"Pentose Phosphate Pathway and Nicotinamide Adenine Dinucleotide Phosphate"

I. Overview

- The pentose phosphate pathway (also known as the hexose monophosphate shunt):
    - Provides ribose 5-phosphate for biosynthesis of nucleotides
    - Is the main source of nicotinamide adenine dinucleotide phosphate (NADPH) in the body

Functions of NADPH

- NADPH is the cellular source of reducing equivalents used for:
    - Biosynthesis of fatty acids
    - Biosynthesis of cholesterol
    - Reduction of hydrogen peroxide (H_2O_2):
        - Formed in response to oxidative stress
        - Also formed as a byproduct of aerobic metabolism

Key Enzyme and Clinical Relevance

- Glucose 6-phosphate dehydrogenase (G6PD):
    - Catalyzes the first and rate-limiting step of the pathway
- G6PD deficiency:
    - Inherited in an X-linked manner
    - Results in insufficient NADPH, especially in red blood cells
    - Leads to susceptibility to lysis during oxidant stress

Energy Involvement

- The pathway does not produce or consume ATP

Location and Phases of the Pathway

- Reactions occur in the cytosol

- The pathway includes:
    - An irreversible oxidative phase
    - Followed by reversible sugar-phosphate interconversions

Oxidative Phase Details

- In this phase:
    - Carbon 1 of a glucose 6-phosphate molecule is released as carbon dioxide (CO_2)
    - Produces:
        - One pentose sugar-phosphate
        - Two reduced NADPHs

Reversible Phase and Metabolic Flexibility

- The rate and direction of the reversible reactions are determined by:
    - The supply of and demand for the intermediates of the pathway

Additional Functions of the Pathway

- Produces ribose 5-phosphate:
    - Required for nucleotide biosynthesis
- Provides a mechanism for the conversion of pentose sugars to:
    - Triose intermediates of glycolysis
    - Hexose intermediates of glycolysis

II. Irreversible Oxidative Reactions

Overview of Oxidative Portion

- The oxidative portion of the pentose phosphate pathway includes three irreversible reactions
- For each molecule of glucose 6-phosphate oxidized, the products are:
    - Ribulose 5-phosphate
    - Carbon dioxide (CO_2)
    - Two molecules of NADPH

Tissues Where the Oxidative Portion is Important

- Liver, lactating mammary glands, and adipose tissue:
    - Site of NADPH-dependent biosynthesis of fatty acids
- Testes, ovaries, placenta, and adrenal cortex:
    - Site of NADPH-dependent biosynthesis of steroid hormones
- Red blood cells:
    - Site of NADPH-dependent reduction of glutathione

A. Glucose 6-Phosphate Dehydrogenation

- Enzyme: Glucose 6-phosphate dehydrogenase (G6PD)
- Reaction catalyzed:
    - Glucose 6-phosphate → 6-phosphogluconolactone
    - $NADP^+$ is reduced to NADPH

- This reaction is:
    - Initial
    - Committed
    - Rate-limiting
    - Regulated step of the pathway

Regulation of G6PD

- NADPH is a potent competitive inhibitor of G6PD
- Under most metabolic conditions:
    - The NADPH/$NADP^+$ ratio is high
    - This substantially inhibits G6PD
- With increased NADPH demand:
    - NADPH/$NADP^+$ ratio decreases
    - Flux through the pathway increases due to enhanced G6PD activity

Hormonal Regulation

- Insulin:
    - Upregulates gene expression of G6PD
    - Therefore, flux through the pathway increases in the absorptive state

B. Ribulose 5-Phosphate Formation

Second Step

- Enzyme: 6-Phosphogluconolactone hydrolase
- Reaction:
    - 6-Phosphogluconolactone is hydrolyzed

Third Step: Oxidative Decarboxylation

- Enzyme: 6-Phosphogluconate dehydrogenase
- Reaction:
    - Oxidative decarboxylation of 6-phosphogluconate

- Products:
    - Ribulose 5-phosphate (a pentose sugar-phosphate)
    - Carbon dioxide (CO_2) — derived from carbon 1 of glucose
    - Second molecule of NADPH

III. Reversible Nonoxidative Reactions

General Characteristics

- Nonoxidative reactions of the pentose phosphate pathway:
    - Occur in all cell types synthesizing nucleotides and nucleic acids
    - Catalyze interconversion of sugars containing three to seven carbons

Functional Importance

- These reversible reactions allow ribulose 5-phosphate (produced by the oxidative portion) to be:
    - Converted to ribose 5-phosphate, needed for nucleotide synthesis

- Or converted to glycolytic intermediates:
  - Fructose 6-phosphate
  - Glyceraldehyde 3-phosphate

Metabolic Need for NADPH vs. Ribose 5-Phosphate

- In many cells performing reductive biosynthetic reactions:
  - There is greater need for NADPH than for ribose 5-phosphate
  - In this case:
    - Transketolase:
      - Transfers two-carbon units
      - Requires thiamine pyrophosphate (TPP)
    - Transaldolase:
      - Transfers three-carbon units
    - These enzymes convert ribulose 5-phosphate (from oxidative phase) to:
      - Glyceraldehyde 3-phosphate
      - Fructose 6-phosphate

Opposite Scenario: Greater Need for Ribose

- When demand for ribose (for nucleotides and nucleic acids) is greater than need for NADPH:
    - Nonoxidative reactions can operate in reverse
    - Provide ribose 5-phosphate from:
        - Glyceraldehyde 3-phosphate
        - Fructose 6-phosphate
    - This occurs without the oxidative steps

Additional Role of Thiamine Pyrophosphate (TPP)

- In addition to transketolase, TPP is also required by the following multienzyme complexes:
    - Pyruvate dehydrogenase
    - α-Ketoglutarate dehydrogenase of the tricarboxylic acid cycle
    - Branched-chain α-keto acid dehydrogenase of branched-chain amino acid catabolism

IV. Uses Of NADPH

Structural Difference Between NADPH and NADH

- NADPH differs from NADH by:
    - The presence of a phosphate group on one of the ribose units
- This small structural change allows NADPH to:
    - Interact with NADPH-specific enzymes
    - Perform unique cellular functions

$NADP^+$/NADPH Ratio and Functional Implication

- In the cytosol of hepatocytes:
    - The $NADP^+$ / NADPH ratio is approximately 0.1
    - This favors the use of NADPH in reductive biosynthetic reactions
- In contrast:
    - The NAD^+ / NADH ratio is about 1,000
    - This favors an oxidative role for NAD^+

Summary of NADPH Roles

- NADPH plays key roles in:
    - Reductive biosynthesis
    - Detoxification reactions

1st Use: Reductive Biosynthesis

- Like NADH, NADPH is a high-energy molecule
- However:
    - NADPH's electrons are used for reductive biosynthesis
    - Not for transfer to the electron transport chain (as with NADH)
- In the pentose phosphate pathway:
    - Part of the energy from glucose 6-phosphate is conserved in NADPH
    - NADPH has a negative reduction potential

- NADPH is used in biosynthetic reactions requiring an electron donor, such as:
    - Fatty acid synthesis
    - Cholesterol synthesis
    - Steroid hormone synthesis

2nd Use: Reduction of H_2O_2

Nature and Source of H_2O_2

- H_2O_2 is part of the reactive oxygen species (ROS) family
- Formed from partial reduction of molecular oxygen (O_2)
- ROS are continuously generated as:
    - Byproducts of aerobic metabolism
    - Through reactions with drugs and environmental toxins
    - When antioxidant levels are diminished
    - These conditions result in oxidative stress

Cellular Damage by ROS

- ROS are highly reactive oxygen intermediates
- Can cause serious chemical damage to:
    - DNA
    - Proteins
    - Unsaturated lipids
- May lead to cell death

ROS in Disease and Immunity

- ROS are implicated in several pathologic processes, including:
    - Reperfusion injury
    - Cancer
    - Inflammatory diseases
    - Aging
- Cells possess protective mechanisms to minimize toxicity of ROS

- ROS are also generated in:
    - Killing of microbes by white blood cells

Defense Against Reactive Oxygen Species (ROS)

1. Enzymes That Catalyze Antioxidant Reactions

- Reduced glutathione (G-SH):
    - A tripeptide-thiol: γ-glutamylcysteinylglycine
    - Present in most cells
    - Can chemically detoxify H_2O_2

- This reaction is:
    - Catalyzed by glutathione peroxidase
    - Converts G-SH to oxidized glutathione (G-S-S-G)
        - G-S-S-G no longer has protective properties

- The cell regenerates G-SH:
    - Reaction catalyzed by glutathione reductase
    - Uses NADPH as the source of reducing equivalents
    - Thus, NADPH indirectly provides electrons for the reduction of H_2O_2

- Additional antioxidant enzymes:
    - Superoxide dismutase
    - Catalase
    - These catalyze the conversion of other ROS to harmless products
- Collectively, these enzymes:
    - Serve as a defense system
    - Protect against the toxic effects of ROS

2. Antioxidant Chemicals

- Several intracellular reducing agents can detoxify ROS in the lab, including:
    - Ascorbate (vitamin C)
    - Vitamin E
    - β-carotene
- Consumption of foods rich in these antioxidants:
    - Correlated with reduced risk for certain cancers
    - Linked to decreased frequency of some chronic health problems

- It is tempting to speculate:
    - That these benefits are partly due to their ability to quench ROS toxicity
- However, clinical trials using antioxidants as dietary supplements have:
    - Failed to show clear beneficial effects
- In the case of β-carotene supplementation:
    - Increased rate of lung cancer in smokers was observed
- Conclusion:
    - The health-promoting effects of fruits and vegetables likely reflect a complex interaction among many naturally occurring compounds
    - These effects have not been duplicated by isolated antioxidant compounds

3rd Use: Cytochrome P450 Monooxygenase System

A. General Features

- Monooxygenases (mixed-function oxidases):
    - Incorporate one atom from O_2 into a substrate (creating a hydroxyl group)
    - The other atom is reduced to water (H_2O)
- In the cytochrome P450 (CYP) monooxygenase system:
    - NADPH provides the reducing equivalents required for the reactions
- The system functions in two separate cellular locations
- Overall reaction catalyzed by a CYP enzyme:
    - $R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$
    - Where R may be a steroid, drug, or other chemical

- CYP enzymes:
    - A superfamily of related heme-containing monooxygenases
    - Participate in a broad variety of reactions
    - The term "P450" reflects the absorbance at 450 nm by the protein

B. Mitochondrial System

- Location: Associated with the inner mitochondrial membrane
- Function: Biosynthesis of steroid hormones
- Tissues involved:
    - Placenta
    - Ovaries
    - Testes
    - Adrenal cortex

- In steroidogenic tissues:
    - Hydroxylates intermediates in the conversion of cholesterol to steroid hormones
    - Makes hydrophobic compounds more water soluble
- In the liver:
    - Used in bile acid synthesis
    - Used in hydroxylation of cholecalciferol to 25-hydroxycholecalciferol (vitamin D_3)
- In the kidney:
    - Hydroxylates vitamin D_3 to its biologically active 1,25-dihydroxylated form

C. Microsomal System

- Location: Associated with the membrane of the smooth endoplasmic reticulum, particularly in the liver
- Function: Detoxification of foreign compounds (xenobiotics)

- Examples of xenobiotics:
    - Drugs
    - Petroleum products
    - Pesticides
- CYP enzymes involved:
    - e.g., CYP3A4
    - Used to hydroxylate toxins (phase I reactions)
- Purpose of these modifications:
    a. May activate or inactivate a drug
    b. Make a toxic compound more soluble, facilitating excretion in urine or feces
- Often, the new hydroxyl group serves as:
    - A site for conjugation with a polar molecule (e.g., glucuronic acid)
    - Conjugation increases compound solubility (phase II)

- Genetic polymorphisms:
    - Variations in CYP enzyme genes can lead to differences in drug metabolism

4th Use: White Blood Cell Phagocytosis And Microbial Killing

A. General Overview

- Phagocytosis:
    - Ingestion by receptor-mediated endocytosis of:
        - Microorganisms
        - Foreign particles
        - Cellular debris
    - Carried out by leukocytes such as:
        - Neutrophils
        - Macrophages (monocytes)

- Biological significance:
    - Important defense mechanism, particularly in bacterial infections

- Mechanisms of bacterial killing:
    - Oxygen-independent
    - Oxygen-dependent

B. Oxygen-Independent Mechanisms

- Use:
    - pH changes in phagolysosomes
    - Lysosomal enzymes
- Function:
    - To destroy pathogens without reactive oxygen species

C. Oxygen-Dependent Mechanisms

- Include enzymes:
    - NADPH oxidase
    - Myeloperoxidase (MPO)
- MPO system:
    - Most potent bactericidal mechanism

D. Mechanism of Action

1. Recognition & Binding:

- Invading bacterium is:
  - Recognized by the immune system
  - Attacked by antibodies
  - Bound to receptors on phagocytic cells

2. Internalization:

- Microorganism is internalized

3. NADPH Oxidase Activation:

- Located in leukocyte cell membrane
- Activated post-internalization
- Reduces O_2 to superoxide (O_2^-) (a free radical ROS)
- Uses NADPH as the electron donor
- Process known as the respiratory burst

- NADPH oxidase complex:
  - Membrane-associated
  - Contains flavocytochrome
  - Includes additional peptides that translocate from the cytoplasm upon activation

- Electron transfer:
  - From NADPH → FAD → Heme → O_2 → generates O_2^-

- Genetic deficiency:
  - Rare NADPH oxidase deficiency causes:
    - Chronic granulomatous disease (CGD)
    - Features:
      - Severe, persistent infections
      - Formation of granulomas (nodular inflammation) to sequester undestroyed bacteria

4. Conversion to Hydrogen Peroxide (H_2O_2):

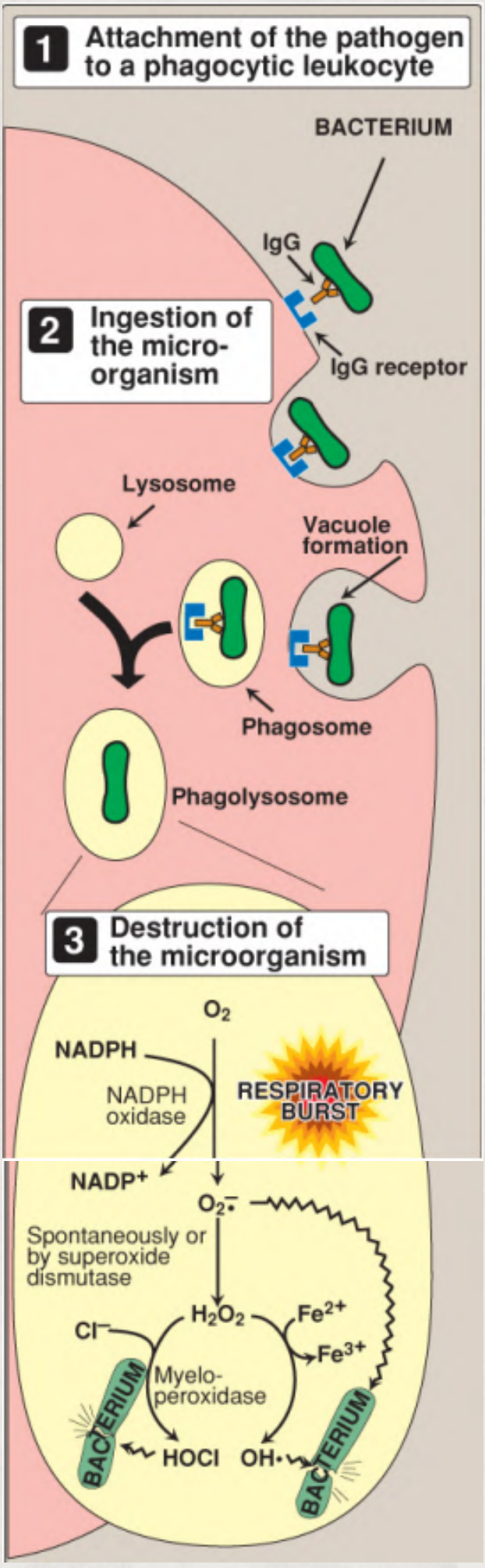
- O_2^- converted to H_2O_2:
  - Either spontaneously
  - Or via superoxide dismutase

5. Myeloperoxidase (MPO) Reaction:

- MPO:
  - A heme-containing lysosomal enzyme
  - Present in the phagolysosome
- Reacts $H_2O_2 + Cl^- \rightarrow$ Hypochlorous acid (HOCl)
  - HOCl = major component of household bleach
  - Strongly bactericidal

6. Other Fates of H_2O_2:

- May be:
  - Partially reduced to hydroxyl radical ($OH^\bullet$) (an ROS)
  - Fully reduced to H_2O by:
    - Catalase
    - Glutathione peroxidase
- MPO deficiency:
  - Does not confer increased susceptibility to infection
  - Because:
    - H_2O_2 from NADPH oxidase is sufficiently bactericidal



5th Use: Nitric Oxide Synthesis

A. General Overview

- Nitric oxide (NO):
    - Recognized as a mediator in many biologic systems
    - Known as the endothelium-derived relaxing factor
    - Causes vasodilation by relaxing vascular smooth muscle
    - Acts as a neurotransmitter
    - Prevents platelet aggregation
    - Plays a role in macrophage bactericidal activity
- Stability:
    - Has a very short half-life in tissues (3 to 10 seconds)
    - Reacts with O_2 to form:
        - Nitrates
        - Nitrites
        - Including peroxynitrite ($O{=}NOO^-$) → a reactive nitrogen species (RNS)

- Chemical nature:
    - NO is a free radical gas
    - Often confused with nitrous oxide (N_2O):
        - "Laughing gas"
        - Used as anesthetic
        - Chemically stable

B. Nitric Oxide Synthase (NOS)

- Substrates:
    - Arginine
    - O_2
    - NADPH
- Products:
    - NO
    - Citrulline
- Cofactors/coenzymes:
    - Flavin mononucleotide (FMN)
    - Flavin adenine dinucleotide (FAD)
    - Heme
    - Tetrahydrobiopterin

- Enzyme: NO synthase (NOS):
    - Located in cytosol
    - 3 isozymes:
        i. eNOS (endothelial) – Ca^{2+}–calmodulin dependent, constitutive
        ii. nNOS (neuronal) – Ca^{2+}–calmodulin dependent, constitutive
        iii. iNOS (inducible) – Ca^{2+}–independent, inducible

- iNOS:
    - Expressed in:
        - Macrophages
        - Neutrophils
        - And many other cells
    - Induced by:
        - Proinflammatory cytokines:
            - TNF-α
            - IFN-γ
        - Bacterial endotoxins:
            - Lipopolysaccharide (LPS)
    - Can produce large amounts of NO over hours to days

C. Nitric Oxide and Vascular Endothelium

- Synthesis site:
  - eNOS in endothelial cells
- Mechanism:
  - NO diffuses to vascular smooth muscle
  - Activates cytosolic guanylyl cyclase to form cGMP
- Analogy:
  - Similar to cAMP formation by adenylyl cyclase
- Effect of cGMP:
  - Activates protein kinase G
  - Phosphorylates Ca^{2+} channels → ↓ Ca^{2+} entry into smooth muscle
  - ↓ Ca^{2+}–CaM activation of myosin light chain kinase → ↓ smooth muscle contraction → Smooth muscle relaxation (vasodilation)

- Pharmacologic relevance:
    - Vasodilator nitrates (e.g., nitroglycerin) are metabolized to NO
        - Causes vascular smooth muscle relaxation
        - Lowers blood pressure
    - NO is considered an endogenous nitrovasodilator

- Under hypoxic conditions:
    - Nitrite (NO_2^-) is reduced to NO
    - NO binds to deoxyhemoglobin
    - NO is then released into blood, causing:
        - Vasodilation
        - ↑ Blood flow

D. Nitric Oxide and Macrophage Bactericidal Activity

- iNOS activity in macrophages:
    - Normally low
    - Strongly stimulated by:
        - Bacterial LPS
        - Cytokines: IFN-γ and TNF-α

- Mechanism:
    - Activated macrophages form radicals
    - Radicals combine with NO
    - Form intermediates
    - Intermediates decompose into:
        - Highly bactericidal OH• radical

E. Additional Functions of NO

- Inhibits platelet adhesion and aggregation:
    - Via cGMP activation pathway
- Functions as a neurotransmitter:
    - In both central and peripheral nervous systems

V. G6PD Deficiency

A. Overview

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency:
    - A hereditary condition that affects mostly males
    - Characterized by hemolytic anemia upon exposure to oxidant stress

- Anemia results from inability of erythrocytes (RBCs) to detoxify oxidizing agents

- Pathophysiology:
  - Less NADPH is available
  - ↓ ability to maintain reduced glutathione (G-SH)
  - ↓ detoxification of H_2O_2 generated under oxidant stress

B. G6PD Role in Erythrocytes

- Adequate G6PD activity:
  - Required for NADPH formation
  - Essential for maintaining the G-SH pool

- Severity in red blood cells:
  - Although deficiency affects all cells, it is most severe in erythrocytes

- Because:
  - Pentose phosphate pathway is the only source of NADPH in RBCs
  - RBCs lack nucleus and ribosomes
    - → Cannot synthesize new enzyme
    - → Vulnerable to unstable G6PD variants

- Other tissues:
  - Can produce NADPH via:
    - $NADP^+$-dependent malate dehydrogenase (malic enzyme)

C. Clinical Application 13.1: Characteristics of G6PD Deficiency

- Inheritance:
  - X-linked trait
  - Mostly affects males

- Prevalence:
  - Most common disease-producing enzyme abnormality in humans
  - Affects over 400 million people worldwide

- Geographic distribution:
    - Highest in individuals of ancestry from:
        - Middle East
        - Tropical Africa
        - Asia
        - Parts of the Mediterranean
- Genetic variability:
    - G6PD deficiency is a family of deficiencies
    - Caused by multiple mutations in the G6PD gene
    - Only some variants cause clinical symptoms

D. Clinical Manifestations

- Hemolytic anemia:
    - Occurs periodically in response to oxidant stress
- Neonatal jaundice:
    - Common manifestation
    - Appears 1 to 4 days after birth
    - May be severe
    - Caused by increased production of unconjugated bilirubin

- Chronic hemolysis:
    - May lead to a somewhat shortened lifespan in severe forms
    - Due to complications from persistent red cell destruction

E. Evolutionary Perspective

- Selective advantage:
    - G6PD deficiency provides increased resistance to malaria
    - Specifically to Plasmodium falciparum

- Mechanism:
    - Infection induces oxidant stress
    - Leads to RBC lysis
    - → Kills parasite
    - → Protects host from developing malaria

Additional Details on G6PD Deficiency

A. Impaired Detoxification and Heinz Body Formation

- Effect of G6PD deficiency:
    - Impairs detoxification of free radicals and peroxides formed within the cell
- Role of reduced glutathione (G-SH):
    - Helps maintain reduced states of sulfhydryl (-SH) groups in proteins, including hemoglobin
- Oxidative damage to proteins:
    - Oxidation of sulfhydryl groups leads to denatured proteins
    - These form insoluble masses called Heinz bodies
        - Heinz bodies attach to red blood cell membranes

- Membrane rigidity:
    - Further oxidation of membrane proteins → RBC membrane becomes rigid (less deformable)
    - These rigid cells are removed by macrophages in the spleen and liver

B. Precipitating Factors in G6PD Deficiency

- Genetics:
    - Males with a G6PD mutation on their single X chromosome are hemizygous
    - These individuals usually remain asymptomatic unless exposed to oxidant stress

- Oxidant stress triggers:
    - Lead to red blood cell lysis and hemolytic anemia in G6PD-deficient individuals

1. Oxidant Drugs

- Categories often begin with letter A:
    - Antibiotics (especially sulfa drugs)
    - Antimalarials
    - Analgesics
    - Antipyretics
- Only specific drugs in each category are problematic
- Drug safety lists are available to prescribers:
    - Include drugs that are safe and those to be avoided in G6PD-deficient individuals

2. Favism

- Fava beans (broad beans) can cause hemolysis in some G6PD-deficient individuals
- Particularly affects those with the Mediterranean variant of G6PD deficiency
- Favism = hemolytic reaction after fava bean ingestion
    - Not seen in all G6PD-deficient individuals
    - But all with favism have G6PD deficiency

3. Infection

- Common precipitating factor
- Inflammatory response generates free radicals in macrophages
    - These radicals diffuse into RBCs
    - → Cause oxidative damage and hemolysis

C. G6PD Gene Variants

- Molecular genetics:
    - Cloning and sequencing of G6PD gene (see Chapter 34) identified >400 variants
    - Only some variants result in enzyme deficiency
- Nature of mutations:
    - Most are missense point mutations

- Effect of mutations:
    - Some ↓ catalytic activity
    - Some ↓ stability
    - Others alter binding affinity for:
        - $NADP^+$
        - Glucose 6-phosphate
- Enzyme structure:
    - Active G6PD exists as a homodimer or tetramer
    - Mutations at subunit interfaces may affect enzyme stability

D. Severity of Hemolytic Anemia and G6PD Variants

- Correlation with enzyme activity:
    - The severity of hemolytic anemia in G6PD deficiency correlates with the residual enzyme activity in red blood cells (RBCs)

1. G6PD A^- Variant (Class III)

- Prototype of moderate form of the disease

- Red blood cells contain:
    - Unstable but kinetically normal G6PD
    - Most enzyme activity is present in reticulocytes and younger RBCs
- Older RBCs:
    - Have lowest G6PD activity
    - Are preferentially removed during hemolytic episodes
- Self-limiting episodes:
    - Because hemolysis spares younger cells

2. G6PD Mediterranean Variant (Class II)

- Prototype of more severe deficiency
- Greater reduction in enzyme activity than Class III

3. Class I Mutations

- Rare and most severe

- Associated with:
    - Chronic nonspherocytic hemolytic anemia
    - Occurs even without oxidative stress

4. Mutation Characteristics

- Both G6PD A^- and G6PD Mediterranean:
    - Result from a single amino acid substitution in the normal enzyme
- No large deletions or frameshift mutations identified
    - Suggests that a complete absence of G6PD activity is likely lethal

Classification of Glucose 6-Phosphate Dehydrogenase (G6PD) Deficiency Variants

Class	Clinical symptoms	Residual enzyme activity
I	Very severe (chronic, nonspher-ocytic hemolytic anemia)	<10%
*II	Severe (acute hemolytic anemia)	<10%
*III	Moderate	10%–60%
IV	None	>60%