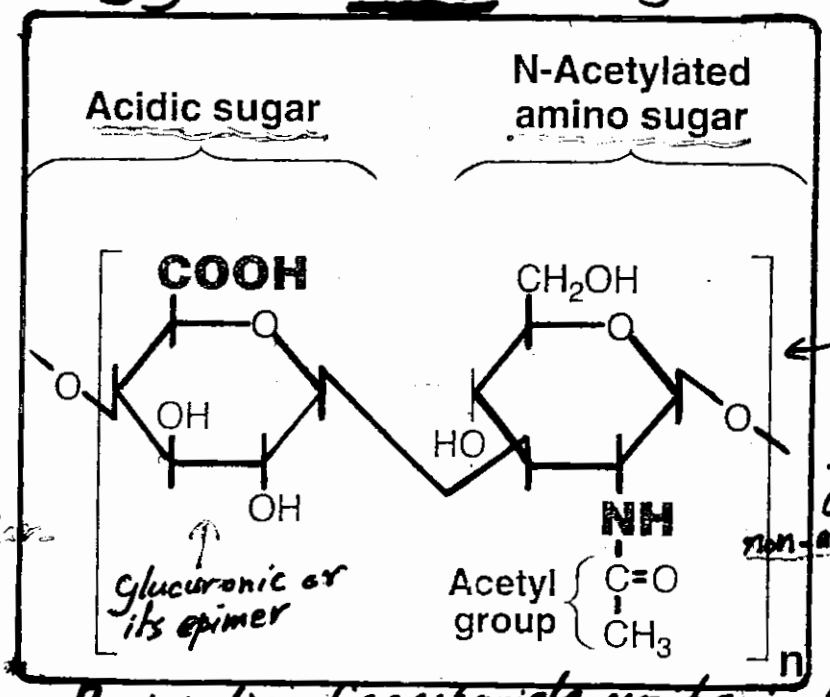
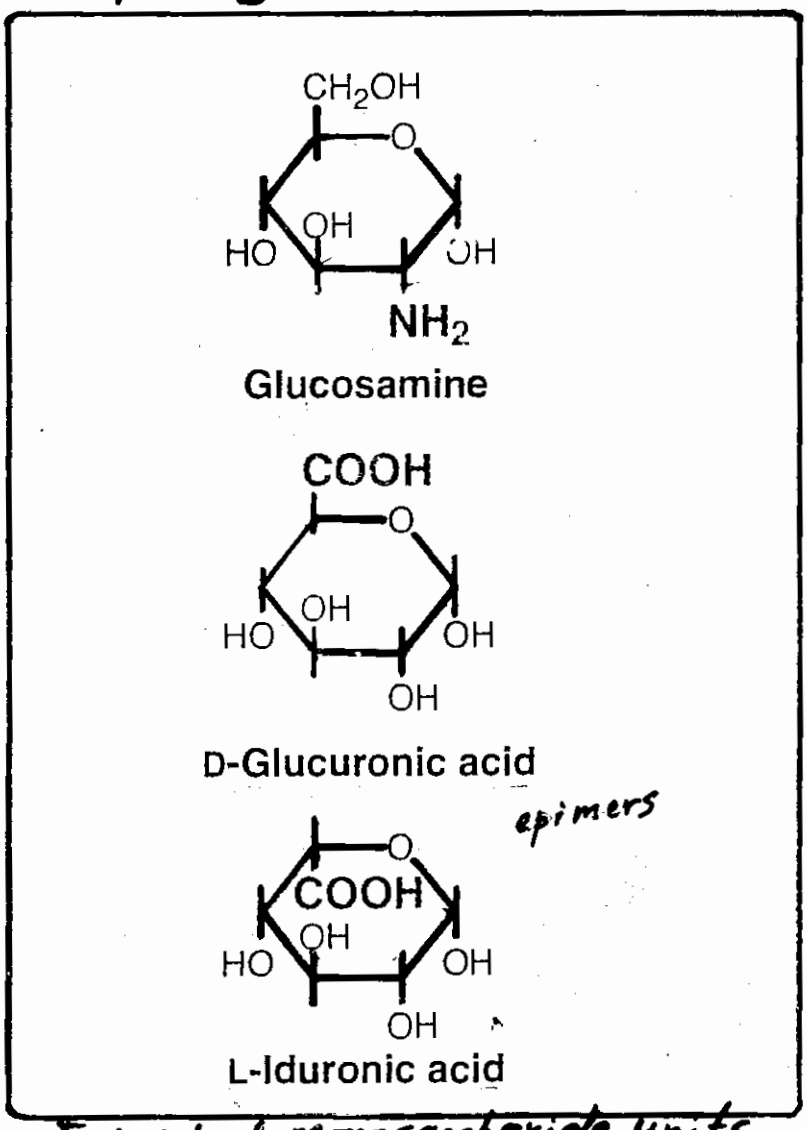
# Glycosaminoglycans (GAG) & Glycoproteins




Repeating disaccharide units in GAG




Individual monosaccharide units in GAG

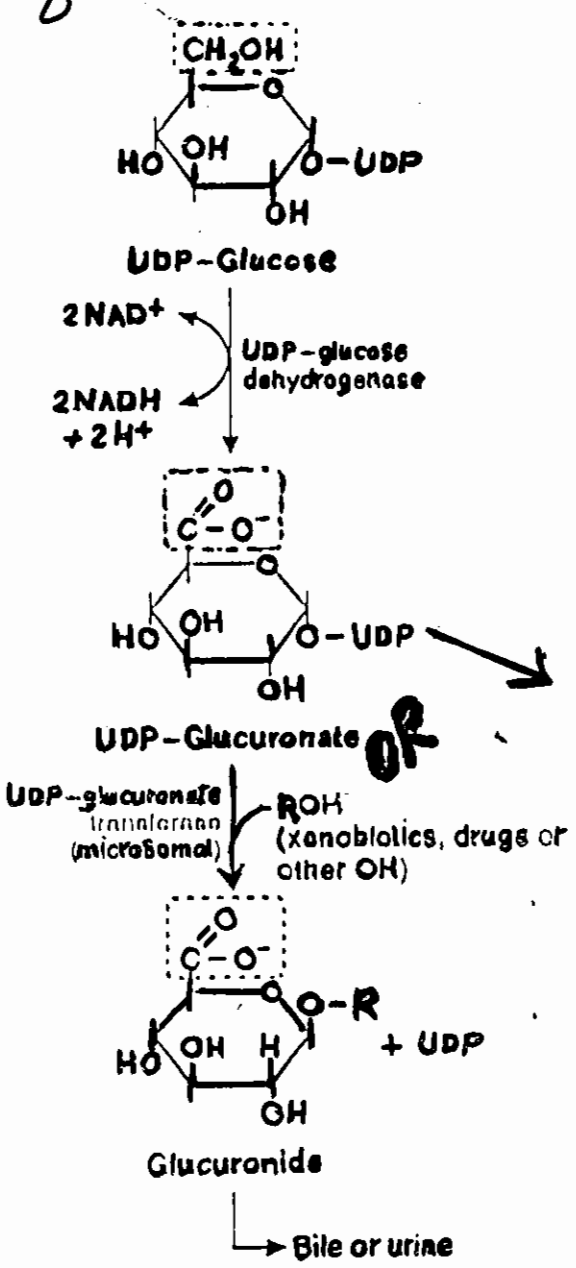
# Glycoproteins



- Oligosaccharides are covalently attached
- 2 to 10 sugar residues
- No serial repeats in structure
- Are mostly branched – mainly D-hexose
  also NANA, fucose in some cases
- May or may not be -ve charged
- Variable amount of carbohydrate
  4% in IgG to 80% human gastric mucin
- Membrane bound glycoproteins – Numerous functions:
  - Cell surface recognition
  - Cell-cell adhesivity
  - [illegible]
  - [illegible] → lubricants
- All globular protein in human plasma are glycoproteins
  Albumin is not a glycoprotein

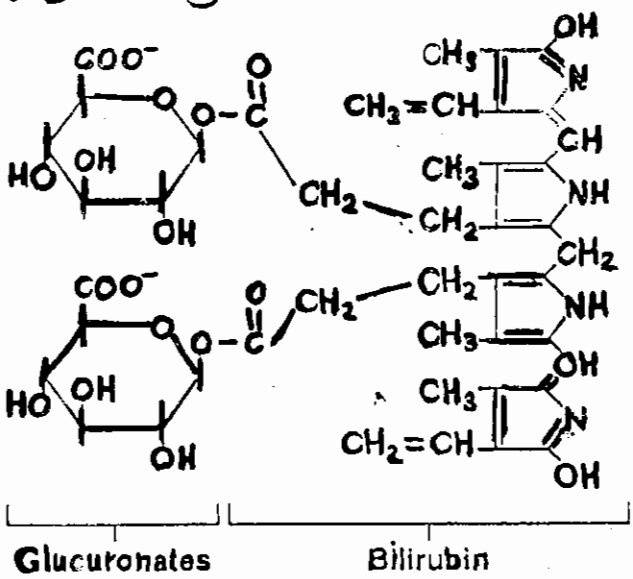
# Formation of Glucuronate






Glycosaminoglycans
"GAG"

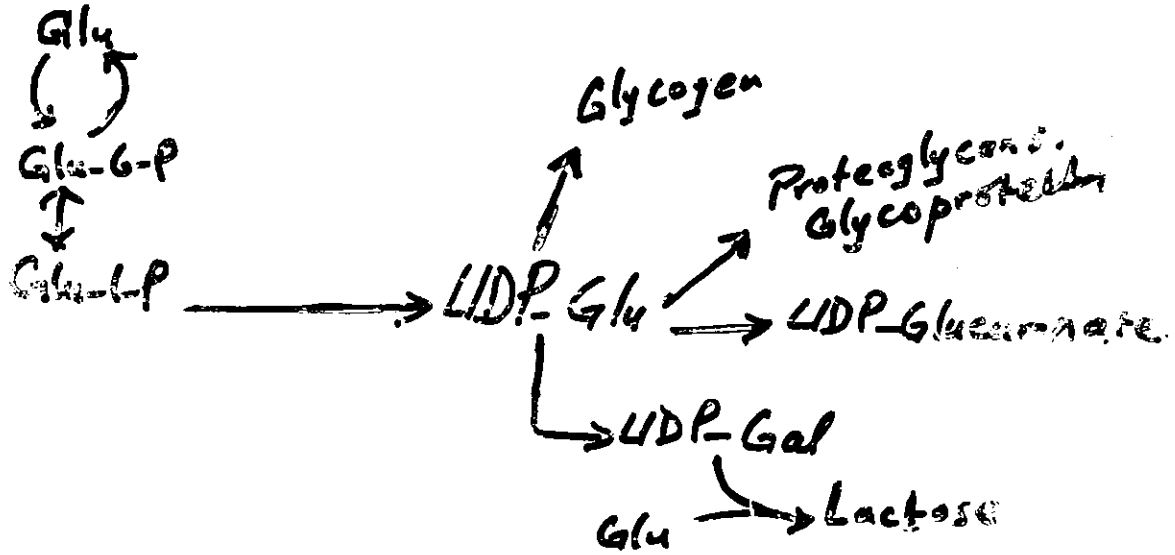
e.g. Bilirubin diglucuronide (Direct bilirubin)

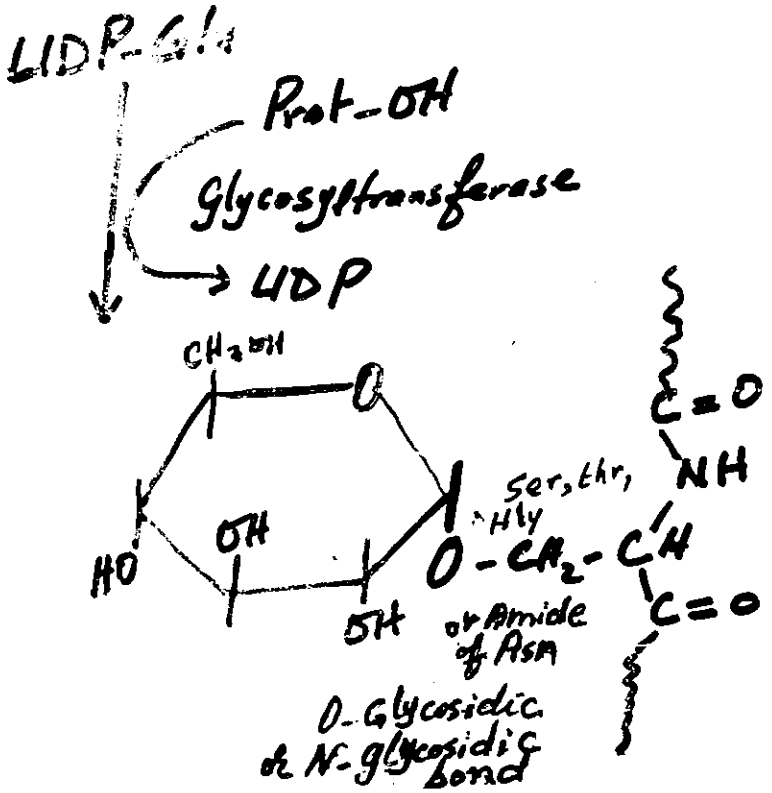


# Interconversions Involving UDP-Sugars

Activated Sugars
UDP-Sugar
→ other sugars
→ Sugar Acid
⟹ Proteins, lipids, other sugars

## Reactions of UDP-Glucose :-



# Properties of the G6PD Variant Enzymes 6

## 1- Classification of G6PD Variants

| Class | Clinical symptoms | Residual enzyme activity |
|---|---|---|
| I | Very severe | <2% |
| II | Severe | <10% |
| III | Moderate | 10–50% |
| IV | None | 60–150% |

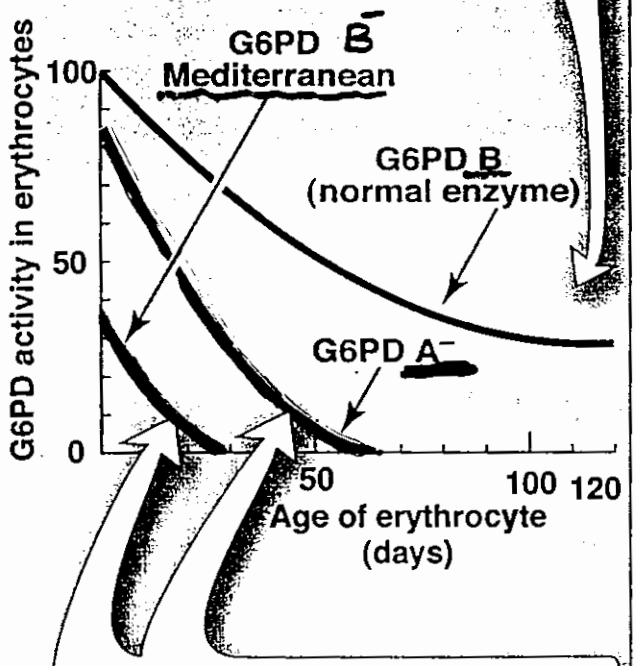
→ Chronic nonspherocytic hemolytic anemia CNSHA $B^-$

e.g. → Med. Variant $B^-$

→ e.g. $A^-$ (African)

## 2- Decline of erythrocyte G6PD activity with cell age for three most common forms:-

Although the activity of the normal enzyme declines as red cells age, even the oldest cells have a sufficient level of activity to provide protection against oxidative damage and hemolysis.




By contrast, very few *G6PD Mediterranean* red cells have sufficient enzyme activity to prevent oxidative damage, whereas a substantial fraction of young *G6PD A⁻* red cells are able to provide protection.

- Precipitating factors in G6PD Deficiency :-

1- Oxidant Drugs

AAA

A = antibiotic e.g. sulfamethoxazole, chloramphenicol

A = Antimalaria primaquine

A : Antipyretics

Acetanalid, but not acetaminophen

2- Favisim

Fava beans → contain the glycosides: vice + convicine → produce the aglycones → divicine* + isouramil*

*oxidants cause rapid decline in GSH

3- Infection

4- Neonatal jaundice

- Properties of the Variant Enzymes

- Molecular Biologe of G6PD

Majority missense mutation → Point mutations

Large deletions or frameshift mutations → not observed

$A^+$ → 376 A → G, 126 Asn → Asp

(B⁻) Med. 563 C → T 188 ser → Phe

$A^-$ → 376 A → G, 126 Asn → Asp + 202 G → A, 68 Val → Met.

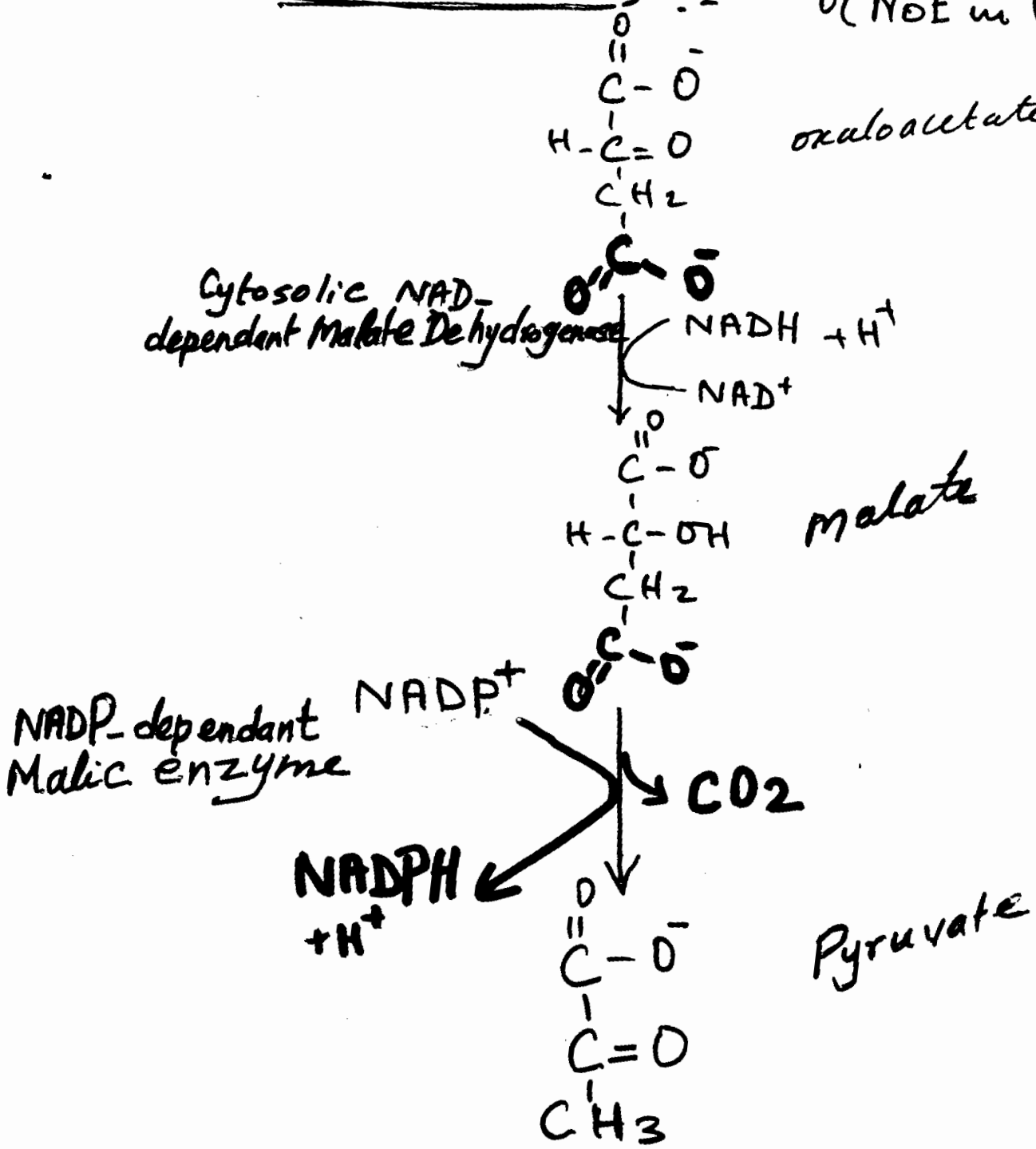
# G6PD Deficiency

- Prevalence and Geographic Distribution
  - 200 to 400 million individuals worldwide
  - Highest prevelance in Middle East, tropical Africa, S.E. Asia & Mediterranean
  - X-linked deficiency
  - Deficiency provides resistance to falciparum malaia

- Variants
  1. Wild type – $B^-$ (class II) Ser → phe < 10% residual activity
  2. Med. variant – 563C → T $B^-$ (class III) 10 – 20%
  3. African variant – two points mutation 376 A → G + 202 G → A Val → Met 80%
  4. African variant $A^-$ – normal activity 376 A → G Asn → Asp < 2%
  5. V. severe deficiency (class I)

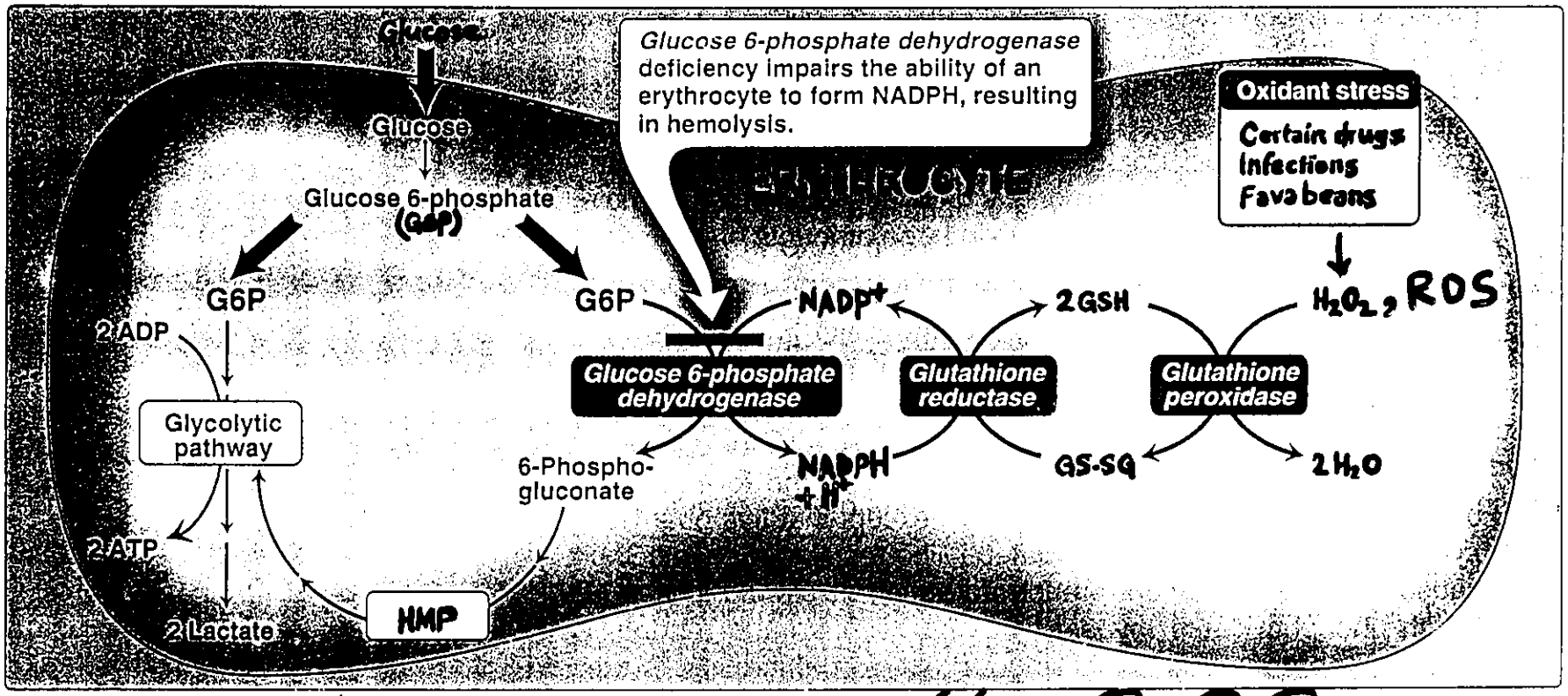
- Role of G6PD in red blood cells
  - Peroxides + GSH $\xrightarrow{\text{Peroxidase}}$ G-S-S-G + 2 $H_2O$
  - G-S-S-G + NADPH $\xrightarrow{\text{GSH reductase}}$ 2 GSH + $NADP^+$
  - G6P + NADP $\xrightarrow{\text{G6PD}}$ NADPH + 6PG

# Alternative Sources of NADPH 3
## in Other Tissues :- (Not in RBC)



# G6PD Deficiency

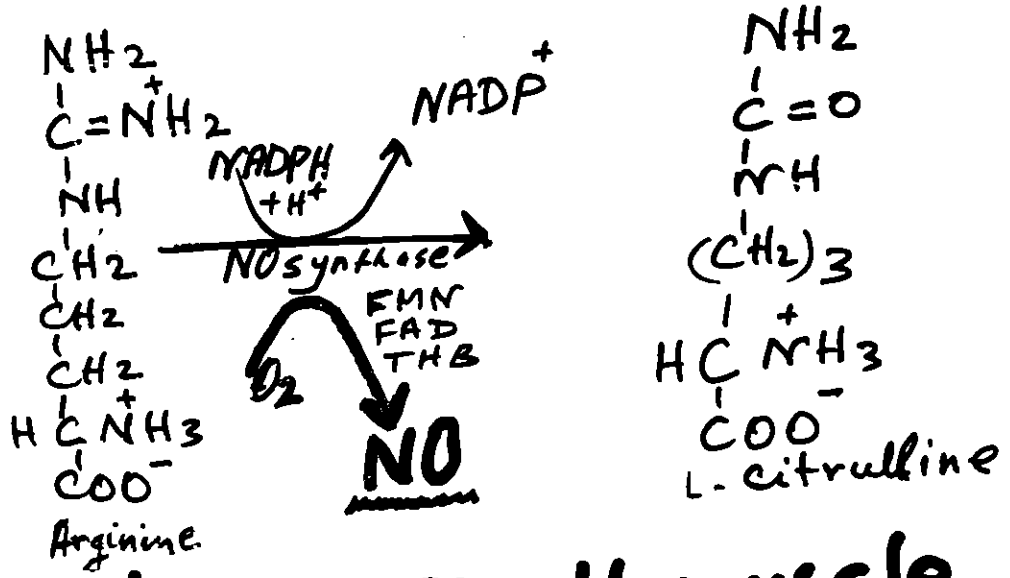
## Pathways of G6P metabolism in the erythrocyte




Hemolysis is caused by ROS

# Synthesis of Nitric Oxide (NO)

2f




- **Relaxes smooth muscle**
  - $GTP \xrightarrow[NO(+)]{Cyclase} cGMP$
  - cGMP activates PKG
  - PKG phosphorylates $Ca^{2+}$ protein channels which decrease $Ca^{2+}$ entry to muscle cells which decrease Ca-calmodulin of myosin light chain kinase ↓ therefore decreasing muscle contraction and favoring relaxation of vascular smooth muscle
- **Prevents platelet aggregation**
- **Neurotransmitter in brain**
- **Mediates tumoricidal and bactericidal actions of macrophages**

# E. NO and RNOS Synthesis 2e

- NO is a free radical, diffuses readily
- Essential to life and toxic
- Neurotransmitter, vasodilator
  Prevents platelet aggregation | at low concentration
- At high concentration combines with $O_2^{\cdot}$ or $O_2$ to form RNOS
  RNOS are involved in neurodegenerative diseases and inflammatory diseases

## NO Synthase

- nNOS - Isoform I (neural)
- eNOS - Isoform III (endothelium)

Both are constitutive enz. produce small amount of NO as neutransmitter + hormone

- iNOS - Isoform II (inducible)

Induction of transcription in many cells of immune system → large amount of NO
↑NO → RNOS to kill invading bacteria

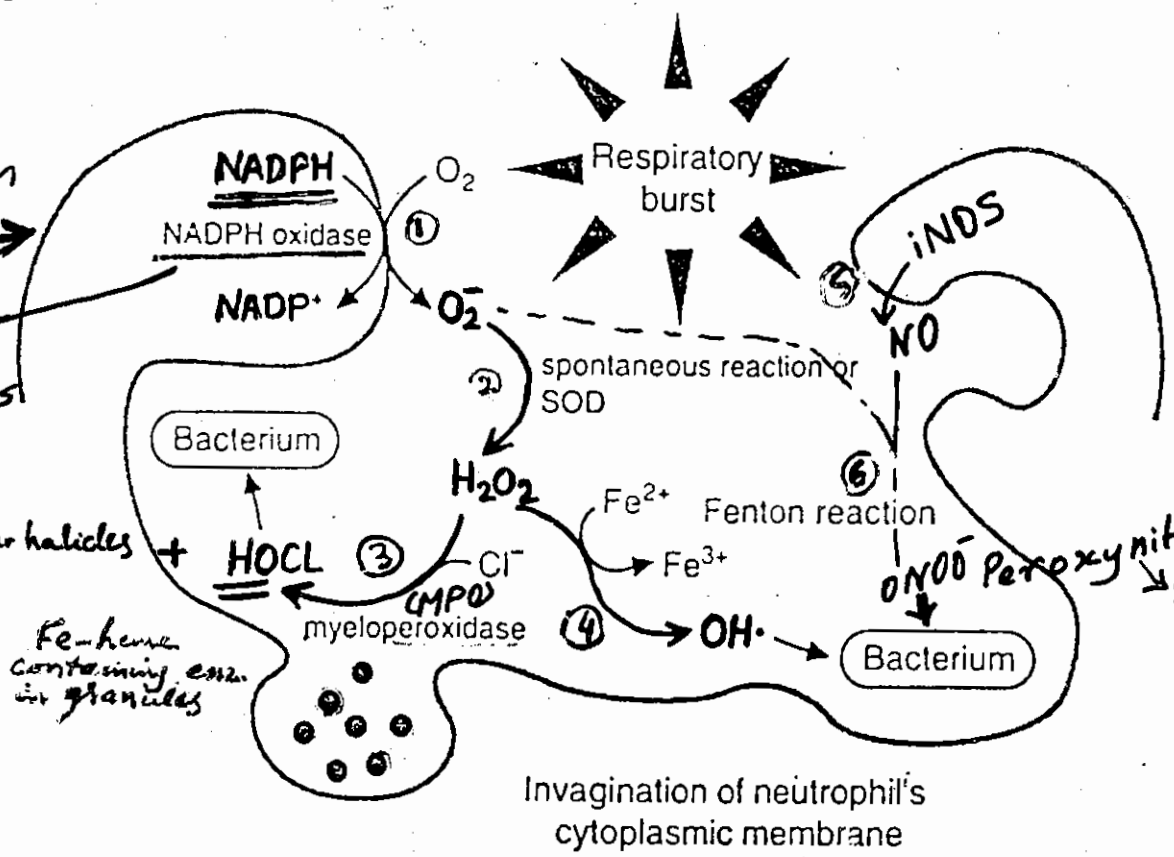
D-PHAGOCYTOSIS BY WHITE BLOOD CELLS

2d 7.

**Production of reactive Oxygen species during the phagocytic Respiratory burst by activated macrophages, neutrophils & eosinophils.**

Activation initiates the burst

Deficiency → chronic granulomatosis




HOCl - hypochlorous acid

powerful toxin - halogenation and ox$^{n}$.

e.g. Fe- & SH- centers ox. decarboxylation, ox. deamination breaking peptide bonds

HOCl hypochlorus acid

other halicles +

Fe-heme containing enz. in granules

generate RNOS

**OH· + HOCl attacks bacterial cells → lysis**

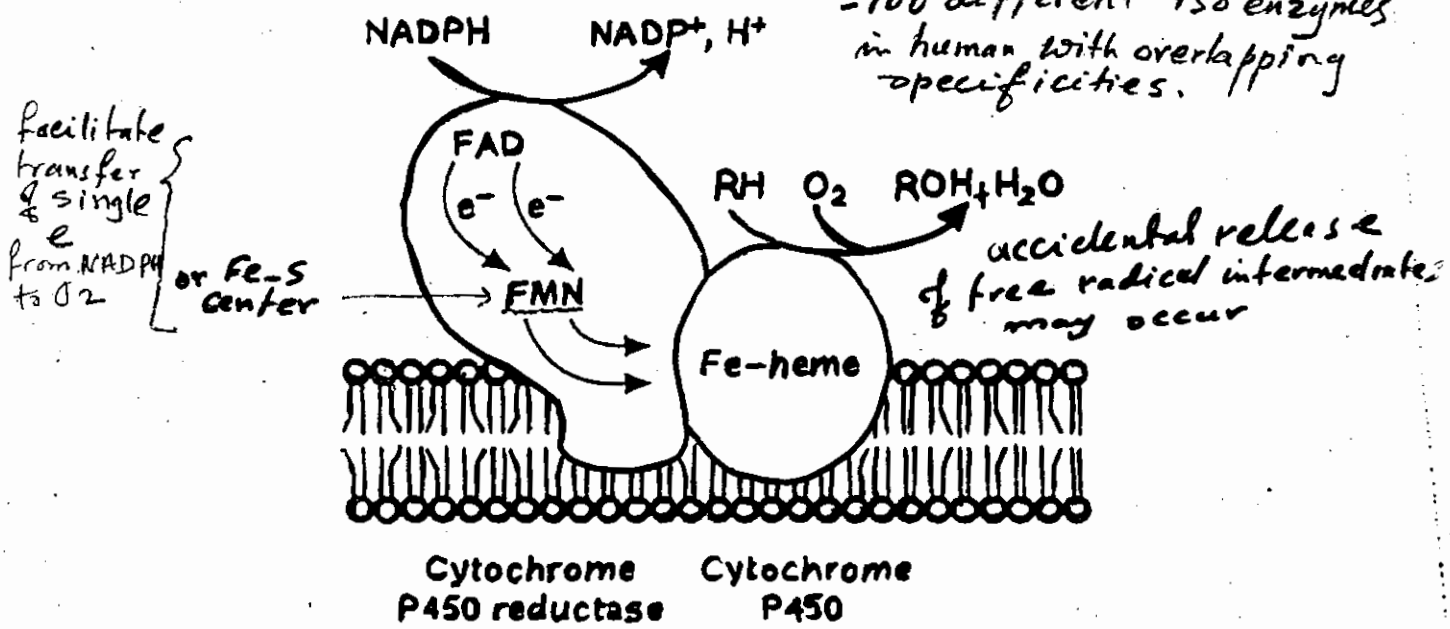
30 - 60 min consumption of $O_2$ } ← Respiratory Burst

free radicals (nitration agent)

Cytochrome P 450 enzymes :-

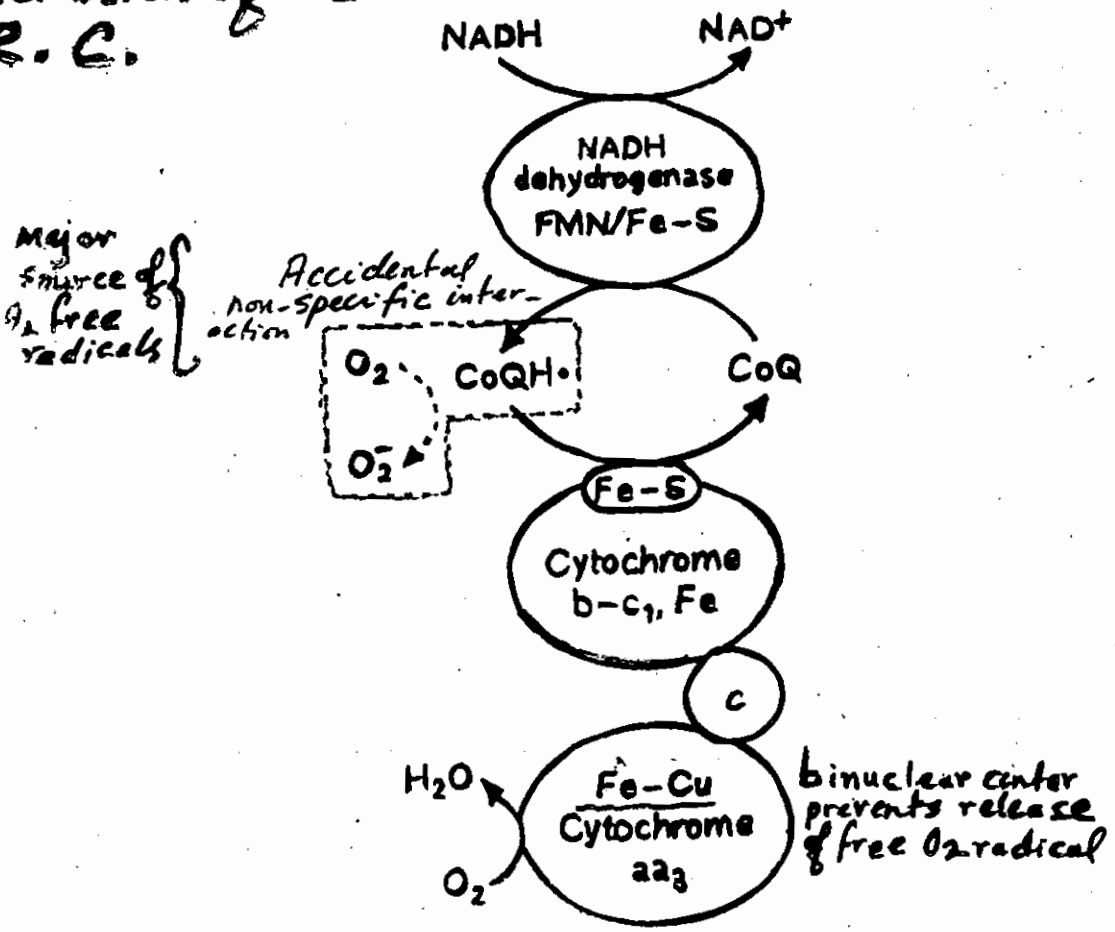
Superfamily of structurally related monooxygenases

- 100 different iso enzymes in human with overlapping specificities.




$$O_2 + S + \text{Electron donor} - XH_2 \longrightarrow H_2O + S-OH + \text{Electron donor} - X$$

## Generation of $O_2^-$ by R.C.



C- Cytochrome $P_{450}$ Monooxygenase
(Mixed Function oxygenase)

$$R\text{-}H + O_2 + NADPH + H^+ \longrightarrow R\text{-}OH + H_2O + NADP$$

R → steroid
→ drug
→ other chemical

1- Mitochondrial System
Hydroxylation of steroids
in steroid hormone-producing tissues
synthesis of bile acids
synthesis of biologically active Vit D

2- Microsomal System
Detoxification of foreign
compounds (xenobiotics)
Activation or inactivation of Drugs
solubilization

# SOURCES of ROS in the cell :-

- Oxidases

$\bar{e} + O_2 \rightarrow H_2O$ or $H_2O_2$

most oxidases (peroxidase) $\rightarrow H_2O_2$ $\xrightarrow{\text{Fenton reaction}}$ $OH^{\cdot}$

Oxidases are confined to sites equiped with protective enzymes

- Oxygenases
  - mono oxygenases (hydroxylases)
  - Dioxygenases
    - → Thromboxanes
    - → PG
    - → Leukotrienes
- Coenzyme Q in R.C.
- Respiratory Burst during phagocytosis → $O_2^{\cdot}$, $H_2O_2$, $OH^{\cdot}$, NO, HOCl
- Ionizing Radiation → $OH^{\cdot}$

# Uses of NADPH



A. Reductive biosynthesis

B. Reduction of $H_2O_2$

$$H_2O_2 + 2\,GSH \xrightarrow[Se^{2+}]{GSH\ Peroxidase} G\text{-}S\text{-}S\text{-}G + 2H_2O$$

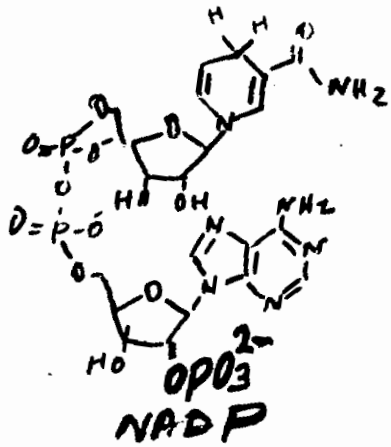
$$NADPH + H^+ + G\text{-}S\text{-}S\text{-}G \xrightarrow[Reductase]{GSH} NADP^+ + 2\,GSH$$

C. Cyt $P_{450}$ monooxygenase system

D- phagocytosis by white blood cells

E. Synthesis of Nitric oxide (NO)

# USES of NADPH




$NADP/NADPH = 0.1$ (In cytosol of hepatocytes)

$NAD/NADH = 1000$

A- Reductive Biosynthesis
Biosynthesis of fatty acids
" " Steroids

B- Reduction of Hydrogen Peroxide, Oxidised Glutathione

aerobic metabolism
Drugs
Enviromental toxins
} → reactive oxygen intermediates (species) ROS
e.g. $H_2O_2$ ; $O_2^{\cdot-}$ ; $OH^{\cdot}$
Oxidants

Chemical Damage:
1) DNA
2) Proteins
3) Unsat. lipids
} → Pathologic Process

1. Enzymes catalyze anti oxidant reactions

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

$$2H_2O_2 \xrightarrow{Catalase} O_2 + 2H_2O$$

$$H_2O_2 + 2GSH \xrightarrow[Peroxidase]{GSH} 2H_2O + \underline{G\text{-}S\text{-}S\text{-}G}$$ oxd. GSH

$$G\text{-}S\text{-}S\text{-}G + \underline{\underline{NADPH}} + H^+ \xrightarrow[reductase]{GSH} 2GSH + NADP$$

2. Antioxidant chemicals
Vit. E
Vit C
Carotenoids

# IRREVERSIBLE OXIDATIVE REACTIONS

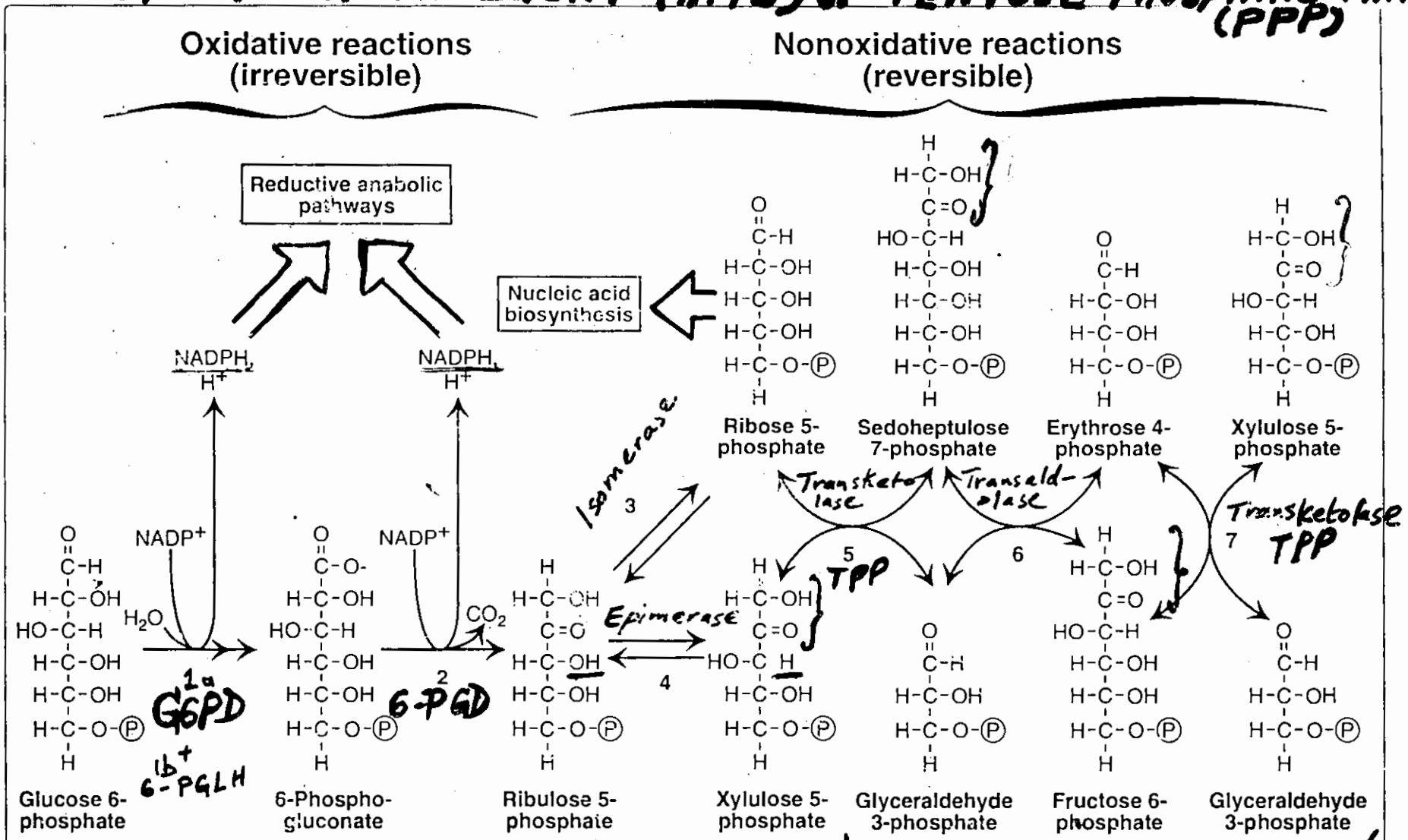
$\longrightarrow$ 2 NADPH

- NADPH-dependent biosynthesis of fatty acids in Liver; lactating mammary glands; adipose tissue

- NADPH-dependent biosynthesis of steroid hormones in the testes; ovaries; placenta and adrenal cortex

- to keep <u>reduced</u> glutathione especially in the red blood cells (RBCs)

# HEXOSE MONOPHOSPHAT SHUNT (HMS) or PENTOSE PHOSPHATE PATHWAY (PPP)

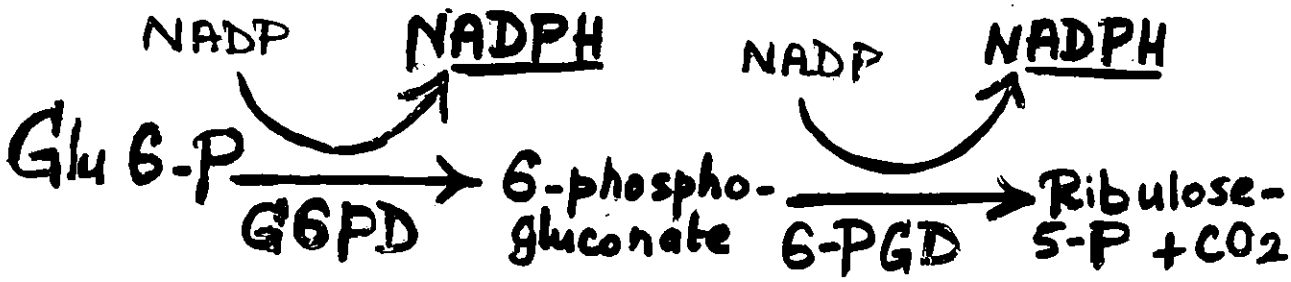



net [ $3\,G6P + 6\,NADP^+ \rightarrow 2\,F6P(G6P) + 3\,CO_2 + 6\,NADPH + 6\,H^+ + GA3P$ ]

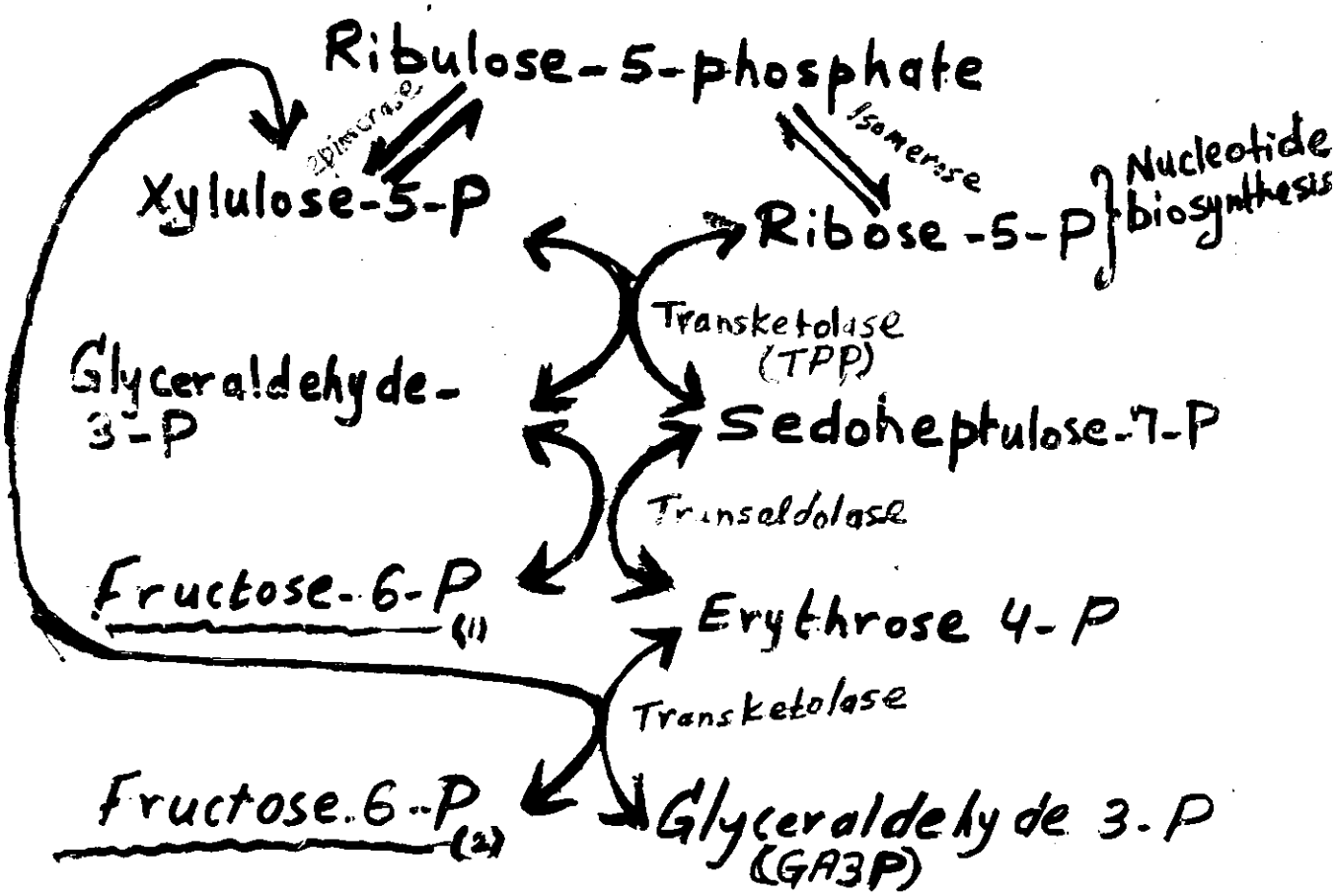
6PGLH = 6-phosphogluconolactone hydrolase

# The Pentose-phosphate Pathway
# The Hexose Monophosphate Shunt "HMS"

## The Oxidative phase - irreversible




## The non oxidative phase




$$3\ G6P + 6\ NADP \rightarrow 2\ G6P + GA3P + 3\ CO_2 + 6H^+ + 6\ NADPH$$

2FBP

# PENTOSE PHOSPHATE PATHWAY

## Functions of the PPP

A: NADPH Production:-

1. NADPH-dependent biosynthesis of fatty acids in liver, lactating mammary gland & adipose

2- NADPH-dependent biosynthesis of steroid hormones in the testes, ovaries, placenta and adrenal Cortex

3- NADPH is required by the RBC ⟶ GSH maintenance

B- Five-carbons sugars metabolism:-

- Ribose-5-phosphate for nucleotides biosynthesis
- Metabolic use of 5-carbon sugars from diet or degradation.