Parasite Biochemistry

Lecture 2

Carbohydrate metabolism in Trichomonads

Trichomonas vaginalis

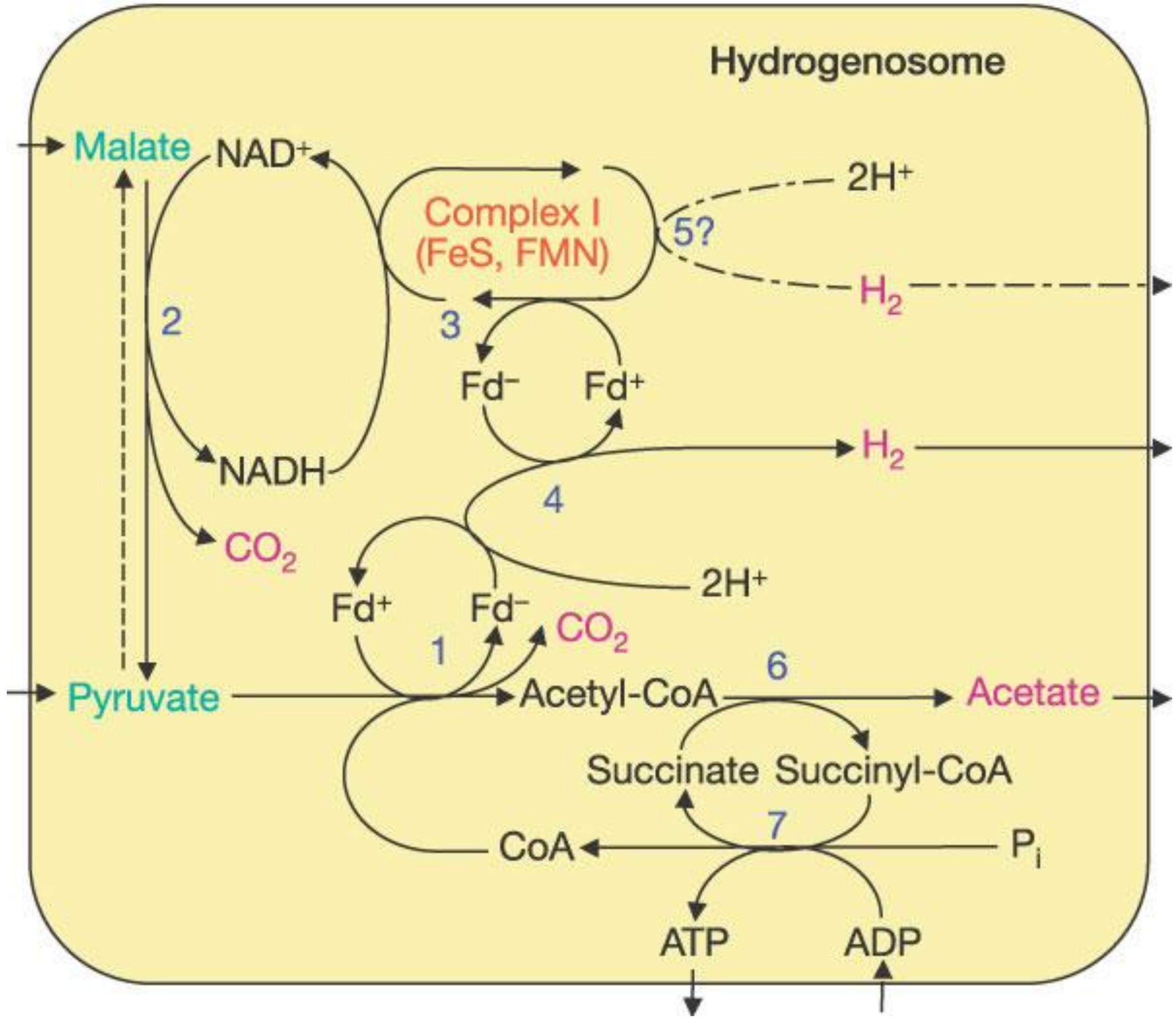
- Trichomonas lack mitochondria hence TCA and Electron transport is not as in other systems.
- But they contain a membrane bound organelle called **hydrogenosome**.
- This hydrogenosome constitutes a separate compartment of energy metabolism as mitochondria perform in other protozoa.
- Below is the metabolic pathway within the trichomonad hydrogenosome.
- H_2 and acetate are the major end products

The hydrogenosome

- These organelles obtained their name because they produce H_2 as metabolic product
- Organelles are predominantly spherical in shape and measure between 0.5-1 µm in diameter.
- They are surrounded by envelop enclosed by closely opposed membranes.
- Unlike mitochondria the inner membrane doesn't fold to form cristae, however like mitochondria the hydrogenosome constitutes a separate compartment of energy metabolism which results in eventual conversion of pyruvate to acetate, malate, CO_2 and H_2
- In *T. vaginalis* this organelle functions under both aerobic and anaerobic conditions.
- However electrons from pyruvate oxidation have different fates depending on presence or absence of O_2
- Under anaerobic conditions H+ serves as the terminal electron acceptor while under aerobic conditions O_2 is the ultimate acceptor

Carbohydrate metabolism in Trichomonads

- Being an anaerobic protozoa, the main source of energy in trichomonads are carbohydrates and their metabolism is fermentative.
- Glucose is phosphorylated by hexokinase
- The produced G-6-P enters glycolytic pathway and is converted to DHAP and G-3-P, just like in mammalian system.
- The latter is further metabolised by classical Embden Meyerhoff pathway to PEP and finally pyruvate.
- In *T. vaginalis* a number of intermediates of glycolytic pathway give rise to glycerol, H_2, CO2 and lactate as end products.
- In *T. foetus* however the major end product is succinate.
- Glycerol is produced by the reduction of DHAP to a product Glycerol-3-Phosphate and Pi.
- In hydrogenosome Pyruvate is oxidatively decarboxylated with the formation of hydrogen and acetate (to a lesser extent malate) as end products.


Hydrogenosome
Malate
NAD+
Complex I
(FeS, FMN)
2H+
5?
H2
2
3
Fd−
Fd+
H2
NADH
4
CO2
2H+
Fd+
Fd−
CO2
1
6
Pyruvate
Acetyl-CoA
Acetate
Succinate
Succinyl-CoA
7
CoA
Pi
ATP
ADP

Enzymes

1. Pyruvate ferredoxin oxidoreductase
2. Malate dehydrogenase
3. NAD: Ferredoxin oxidoreductase
4. H_2: Ferredoxin oxidoreductase
6. Acetate: Succinyl CoA transferase
7. Succinate thiokinase

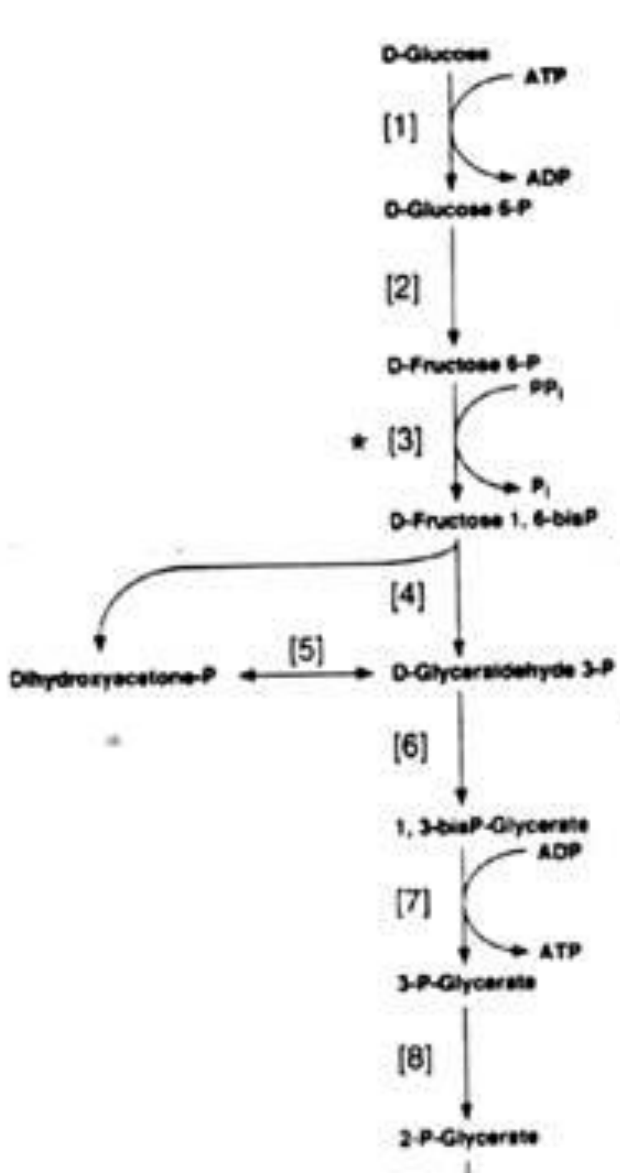
- In *T. vaginalis* oxidative decarboxylation of Pyruvate to Acetyl CoA is catalyzed by a reversible enzyme called **Pyruvate:Ferredoxin oxidoreductase** instead of the irreversible Pyruvate DH of most organisms (including mammals)
- It uses a sulfur-protein known as ferredoxin
- In addition to acetate, ethanol is produced by a pathway similar to that utilized by *Entamoeba histolytica*.
- In the cytosol, pyruvate can further give rise to lactate by the enzyme Lactate dehydrogenase.

Carbohydrate metabolism in Entamoeba

- Like in trichomonads, *Entamoeba histolytica* lack mitochondria. In addition they also lack hydrogenosome.
- Similar to most other anaerobic parasites, *E. histolytica* utilises carbohydrates as its major energy source.
- Glucose is taken up mainly by a carrier mediated system present in the membrane.
- *Entamoeba histolytica* therefore obtains its energy by a glycolytic pathway i.e. Embden Meyerhoff pathway.
- The parasite **lacks** the enzyme **LDH**, therefore **Lactate is not** an end product of its carbohydrate metabolism
- Instead of Pyruvate → Lactate, it is converted to ethanol and CO_2 which are the main end products of anaerobic metabolism.

- D-Galactose can substitute for glucose and supports growth in axenic culture.
- Several key enzymes concerned with glycogen synthesis have been found but the chief enzyme i.e. **glycogen synthase is absent**.
- Because the **mitochondria is absent**, *E. histolytica* lacks a functional kreb's cycle.
- The unique feature of glycolysis in *E. histolytica* is that the general reaction beyond PEP are catalyzed by ppi-dependent enzyme called **Pyruvate Phosphate dikinase**.

  PEP + AMP + Ppi → Pyruvate + ATP + Pi
- This forward reaction predominates resulting in the formation of pyruvate with a net yield of ATP.
- An alternative route for the formation of Pyruvate from PEP has been postulated. This requires a unique enzyme called PEP carboxyphosphotransferase
- Under aerobic conditions both **ethanol and acetate** are formed as well as CO_2
- Under anaerobic conditions only ethanol and CO_2 are formed.



Enzymes

1, Glucokinase

2, phosphoglucose isomerase

3, phosphofructokinase

4, Aldolase

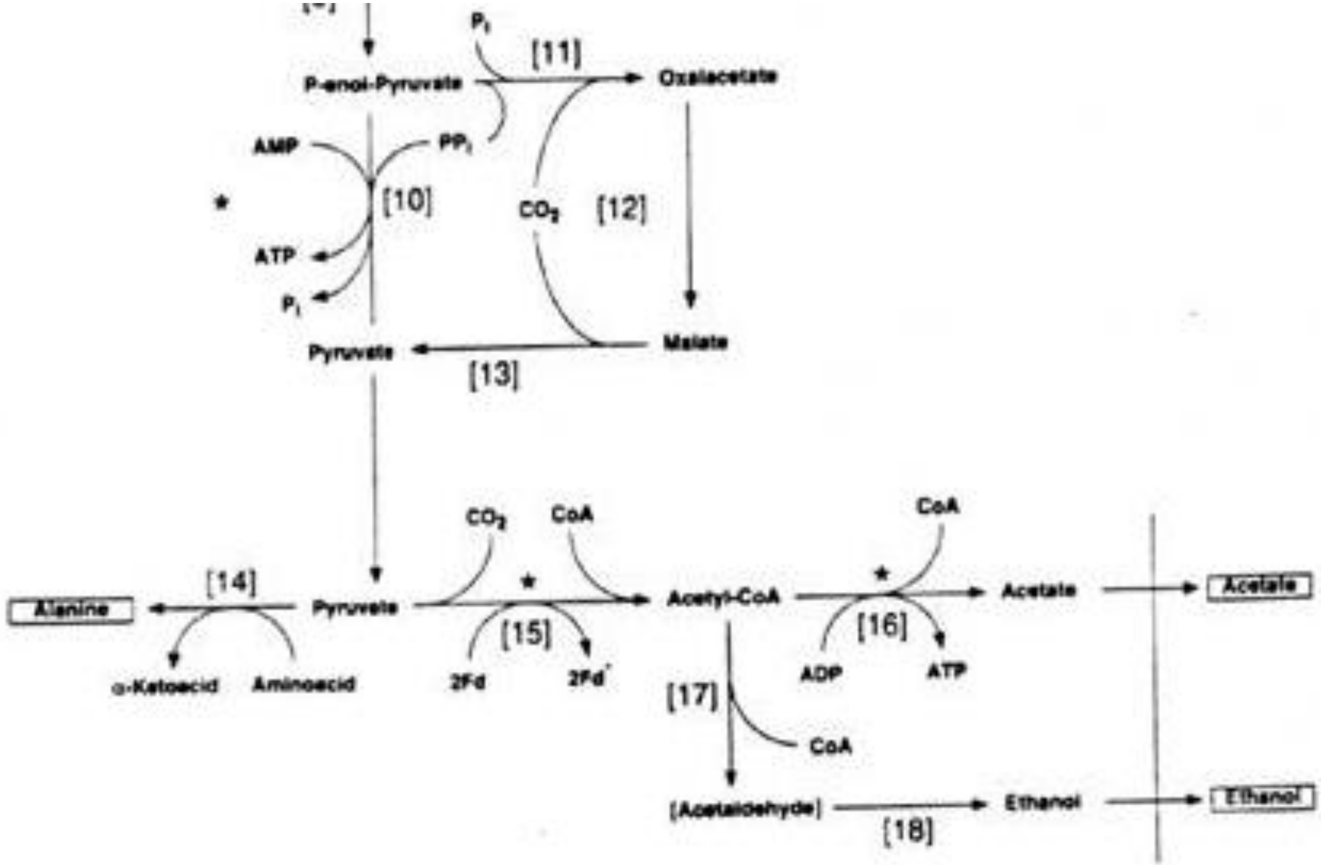
5, triosephosphate isomerase

6, glyceraldehyde-3-P DH

7, Phosphoglycerate kinase

8, phosphoglyceromutase

9, Enolase



Enzymes

10, **Pyruvate phosphate dikinase**

11, PEP carboxyphosphotransferase

12, Malate DH

13, Malic enzyme (decarboxylating)

14, Alanine aminotransferase

15, **Pyruvate:Ferredoxin oxidoreductase**

16, Thiokinase

17, Acetyl CoA reductase-

18, Alcohol dehydrogenase

Carbohydrate metabolism in *Leishmania*

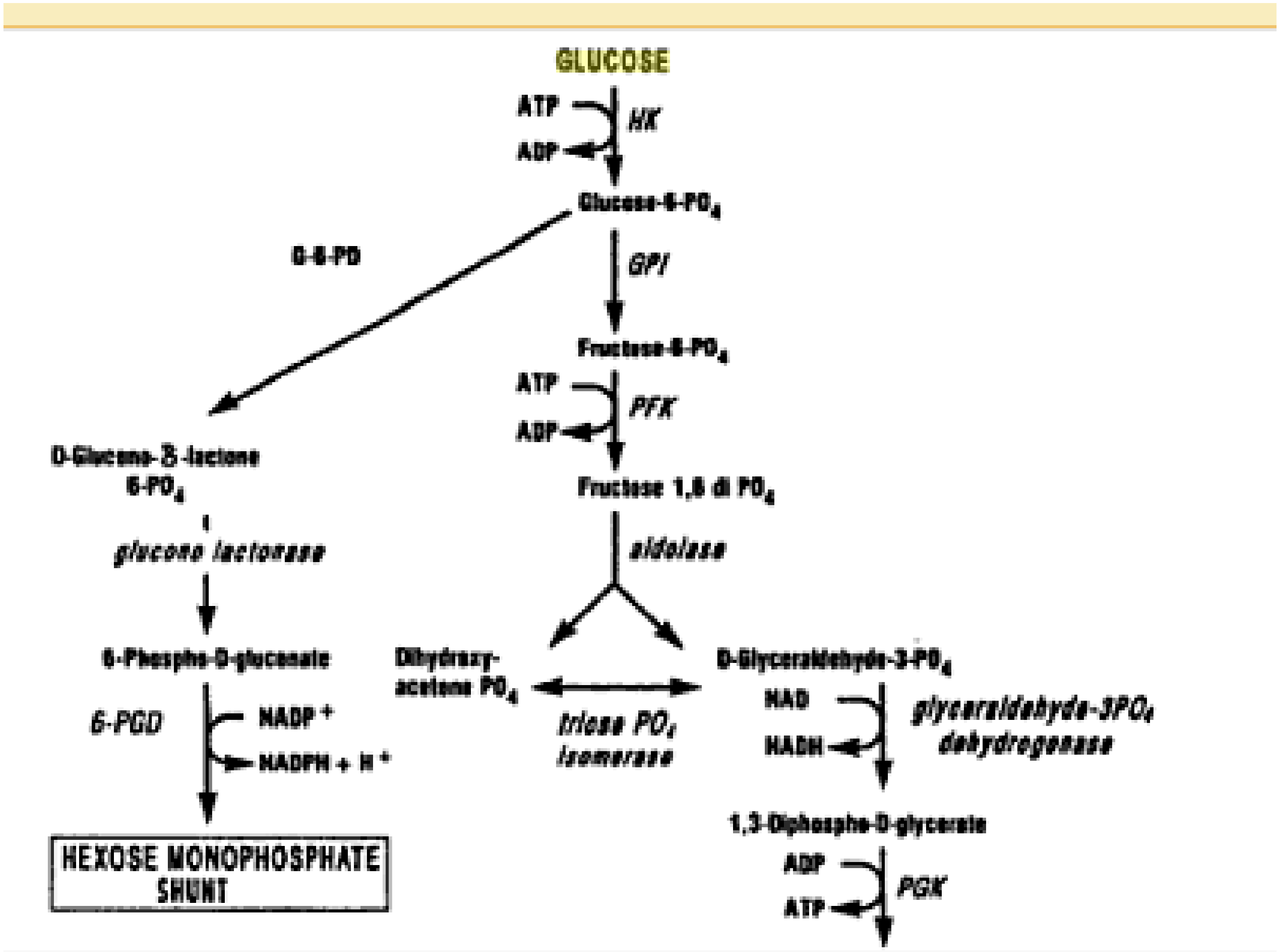
- *Leishmania* have a glycosome and mitochondria
- The glycosome is thought to play a major role in carbohydrate metabolism and like the glycosome of trypanosomes, Leishmania glycosome may contain all the early enzymes of glycolytic pathway.
- Carbohydrate metabolism follows early stages of glycolysis in *T. brucei* up to the Pyruvate stage, but Pyruvate is metabolized further in Leishmania.
- The products of glucose metabolism have been identified as **succinate, glycerol, Lactate, Pyruvate and Alanine.**

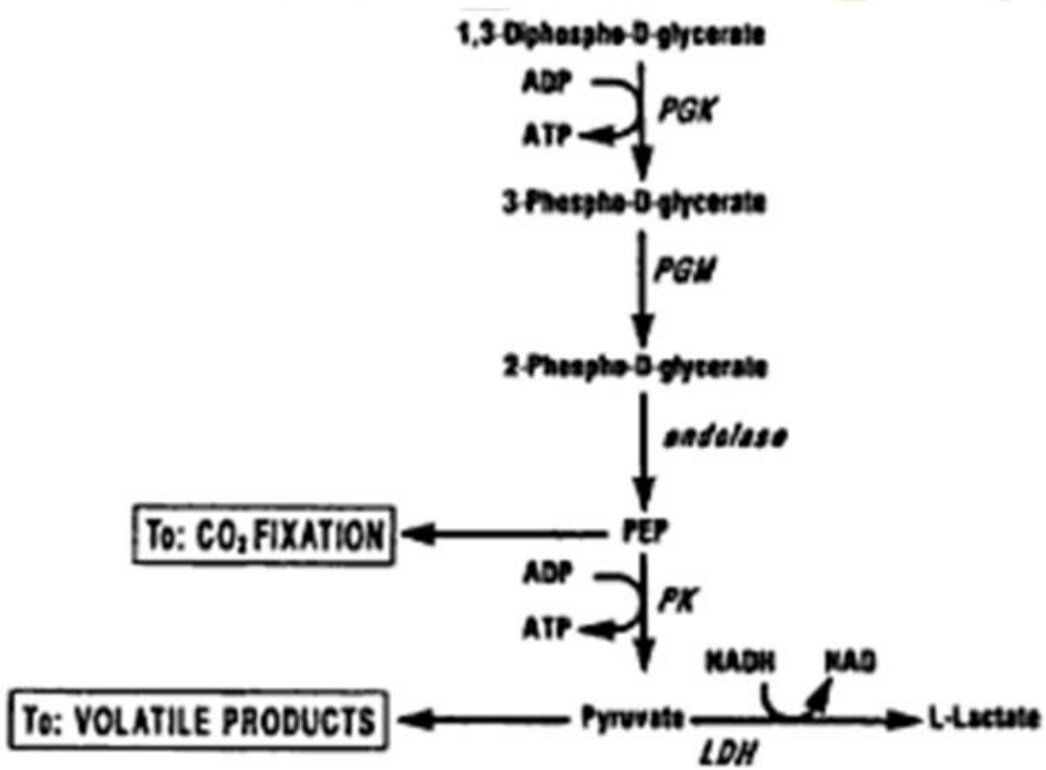
Carbohydrate metabolism in *Plasmodia*

Glycolysis

- As in trypanosomes, intraerythrocytic stages of *Plasmodium* lack carbohydrate reserves and consequently their primary source of energy is glucose from the blood stream.
- Although the partial pressure of O_2 in the blood is high, *P. falciparum* doesn't oxidise glucose completely to CO_2 and H_2O.
- Glucose is catabolized via the glycolytic pathway but **Pyruvate is not the end product**.
- Most of the pyruvate is converted to volatile products such as **formate and acetate.**
- Pyruvate is also converted to **lactate** which is one of the major end products.
- In *P. falciparum* the schizont stages produce most of the lactate
- The lactate produced has a marked inhibitory effect on growth *in vitro;* this emphasizes the importance of replenishing the medium frequently in cultures.

Pathways of the glycolytic conversion of glucose in *Plasmodium*





Enzymes

HK = hexokinase

GPI= glucose phosphate isomerase

PFK = phosphofructokinase

PGK = phosphoglycerate kinase

PGM = phosphoglyceromutase

PK = pyruvate kinase

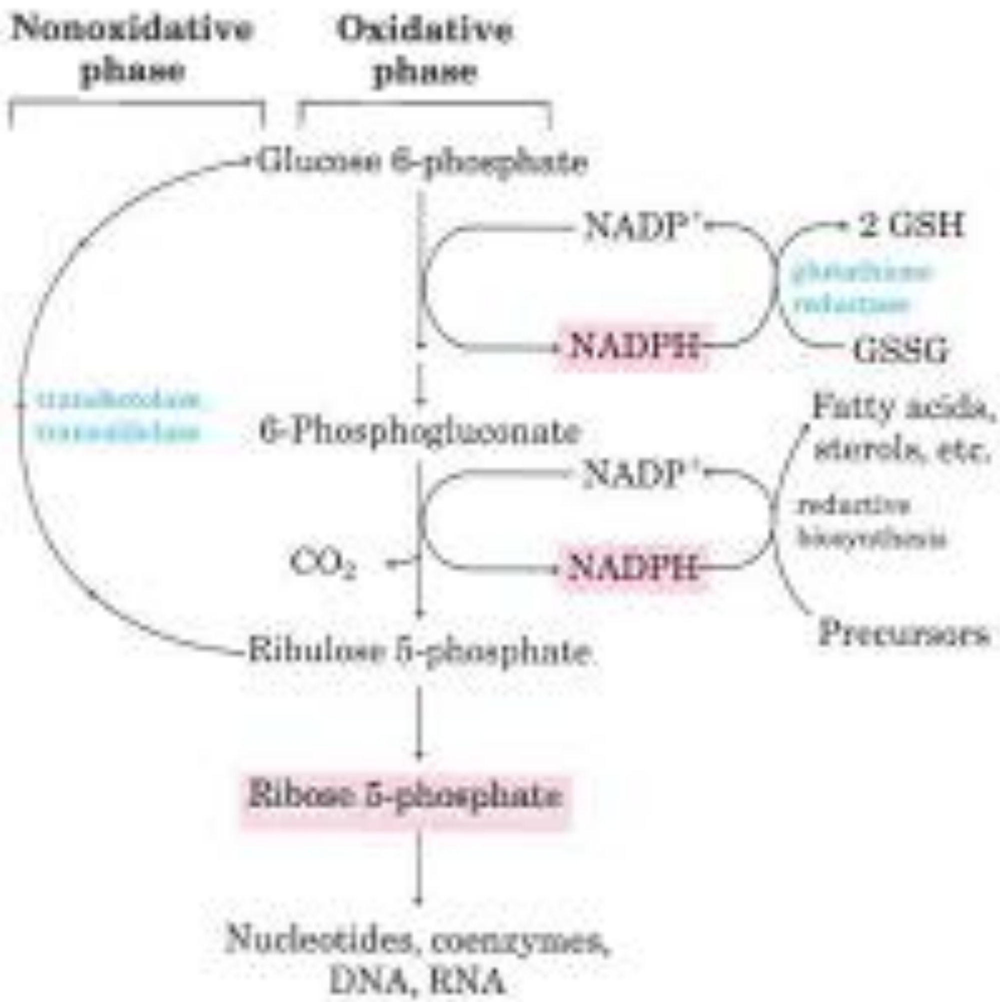
LDH= lactate dehydrogenase

6-PGD = 6-phosphogluconate dehydrogenase

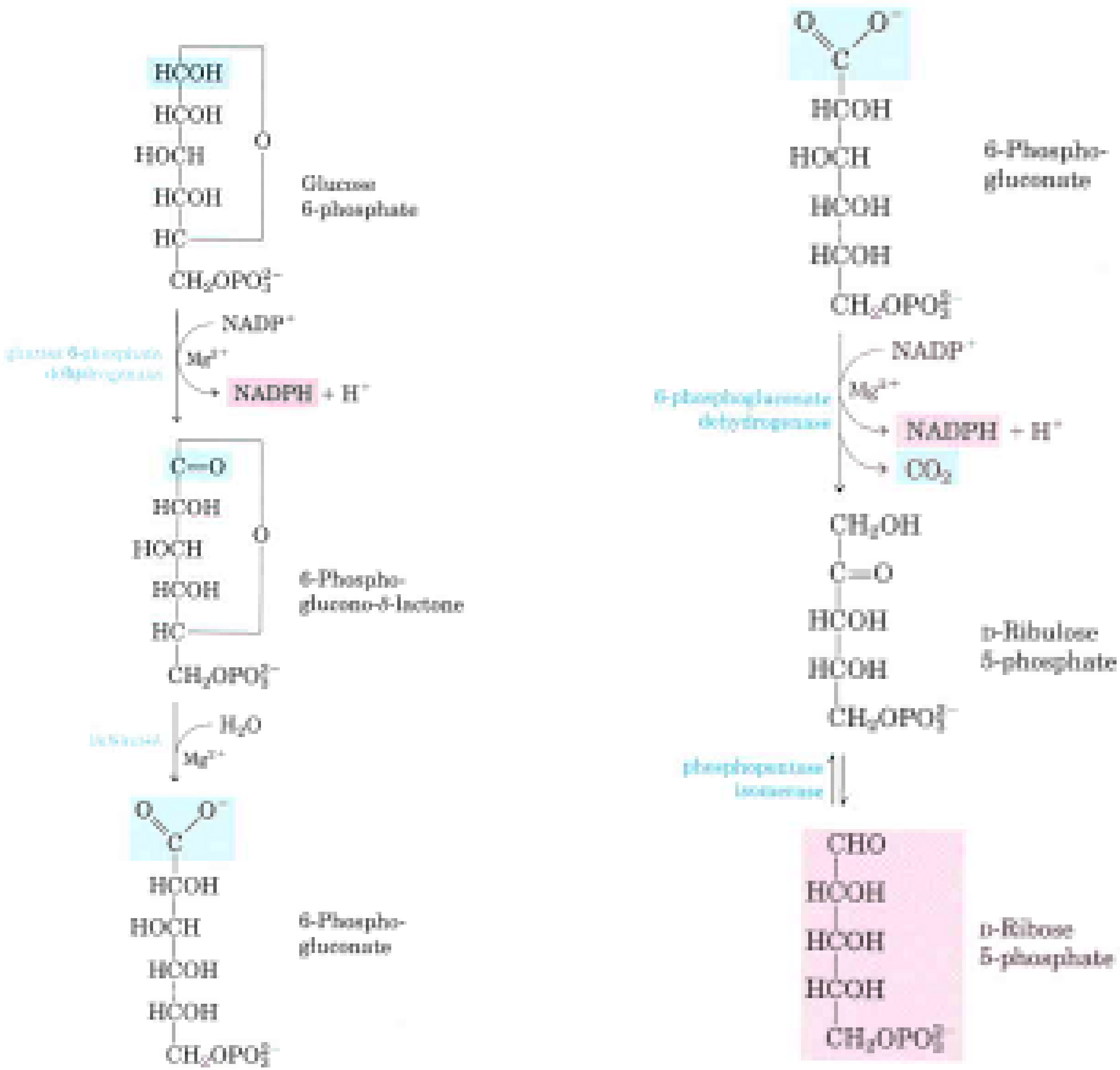
Pentose phosphate pathway

- Also called Hexose Monophosphate (HMP) pathway or phosphogluconate pathway
- Glucose-6-phosphate dehydrogenase (DH) has been identified in *Plasmodium* (including *P. falciparum*, *P. knowlesi* and *P. berghei*)
- *Plasmodium* glucose-6-phosphate DH has higher affinity for glucose-6-phosphate than does the host enzyme.
- Glucose-6-P DH deficiency protects against falciparum malaria.
- The parasites causing this disease require reduced glutathione and the products of the hexose monophosphate shunt (pentose phosphate pathway) for optimal growth.

General scheme of the HMP pathway

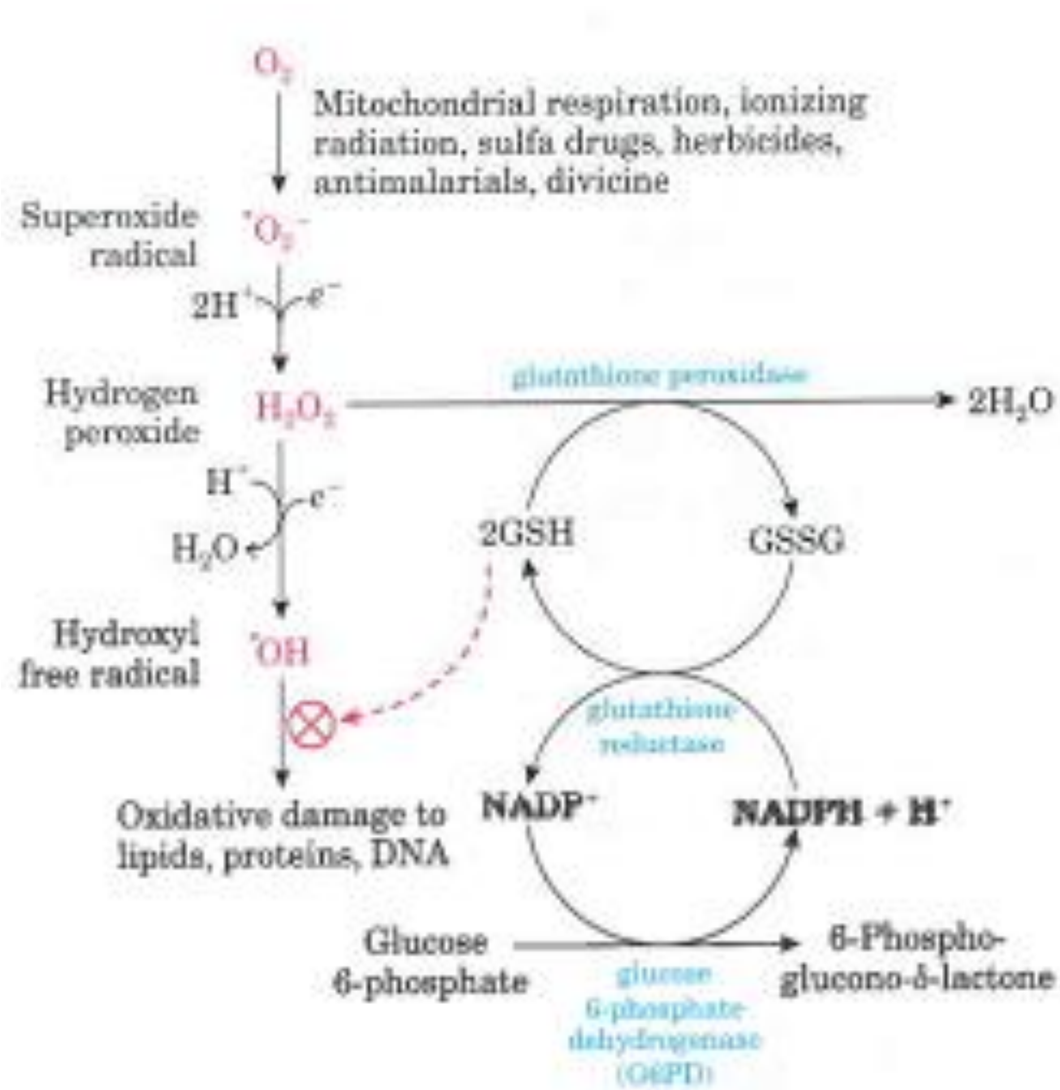


Oxidative reactions of the HMP pathway



- 1st reaction is the oxidation of glucose-6-phosphate by glucose-6-phosphate dehydrogenase (G6PDH) to form 6-phosphoglucono-δ-lactone.
- The lactone is hydrolyzed to the free acid 6 -phosphogluconate by a specific lactonase
- 6-phosphogluconate undergoes oxidation and decarboxylation by 6-phosphogluconate dehydrogenase to form the ketopentose ribulose 5-phosphate
- Phosphopentose isomerase converts ribulose 5-phosphate to its aldose isomer, ribose 5-phosphate.

Role of NADPH and glutathione in protecting cells against highly reactive oxygen derivatives



Carbon dioxide fixation

- All the species of Plasmodium, so far studied, are capable of CO_2 fixation.
- The end products have been identified as alanine, aspartate, glutamate, and citrate with α-ketoglutarate and oxaloacetate as intermediate products

Kreb's cycle

- Plasmodium lack a functional Kreb's cycle.
- α-ketoglutarate dehydrogenase activity is absent

Electron transport

- Plasmodium has a classical electron transport chain, perhaps for other processes not necessarily energy production
- P. berghei and P. knowlesi utilise O2 and respiration is sensitive to CN^- in *P. berghei* and CO in *P. knowlesi*