

Pathologic Effects of Free Radicals

Mechanisms of oxidative cellular injury

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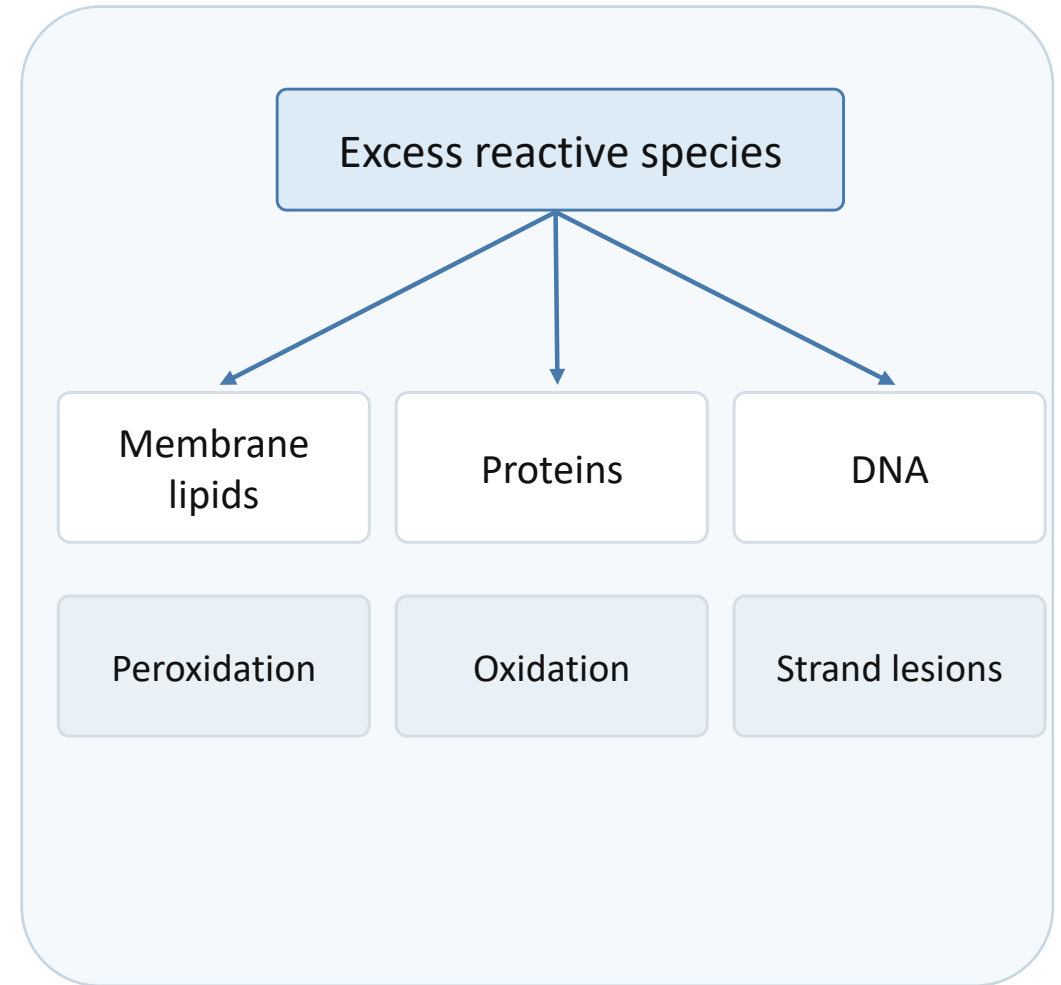
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Learning objectives

- Identify the three principal macromolecular targets of free radical injury.
- Explain how lipid peroxidation amplifies damage through chain propagation.
- Relate protein and DNA oxidation to cellular dysfunction and outcomes.
- Integrate antioxidant defenses with representative clinical injury mechanisms.

Three major targets account for diverse effects

- Membrane lipids undergo peroxidation, threatening membrane integrity.
- Proteins acquire oxidative changes that alter structure, activity and turnover.
- DNA develops breaks, cross-links and adducts with potential long-term effects.

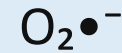


A radical is defined by its electron configuration

- A free radical contains an unpaired electron in an outer orbital.
- The unpaired electron can favor reaction with nearby cellular molecules.
- In some reactions, the attacked molecule becomes reactive and propagates injury.

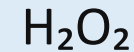
Reactive species are not all free radicals

- Superoxide ($\text{O}_2\bullet^-$) and hydroxyl ($\bullet\text{OH}$) are oxygen-centered radicals.
- Hydrogen peroxide (H_2O_2) lacks an unpaired electron but is an important ROS.
- Peroxynitrite (ONOO^-) is a reactive nitrogen/oxygen species; Terminology should distinguish reactivity from radical status.



Superoxide

Radical



Hydrogen peroxide

Non-radical ROS



Hydroxyl radical

Radical

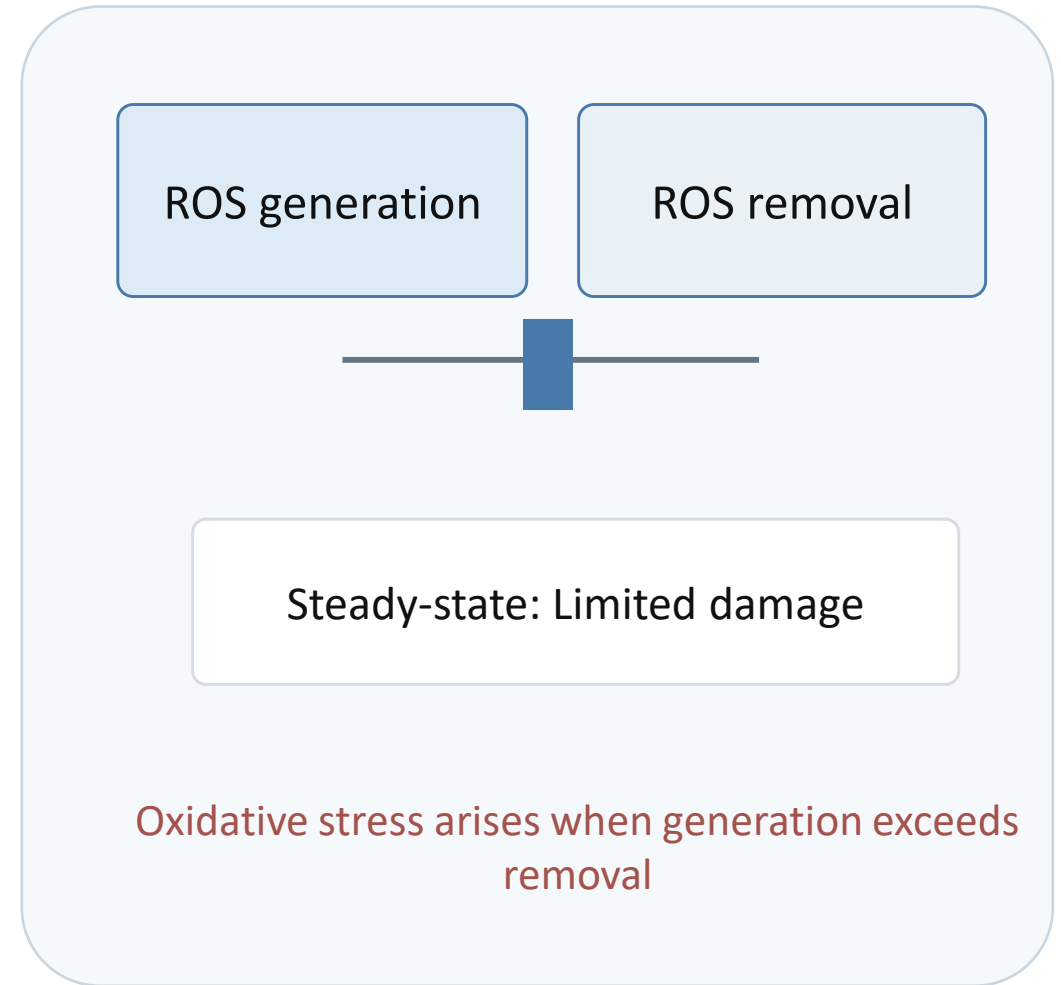


Peroxynitrite

Reactive N/O species

Oxidant production and clearance are normally balanced

- Cells generate some ROS during ordinary metabolism.
- Intracellular antioxidants and enzymes limit accumulation and damage.
- An excess of production over scavenging results in oxidative stress.

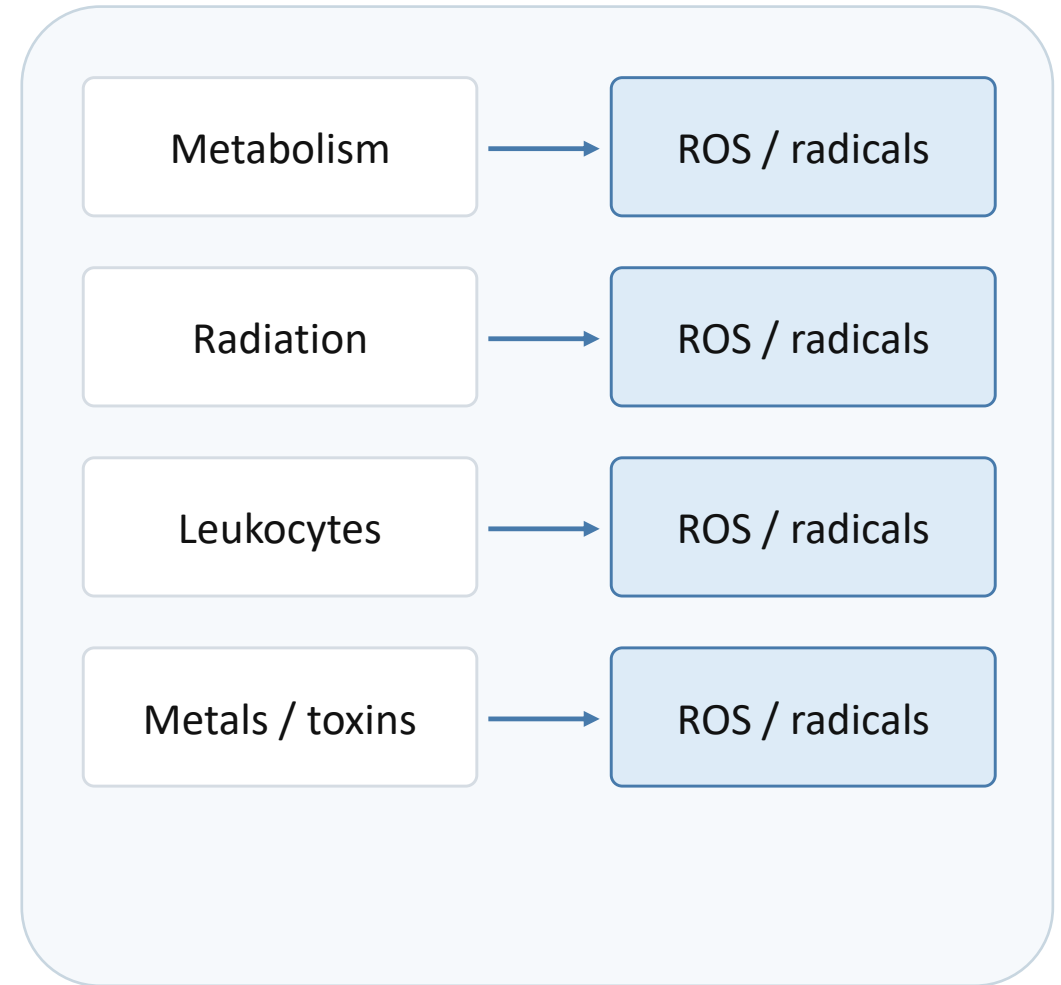


Oxidative stress is a mechanism, not one diagnosis

- An oxidant imbalance can contribute to chemically induced or radiation-induced injury.
- It also participates in ischemia-reperfusion injury and cellular aging.
- The tissue outcome depends on the target molecules and other concurrent injury pathways.

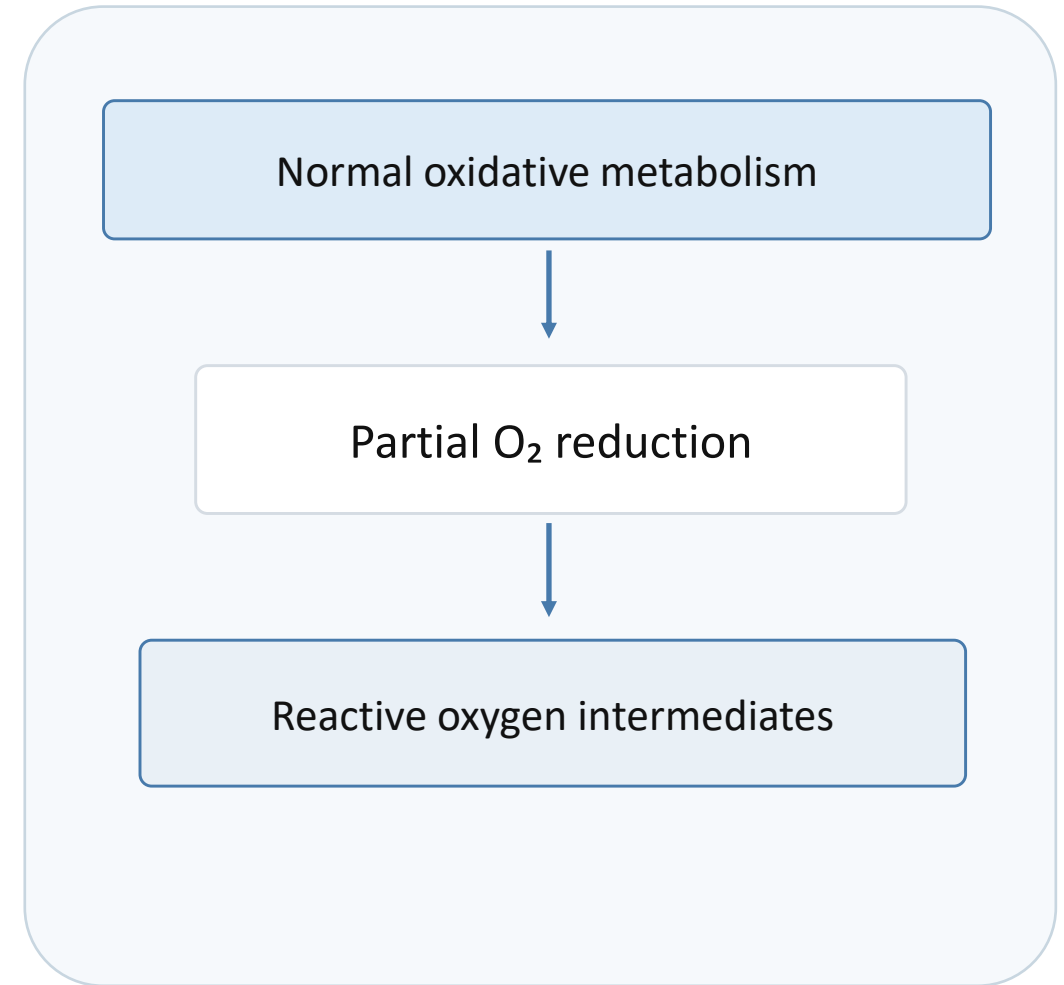
ROS generation occurs in several settings

- Endogenous metabolism generates partially reduced oxygen intermediates.
- Radiant energy can produce radicals from water.
- Inflammatory leukocytes intentionally generate ROS, while metals and chemicals can intensify radical reactions.



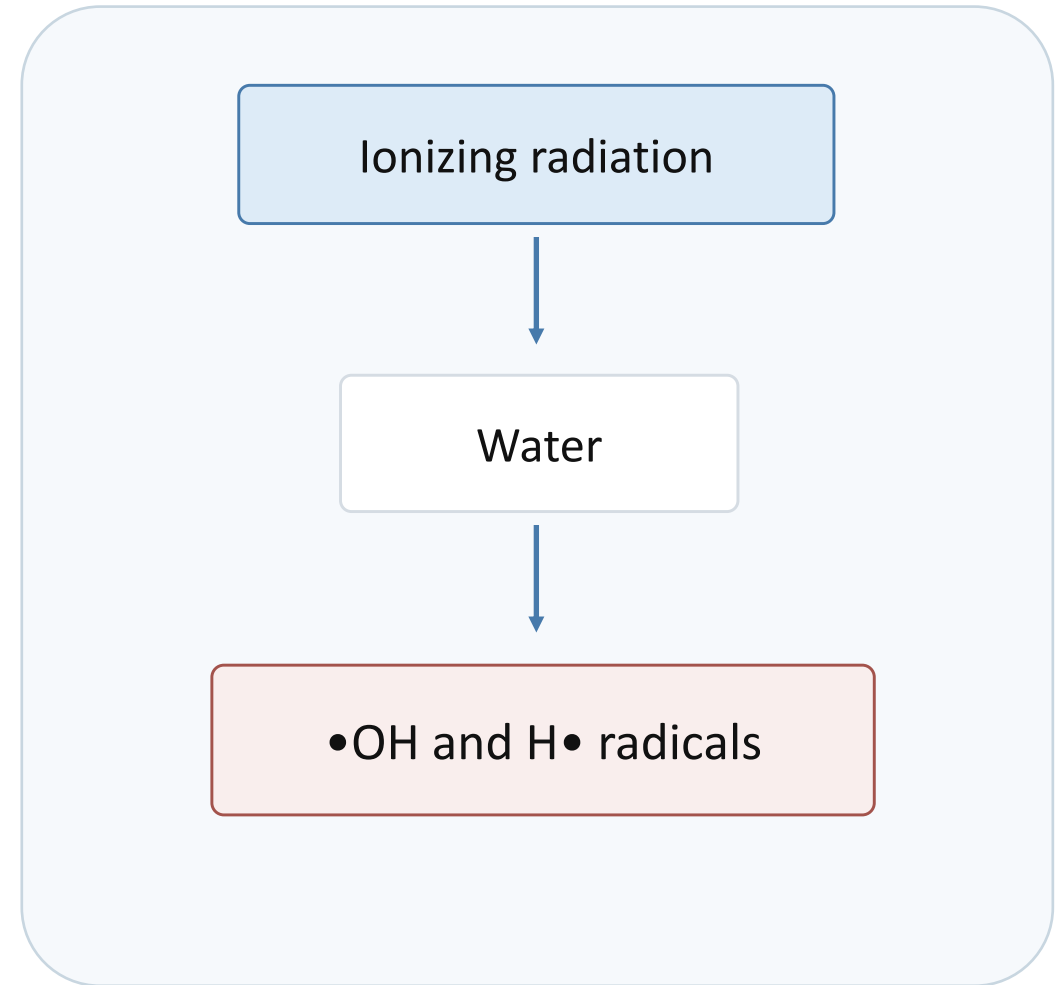
Normal respiration generates reactive intermediates

- Molecular oxygen is normally reduced during cellular respiration.
- Partial oxygen reduction produces intermediates, including superoxide and hydrogen peroxide.
- These species are continuously managed by enzymatic scavengers.



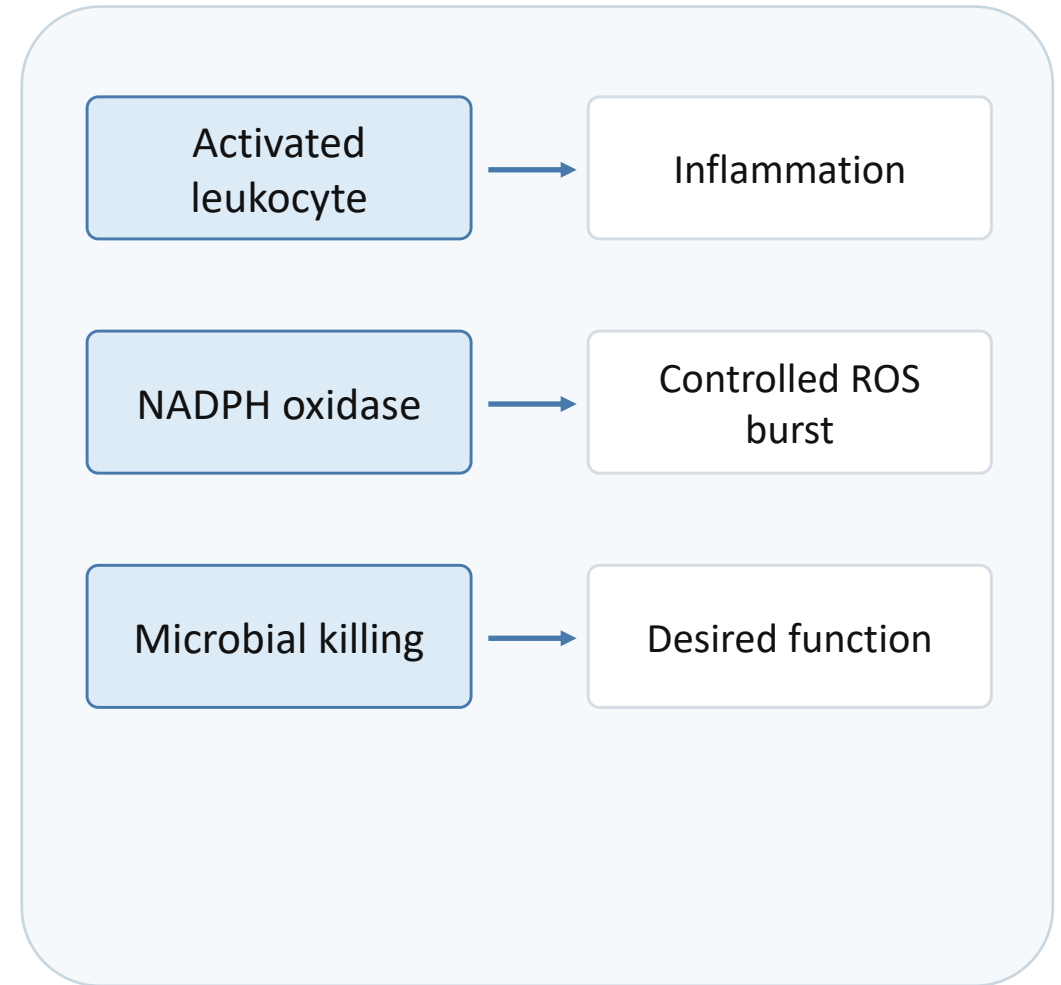
Radiation can generate radicals from water

- Radiant energy is one source of free radical generation.
- Ionizing radiation can produce hydroxyl and hydrogen radicals by radiolysis of water.
- Radicals can then interact with lipids, proteins and nucleic acids.



The respiratory burst has a physiological purpose

- Activated neutrophils and macrophages generate abundant reactive oxygen species.
- A membrane-associated NADPH oxidase complex controls this response.
- The products help destroy microbes, but excessive or misdirected oxidant production can contribute to tissue injury.

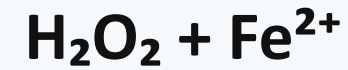


Other oxidases and xenobiotics generate radicals

- Intracellular oxidases such as xanthine oxidase can generate superoxide.
- Enzymatic metabolism of certain foreign chemicals generates reactive radical intermediates.
- Not every injurious radical is oxygen-derived: The trichloromethyl radical is one example.

Iron can amplify radical formation

- Transition metals catalyze electron-transfer reactions associated with radical formation.
- The Fenton reaction uses hydrogen peroxide and ferrous iron to generate hydroxyl radicals.
- Superoxide can facilitate iron reduction, allowing radical-generating processes to interact.



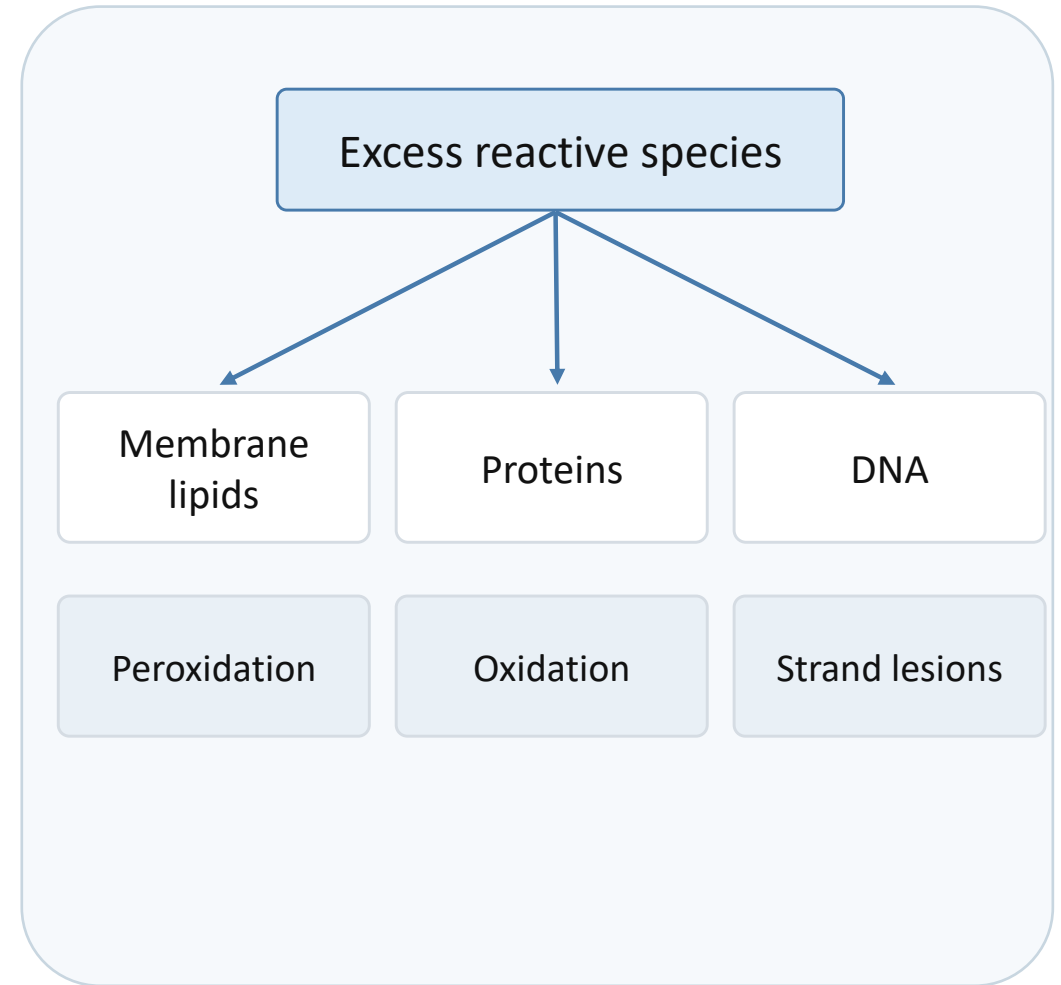
Transition-metal availability influences radical formation

Nitric oxide connects oxygen and nitrogen chemistry

- Nitric oxide is formed by several cell types, including endothelium and leukocytes.
- Its reactions may generate highly reactive nitrogen-containing products, including peroxynitrite.
- Reactive nitrogen and oxygen species participate in injury, particularly during reperfusion.

Different sources converge on common macromolecules

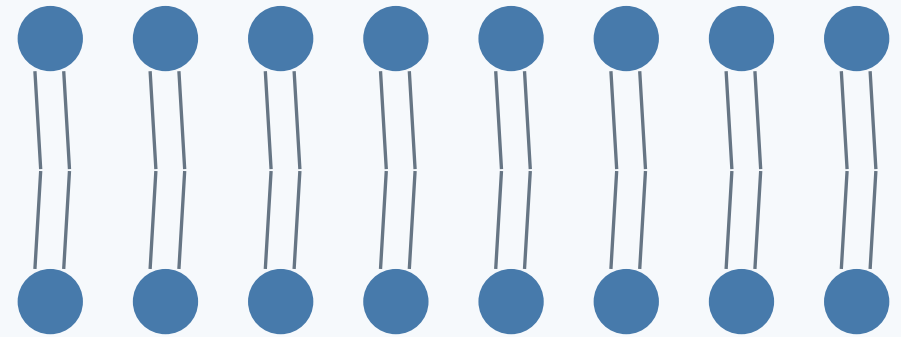
- The source of a reactive species does not uniquely determine the injured molecule.
- Membrane lipids, proteins and DNA are all potential oxidative targets.
- The site and severity of oxidant generation influence the pattern of injury.



Lipid peroxidation affects plasma and organelle membranes

- Membranes are lipid-rich structures that contain susceptible fatty-acid chains.
- Oxidant reactions can occur in plasma membranes and membranes of intracellular organelles.
- Damage to membrane components can compromise membrane function.

Membrane phospholipid bilayer — schematic

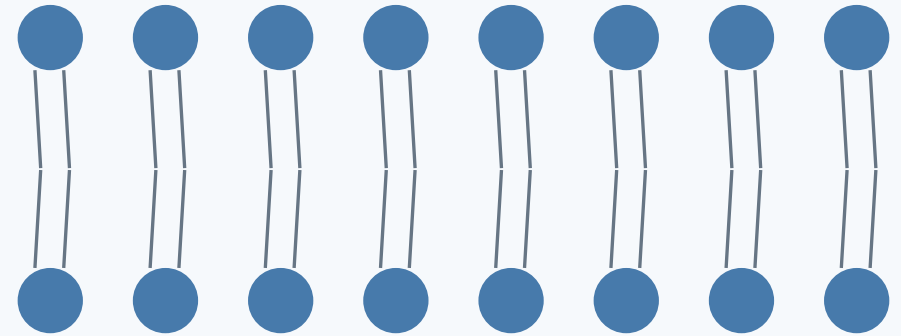


Unsaturated lipid tails provide targets for radical attack

Unsaturated fatty acids are vulnerable targets

- Oxidative attack involves the double bonds of unsaturated fatty acids within membrane lipids.
- Reaction of these lipids with radicals generates chemically reactive intermediates.
- The architecture of the lipid bilayer makes widespread damage functionally important.

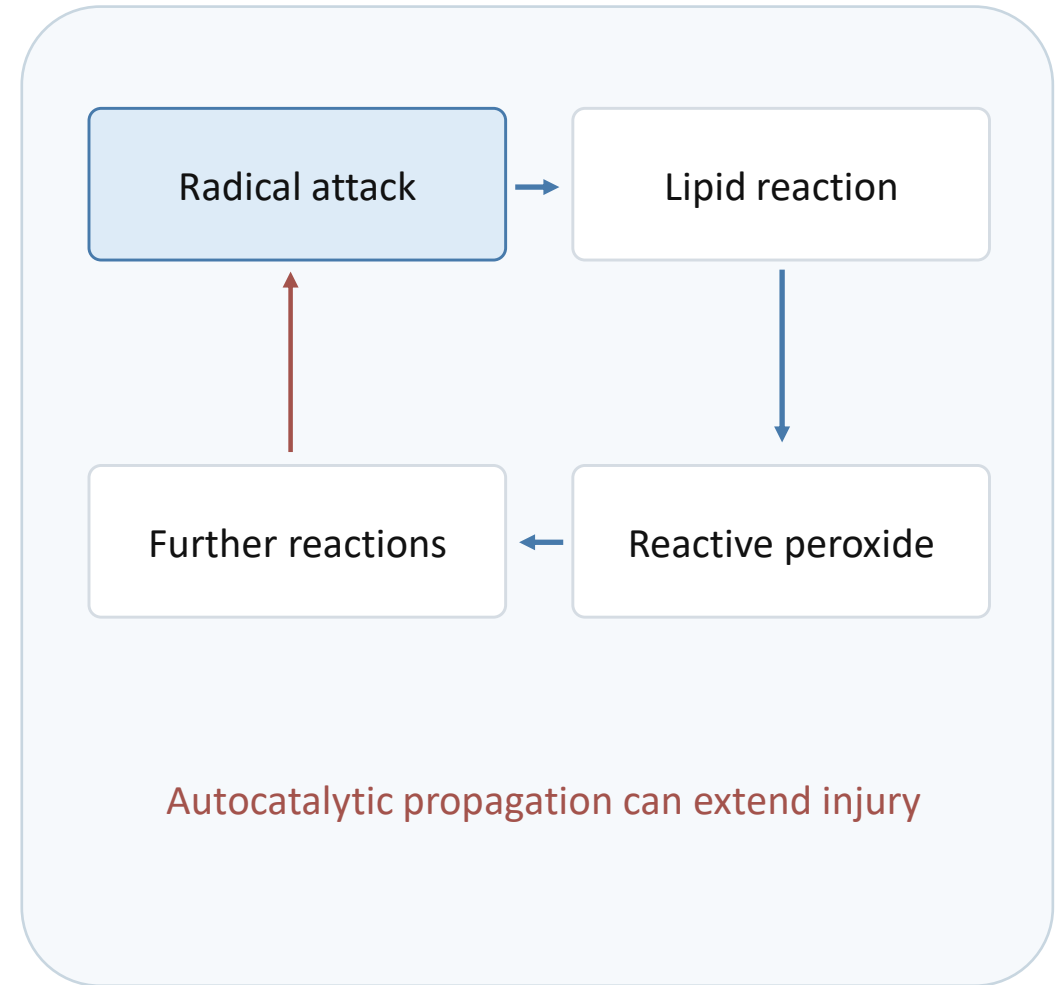
Membrane phospholipid bilayer — schematic



Unsaturated lipid tails provide targets for radical attack

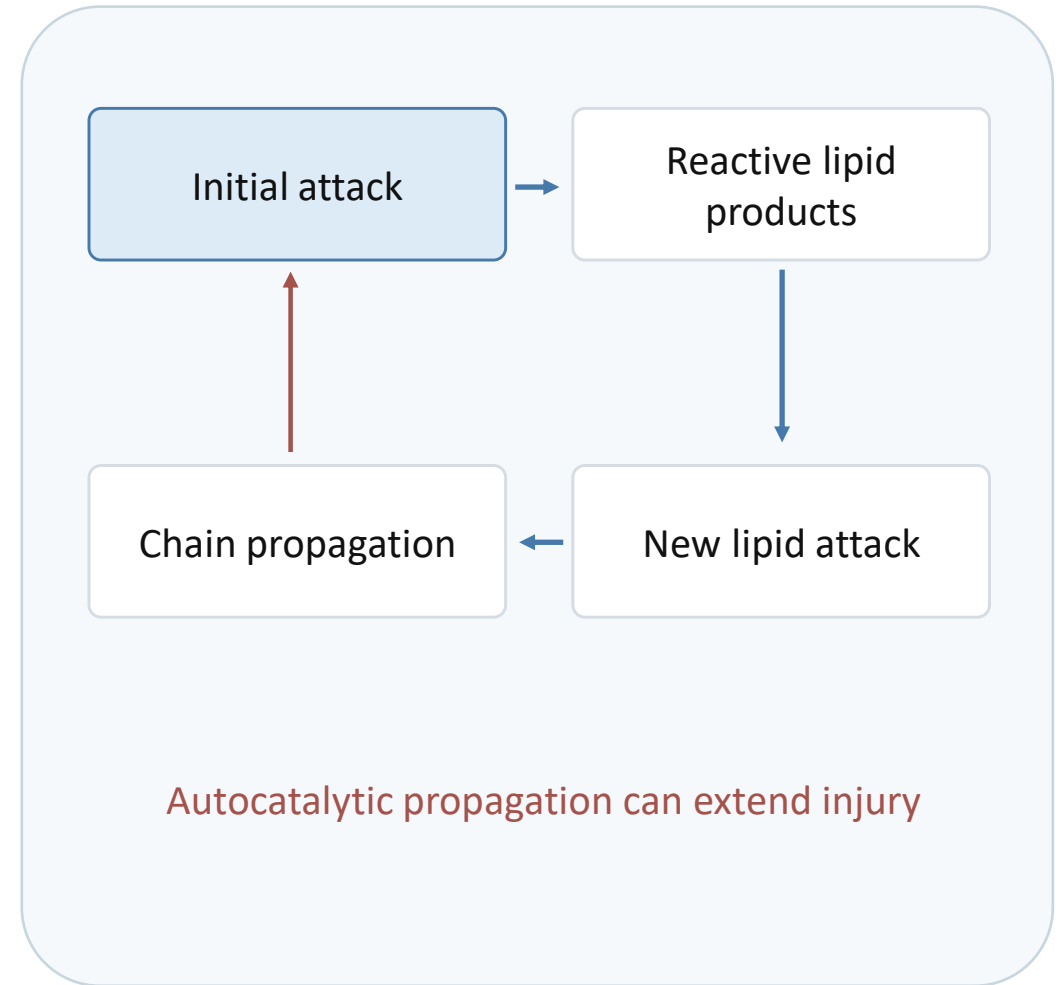
Initiation begins with radical attack on a lipid

- An oxygen-derived radical interacts with an unsaturated membrane lipid.
- The attacked lipid becomes part of a reactive sequence rather than remaining inert.
- This initiation step permits subsequent reactions with neighboring lipids.



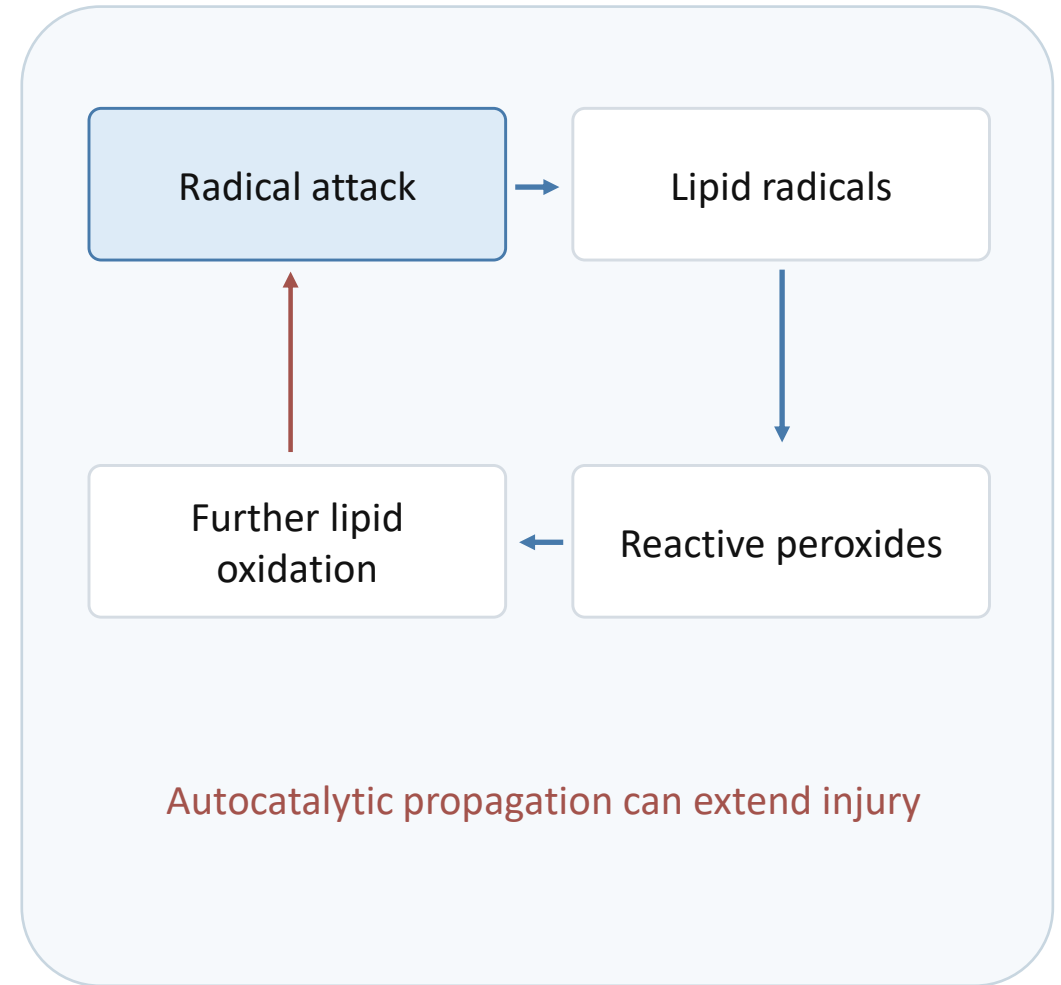
Propagation turns a local reaction into a chain

- Lipid–radical interactions yield peroxides that are themselves reactive and unstable.
- These reactive products support further lipid oxidation.
- The propagation process can extend damage beyond the initial point of attack.



The chain reaction can amplify membrane damage

- Lipid peroxidation is described as autocatalytic because products can sustain additional reactions.
- A discrete initial radical reaction may therefore evolve into more extensive injury.
- The ultimate pathological consequence is loss of membrane integrity or function.

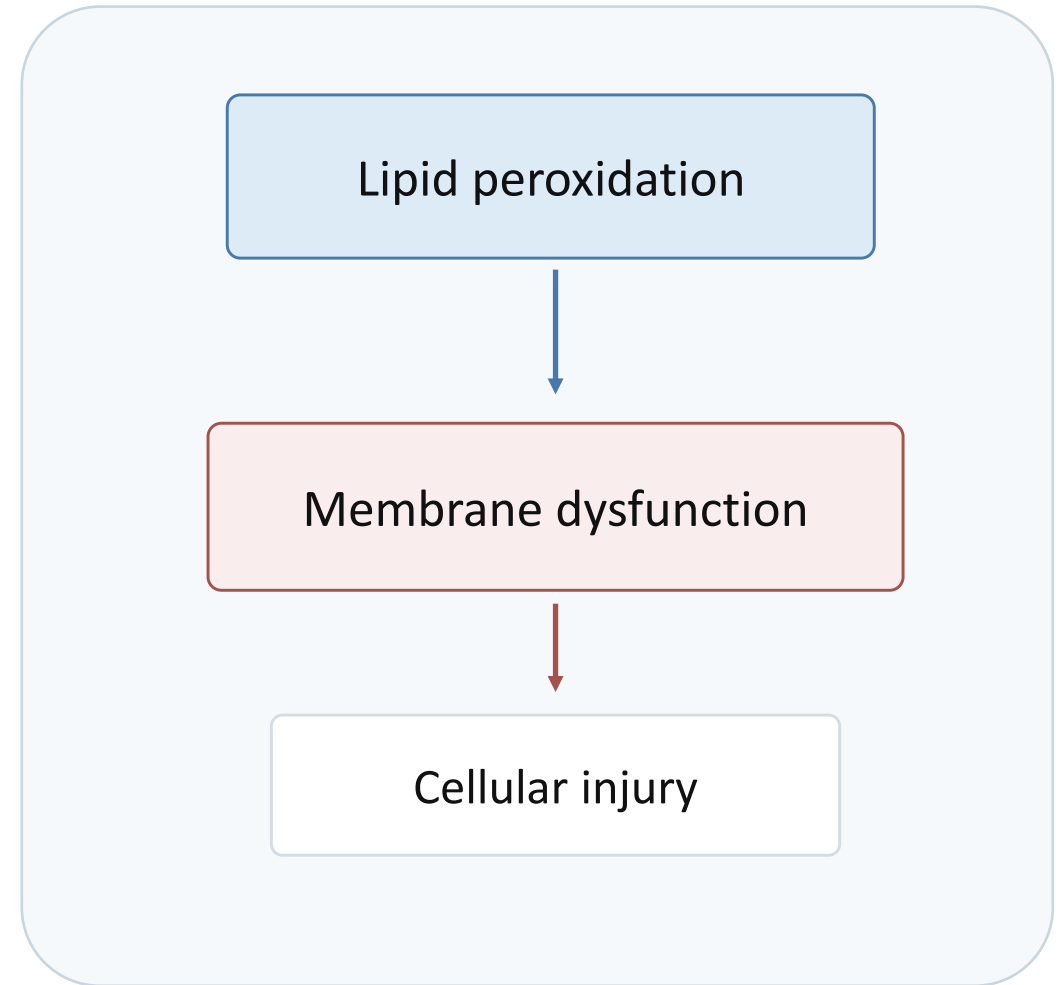


Lipid peroxides are not chemically inert products

- Formation of a lipid peroxide does not necessarily terminate damage.
- Instability and reactivity of peroxidation products contribute to chain propagation.
- This distinguishes peroxidation from a single isolated covalent modification.

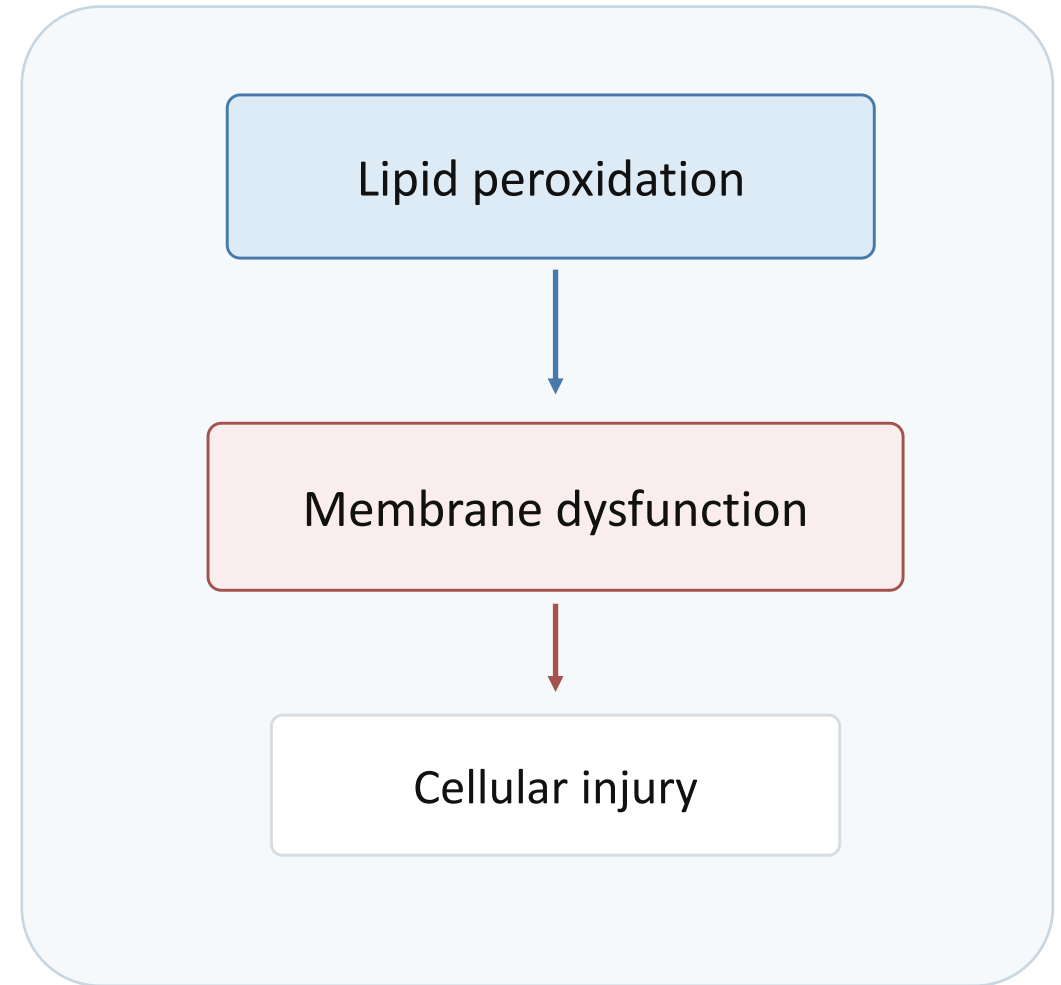
Plasma membrane injury compromises cell integrity

- Peroxidation of the plasma membrane is one biochemical route to membrane damage.
- Loss of normal selective permeability is an important event in cell injury.
- Severe membrane defects contribute to irreversible injury and cell death.



Oxidative injury also affects organellar membranes

- Lipid peroxidation need not be restricted to the cell surface.
- Organellar membranes contain lipids vulnerable to oxidative reactions.
- Disruption of mitochondrial membranes can cooperate with impaired ATP production and cell-death pathways.



Membrane damage is mechanistically heterogeneous

- ROS-induced lipid peroxidation is one mechanism of membrane injury.
- ATP depletion can reduce phospholipid synthesis.
- Calcium-dependent phospholipases accelerate phospholipid breakdown; Cytoskeletal defects also weaken membrane stability.

Oxidative lipid injury interacts with other pathways

- Impaired energy production can make membrane maintenance more difficult.
- Calcium dysregulation can activate phospholipid-degrading enzymes.
- Free radical injury may therefore act alongside rather than replace ATP depletion and calcium-mediated injury.

Lipids → membrane disruption

Proteins → altered cell function

DNA → genomic lesions

Protein oxidation comprises several distinct reactions

- Oxidative modification can involve amino acid side chains.
- Free radicals can produce covalent cross-links between protein molecules.
- The protein backbone is another site of oxidative modification.

Side-chain oxidation

Protein cross-links

Backbone oxidation

Conformational change

Amino acid side-chain oxidation changes protein chemistry

- Protein side chains contain chemical groups that can be modified by oxidants.
- Side-chain oxidation can alter interactions necessary for protein structure and function.
- The effect depends on the location and role of the modified residue.

Side-chain oxidation

Protein cross-links

Backbone oxidation

Conformational change

Covalent cross-links can alter protein organization

- Oxidative stress can promote protein–protein covalent cross-linking.
- Disulfide bond formation is an example cited in the source.
- Inappropriate cross-linking may alter the conformation and behavior of cellular proteins.

Protein oxidation

Covalent cross-links

Altered folding

Functional disturbance

Oxidation may involve the protein backbone

- Free radical reactions are not limited to amino acid side chains.
- Oxidation of the protein backbone can disturb protein integrity.
- This expands the range of possible structural and biochemical consequences.

Side-chain oxidation

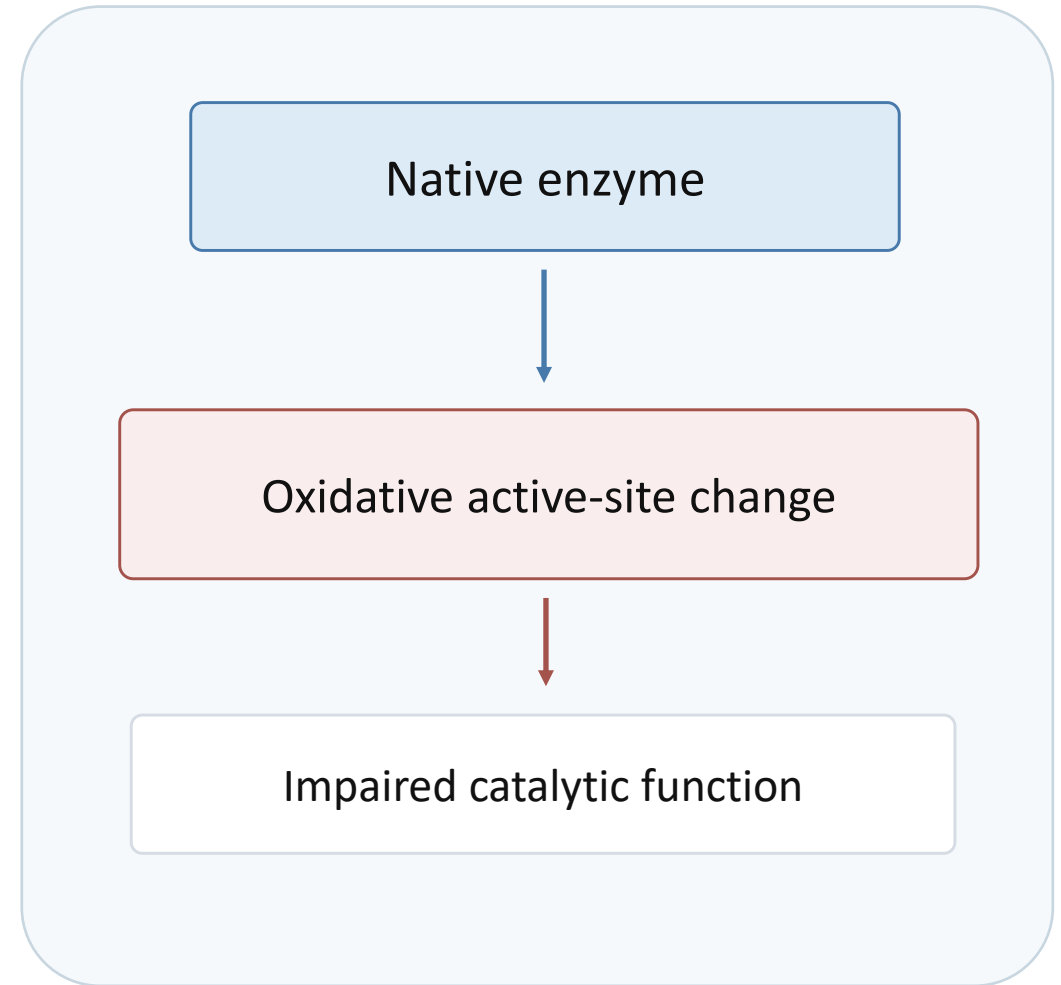
Protein cross-links

Backbone oxidation

Conformational change

Oxidized enzyme active sites may lose function

- The catalytic activity of an enzyme depends on correct active-site structure.
- Oxidative modification of active sites can disrupt that structure.
- Impaired enzyme function can propagate metabolic disturbances throughout the cell.



Structural proteins depend on stable conformation

- Structural proteins contribute to cellular organization and mechanical integrity.
- Oxidative modification may change their three-dimensional conformation.
- Such changes can compromise normal interactions and structural roles.

Structural protein

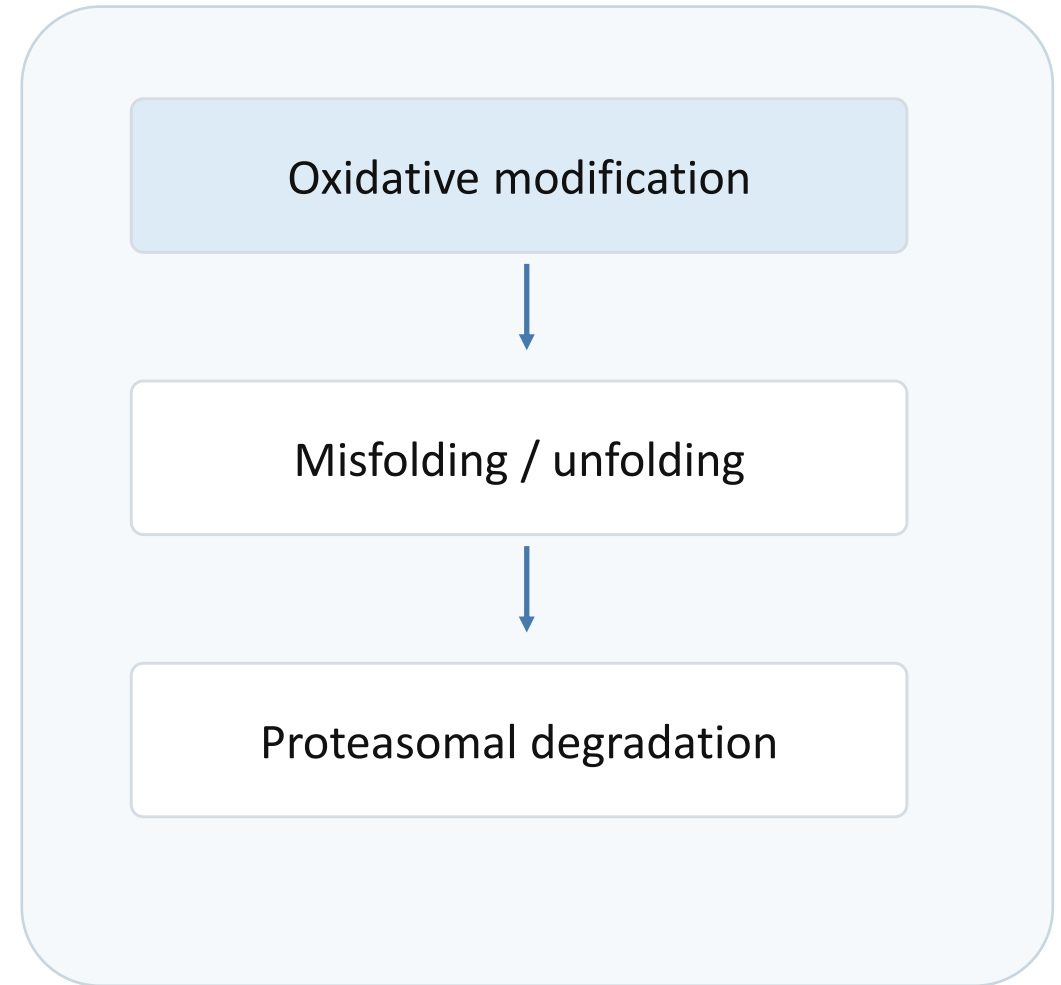
Oxidative change

Conformation altered

Altered cellular function

Oxidative injury increases the burden on proteostasis

- Oxidized proteins can unfold or misfold.
- Proteasomal degradation of abnormal proteins may increase.
- Protein oxidation therefore disrupts both function and protein-quality control.



Protein injury extends beyond individual molecules

- Oxidative changes can affect enzymes, structural proteins and protein-turnover pathways simultaneously.
- The resulting consequences can be biochemical, structural and regulatory.
- Global cellular dysfunction can emerge without a single uniquely diagnostic protein lesion.

Lipids → membrane disruption

Proteins → altered cell function

DNA → genomic lesions

DNA is a major molecular target of oxidants

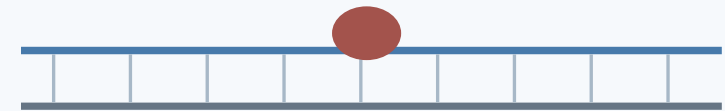
- Free radicals react with nucleic acids as well as proteins and lipids.
- Strand breaks, cross-linking and adduct formation as major DNA lesions.
- These processes can have immediate and persistent biological consequences.



Single-strand break



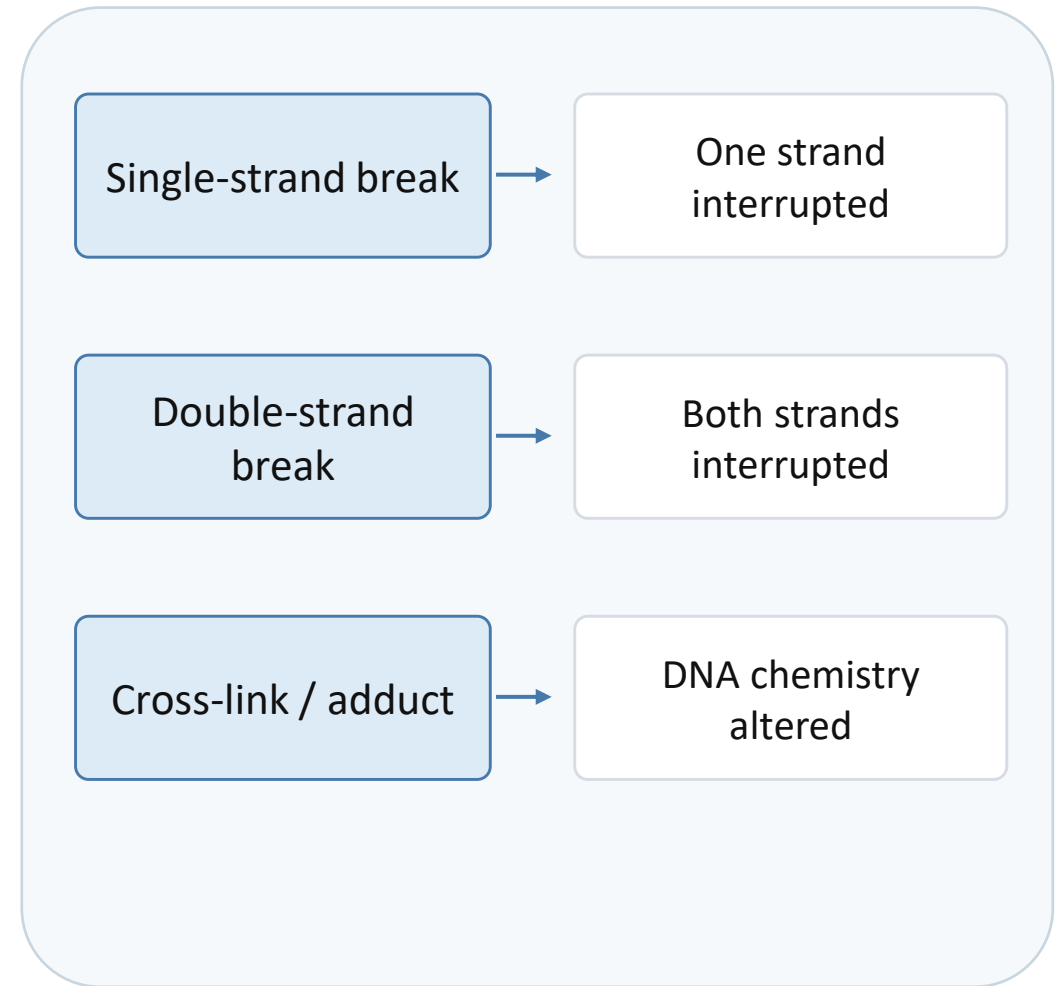
Cross-link



Adduct

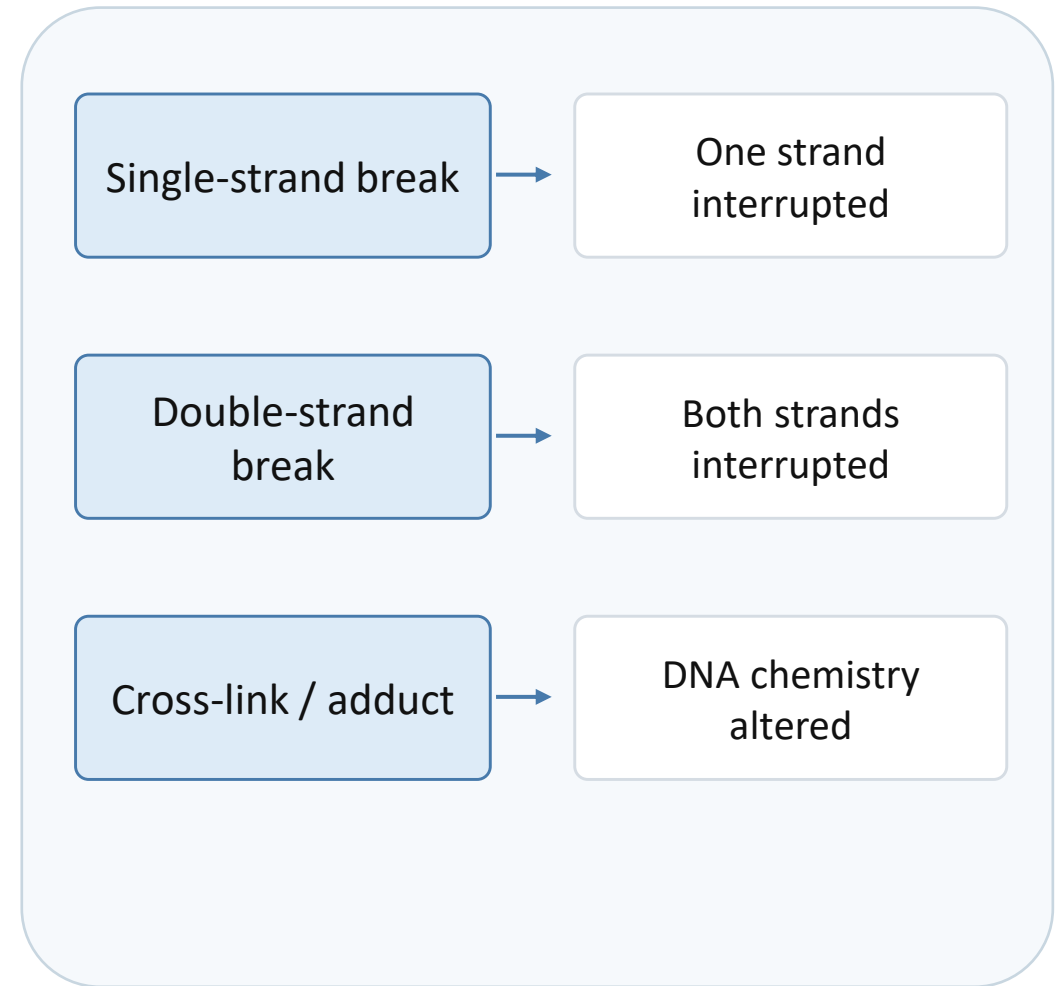
Single- and double-strand breaks are distinct lesions

- A single-strand break interrupts one DNA strand.
- A double-strand break affects both strands of the double helix.
- Both forms are included among the DNA lesions induced by free radicals.



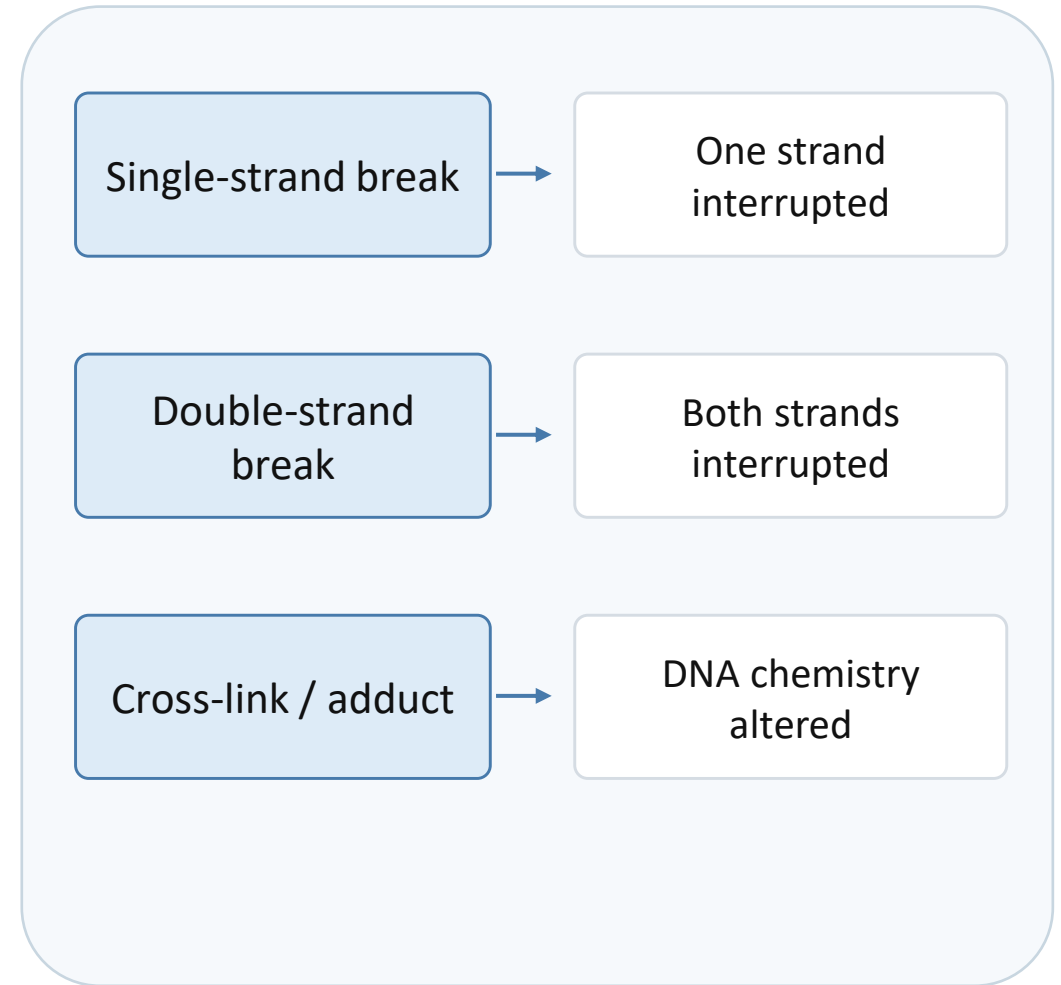
DNA cross-linking creates abnormal covalent connections

- Free radicals can produce cross-linking involving DNA strands.
- Such lesions chemically link components that are normally able to separate.
- Cross-linking is mechanistically different from direct strand breakage.



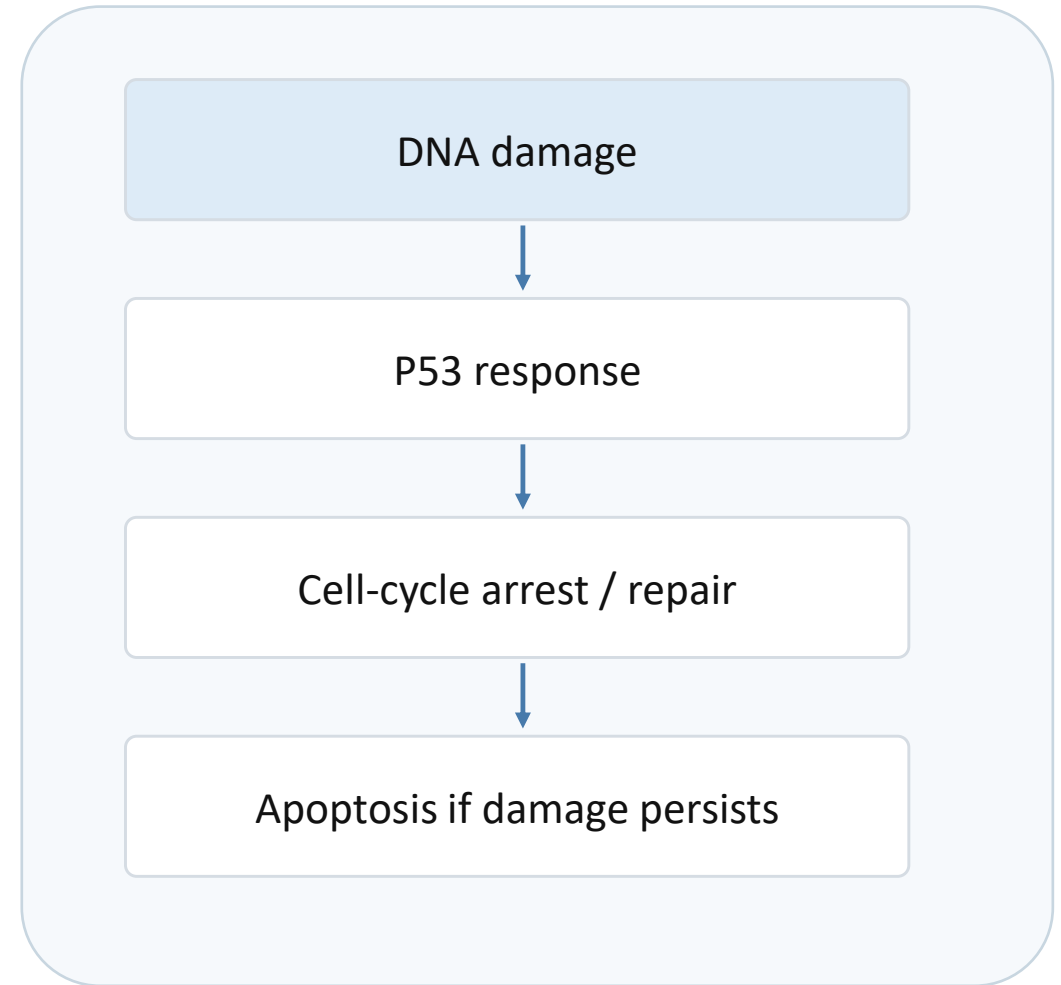
Adduct formation chemically modifies DNA

- An adduct is a chemical modification formed when another group becomes attached to DNA.
- Oxidative reactions can create DNA adducts in addition to strand breaks.
- The specific downstream effect depends on how the lesion is processed by the cell.



DNA damage activates protective cell responses

- In the surrounding Robbins discussion, DNA damage activates p53-dependent responses.
- Cell-cycle arrest and DNA repair help prevent propagation of damaged genetic material.
- When damage is not successfully corrected, p53 can initiate apoptotic signaling.



Oxidative DNA injury has been linked to cellular aging

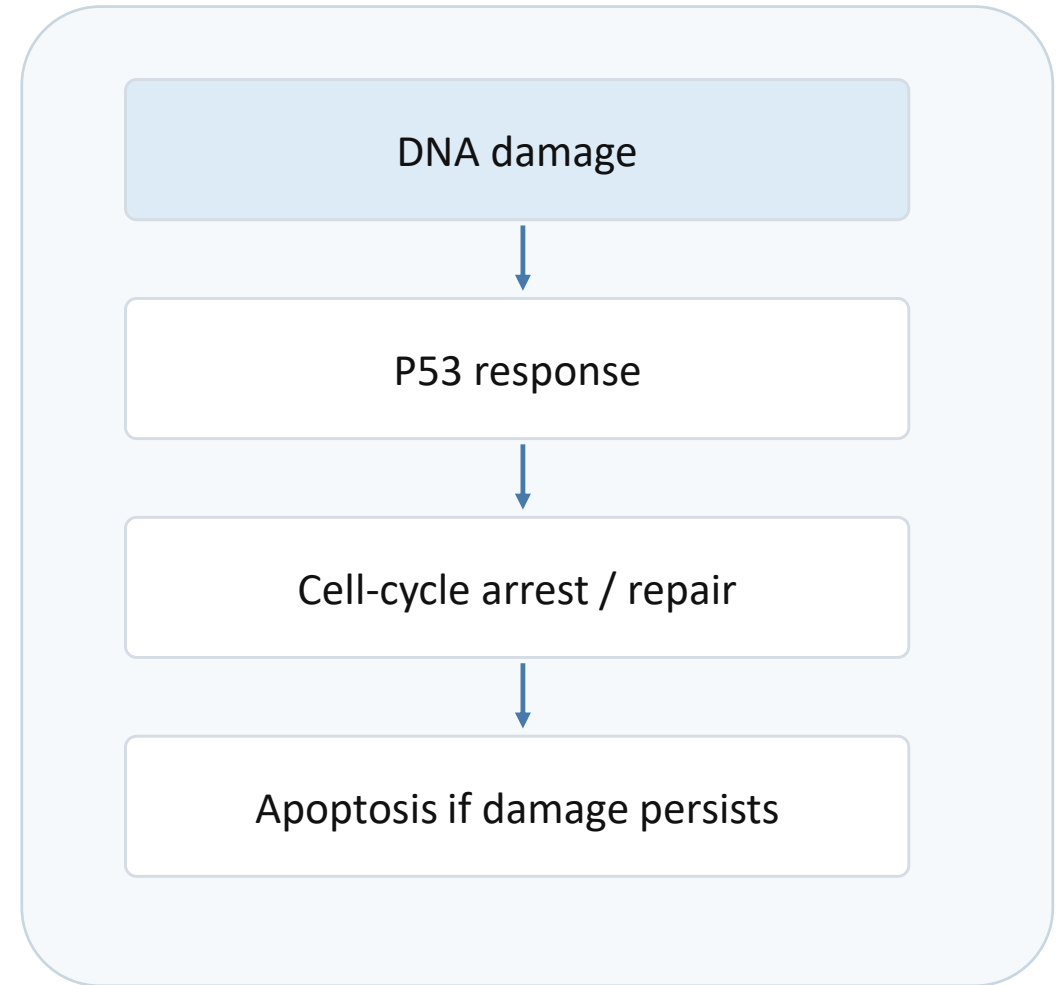
- The supplied text implicates oxidative DNA damage in cellular aging.
- Accumulated nucleic-acid injury provides one mechanistic connection between stress and age-associated change.
- This is an implicated contribution, not a claim that ROS explain all biological aging.

Oxidative DNA injury has oncogenic implications

- Oxidative DNA damage is implicated in malignant transformation.
- Persistent or improperly resolved genetic lesions are relevant to abnormal cell survival and proliferation.
- The relationship is mechanistic; It does not mean that every oxidatively injured cell becomes malignant.

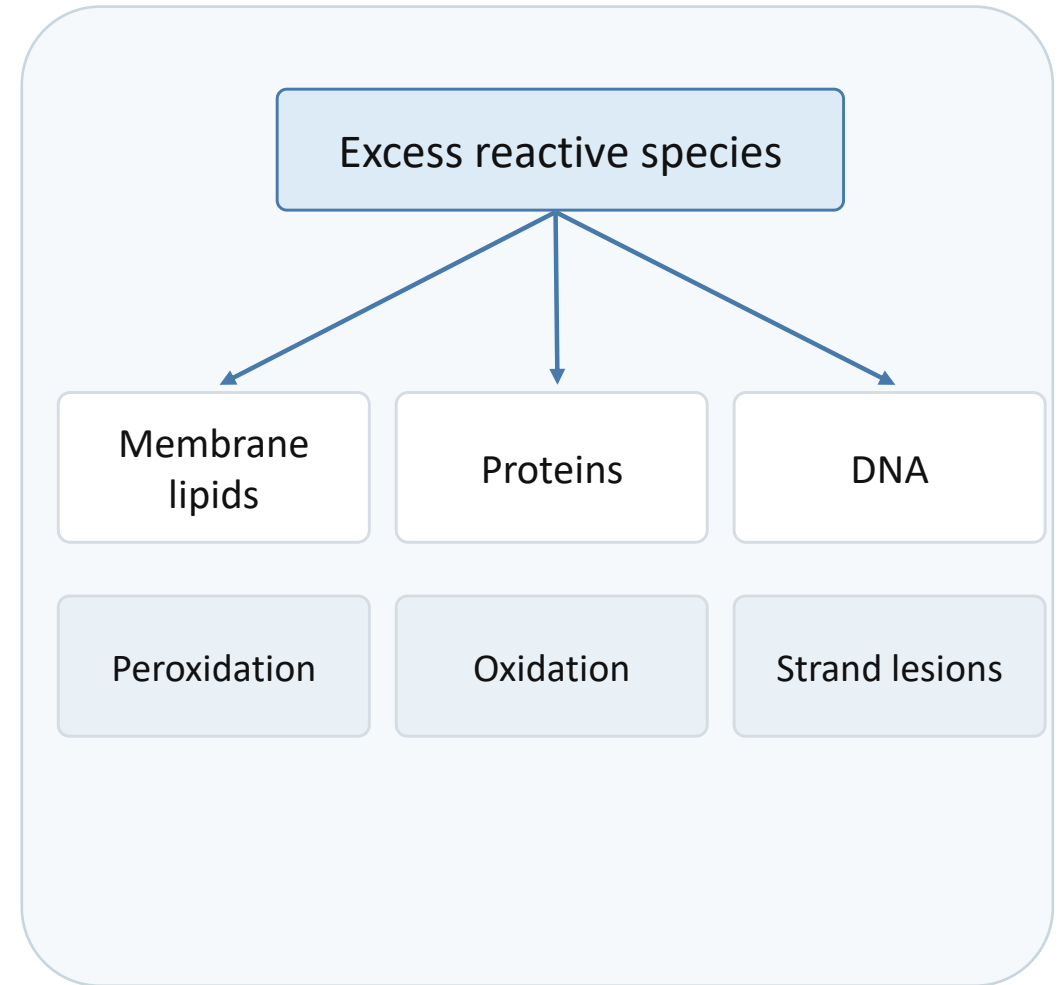
Apoptosis can prevent persistence of damaged DNA

- The parent discussion emphasizes cell-cycle control and DNA repair after injury.
- If damage cannot be corrected, apoptosis removes cells carrying potentially dangerous DNA changes.
- This protective response illustrates why DNA injury can result in cell death rather than mutation survival.



Lipid, protein and DNA injury differ in consequences

- Lipid injury primarily threatens membrane properties.
- Protein oxidation can impair catalysis, architecture and turnover.
- DNA lesions threaten genomic integrity and engage damage-response pathways.



The three macromolecular targets often coexist

- A given oxidant exposure can affect more than one class of cellular macromolecule.
- Membrane failure, protein dysfunction and DNA lesions may interact in severe injury.
- Understanding the dominant target explains how radical chemistry becomes cellular pathology.

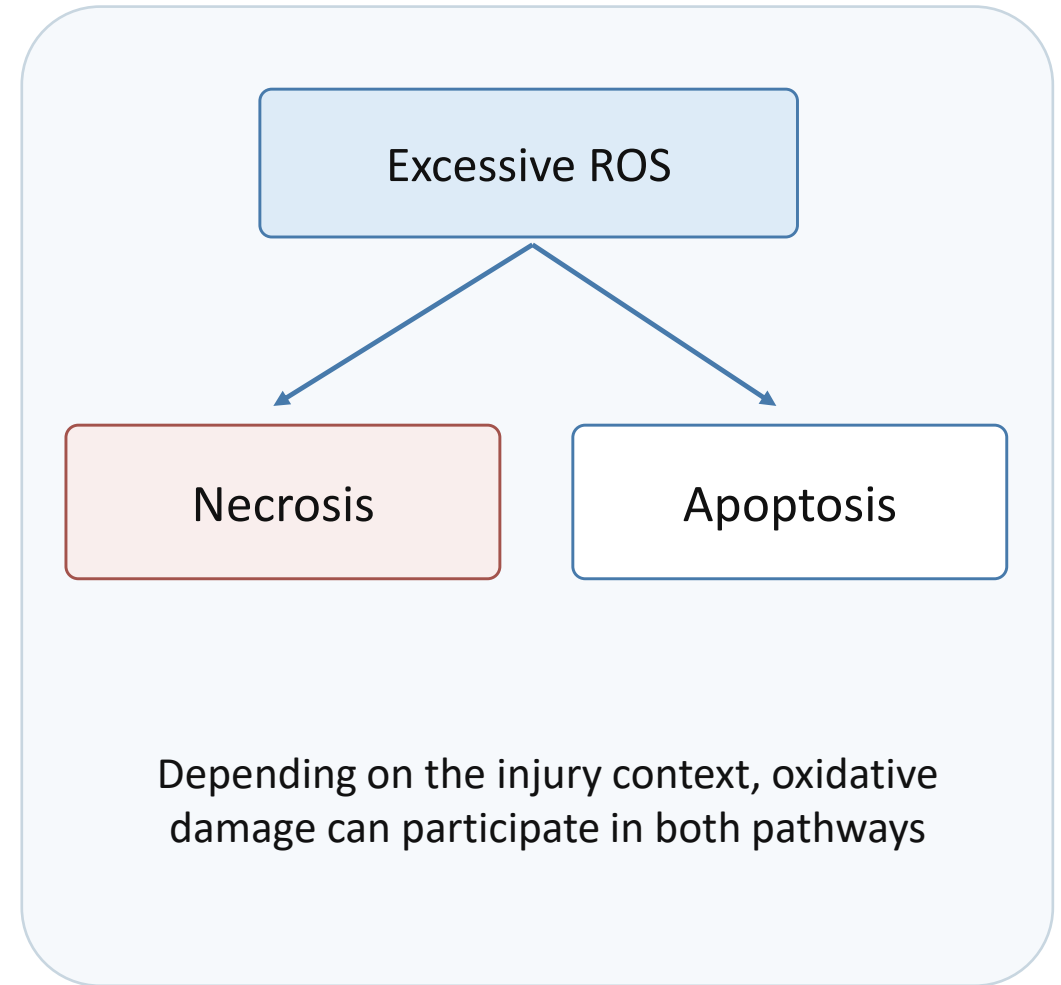
Lipids → membrane disruption

Proteins → altered cell function

DNA → genomic lesions

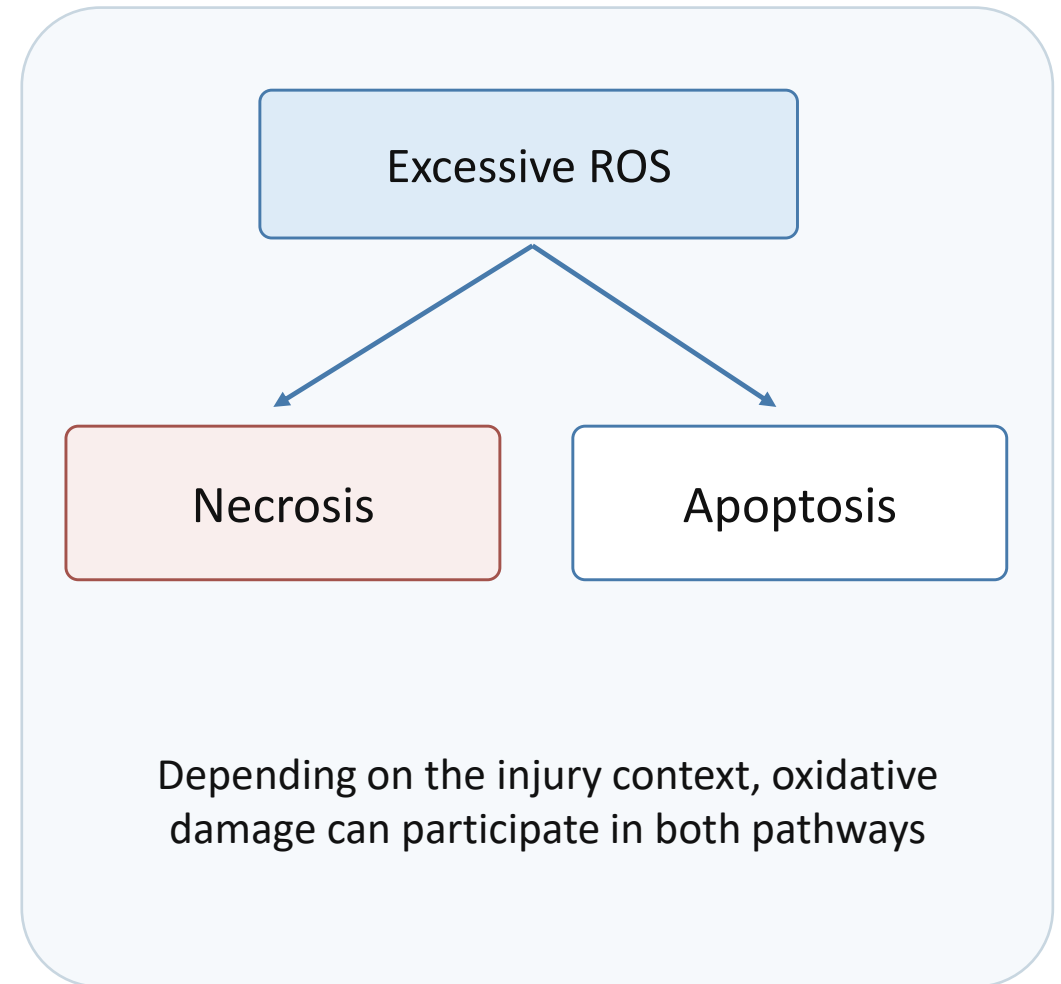
Oxidative injury can contribute to necrosis

- Historically, free radical injury was commonly linked to necrotic cell death.
- Extensive membrane injury is especially relevant to loss of cellular integrity.
- Oxidative injury frequently operates alongside ATP depletion and other necrosis-promoting processes.



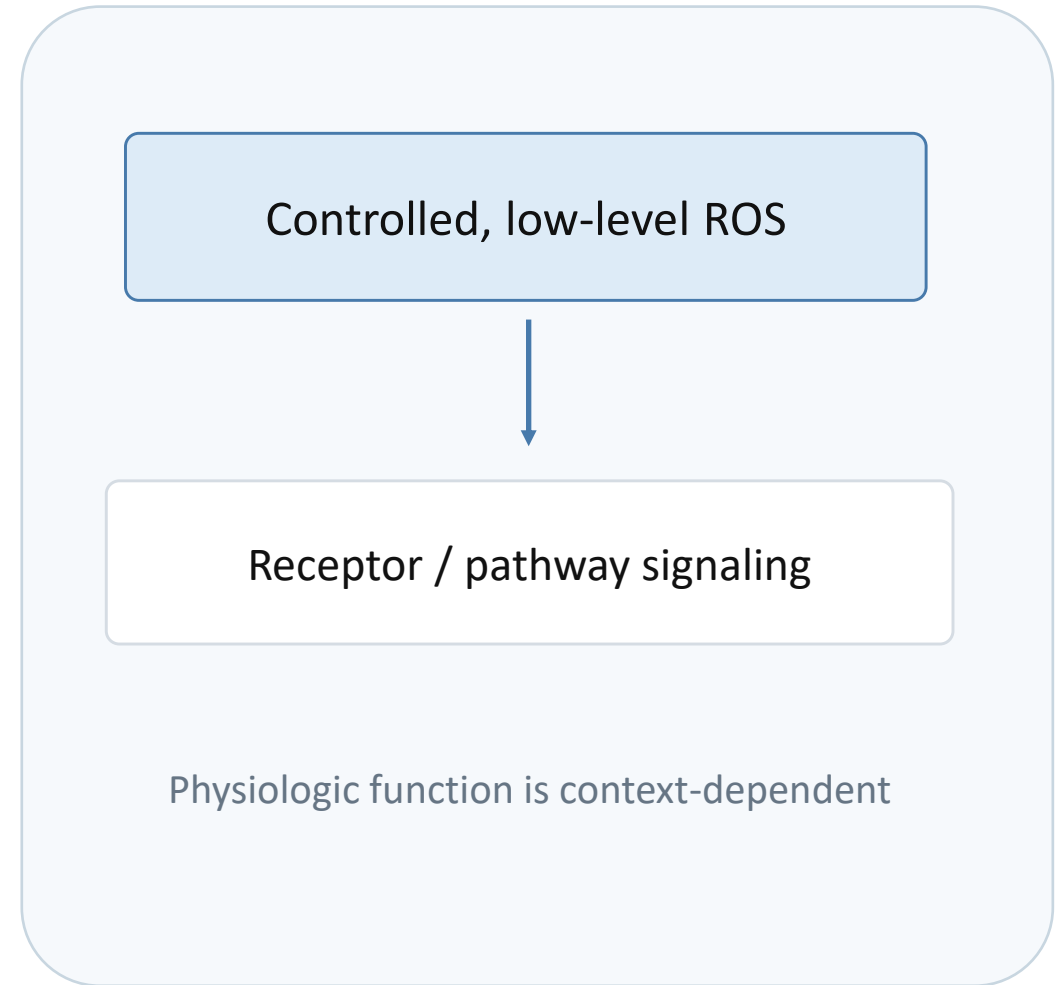
Free radicals can also trigger apoptosis

- Robbins explicitly identifies apoptosis as another possible consequence of radical injury.
- DNA damage and mitochondrial pathways offer routes from injurious stress to apoptotic responses.
- Thus necrosis is not the only fate of cells exposed to excessive reactive species.



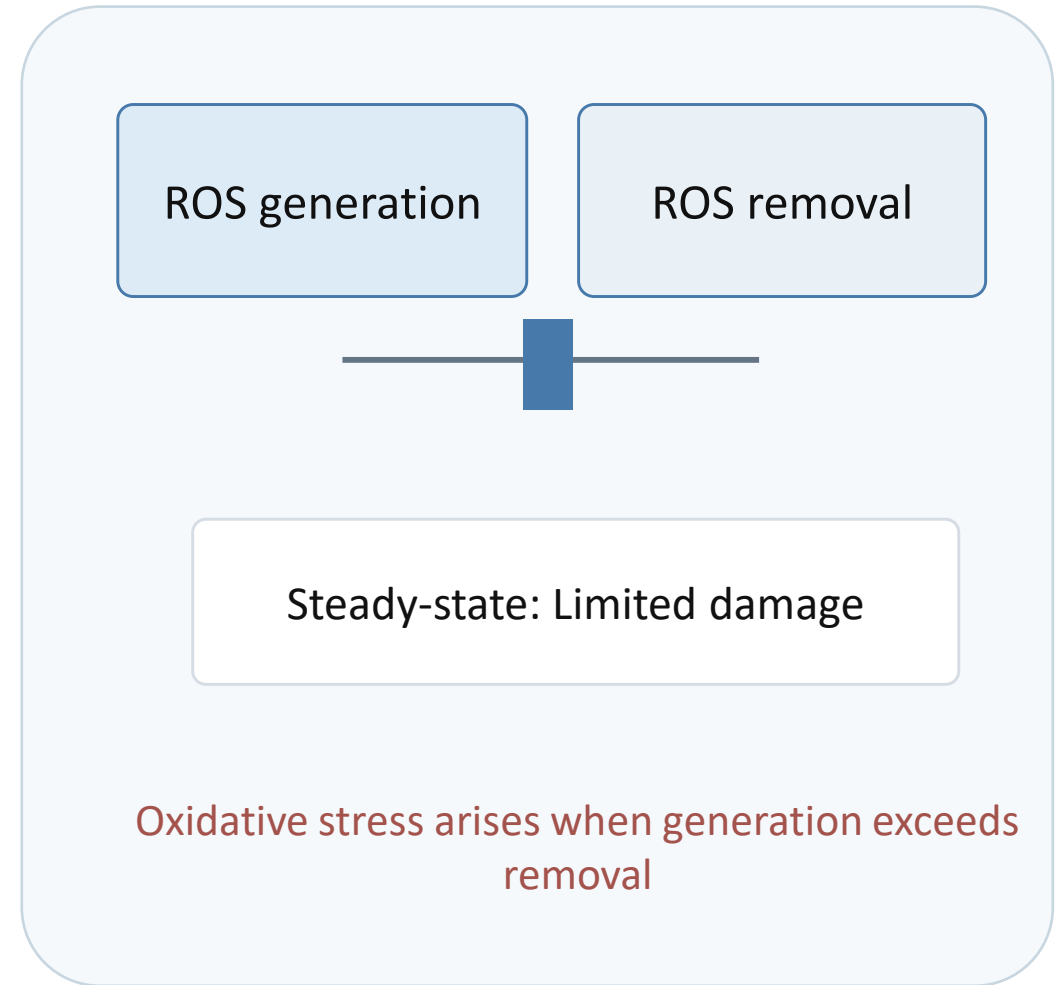
Reactive species are not uniformly pathological

- Free radicals produced under controlled conditions may serve physiological signaling roles.
- Their effects are influenced by dose, cellular context and local regulation.
- The distinction is regulated signaling versus damaging oxidative excess.



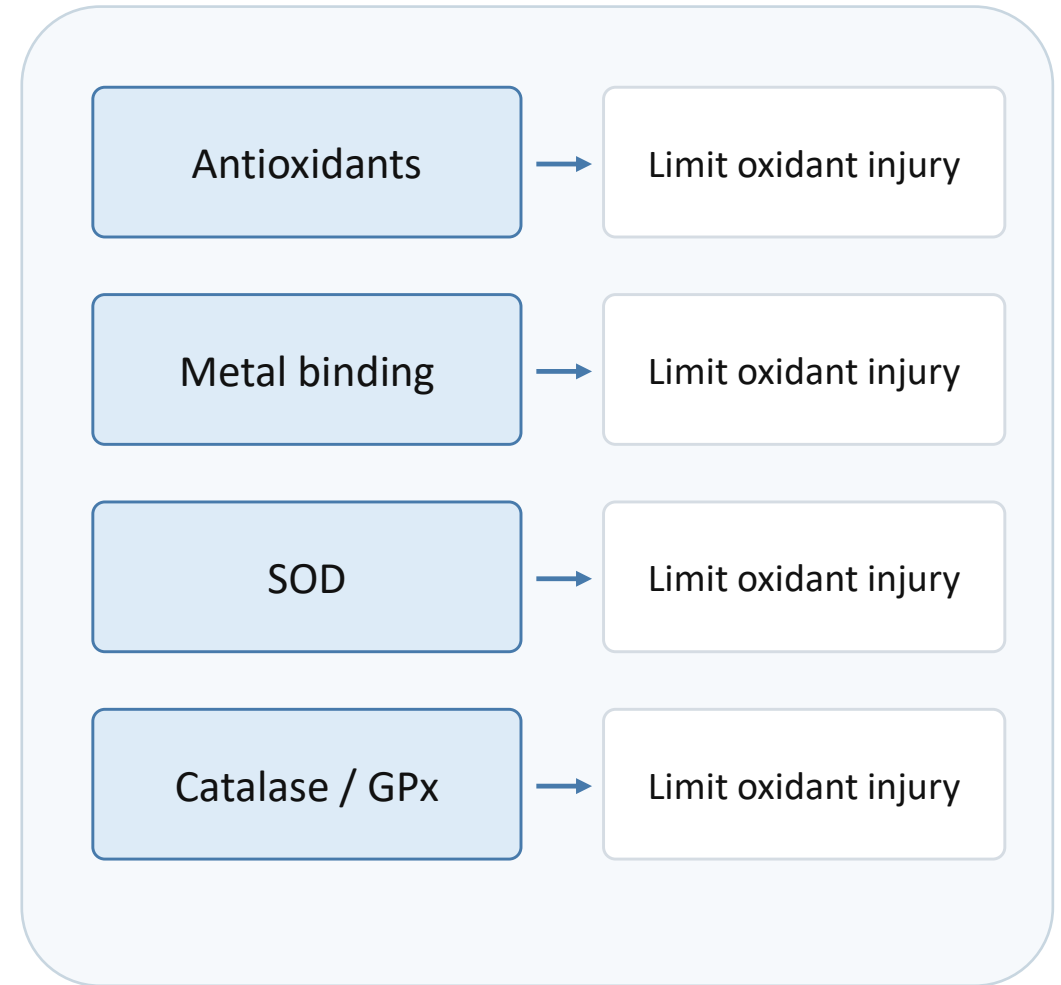
Cellular outcome reflects context rather than ROS alone

- Controlled generation can support signaling or microbial killing.
- Excessive generation or inadequate detoxification promotes macromolecular injury.
- The severity and reversibility of the associated cellular disturbance shape final outcomes.



Cells have overlapping antioxidant defenses

- Free radicals may decay spontaneously and can be scavenged by specific molecules.
- Cells also limit participation of redox-active metals in harmful reactions.
- Specialized enzymes detoxify superoxide and hydrogen peroxide near their sources.

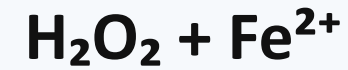


Small-molecule antioxidants limit radical injury

- The text lists vitamin E, vitamin A, ascorbic acid and glutathione among protective antioxidants.
- These agents can block radical formation or inactivate reactive species.
- Protection is one component of the broader enzymatic and metal-binding network.

Metal-binding proteins reduce catalytic availability

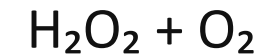
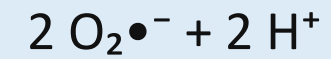
- Free iron and copper can participate in radical-forming reactions.
- Proteins including transferrin, ferritin, lactoferrin and ceruloplasmin sequester reactive metals.
- Metal sequestration limits the opportunity for radical amplification.



Transition-metal availability influences radical formation

Superoxide dismutase converts superoxide

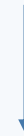
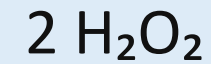
- Superoxide dismutase (SOD) catalyzes conversion of superoxide to hydrogen peroxide and oxygen.
- Manganese-SOD is localized in mitochondria; Copper-zinc-SOD occurs in the cytoplasm.
- Hydrogen peroxide still requires additional detoxification.



Catalase / GPx then remove hydrogen peroxide

Catalase detoxifies hydrogen peroxide

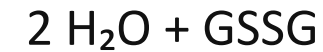
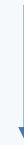
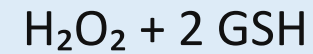
- Catalase is localized in peroxisomes.
- It decomposes hydrogen peroxide into water and oxygen.
- This limits hydrogen peroxide accumulation and its participation in further oxidant reactions.



Catalase acts in peroxisomes

Glutathione peroxidase consumes reducing capacity

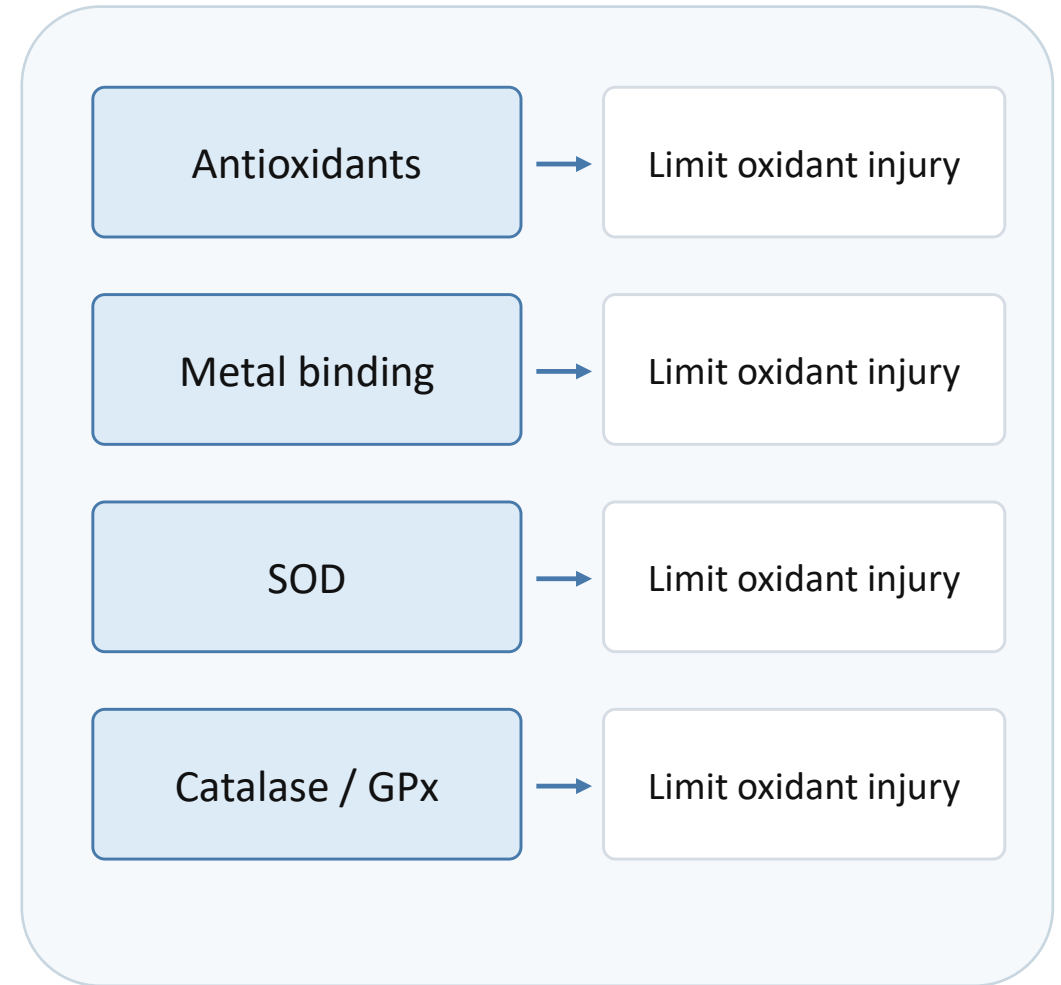
- Glutathione peroxidase detoxifies hydrogen peroxide using reduced glutathione (GSH).
- Oxidized glutathione (GSSG) is produced as hydrogen peroxide is converted into water.
- The GSSG-to-GSH relationship reflects the cellular oxidative state.



Reduced glutathione is consumed as oxidants are detoxified

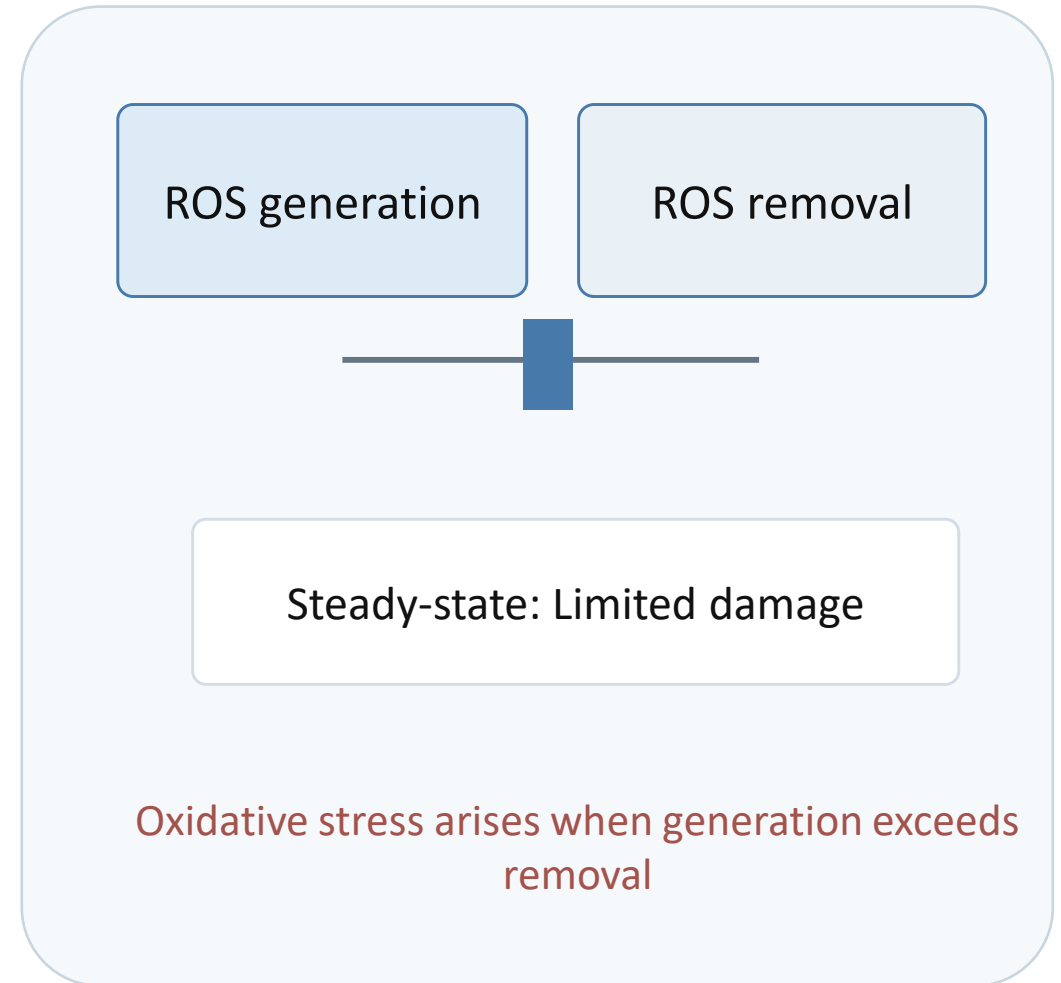
Antioxidant systems form a coordinated sequence

- SOD converts superoxide into hydrogen peroxide.
- Catalase and glutathione peroxidase help remove hydrogen peroxide.
- Metal sequestration and molecular antioxidants act through complementary mechanisms.



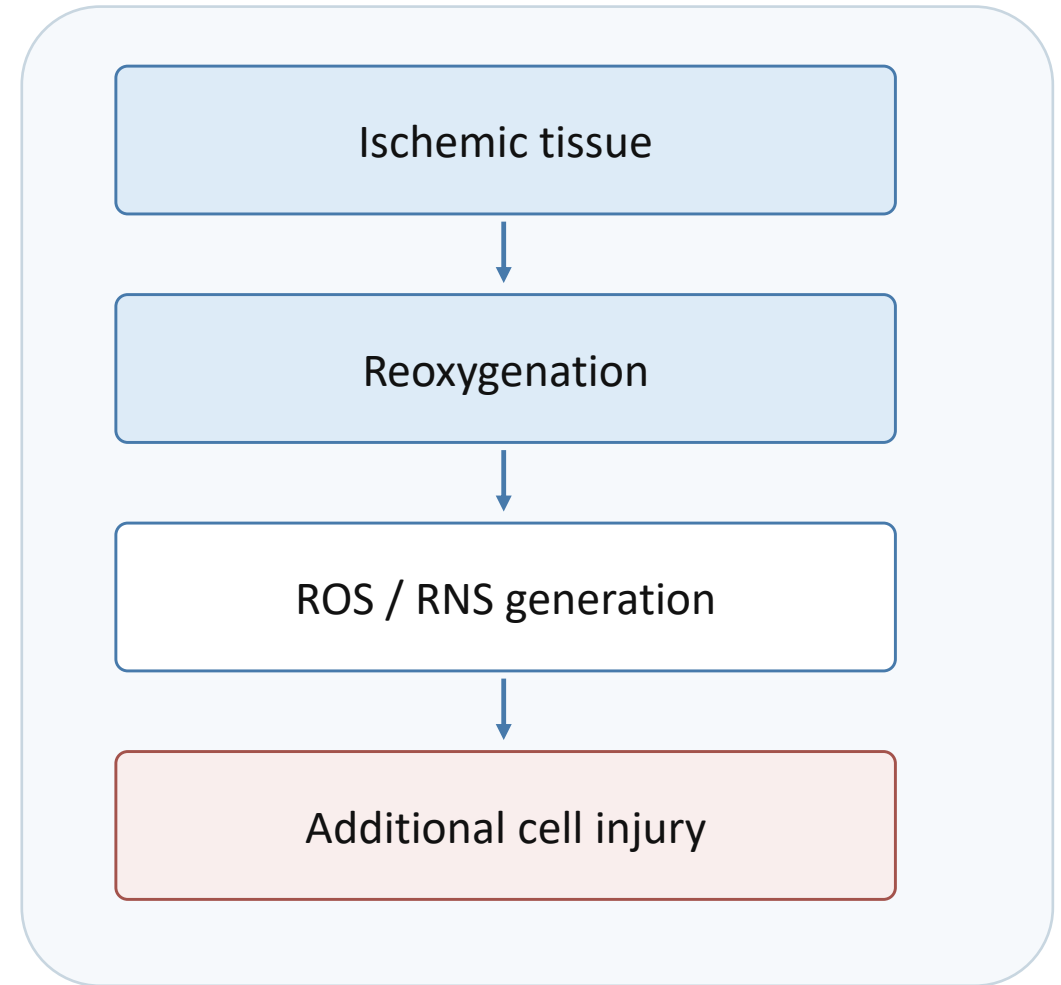
Oxidative stress occurs when protection is overwhelmed

- Increased ROS generation can exceed the capacity of scavenging systems.
- Alternatively, weakened or depleted defenses can increase susceptibility to injury.
- The relevant abnormality is the imbalance between the two processes.



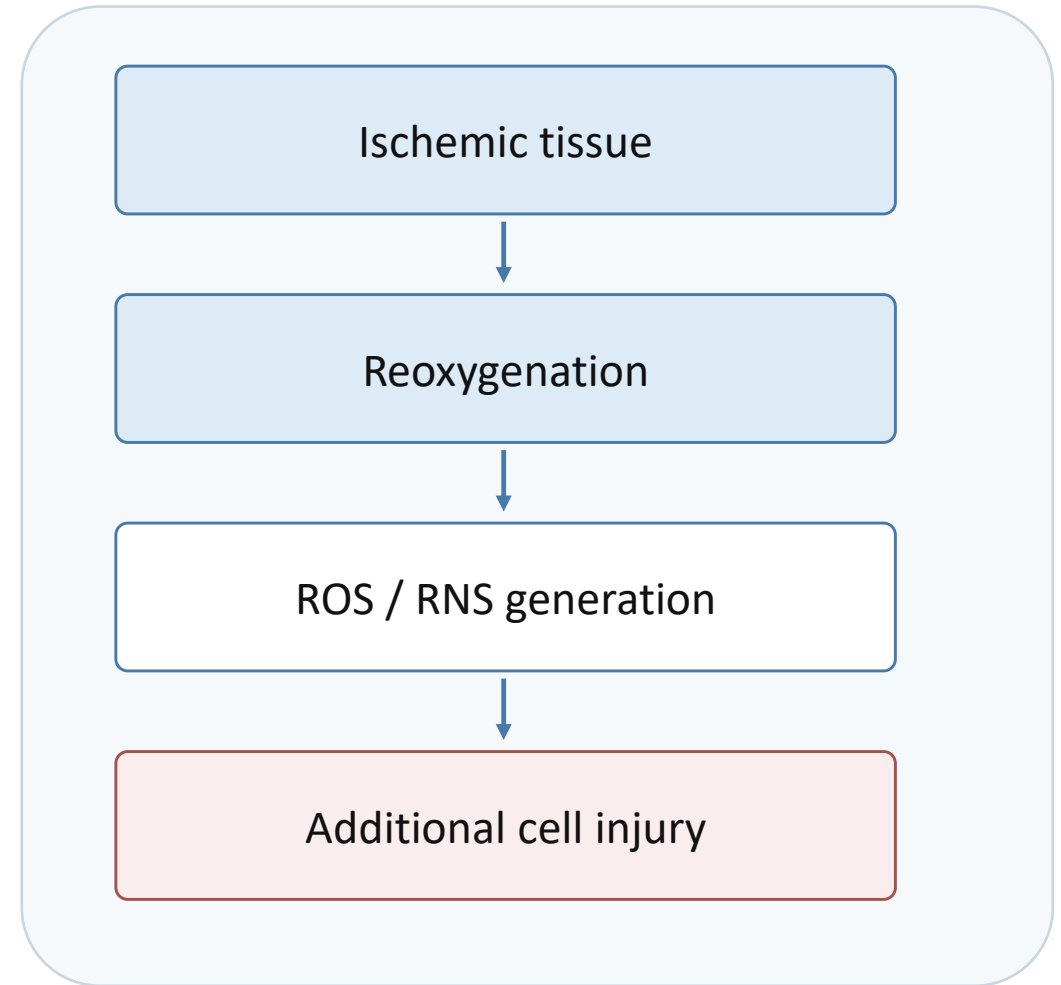
Reperfusion can both rescue and injure tissue

- Restoring blood flow can recover reversibly ischemic cells.
- Reoxygenation can also initiate new injurious processes in vulnerable tissue.
- Ischemia-reperfusion injury is especially relevant to myocardial and cerebral infarction.



ROS and RNS contribute to reperfusion injury

- Reoxygenated leukocytes, endothelial cells and parenchymal cells can generate reactive species.
- Antioxidant defenses may already be compromised by preceding ischemia.
- The resulting oxidative stress is one component of reperfusion-associated cell loss.

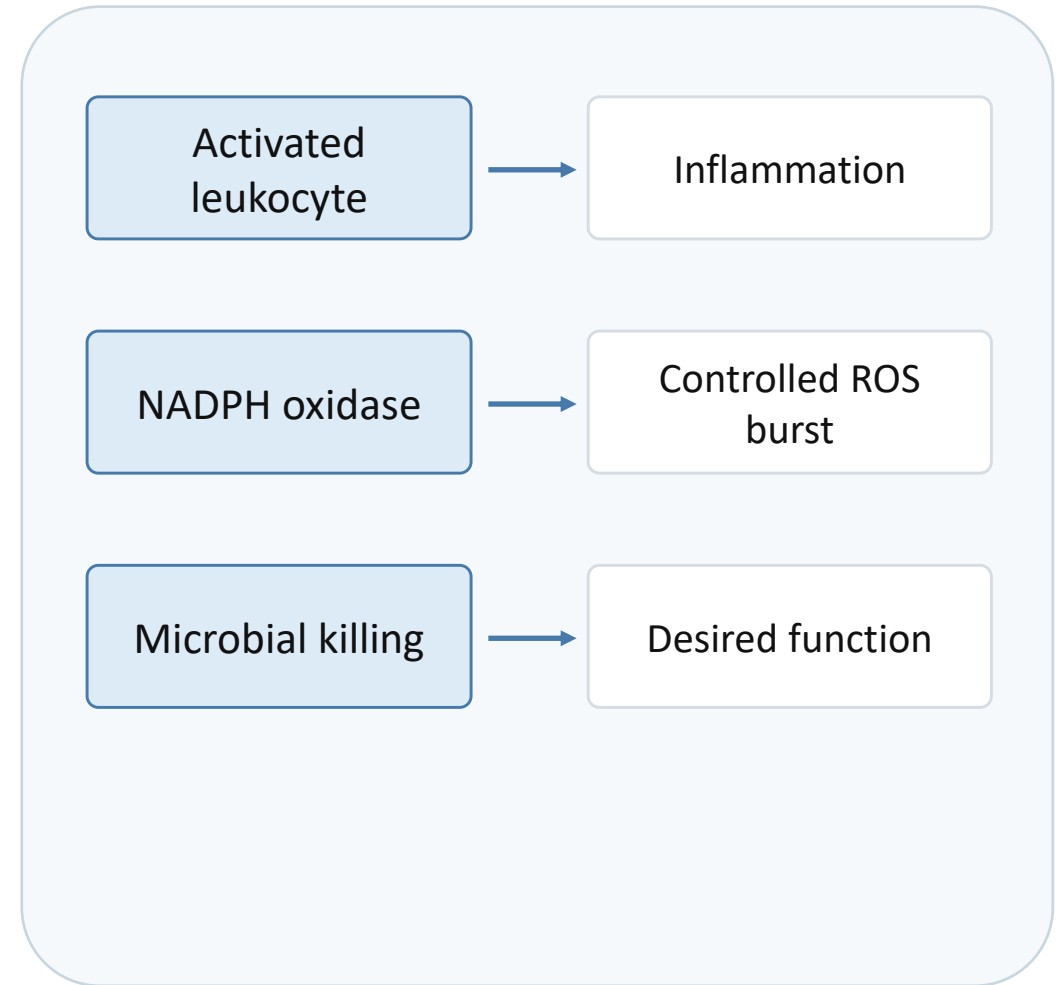


Reperfusion injury involves interacting pathways

- Oxidative stress may coexist with intracellular calcium overload.
- Calcium excess promotes mitochondrial dysfunction and further cellular injury.
- Inflammatory-cell recruitment and complement activation also contribute to tissue damage.

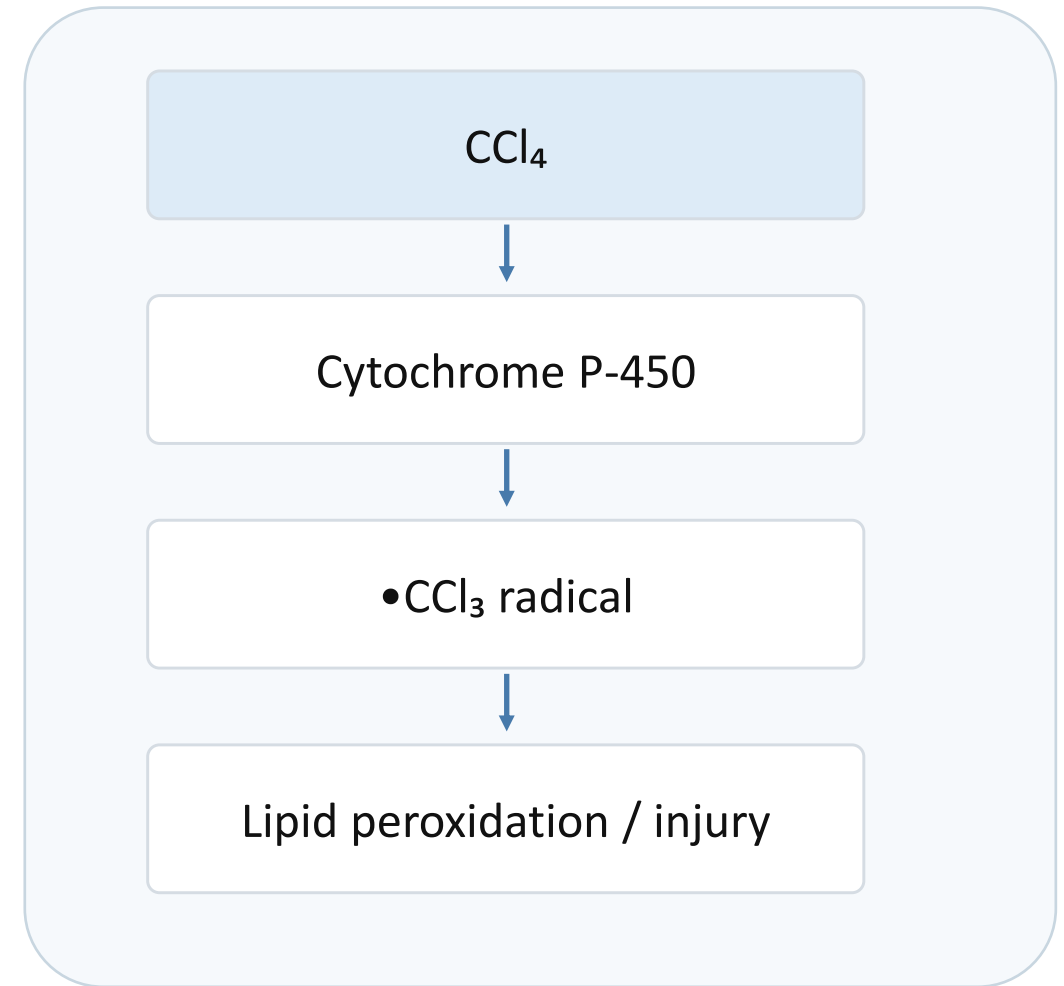
Leukocyte ROS have both benefit and risk

- Phagocytes deliberately generate ROS to kill microbes and clear damaged material.
- ROS also contribute to collateral tissue injury in some inflammatory contexts.
- The pathologic significance depends on localization, regulation and tissue susceptibility.



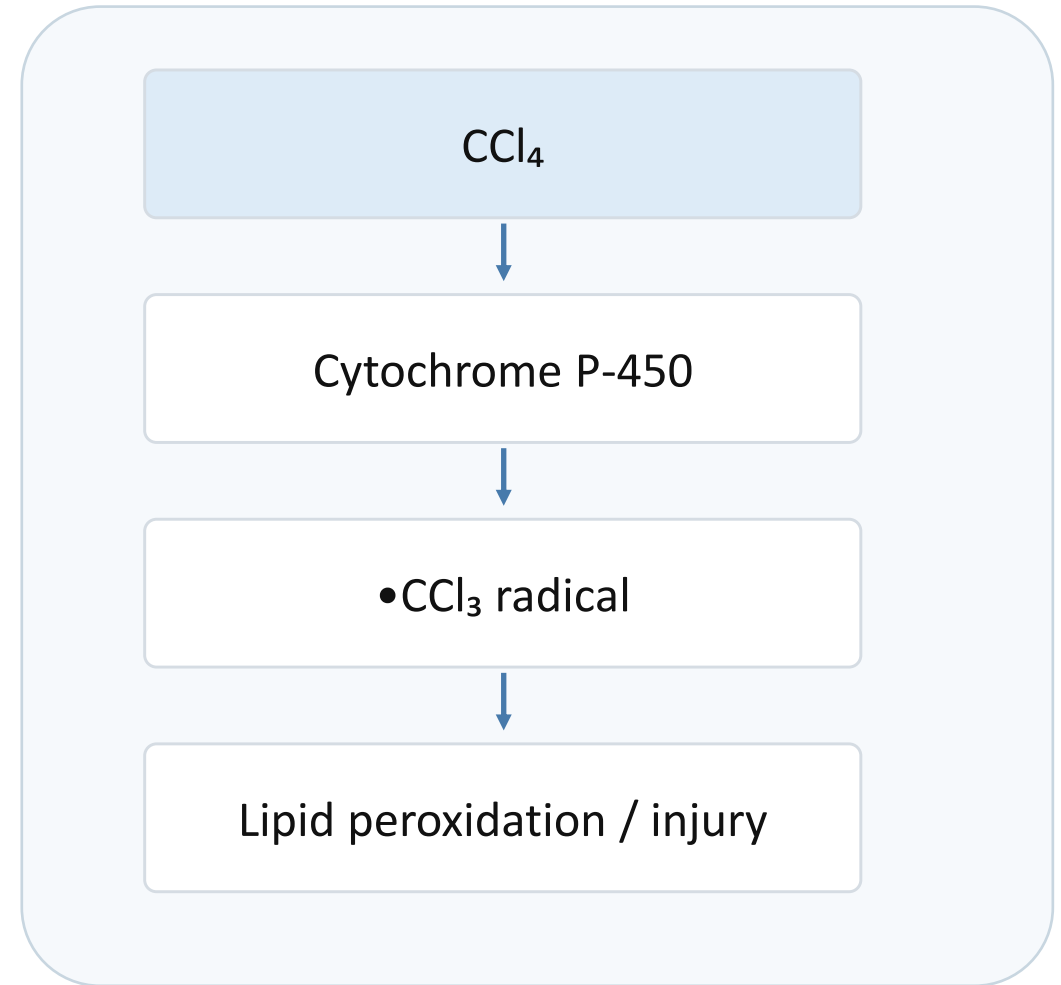
Carbon tetrachloride illustrates radical-mediated toxicity

- Cytochrome P-450 metabolizes carbon tetrachloride (CCl_4) to a reactive trichloromethyl radical ($\bullet\text{CCl}_3$).
- That radical initiates lipid peroxidation and damage to cellular structures.
- It demonstrates how chemical metabolism can generate radical-mediated cell injury.



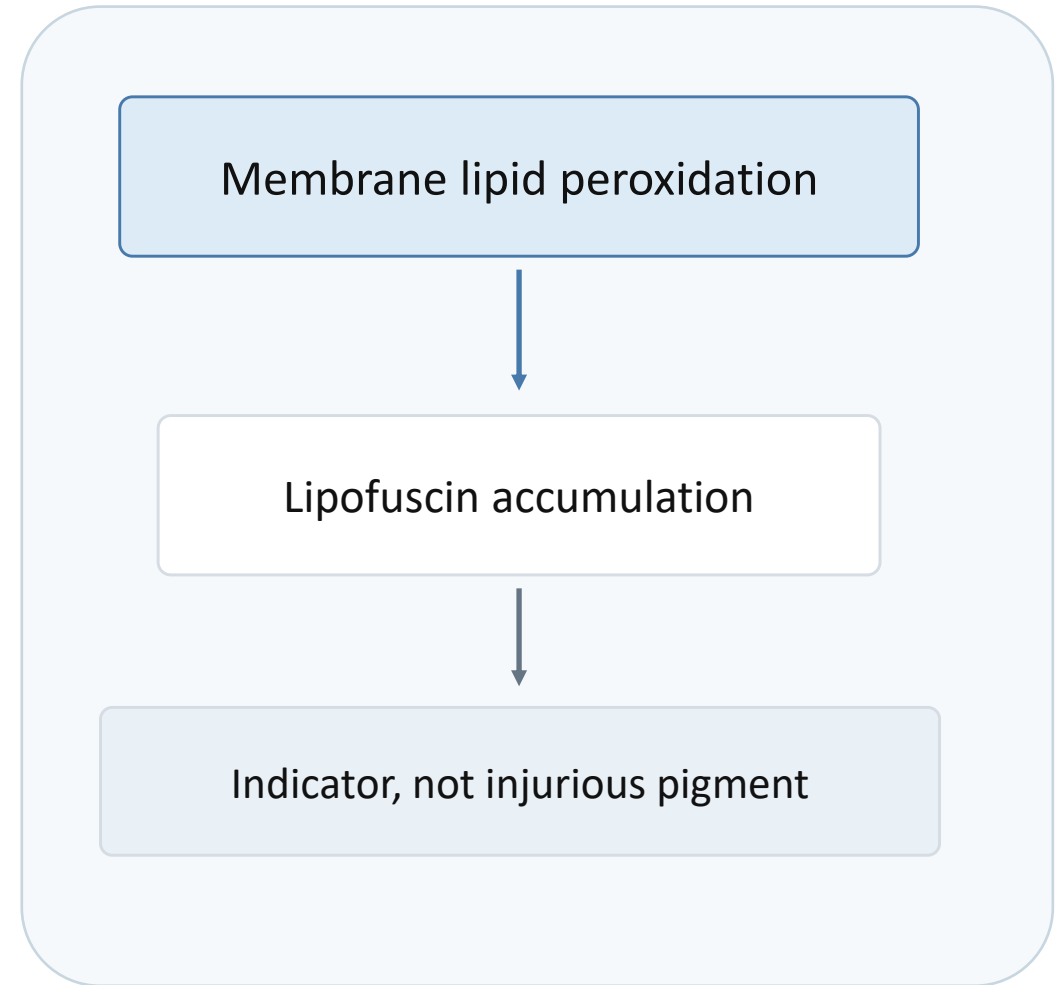
Reactive metabolites explain one form of chemical injury

- Many toxic compounds must first undergo enzymatic conversion to reactive metabolites.
- Radical formation and subsequent lipid peroxidation are major mechanisms for some chemicals.
- Other toxic mechanisms, including direct covalent binding, may coexist.



Lipofuscin is a morphologic clue to lipid oxidation

- Lipofuscin is a yellow-brown cytoplasmic pigment related to lipid peroxidation products.
- It is particularly evident in aging or chronically stressed tissues such as heart and liver.
- The pigment itself is not considered injurious; It indicates prior free radical injury.

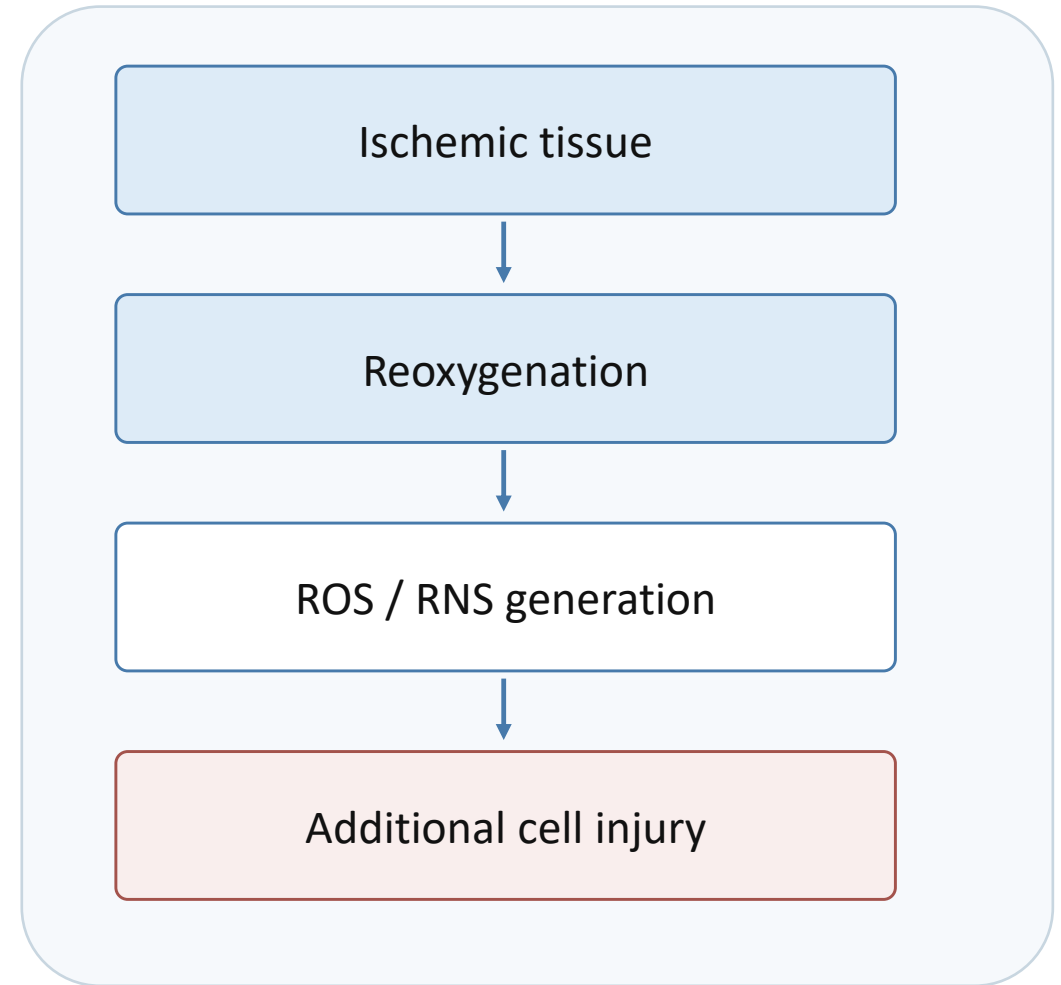


Do not infer ROS injury from morphology alone

- Lipid peroxidation, protein oxidation and DNA lesions are biochemical processes.
- A nonspecific pattern of cell injury does not identify a unique free radical mechanism.
- Interpret oxidative stress in the context of the cause, tissue response and competing injury pathways.

Clinical application: Tissue injury after reperfusion

- A patient undergoes restoration of coronary blood flow after prolonged ischemia.
- Some jeopardized cells recover; Additional injury may nevertheless occur after reperfusion.
- Which source of ROS and which antioxidant vulnerability help explain this paradox?

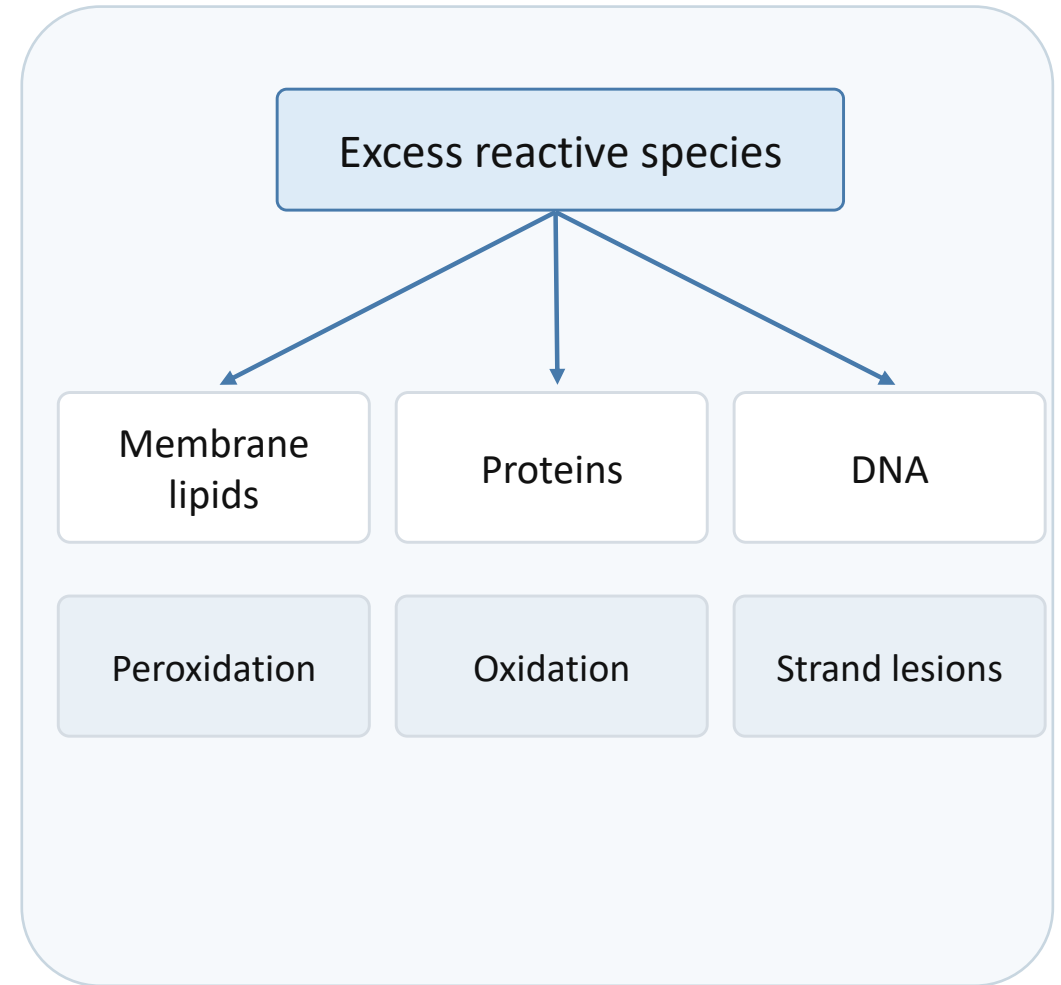


Reperfusion: Mechanistic interpretation

- Reactive oxygen and nitrogen species are generated during reoxygenation.
- Leukocytes and previously injured endothelial and parenchymal cells can contribute.
- Compromised antioxidant defenses amplify the impact; Calcium overload and inflammation add injury.

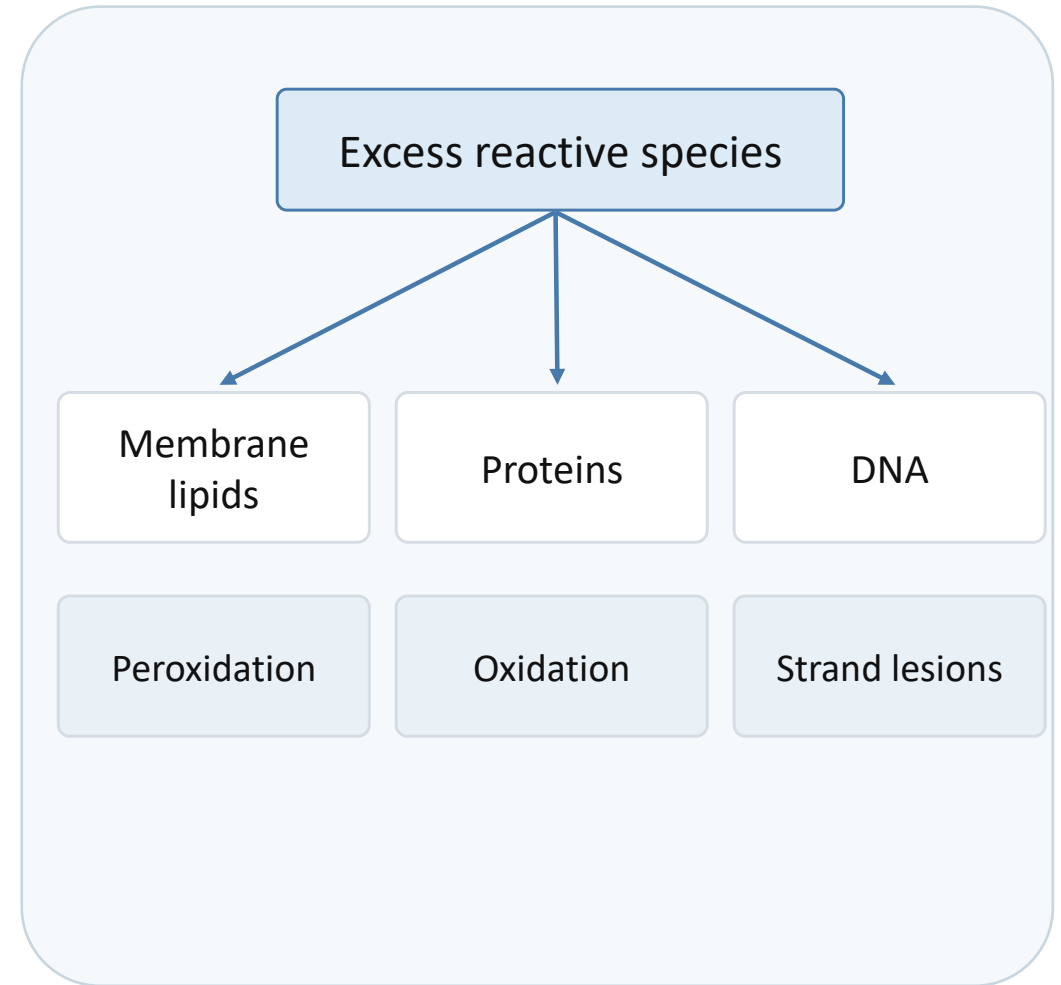
Mechanism exercise: Identify the primary target

- Loss of membrane function after an autocatalytic chain reaction points to lipid peroxidation.
- Inactivated enzymes and increased abnormal-protein turnover point to protein oxidation.
- DNA strand breaks, cross-links and adducts point to nucleic-acid damage.



Integrative model of free radical pathology

- A shift in oxidant production relative to detoxification establishes oxidative stress.
- Reactive species modify membrane lipids, proteins and DNA through distinct chemical reactions.
- Combined macromolecular damage can trigger dysfunction, necrosis or apoptosis; Controlled ROS also signal physiologically.



Primary reference

Robbins, Cotran & Kumar

Pathologic Basis of Disease · 11th edition (2026)

Chapter 2: Cell Injury, Cell Death, and Adaptations

Relevant sections: oxidative stress, generation and removal of free radicals, pathologic effects, membrane and DNA injury, and selected clinical correlations.