# Parasite Biochemistry

Lecture 1
Lecture slides

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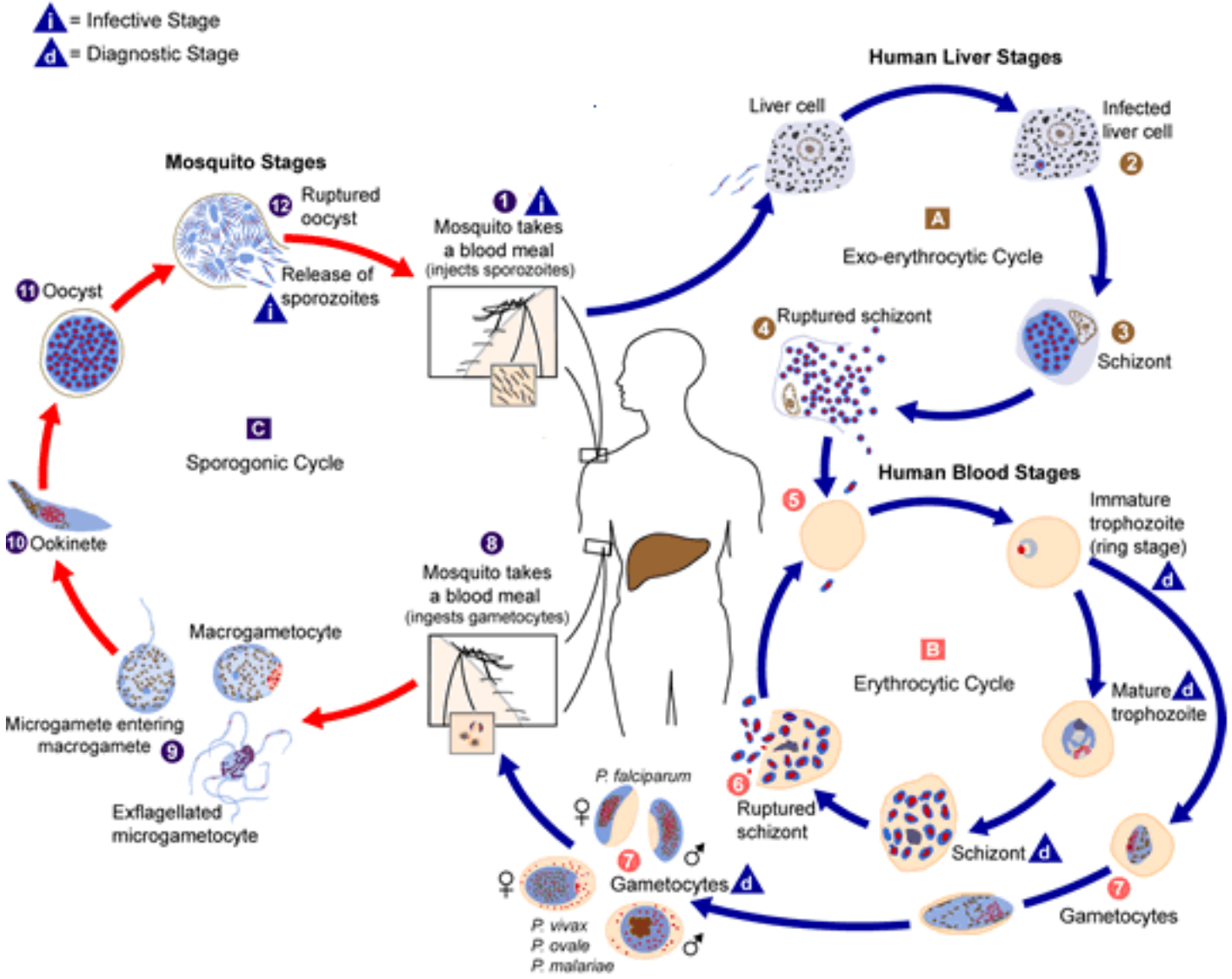
**Module Objective**

- The objective of this module is to understand biochemistry of eukaryotic parasites. The lecture will feature unique biochemical pathways found in parasites and their potential for exploitation as chemotherapeutic targets

# Protozoan parasites

## Life cycle of *Plasmodium*

- Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium*, and transmission is achieved through a mosquito vector of the genus *Anopheles*.
- Plasmodium species usually have strong specificity for the host species they infect .
- Four species of Plasmodium parasites predominantly infect humans: *P. falciparum*, *P. ovale*, *P. vivax* and *P. malariae*. Besides these, *P. knowlesi* which is a natural parasite of macaque monkeys has been found to infect humans commonly in some parts of Southeast Asia.


i = Infective Stage
d = Diagnostic Stage
Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
3
Schizont
4
Ruptured schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
d
Mature trophozoite
d
B
Erythrocytic Cycle
6
Ruptured schizont
Schizont
d
7
Gametocytes
Gametocytes
d
P. falciparum
P. vivax
P. ovale
P. malariae
8
Mosquito takes a blood meal
(ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
9
Exflagellated microgametocyte
10
Ookinete
11
Oocyst
12
Ruptured oocyst
Release of sporozoites
Mosquito Stages
1
Mosquito takes a blood meal
(injects sporozoites)
C
Sporogonic Cycle

- Infected anopheline mosquito injects sporozoites into the human host (1). Sporozoites move through the dermis and then the circulatory system to infect hepatocytes (2) where they undergo multiple rounds of replication generating thousands of merozoites and the infected hepatocytes mature into schizonts (3), which rupture and release merozoites (4).
- After this initial exo-erythrocytic replication cycle [A], the parasites undergo asexual multiplication in the erythrocytes [B].
- The intra-erythrocytic cycle begins with the invasion of merozoites into the erythrocytes (5).
- The ring stage trophozoites mature into schizonts, which rupture releasing merozoites (6).
- Some parasites differentiate into blood stage nonreplicating sexual forms called gametocytes (7).

- Male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are ingested by an anopheline mosquito during a blood meal (8).
- The parasite undergoes multiplication in the mosquito, the sporogonic cycle [C].
- While in the mosquito's stomach, the microgametes penetrate the macrogametes (9) generating diploid zygotes.
- The zygotes become motile and elongated (ookinetes) (10) which invade the midgut wall of the mosquito where they develop into oocysts (11).
- Mature oocysts rupture and release sporozoites (12), which may reinvade a host following an infectious bite (1).

- Haploid asexual reproduction of the parasite takes place throughout the life cycle except for a brief diploid sexual phase in the female *Anopheles* mosquito
- During mosquito blood feeding an infected mosquito inoculates sporozoites into the human host. Majority of the sporozoites are deposited under the skin and not directly injected into the circulation
- A significant proportion of sporozoites remain in the dermis. Sporozoites use gliding motility to migrate through the dermis tissue and to the liver via the circulatory system, and invade hepatocytes.
- About 30% of the sporozoites that leave the dermis take an alternative route through the lymphatic system. However, most of the sporozoites that invade the lymphatic vessels are trapped in the lymph nodes and despite some of them undergoing partial development similar to the liver stage of the parasite; they are eventually destroyed

- Depending on *Plasmodium* species, over a period of 5-16 days each parasite grows and divides mitotically into tens of thousands of merozoites within schizonts, which rupture and release into the blood circulation and then infect erythrocytes.
- Alternatively, some *P. vivax* and *P. ovale* parasites can remain dormant (hypnozoites) and persist in the liver, and these may cause relapses by invading the bloodstream weeks or years later.
- Blood stage parasites are responsible for the clinical manifestations (for instance, fevers and chills) of the disease.
- Erythrocyte-stage Plasmodium parasites undergo repetitive rounds of invasion, growth, and mitotic division every 24 hours (P. knowlesi), 48 hours (*P. falciparum*, *P. vivax*, and *P. ovale*), or 72 hours (*P. malariae*).
- In the erythrocytes the ring stage trophozoites mature into schizonts, which rupture releasing merozoites.

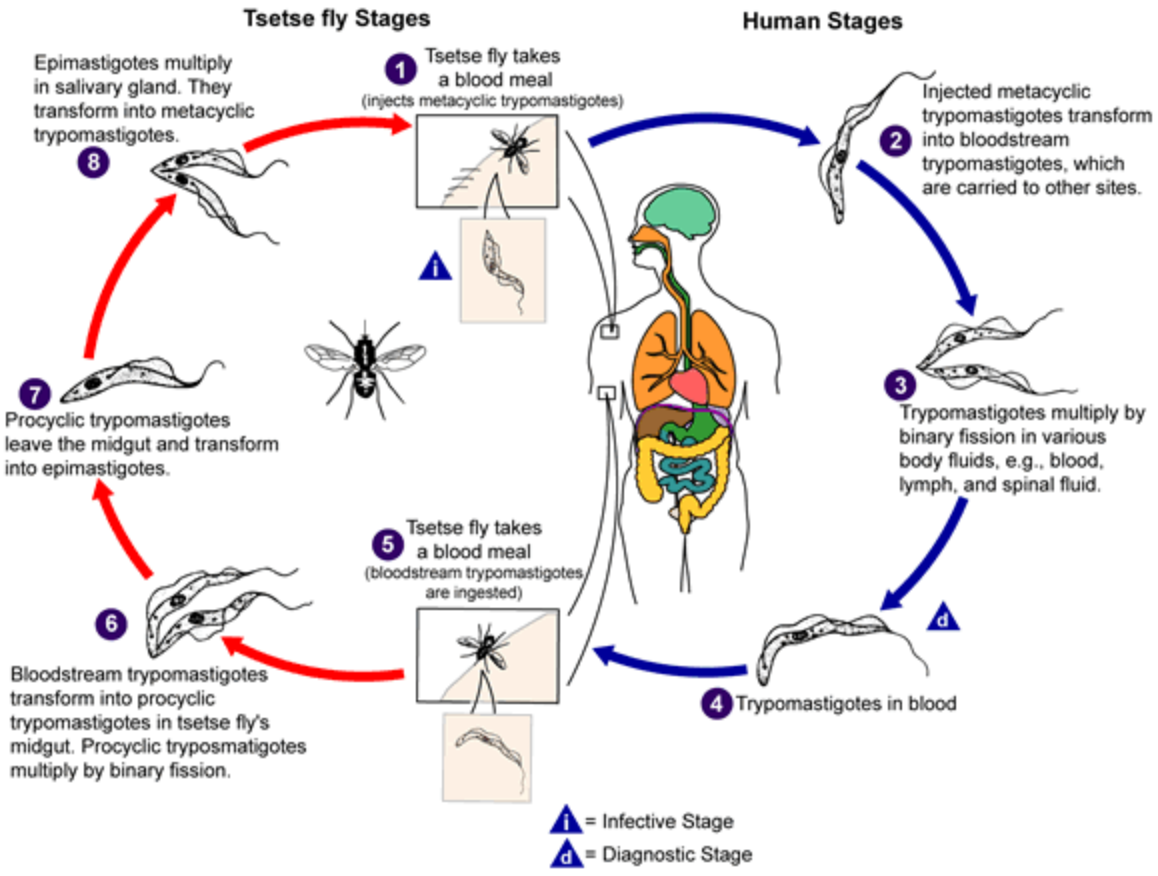
- Some parasites differentiate into sexual erythrocytic stages (gametocytes).
- The commitment to differentiate into male or female occurs in the cycle before the one in which gametocytes differentiate; as the progeny of a single schizont are all either male or female.
- The male gametocytes (microgametocytes) and female gametocytes (macrogametocytes) are ingested by an *Anopheles* mosquito during a blood meal.
- While in the mosquito's stomach, microgametocytes undergo exflagellation to form microgametes which penetrate the macrogametes generating diploid zygotes.

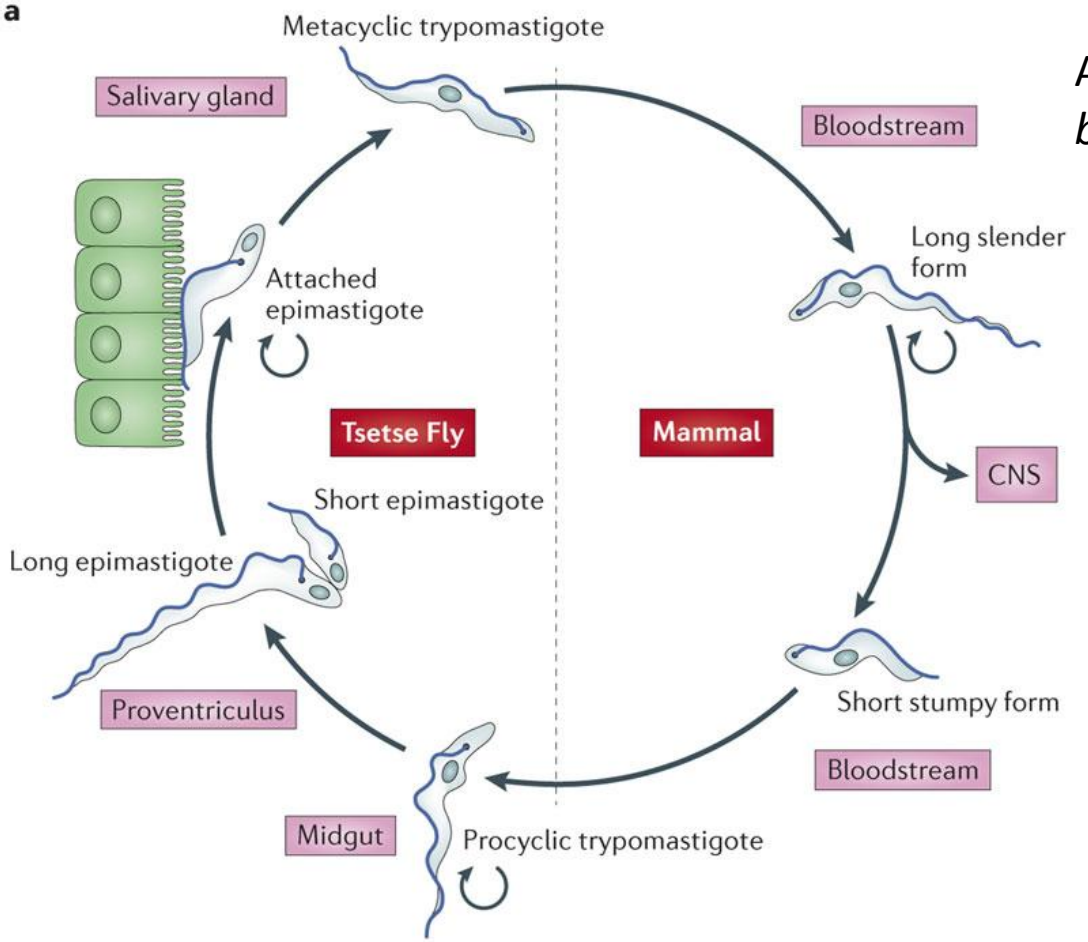
- These zygotes may be a product of fertilization between either genetically different or identical male and female gametes, when there is more than one genotype of parasite in the blood meal.
- The timing of the process of gametogenesis in female gametocytes is critical for fertilization to take place since the male gamete is short-lived.
- Gene disruption of female gametocyte-specific gene that is involved in gametocytes egress from erythrocytes while in the mosquito vector, undermines gametogenesis and may block infection.
- The zygotes formed after fusion of gametes become motile and elongated forming diploid ookinetes which invade the midgut wall of the mosquito where they develop into oocysts.
- Ookinetes divide meiotically to give haploid progeny that then replicate asexually with mitotic divisions within oocysts.

- The oocysts grow, rupture, and release haploid sporozoites that travel to the mosquito salivary glands.
- Inoculation of the sporozoites into a new human host during feeding initiates another infection.

# Life cycle of Trypanosomes

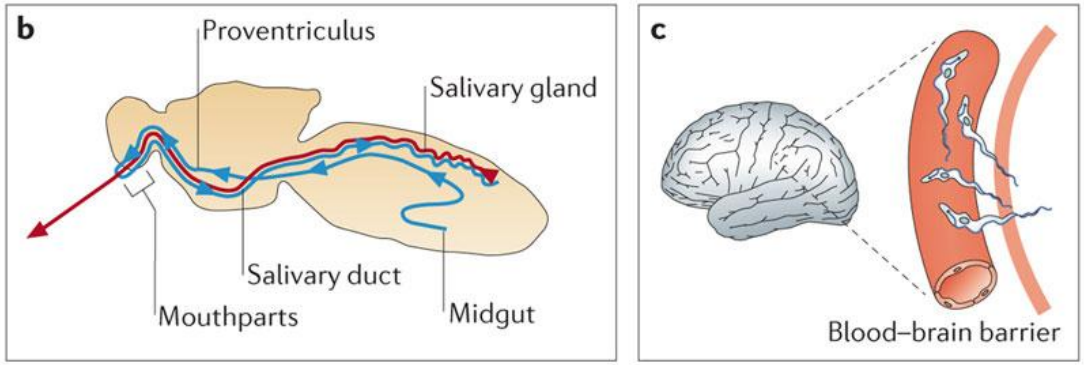
- These are Protozoan hemoflagellates belonging to the complex *Trypanosoma brucei*.
- The African Salivarian trypanosomes are the causative agents of both Human African Trypanosomiasis, or sleeping sickness, and Animal African Trypanosomiasis, more widely known as Nagana
- Two subspecies that are morphologically indistinguishable cause distinct disease patterns in humans: *T. b. gambiense* causes West African sleeping sickness and *T. b. rhodesiense* causes East African sleeping sickness.
- A third member of the complex, *T. b. brucei*, under normal conditions does not infect humans
- The animal infective species *T. congolense* and *T. vivax* are responsible for millions of livestock and wild animal infections across the continent.


Tsetse fly Stages
Human Stages
Epimastigotes multiply in salivary gland. They transform into metacyclic trypomastigotes.
8
1 Tsetse fly takes a blood meal
(injects metacyclic trypomastigotes)
i
2 Injected metacyclic trypomastigotes transform into bloodstream trypomastigotes, which are carried to other sites.
3 Trypomastigotes multiply by binary fission in various body fluids, e.g., blood, lymph, and spinal fluid.
d
4 Trypomastigotes in blood
5 Tsetse fly takes a blood meal
(bloodstream trypomastigotes are ingested)
6 Bloodstream trypomastigotes transform into procyclic trypomastigotes in tsetse fly's midgut. Procyclic tryposmatigotes multiply by binary fission.
7 Procyclic trypomastigotes leave the midgut and transform into epimastigotes.
i = Infective Stage
d = Diagnostic Stage




A generalized life cycle of *Trypanosoma brucei*

Can invade extravascular tissues, including the central nervous system (CNS)



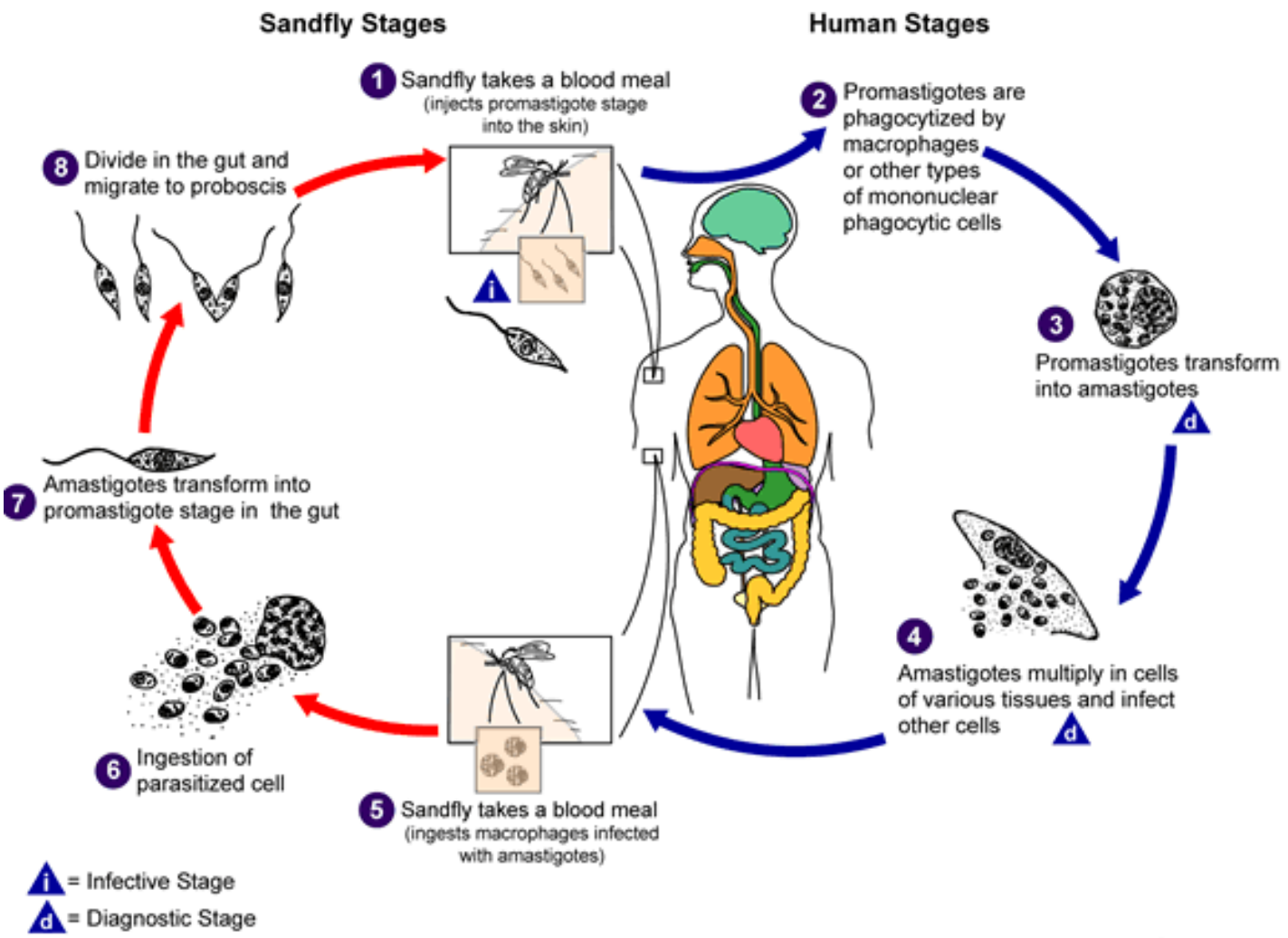

Parasites move out of blood vessels, penetrating the BBB to enter the CNS

- During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue.
- The parasites enter the lymphatic system and pass into the bloodstream .
- Inside the host, they transform into bloodstream trypomastigotes , are carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission .
- The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host.
- In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission , leave the midgut, and transform into epimastigotes .

- The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission .
- The cycle in the fly takes approximately 3 weeks.
- Humans are the main reservoir for *Trypanosoma brucei gambiense*, but this species can also be found in animals.
- Wild game animals are the main reservoir of *T. b. rhodesiense*.

# Life cycle of Leishmania

- Leishmaniasis is a vector-borne disease that is transmitted by sandflies and caused by obligate intracellular protozoa of the genus *Leishmania*.
- Human infection is caused by about 21 of 30 species that infect mammals.
- These include the *L. donovani* complex with 2 species (*L. donovani*, *L. infantum* ); the *L. mexicana* complex with 3 main species (*L. mexicana*, *L. amazonensis*, and *L. venezuelensis*); *L. tropica*; *L. major* and *L. aethiopica*.
- The different species are morphologically indistinguishable, but they can be differentiated by isoenzyme analysis, molecular methods, or monoclonal antibodies.


Sandfly Stages
Human Stages
1 Sandfly takes a blood meal
(injects promastigote stage into the skin)
2 Promastigotes are phagocytized by macrophages or other types of mononuclear phagocytic cells
3 Promastigotes transform into amastigotes
4 Amastigotes multiply in cells of various tissues and infect other cells
5 Sandfly takes a blood meal
(ingests macrophages infected with amastigotes)
6 Ingestion of parasitized cell
7 Amastigotes transform into promastigote stage in the gut
8 Divide in the gut and migrate to proboscis
i = Infective Stage
d = Diagnostic Stage

- Leishmaniasis is transmitted by the bite of infected female phlebotomine sandflies.
- The sandflies inject the infective stage (i.e. promastigotes) from their proboscis during blood meals .
- Promastigotes that reach the puncture wound are phagocytized by macrophages and other types of mononuclear phagocytic cells.
- Progmastigotes transform in these cells into the tissue stage of the parasite (i.e. amastigotes) , which multiply by simple division and proceed to infect other mononuclear phagocytic cells .
- Parasite, host, and other factors affect whether the infection becomes symptomatic and whether cutaneous or visceral leishmaniasis results.
- Sandflies become infected by ingesting infected cells during blood meals .

- In sandflies, amastigotes transform into promastigotes, develop in the gut (in the hindgut for leishmanial organisms in the *Viannia* subgenus; in the midgut for organisms in the *Leishmania* subgenus), and migrate to the proboscis .

# Comparative carbohydrate metabolism in Trypanosomes

- The blood stream trypomastigotes forms of *T. brucei* possess no energy stores, are unable to oxidize amino acids or fatty acids and are thus entirely dependent on an ~~exogenous~~ supply of carbohydrate for their energy production.
- In a pleomorphic *T. rhodesiense* infection, 2 morphological forms of the parasite exist: Long slender (LS) and short stumpy (SS).
- The ~~incomplete~~ oxidation of glucose is most marked in LS forms of an infection.
- These organisms catabolize glucose aerobically to ~~pyruvate~~, with traces of ~~$CO_2$~~ and ~~glycerol~~.
- Pyruvate is not catabolized further as the LS form lacks the enzymes ~~pyruvate dehydrogenase~~ and ~~Lactate dehydrogenase~~ (LDH)
- However, in the SS forms of *T. rhodesiense*, active enzyme systems for the oxidative decarboxylation of pyruvate have developed.

- These organisms produce a much more varied series of end products from glucose catabolism including ~~$CO_2$~~, ~~acetate~~ and ~~succinate~~.
- In *T. brucei* molecular oxygen serves as the terminal electron acceptor, resulting in the formation of $H_2O$.
- ~~Glycerol-3-phosphate~~ shuttle transports the reducing equivalents from NADH in the cytosol into the mitochondrion, where via a glycerophosphate oxidase system, it passes to $O_2$. This enzyme appears to be unique to salivarian trypanosomes.
- Under anaerobic conditions, LS blood stream forms continue to utilise glucose, as under aerobic conditions, but because the glycerophosphate oxidase is inoperative, the glucose is metabolized into equimolar amounts of pyruvate and glycerol.
- The procyclic (vector) forms undergo a metabolic switch, with the development of a fully developed mitochondrion with cristae and most of the Kreb's cycle enzymes although citrate synthase appears to be missing.
- In cultured procyclic forms, glucose is consumed at a slower rate than in the blood stream forms and pyruvate is oxidized further, the main end product being $CO_2$ , acetate and citrate.

- The enzyme NADH-fumarate reductase (FR) which can reverse the Kreb's cycle by reducing fumarate to succinate is present, a situation that also occurs in parasitic helminths.
- Succinate is also an end product of proline catabolism in the midgut stages of *T. brucei* which closely resemble procyclic culture forms.
- Utilisation of proline correlates well with environment provided by the tsetsefly as the haemolymph contains excessively high levels of proline although deficient in carbohydrates.
- In tsetseflies, therefore trypanosomes depend largely on proline for their energy, by developing NADH-fumarate reductase, they are able to produce succinate which may be used as the main respiratory substrate or as an electron sink to be excreted outside the cell under anaerobic conditions.

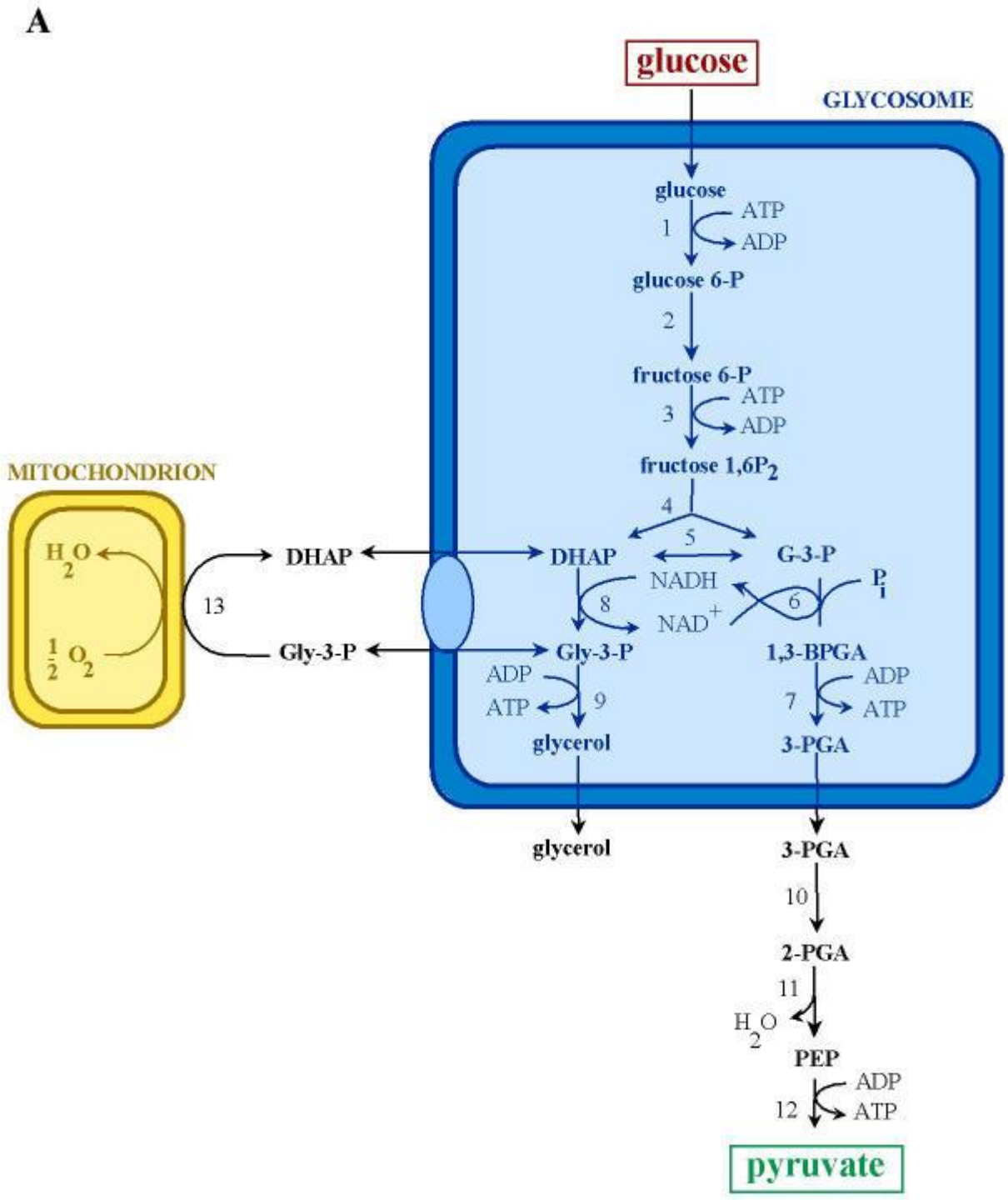



Figure A
The energy metabolism of bloodstream-form of *T. brucei*

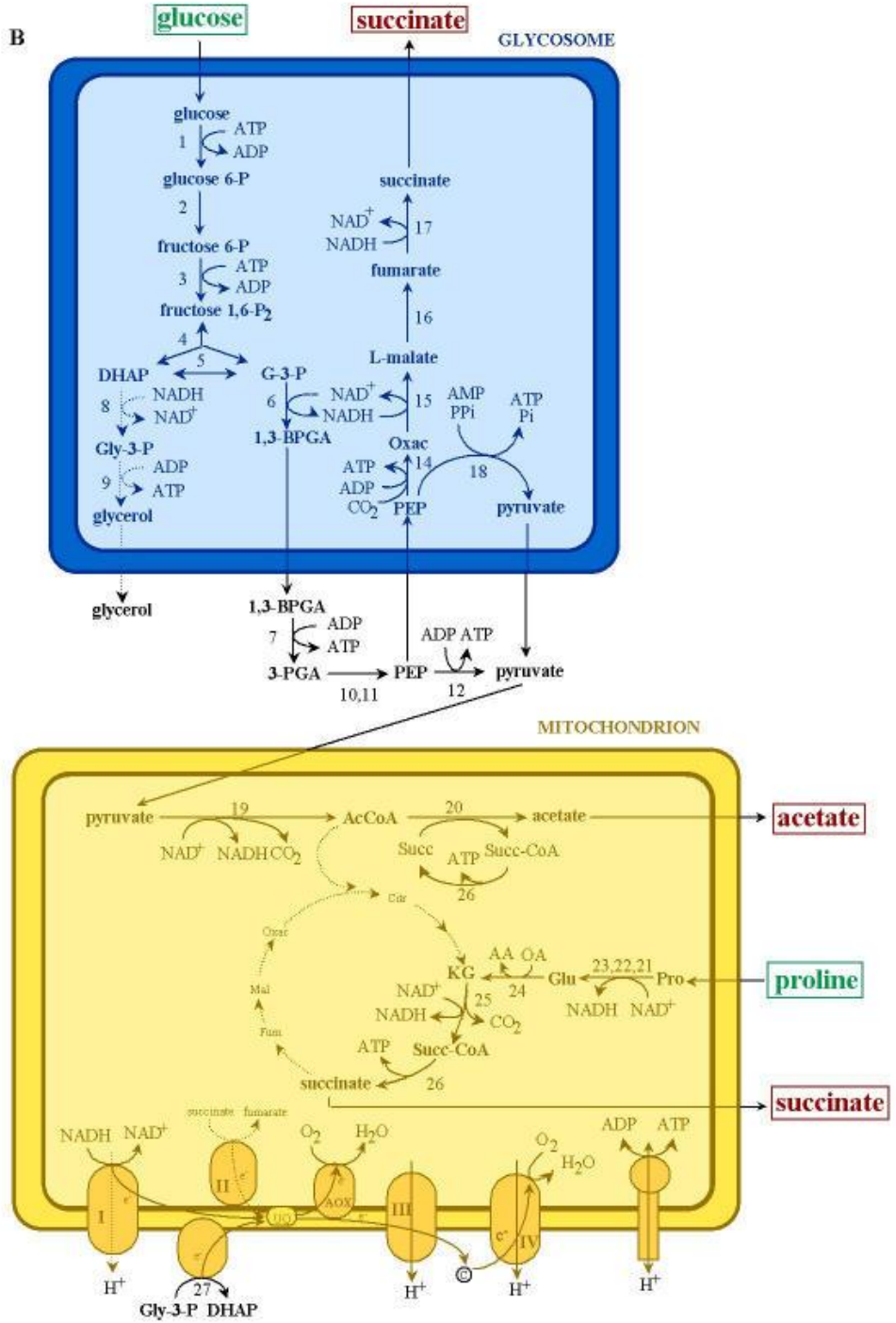



Figure B
The energy metabolism of Procyclic (vector) form of *T. brucei*

Abbreviations:
AA, amino acid; AcCoA, acetyl-CoA; 1,3-BPGA, 1,3-bisphosphoglycerate;
c, cytochrome c; Citr, citrate; DHAP, dihydroxyacetone phosphate;
Fum, fumarate; G-3-P, glyceraldehyde 3-phosphate;
Glu, glutamate; Gly-3-P, glycerol 3-phosphate; KG, α-ketoglutarate; Mal, malate; OA, 2-oxoacid; Oxac, oxaloacetate; PEP,phosphoenolpyruvate; 3-PGA, 3-phosphoglycerate; Succ, succinate; Succ-CoA, succinyl-CoA; UQ, ubiquinone.

# Enzymes

1, hexokinase;

2, glucose-6-phosphate isomerase;

3, phosphofructokinase;

4, aldolase;

5, triosephosphate isomerase;

6, glyceraldehyde-3-phosphate dehydrogenase;

7, phosphoglycerate kinase;

8, glycerol-3-phosphate dehydrogenase;

9, glycerol kinase;

10, phosphoglycerate mutase;

11, enolase;

12, pyruvate kinase;

13, glycerol-3-phosphate oxidase;

14, phosphoenolpyruvate carboxykinase;

15, L-malate dehydrogenase

16, fumarase;

17, fumarate reductase;

18, pyruvate phosphate dikinase;

19, pyruvate dehydrogenase complex;

20, acetate:succinate CoA transferase;

21, proline oxidase;

22, Δ'-pyrroline-5-carboxylate reductase;

23, glutamate semialdehyde dehydrogenase;

24, glutamate dehydrogenase;

25, α-ketoglutarate dehydrogenase;

26, succinyl CoA synthetase;

27, FAD-dependent glycerol-3-phosphate dehydrogenase.

- Substrates and secreted end-products are indicated in green and red, respectively, and boxed.
- Enzymes involved in reactions represented by dashed lines are present, but experiments indicated that no significant fluxes occurred through these steps
- A complex II is depicted because succinate dehydrogenase activity and succinate-dependent respiration have been demonstrated in mitochondria of *T. brucei* procyclics and *T. cruzi* epimastigotes. However the role of succinate respiration in the overall metabolism of these cells remains to be clarified.
- No evidence has been reported that electron transfer through complex I of the respiratory chain of trypanosomatids is coupled to proton expulsion. The mitochondrion contains two membranes; the inner membrane containing the respiratory chain and $H^+$-ATPase has been drawn in the figure above.

# Carbohydrate metabolism in Trichomonads

**Trichomonas vaginalis**

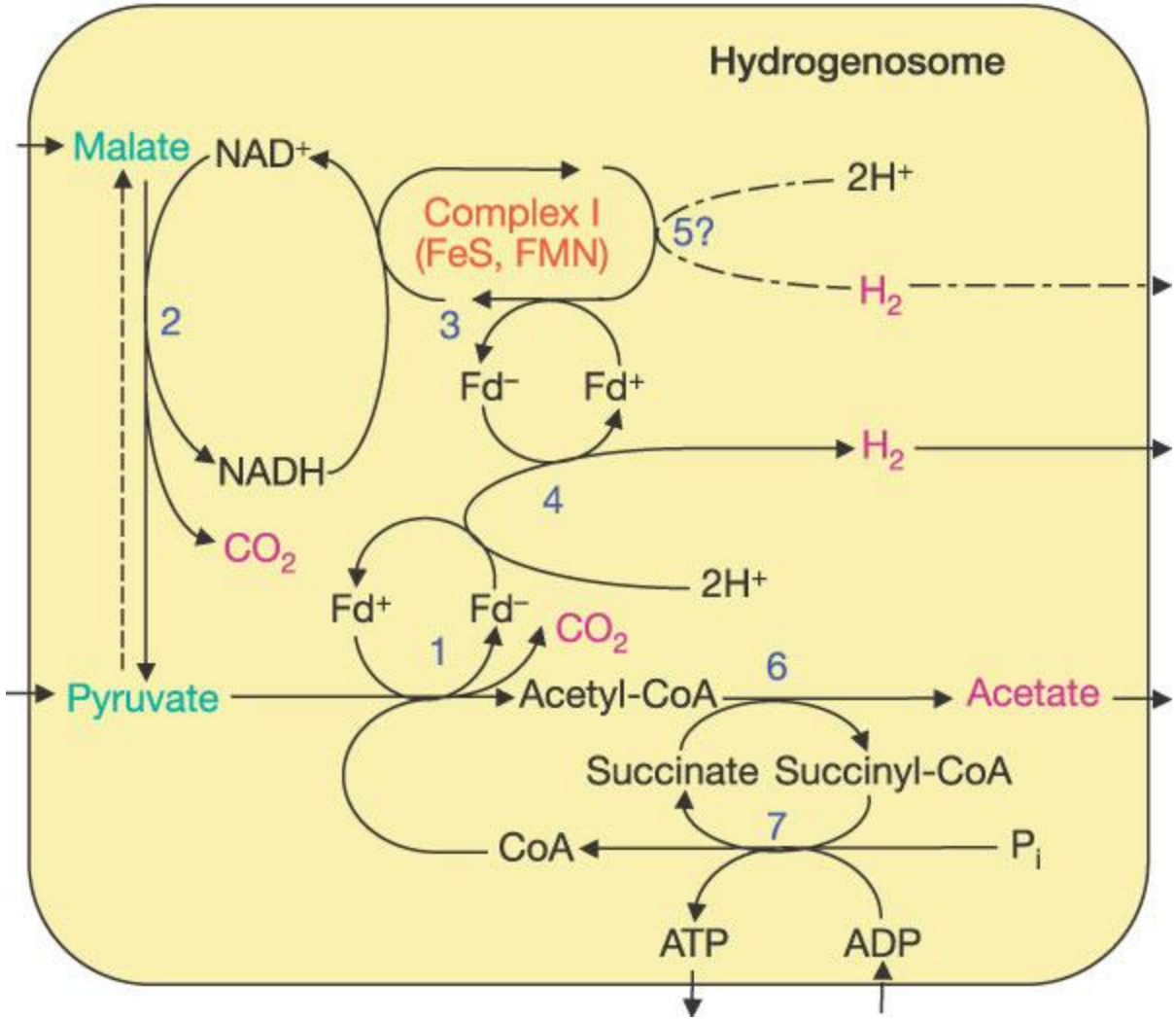
- Trichomonas lack mitochondria hence TCA and Electron transport is not as in other systems.
- But they contain a membrane bound organelle called hydrogenosome.
- This hydrogenosome constitutes a separate compartment of energy metabolism as mitochondria perform in other protozoa.
- Below is the metabolic 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+
NADH
H2
4
CO2
2H+
Fd+
Fd−
CO2
1
6
Pyruvate
Acetyl-CoA
Acetate
Succinate
Succinyl-CoA
7
CoA
Pi
ATP
ADP

# Enzymes

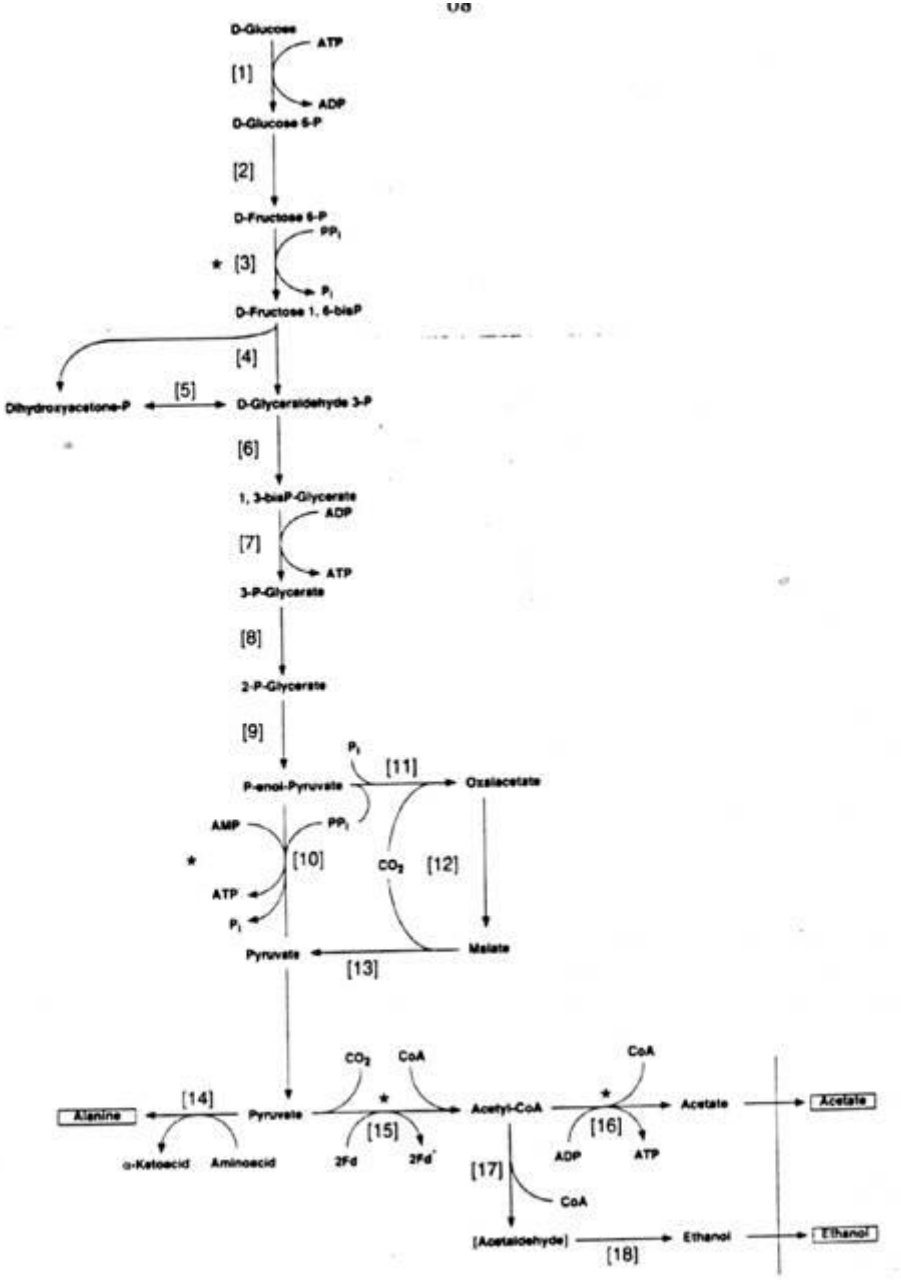
1. Pyruvate ferredoxin oxidoreductase
2. Malate dehydrogenase
3. NAD: Ferredoxin oxidoreductase
4. $H_2$: Ferredoxin oxidoreductase
6. Acetate: Succinyl CoA  transferase
7. Succinate thiokinase

- In *T. vaginalis* oxidative decarboxylation of Pyruvate to Acetyl CoA is catalyzed by a reversible enzyme called Pyruvate:Ferredoxin oxidoreductase instead of the reversible 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

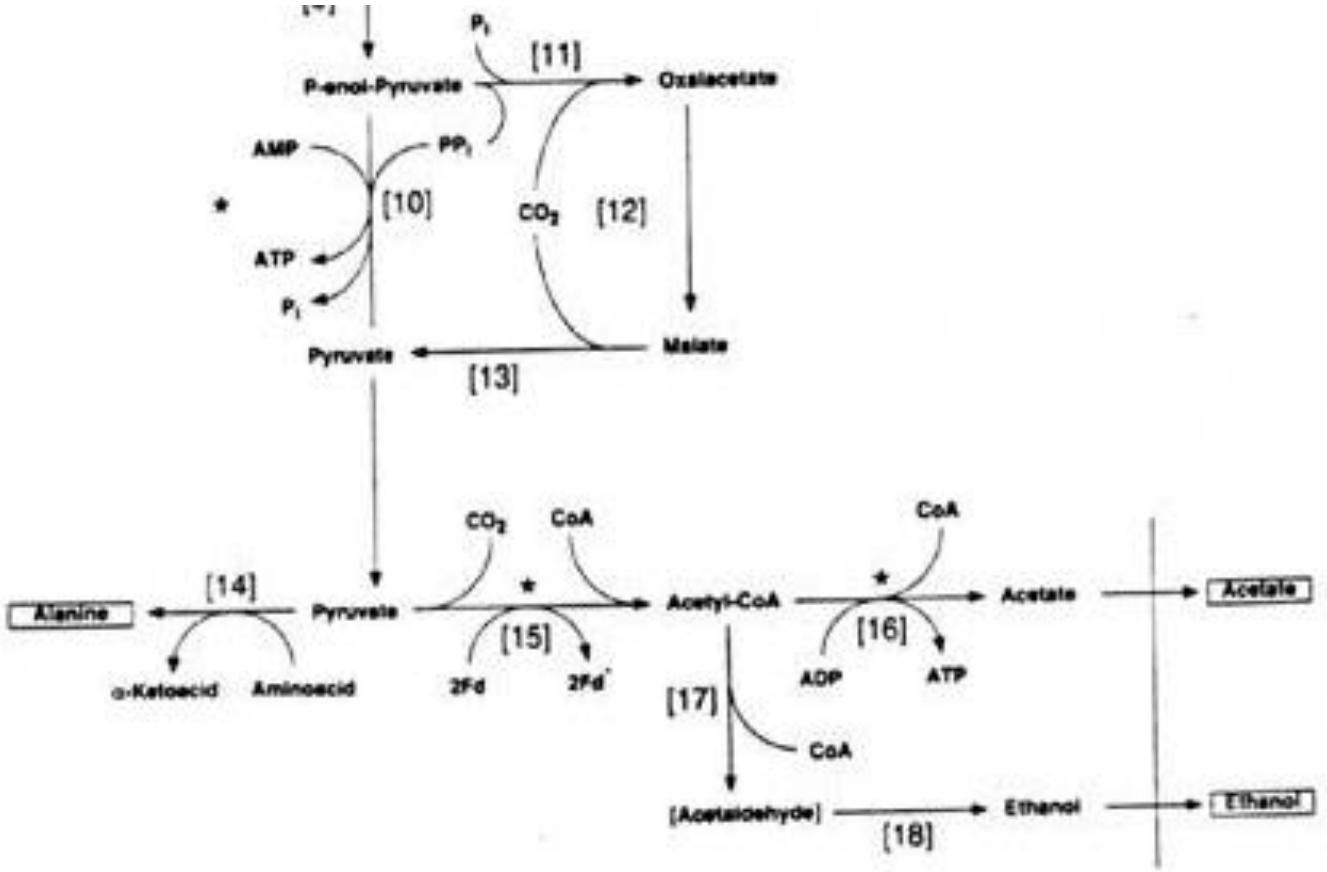
6, glyceraldehyde-3-P DH

7, Phosphoglycerate kinase

8, phosphoglyceromutase

9, Enolase

10, Pyruvate phosphate dikinase




Enzymes

10, Pyruvate phosphate dikinase

11, PEP carboxyphosphotransferase

12, Malate DH

13, Malic enzyme (decarboxylating)

14, Alanine aminotransferase

15, Pyruvate:Ferredoxin oxidoreductase

16, Thiokinase

17-18 Acetyl CoA reductase-alcohol dehydrogenase

# Carbohydrate metabolism in Leishmania

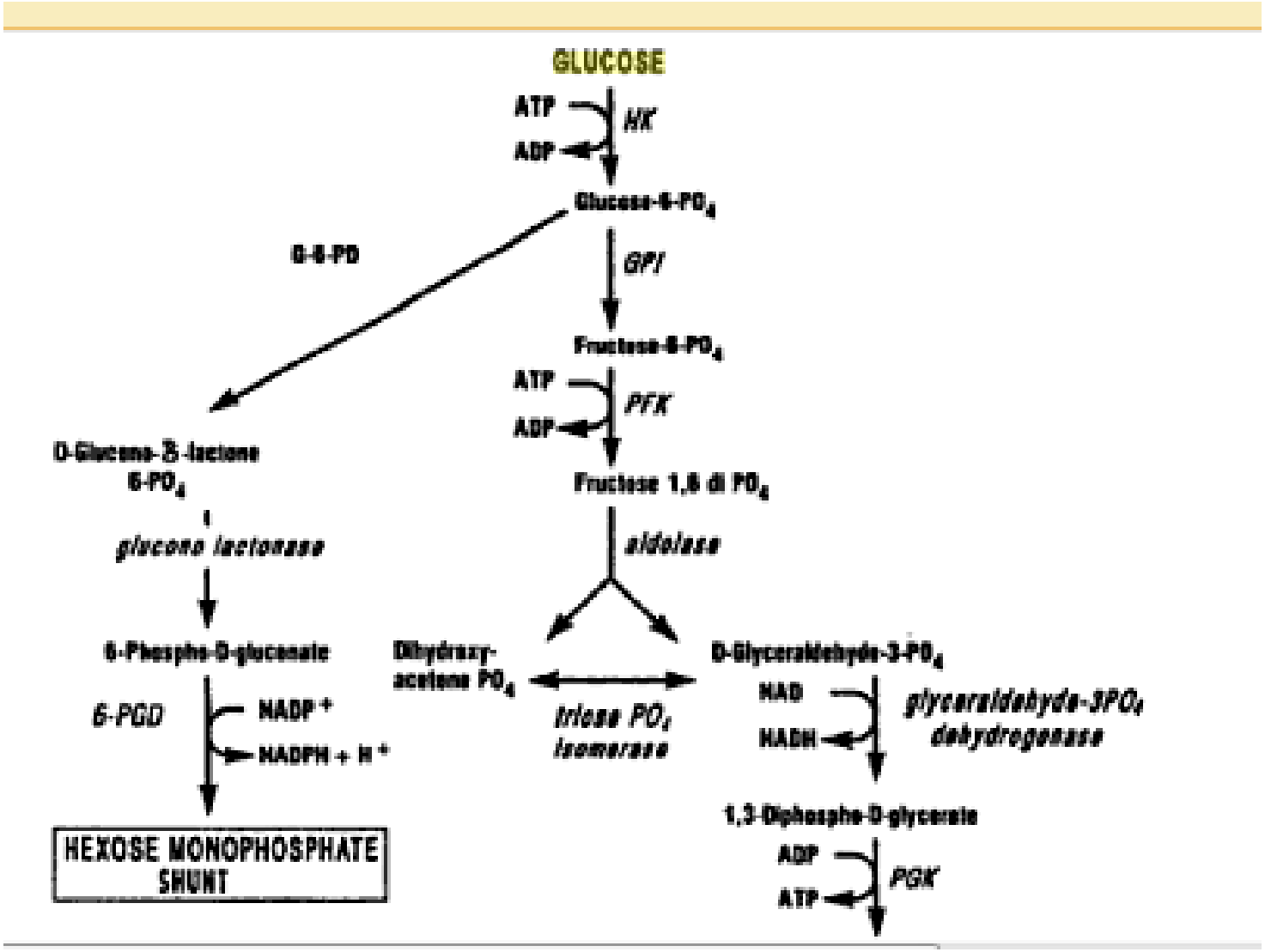
- Leishmania have a glycosome and mitochondria
- The glycosome is thought to play a major role in carbohydrate metabolism and like the glycosome of trypanosomes, Leishmania glycosome may contain all the early enzymes of glycolytic pathway.
- Carbohydrate metabolism follows early stages of glycolysis 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.~~

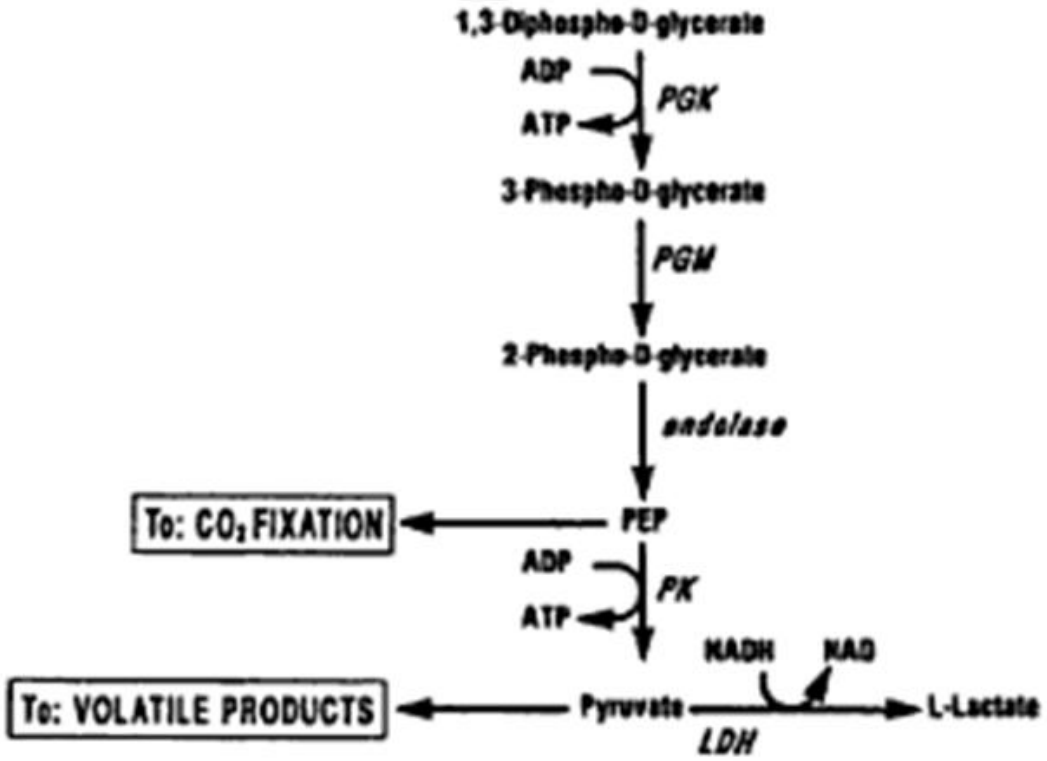
# Carbohydrate metabolism in *Plasmodia*

**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

- 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.

# 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*

# Nucleic acid metabolism

**Pyrimidine biosynthesis**

- Unlike some flagellate protozoa (e.g. Trichomonas), *Plasmodium* is able to synthesise pyrimidines *de novo*, although the erythrocyte is unable to do so.
- Salvage mechanism is unnecessary for survival

**~~Purine salvage~~**

- A functional *de novo* biosynthesis pathway for purines has not been identified in any Plasmodium species
- A purine salvage pathway operates
- The purine preferred by *P. falciparum* is hypoxanthine

# Purines, Pyrimidines and their salvage

- There is almost no evidence to suggest that either the parasitic protozoa or the parasitic helminths are capable of synthesizing the purine ring out of simple precursors (i.e. are not able to synthesize purines *de novo*).
- The precursors of purine synthesis in organisms that do have the capacity are: ~~CO2, formate, glycine, aspartate and~~ glutamine.
- In the absence of de novo synthesis, it is not very surprising to discover that parasites have elaborate systems for securing and interconverting purine derivatives of host origin.
- The reactions that result in the interconversion of purine derivatives are collectively known as salvage pathways.
- Analysis of extracts of parasites (studies in malaria parasites and schistosomes) show that they contain the same range of purines and purine derivatives that are found in free-living organisms.

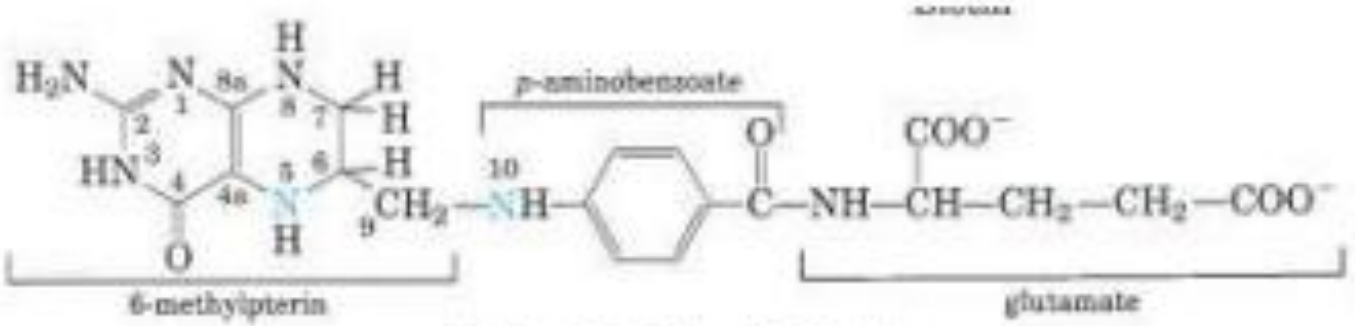
- The processes of purine salvage and the loss of *de novo* purine synthetic activity are parasitic adaptations that release the organism from the necessity of maintaining a large suite of enzymes to sustain a highly energy demanding synthetic pathway.
- Most parasitic protozoa and all helminths seem to be capable of synthesizing pyrimidines de novo from carbamoyl phosphate and aspartate
- Carbamoyl phosphate synthase is a key enzyme in pyrimidine synthesis.

# Metabolism of cofactors
# Folic Acid Metabolism

- Folic acid is very important in metabolism because it is involved in transfer of one carbon group from one compound to another during biosynthetic processes.
- The one carbon groups carried include: ~~methyl, methylene, methenyl, formyl and formimino groups.~~

**Structure**

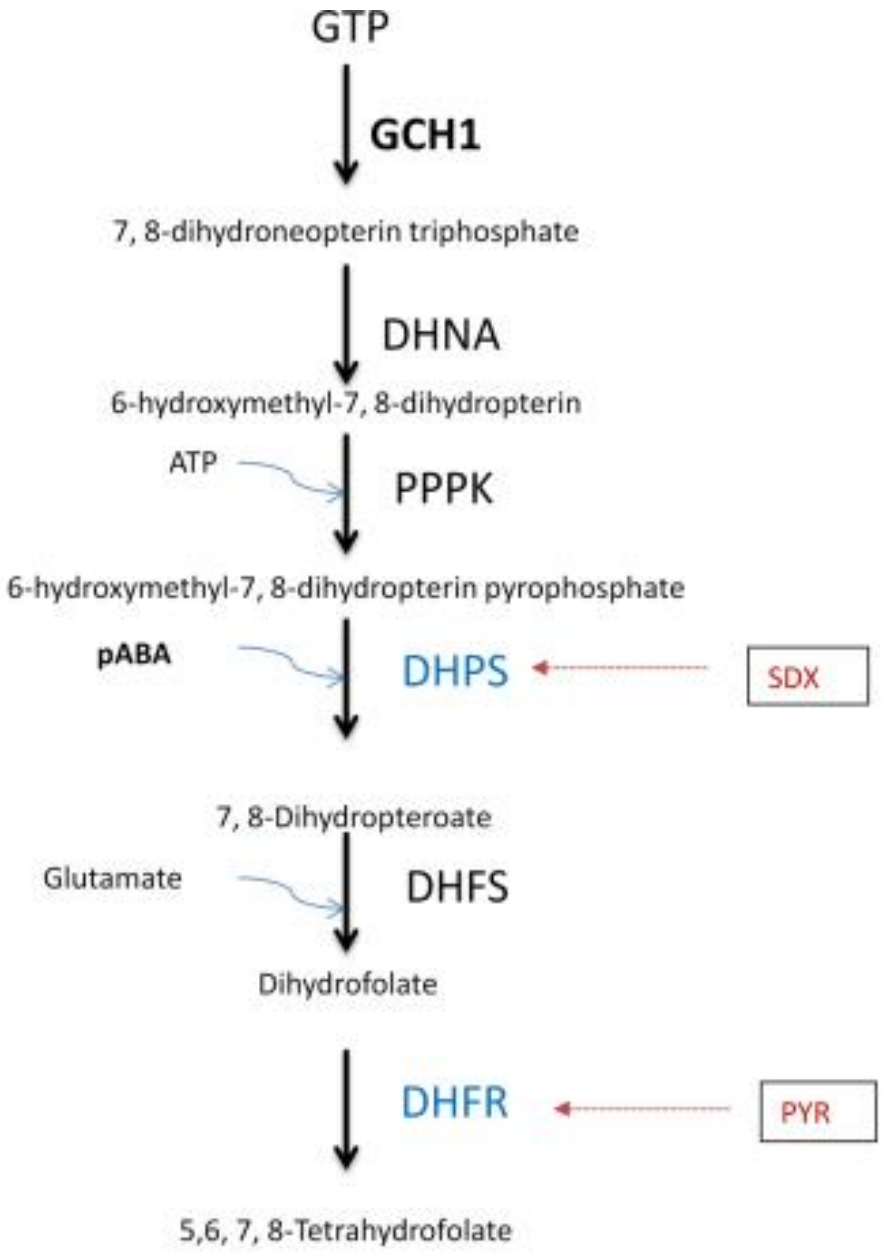
Tetrahydrofolate (also called tetrahydropteroylglutamate), consists of three groups: a ~~substituted pteridine, p-aminobenzoate, and a glutamate residue.~~




Tetrahydrofolate ($H_4$ folate)

# Role

- ~~Folic acid is required for purine and pyrimidine metabolism~~
- Folate metabolism is extremely important for metazoans and is highly conserved in all metazoans.
- An important difference in folic acid metabolism and mammalian host is in Filariae.
- Filaria can oxidise 5-methyl Tetrahydrofolate to 5,10 Methylene Tetrahydrofolate. This reaction doesn't occur in the host.
- This may be an adaptation with the parasite to provide them with an ~~additional substrate~~ other than folate.




GCH1, GTP cyclohydrolase I ;
DHNA, dihydroneopterin aldolase;
PPPK, hydroxymethyldihydropterin pyrophosphokinase ;
DHPS, dihydropteroate synthase ;
DHFS, dihydrofolate synthase ;
DHFR, dihydrofolate reductase.

- 5,10 Methylene Tetrahydrofolate is a substrate for thymidylate synthetase, which is involved in the conversion of dUMP to dTMP
- dTMP can be converted to dTTP required for nucleic acid synthesis.
- The reaction is very important for the parasites that cant synthesise the nucleotide *de novo* or salvage it.
- However, parasites employing de novo purine synthesis require 5,10 Methylene Tetrahydrofolate and such parasites include Filariae.
- Because of this importance of purine metabolism, interruption of folate supply in metabolism will inhibit growth and reproduction in parasites.
- Intracellular protozoa and pathogenic bacteria synthesise Tetrahydrofolate de novo from ~~GTP, PABA and glutamate,~~ whereas mammalian host don't have this pathway.

- Mammals get folate from their diet which they directly reduce to tetrahydrofolate.
- This substantial difference in the source of tetrahydrofolate has proved useful in chemotherapy since analogs of ~~PABA competitively inhibit the enzyme dihydropteroate synthase (DHPS) and this diminishes the rate of tetrahydrofolate~~ synthesis.
- ~~Sulfonamides~~ e.g. sulfathiazole, sulfaquinidine, sulfanilamide, sulfadoxine etc and sulfones e.g. dapsone, will inhibit the enzyme in Plasmodia spp as well as other intracellular species of sporozoans such as Toxoplasma and Eimeria.
- Another important chemotherapeutic target in folic acid metabolism is the enzyme dihydrofolate reductase.
- Methotrexate which is a structural analog of pteridine derivative inhibits ~~dihydrofolate reductase (DHFR).~~
- Methotrexate is used in cancer chemotherapy. Since the drug is moderately selective it causes toxicity problems in the host.

- The drug is not generally taken up by most bacteria or protozoa because this organisms don't have a specific transport mechanism for the drug.
- However this drug is a potent inhibitor of *P. berghei* DHFR. It will also inhibit the growth of Leishmania species *in vitro*.
- Pyrimethamine is a derivative of 2, 4 diamino pyrimidinyl moiety of methotrexate. It is a powerful antimalarial which is highly selective and therefore widely used.
- Plasmodial DHFR is a 1000× more sensitive to inhibition to this compound compared to mammalian enzyme. However, Leishmanial enzyme is less sensitive.
- Other inhibitors of DHFR used against Plasmodia include: Trimethoprim, Proguanil, Clociguanil etc
- Trimethoprim is also active against trypanosomes *in vitro* but not in vivo. Because trypanosomes in vivo seemingly have a higher concentration of DHFR is their cells.

- Suramin inhibits DHFR of *T. brucei brucei*.
- The enzyme from filaria worms O. volvulus appears to be different among metazoans because it is particularly sensitive to suramin.
- Combination of agents that attack both of these enzymes in the folate metabolism act synergistically because pathway inhibition is achieved by inhibiting two sequential sites in a reaction series.
- Important malarial treatments employing this principle are: Maloprim (pyrimethamine and dapsone), Fansidar (Pyrimethamine and sulfadoxine) and Metakelfin.
- Toxoplasmosis has also been effectively treated with high concentrations of pyrimethamine and sulfonamide. In this case the host is protected from inhibition of the DHFR by supplementation of Leucovorin (reduced folate)
- Leucovorin cant be utilised by the toxoplasma but can only be utilised by mammalian cells.

# Potential Drug Targets

- Parasitic protozoa multiply rapidly in the host. Most undergo differentiation and maturation in the host.
- Metabolism associated with cell division e.g. Nucleic acid metabolism and compounds involved in other metabolism e.g. polyamines hence these are potential targets.
- Most helminths invade the host after their last stage of reproduction hence no proliferation.
- In helminths proliferation takes place in specialized reproductive tissues and inhibiting such proliferation would not necessarily affect the disease process, though it may interrupt the life cycle.
- Some useful targets in helminths are like muscular activity and neuromuscular coordination, nutrient uptake.
- Other targets include: interparasite communication, essential energy metabolism etc.

- Helminths are close to humans than in protozoa hence protozoa will be having more unique receptors than helminths

**Drug Targets in Parasites**

- In a broad sense the type of drug targets can be categorised according to the kind of metabolism that can be targeted.
- Complication is that many compounds affect more than one process. e.g. Suramin has multi-targets
- In addition many compounds attack different taxonomic groups hence no justification that they will work in the same way in all the groups.

**Drug targets in energy metabolism**

- Because of heavy reliance in glycolysis for the provision of energy, the enzymes involved are different.
- Hexokinase and PFK are rate limiting steps and feedback inhibition targets them but in *T. brucei* there are no such feedback inhibitions.

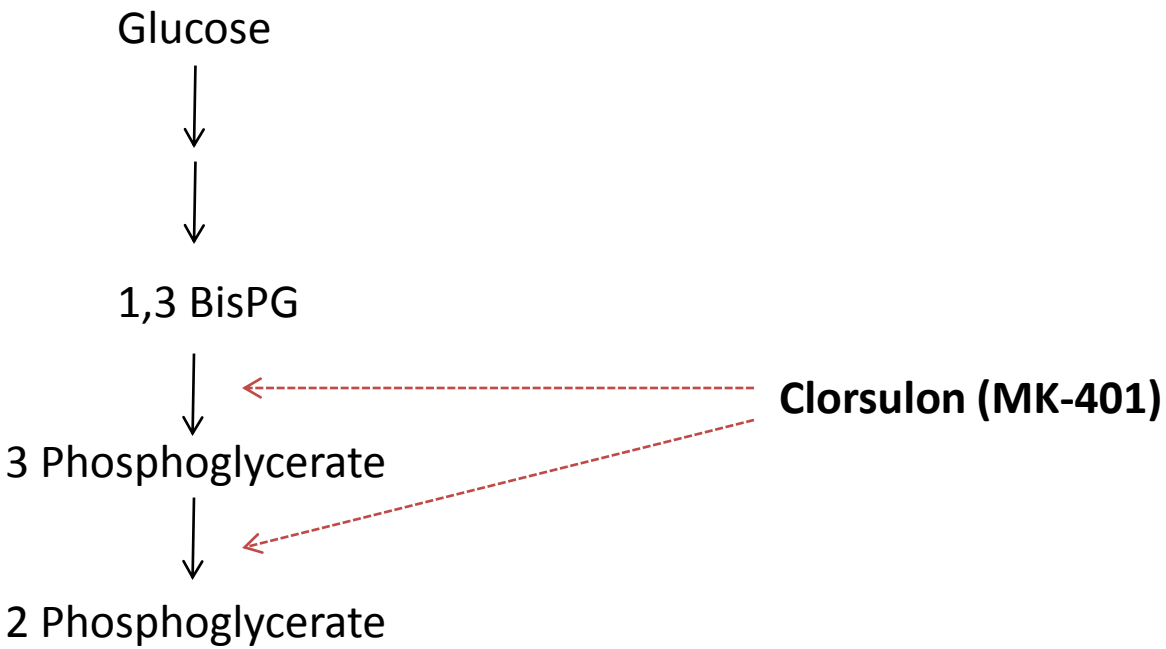
# Drug targets in carbohydrate metabolism

- It is possible to design a molecule that specifically inhibits glycolytic pathway in the parasites.
- Since TCA cycle is absent or restricted hence inhibition of glycolytic pathway will be detrimental.
- Rate controlling enzymes (RCE) are very attractive targets for attack.
- ~~In parasites it will be the glucose transport system from the environment or for parasites that store glycogen it will be the 1st step of breakdown of glycogen i.e. glycogen phosphorylase.~~
- ~~Glycogen phosphorylase is controlled by covalent~~ modification which is affected by external signals.
- A number of chemotherapeutic agents affect the glycogen phosphorylase

e.g. In schistosoma mansoni, a drug Niridazole affects glycogen phosphorylase phosphatase (enzyme responsible for inactivating glycogen phosphorylase).

- Because of this inhibition there is disruption of glycogenolysis and this leads to increase in rate of the glycogen breakdown hence the reserves will be depleted hence the parasite starves.
- ~~Levamisole~~ is antihelminthic drug which affects the control of glycogen metabolism. It has disruptive effects on nematode *in vitro.* It is used in treatment in *L. carinii* and *A. suum.*
- Levamisole increases glycogen synthase activity and inhibits glycogen phosphorylase activity. Therefore, the mobilisation of glycogen is affected at a time when ATP levels are diminished as a result of initial muscular paralysis induced by Levamisole.
- The precise molecular site of action has not been identified

- **~~Clorsulon (MK-401)~~** is a structural analog of 1,3 Bisphosphoglycerate
- It inhibits 2 enzymes of glycolysis in *F. hepatica***.** The enzymes are ~~phosphoglycerate kinase and phosphoglycerate mutase.~~




- The net result is the inhibition of glucose utilisation and end product formation.

- In the host MK-401 binds to the carbonic anhydrase in RBC. The blood feeding parasites ingest the RBC and liberate the compounds which are active during digestion hence affected more than the host.
- ~~Glycolytic kinases are particularly sensitive to organic antimony compounds. e.g. 6-phosphofructokinase of *S. mansoni* is a lot more sensitive to two of these antimony compounds i.e. Stibophen and Antimony potassium tartarate~~.
- Parasite from adult filarial worms is also sensitive to Stibophen.
- Antimony organic compounds, Melarsen and Melarsen oxide inhibit pyruvate kinase of African trypanosomes both *in vivo* and *in vitro*.
- In Leishmania mexicana Hexokinase, pyruvate kinase, PFK and malate DH are inhibited by Melarsen oxide