence sites beyond its initial production compartment.
- The production–removal balance determines net exposure.

Cytosolic oxidases are additional ROS sources

Cytosol

Soluble oxidoreductases

Substrate

O₂ accepts electrons

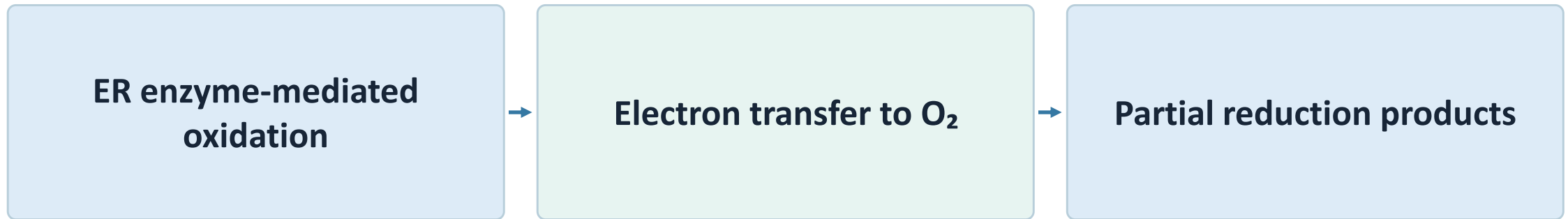
Products

O₂•⁻ or H₂O₂

- Cytosolic oxidative enzymes among sources of intermediates.
- Enzyme identity and substrate availability govern product formation.

Endoplasmic-reticulum redox reactions may generate ROS

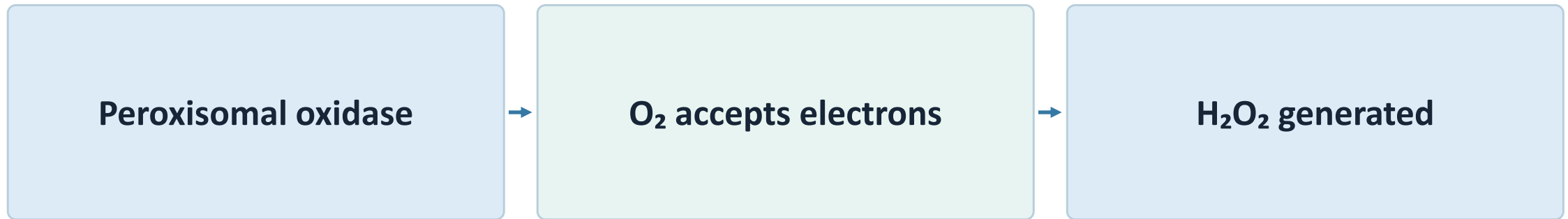
Sequential mechanism



- The ER is part of the intracellular oxidative-enzyme network.
- Oxidative drug metabolism offers an important related clinical example.

Peroxisomal oxidases contribute to hydrogen peroxide

Sequential mechanism



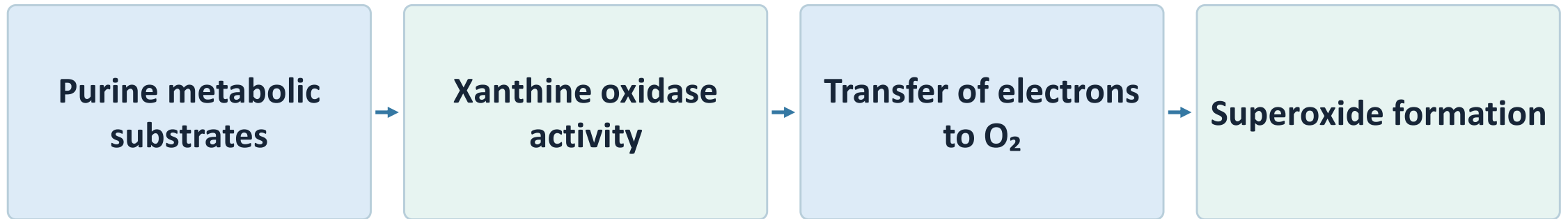
- Peroxisomes contain enzymes that create H₂O₂ during substrate oxidation.
- Peroxide production and peroxide detoxification coexist within the organelle.

Lysosomes are included among cellular redox compartments

- The source chapter lists lysosomal oxidative enzymes among cellular sources.
- The lysosomal environment is relevant to degradative cell functions.
- Do not assume all lysosomal ROS originate from mitochondrial leakage.
- Exact enzyme-specific pathways require separate mechanistic evidence.

Xanthine oxidase is a named intracellular oxidant source

Oxidase-mediated production



- Robbins explicitly identifies xanthine oxidase as an intracellular O₂•⁻-generating oxidase.
- The source illustrates enzyme-driven generation beyond mitochondrial respiration.

Basal metabolic ROS versus deliberate immune-cell ROS

Ordinary metabolism

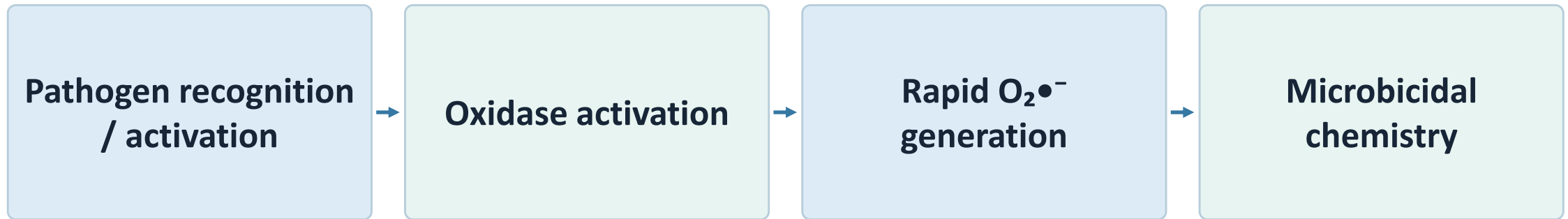
- Redox enzymes function continuously.
- Small reactive intermediate quantities may appear.
- Exposure is usually constrained by cellular defenses.

Leukocyte respiratory burst

- Activation rapidly elevates oxidant production.
- A multiprotein NADPH oxidase complex is involved.
- Oxidants support microbial killing.

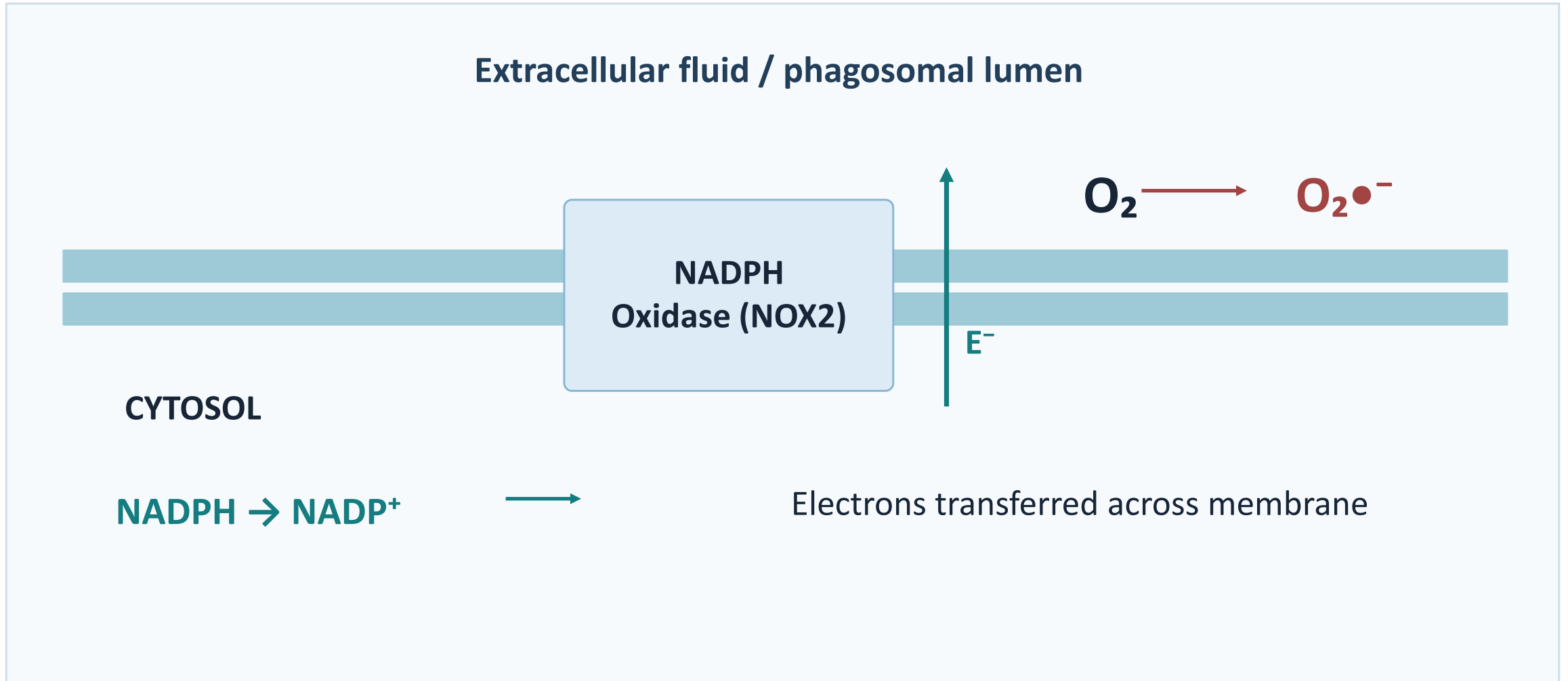
Activated phagocytes intentionally generate oxidants

Sequential mechanism



- Inflammation recruits neutrophils and macrophages that produce reactive oxygen species.
- The burst is a controlled host-defense program, not simply metabolic leakage.

NADPH oxidase is a membrane-associated electron-transfer system



NADPH supplies reducing equivalents for phagocyte oxidase

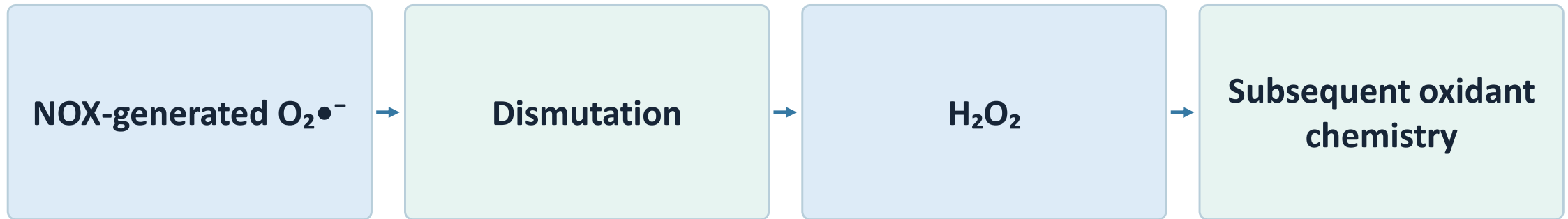
NOX reaction — simplified



- The oxidase transfers electrons from cytosolic NADPH to molecular oxygen.
- Superoxide is generated on the membrane-opposite side of electron donation.
- This schematic omits regulatory subunits and proton-coupled steps.

Respiratory burst connects superoxide to other oxidants

Sequential mechanism



- H₂O₂ is a downstream product of superoxide conversion.
- Specific microbial killing pathways depend on phagocyte enzymes and local conditions.

The location of oxidase assembly matters

Phagosomal membrane

- Electron transfer can create oxidants in the phagosomal lumen.
- Reactive intermediates confront ingested microbes.

Cell surface membrane

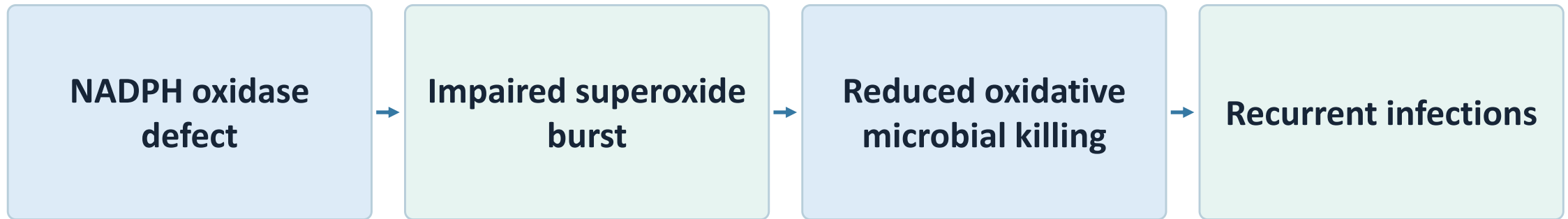
- Activated oxidase can release oxidants extracellularly.
- Surrounding host tissue may be exposed during inflammation.

Leukocyte oxidant formation is controlled and inducible

- Inflammatory activation precedes the rapid increase in oxidant production.
- A multiprotein NADPH oxidase system mediates the response.
- The respiratory burst is separable from mitochondrial respiration.
- Defects in this pathway compromise a major antimicrobial mechanism.

Defective superoxide production: Chronic granulomatous disease

Sequential mechanism



- Chronic granulomatous disease as the classic clinical consequence.
- Not all leukocyte antimicrobial functions are lost.

Clinical application: Failure of respiratory burst

CASE / APPLICATION

A child has recurrent bacterial and fungal infections. Neutrophils phagocytose organisms, but the oxidative burst is defective.

Which reaction is primarily compromised?

- A. NADPH oxidase-mediated $O_2\bullet^-$ production
- B. Peroxisomal catalase breakdown of H_2O_2
- C. Fe^{2+} -mediated peroxide cleavage

Radiant energy can initiate radical chemistry

Energy input

Radiant energy absorbed

Primary target

Water and other molecules

Reactive products

Radical intermediates

- Ultraviolet light and x-rays among relevant radiant energy sources.
- For ionizing radiation, radiolysis of water is a well-established route to $\bullet\text{OH}$.

Radiolysis of water produces reactive radical fragments

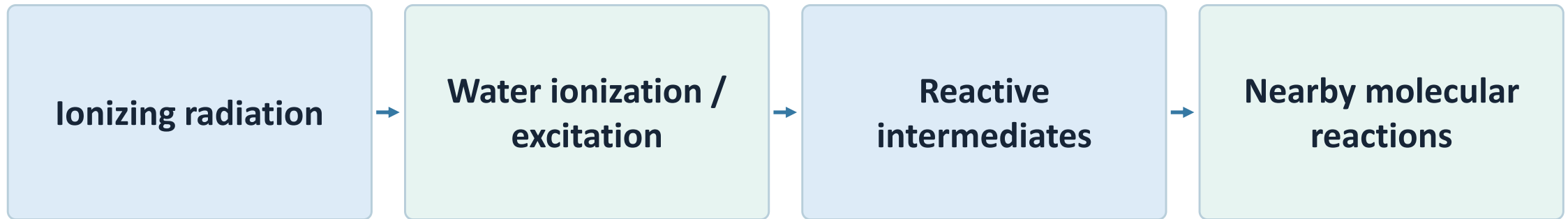
Simplified radiolysis



- This is an illustrative net representation, not a full radiation-chemistry reaction set.
- Hydroxyl radicals formed locally can attack nearby cellular targets.

Ionizing radiation and water radiolysis

Sequential mechanism



- Water is abundant in cells and serves as a major radiation target.
- Short-lived radicals can mediate an indirect component of radiation injury.

UV irradiation and x-rays are not mechanistically identical

Ultraviolet light

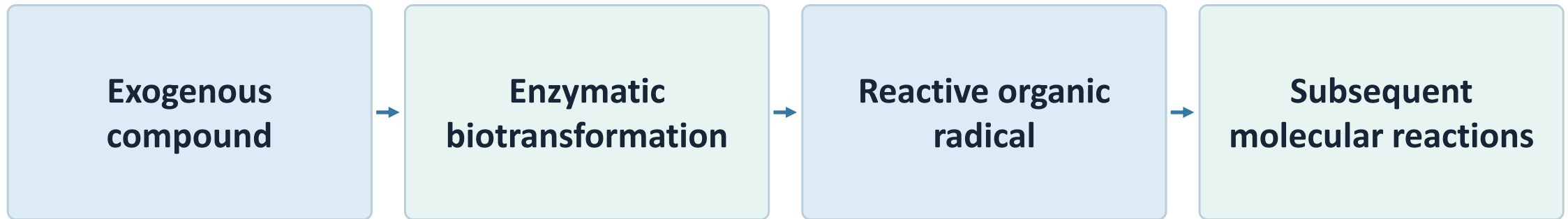
- Can initiate photochemical cellular damage.
- Wavelength and target determine the pathway.
- Not every UV effect is explained by water radiolysis.

X-rays / ionizing radiation

- Can ionize water and generate free radicals.
- •OH is among important products.
- Radical and direct ionization effects may coexist.

Drug and chemical metabolism can generate non-ROS radicals

Sequential mechanism



- Free radicals are not limited to oxygen-centered species.
- Carbon tetrachloride as a chemical example.

Carbon tetrachloride: A classic radical-forming xenobiotic

Reductive bioactivation —
schematic



- Reductive metabolism forms the trichloromethyl radical ($\bullet\text{CCl}_3$).
- Further reactions can propagate damage to cellular membranes.
- An organic radical can initiate downstream ROS-linked injury.

Radical formation and oxidative damage are not the same step

Initiation

- CCl_4 is converted enzymatically to $\bullet\text{CCl}_3$.
- The newly formed radical is not itself an ROS.

Propagation

- Reactive intermediates can attack neighboring molecules.
- A radical chain can amplify the initial chemical event.

Chemical exposure: Identify the initiating species

CASE / APPLICATION

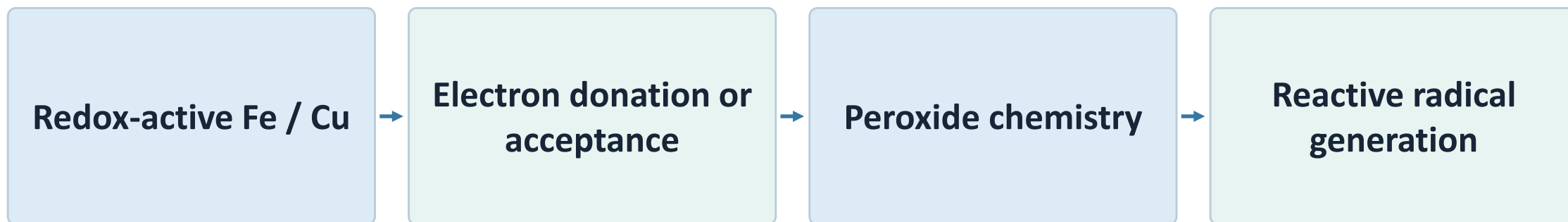
A halogenated solvent undergoes enzymatic reductive metabolism in hepatocytes, generating a carbon-centered free radical.

What is the principal initiating product in the Robbins example?

- A. Trichloromethyl radical ($\bullet\text{CCl}_3$)
- B. Superoxide ($\text{O}_2\bullet^-$) as the only product
- C. Peroxynitrite (ONOO^-)

Transition metals catalyze radical production

Sequential mechanism



- Iron and copper can move between oxidation states during redox reactions.
- Metal availability changes which peroxide reactions proceed.

Fenton chemistry generates hydroxyl radicals

Fenton reaction



- Ferrous iron (Fe^{2+}) participates directly.
- Hydrogen peroxide is converted into hydroxyl radical plus hydroxide.
- Ferric iron (Fe^{3+}) is the oxidized iron product.

Fe^{3+} must be reduced to Fe^{2+} to sustain Fenton chemistry



Electron donation / peroxide cleavage



Highly reactive hydroxyl radical

Superoxide can facilitate $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ recycling

$\text{O}_2\bullet^-$ present



Fe^{3+} reduction



Fe^{2+} regenerated



Further Fenton chemistry

Superoxide can support Fe³⁺ to Fe²⁺ recycling

Conceptual metal cycling



- This electron-transfer reaction replenishes ferrous iron.
- Superoxide and peroxide may therefore cooperate in •OH production.
- The Fenton reaction itself consumes Fe²⁺ and generates Fe³⁺.

Redox-active copper provides a related catalytic route

Metal redox flexibility

Copper also alternates between oxidation states while donating or accepting electrons.

Biologic implication

The availability of poorly bound, reactive metal ions can accelerate oxidant-generating reactions.

The core Robbins passage identifies both iron and copper as redox-active catalysts.

Metal-binding proteins restrict the reactive metal pool

Iron binding

Ferritin / transferrin

Copper binding

Ceruloplasmin

Inflammatory sites

Lactoferrin

- Tightly bound metal is less available to catalyze free-radical formation.
- This is preventive context; Removal of radicals is treated in the next Robbins topic.

Why peroxide plus labile iron is a hazardous combination

CASE / APPLICATION

A cell accumulates hydrogen peroxide and an expanded pool of redox-active ferrous iron. Antioxidant buffering is overwhelmed.

Which species is directly generated by the Fenton reaction?

- A. Hydroxyl radical ($\bullet\text{OH}$)
- B. Nitric oxide ($\text{NO}\bullet$)
- C. Molecular oxygen (O_2) alone

Nitric oxide is a free radical generated by several cell types

Endothelium

NO supports vascular signaling

Macrophages

NO participates in host defense

Neurons

NO functions as a signaling mediator

- NO as an important chemical mediator and a free radical.
- Its chemistry becomes particularly important in the presence of superoxide.

Nitric oxide differs chemically from peroxynitrite

Nitric oxide: NO•

- Nitrogen-centered radical.
- Synthesized in multiple cell types.
- Acts as a mediator and reacts with $O_2^{\bullet-}$.

Peroxynitrite: ONOO⁻

- Formed from NO• and $O_2^{\bullet-}$.
- Reactive nitrogen-containing oxidant.
- Not itself a free radical.

Superoxide reacts with nitric oxide to form peroxynitrite

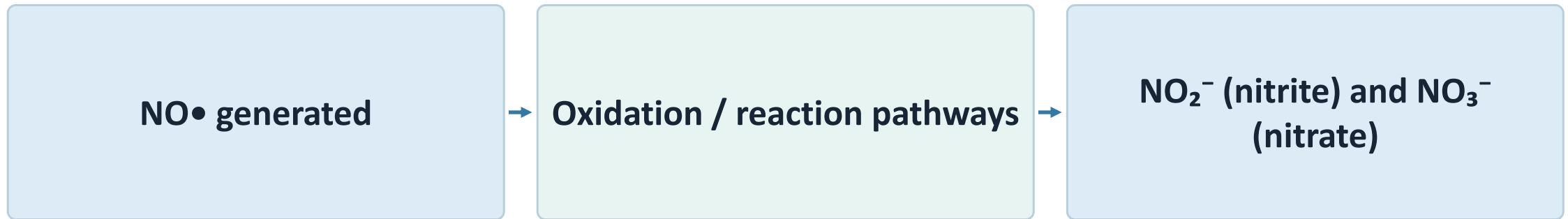
ROS–RNS coupling



- The reaction links sources of nitric oxide with superoxide production.
- Peroxynitrite is a highly reactive product.
- The location and coincidence of both reactants matter.

NO-derived chemistry includes nitrite and nitrate

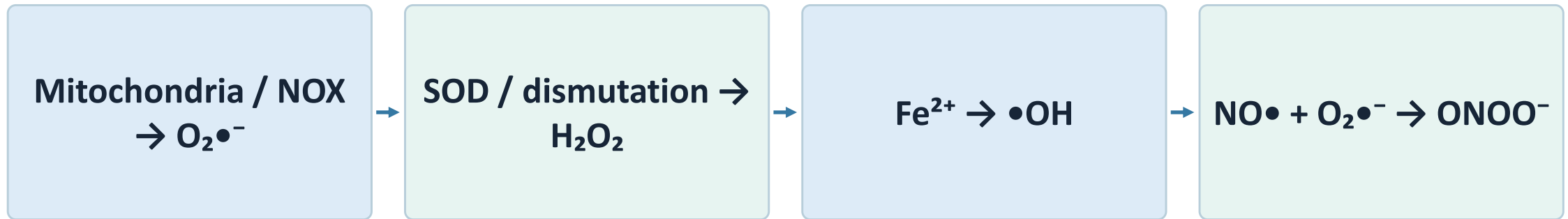
Sequential mechanism



- These compounds are nitrogen oxide metabolites.
- They should not be depicted as identical to short-lived radical intermediates.

Generation network: Oxygen radicals meet nitrogen chemistry

Sequential mechanism



- This is a source-to-product map; Not every arrow is a single uninterrupted pathway.
- The terminal chemistry depends on which reactants coexist.

Source-to-species crosswalk

Principal superoxide pathways

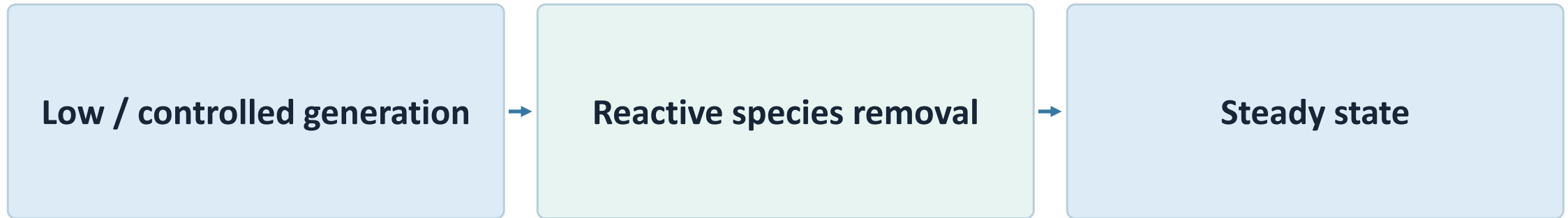
- Mitochondrial partial O_2 reduction
- Phagocyte NADPH oxidase
- Xanthine oxidase and other oxidases
- Possible Fe^{3+} reduction by $O_2\bullet^-$

Other reactive species pathways

- Radiolysis $\rightarrow \bullet OH$ and $H\bullet$
- Fenton reaction $\rightarrow \bullet OH$
- CCl_4 metabolism $\rightarrow \bullet CCl_3$
- $NO\bullet + O_2\bullet^- \rightarrow ONOO^-$

Oxidant production and oxidative stress are not equivalent

Sequential mechanism



- An increase in production can cause oxidant accumulation.
- A decrease in clearance can yield a similar imbalance.
- Free-radical formation also performs physiologic signaling and host defense.

Clinicopathologic contexts have different initiation routes

Chemical / physical insults

- X-rays $\rightarrow$ water radiolysis
- $\text{CCl}_4 \rightarrow$ trichloromethyl radical
- Reactive metal pool $\rightarrow$ Fenton chemistry

Cellular / inflammatory sources

- Mitochondrial respiration $\rightarrow \text{O}_2\bullet^-$
- Activated phagocytes $\rightarrow$ NOX burst
- $\text{NO}\bullet$ with $\text{O}_2\bullet^- \rightarrow$ peroxynitrite

Application: Match the initiating route with its product

CASE / APPLICATION

During inflammation, nitric oxide and superoxide are produced in the same microenvironment. Both react before superoxide is cleared.

Which reactive product is most directly expected?

- A. Peroxynitrite (ONOO^-)
- B. Trichloromethyl radical ($\bullet\text{CCl}_3$)
- C. Hydroxide ion (OH^-) as the defining oxidant

Mechanistic synthesis: Six experimentally distinguishable origins

Redox enzymes

Mitochondria / oxidases

Host defense

NADPH oxidase burst

External triggers

Radiation / xenobiotics

- Iron and copper catalysis and NO-dependent chemistry add further pathways.
- Identify the source, immediate reactive species and subsequent reaction separately.

Essential distinctions for interpreting free-radical mechanisms

- $\text{O}_2\bullet^-$ and $\bullet\text{OH}$ are radicals; H_2O_2 and ONOO^- are nonradical oxidants.
- Physiologic O_2 reduction to water can leak partially reduced intermediates.
- NOX-driven leukocyte production is deliberately inducible.
- Radiation, chemical metabolism and transition metals generate distinct radical products.
- $\text{NO}\bullet$ plus superoxide couples oxygen and nitrogen reactive chemistry.

Primary reference and scope

- Robbins, Cotran & Kumar: Pathologic Basis of Disease, 11th edition (2026).
- Chapter 2: Cell Injury, Cell Death, and Adaptations.
- Sections: Oxidative Stress—Generation of Free Radicals; Table 2.3.
- Companion topics in this series address removal of radicals and their pathologic effects.