Anaerobic Protist Metabolism

***Giardia*, *Entamoeba* and Trichomonads**

Review glycolysis and oxidative decarboxylation

Parasite Biochemistry (in general)

- Parasitic life style = ADAPTATIONS
- Specific niche = diversity in adaptations
  - Anaerobic vs. aerobic
- All parasites still require a supply of energy for biosynthesis of macromolecules, growth, mechanical activity, reproduction etc.
- A major nutritional requirement is supplied by the host.
- Limited range of biosynthetic pathways - evolution of salvage pathways - no purine (A, G) biosynthetic pathway in any parasites - only salvage pathways.

Amitochondriate Biochemistry

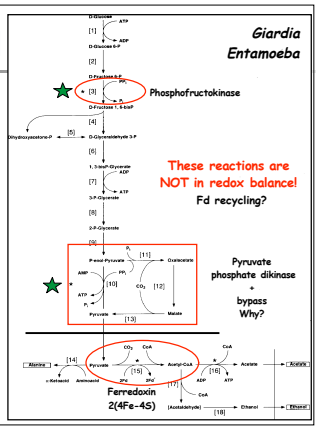
- Lumen dwelling - environment is O_2 low
- Anaerobic or microaerophilic organisms
  - Do not require O_2 for survival and multiplication
  - O_2 is not the terminal electron acceptor
  - Can tolerate low O_2 conc., growth is inhibited under higher O_2 conc. (aerotolerant anaerobes)
- Fermentative metabolism
  - In the presence or absence of O_2
  - Different end products
- Metabolic oddities
  - PPi-linked enzymes (instead of ATP)
  - Fe-S based carbohydrate catabolism

Synthesis of ATP

- Oxidative phosphorylation - coupling of ATP formation to the respiratory chain (electron transport, membrane associated, O_2 as final e- acceptor). As electrons move through complexes, a proton gradient is generated which drives ATP formation. **Chemiosmotic theory - P. Mitchell, 1978.**
- Substrate level phosphorylation - direct phosphorylation of ADP via the transfer from a high-energy intermediate.

Glycolysis I

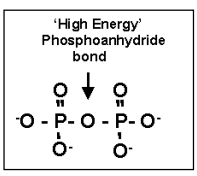
- Overall scheme is similar to aerobic eukaryotes
- Enzymes that utilize PPi instead of ATP
- Glycolytic control points are lacking - high flux through glycolysis
- 2 routes to produce pyruvate: direct + bypass
- Fe-S proteins for pyruvate decarboxylation
- Fd more like anaerobic bacteria



PPi vs. ATP

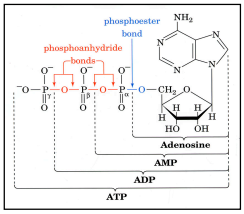
Inorganic pyrophosphate

'High Energy' Phosphoanhydride bond



PPi

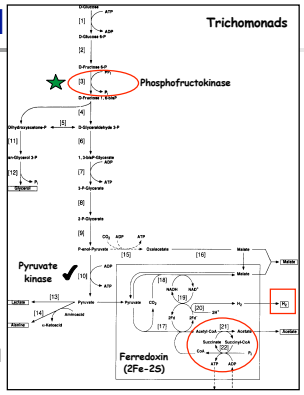
Adenosine Triphosphate



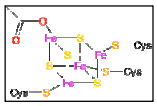
ATP

Glycolysis II

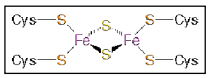
- PPi phosphofructokinase
  - No regulation
- 2 routes to produce pyruvate: direct + bypass
  - Slightly different
- PEP is a key metabolite instead of acetyl-CoA
- Pyruvate catabolism is compartmentalized
- Fe-S proteins for pyruvate decarboxylation
- Fd more like mitochondrial



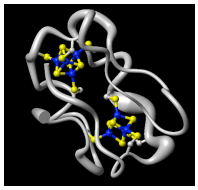
Differences in ferredoxins



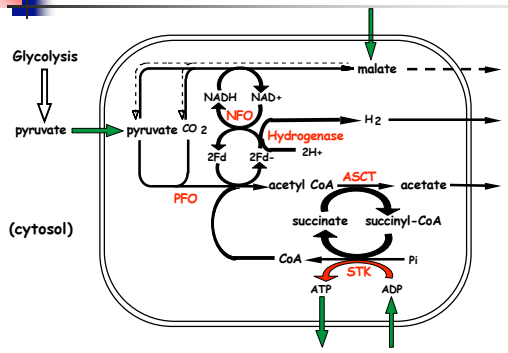
2 [4Fe-4S]



[2Fe-2S]



Hydrogenosomal Metabolism

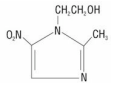


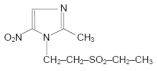
Important Enzymes

- PFO - pyruvate ferredoxin oxidoreductase
  - pyruvate + CoA + (ox)ferredoxin = acetyl-CoA + CO_2 + (red) ferredoxin
- NFO - NAD(P)H ferredoxin oxidoreductase
  - reduced ferredoxin + NAD+ = oxidized ferredoxin + NADH + H+
- Hydrogenase
  - reduced ferredoxin + 2 H+ = oxidized ferredoxin + H_2
- Acetate:succinate CoA transferase
  - Acetyl-CoA + succinate = succinyl- CoA + acetate
- Succinate thiokinase (succinyl-CoA synthetase)
  - ADP + phosphate + succinyl-CoA = succinate + CoA + ATP

Metronidazole

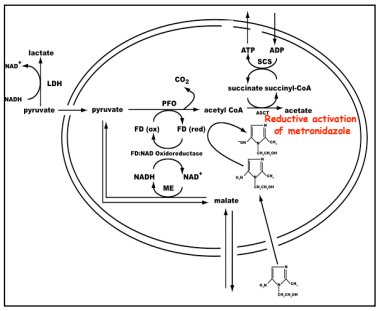
- Rare example of a drug developed for parasitic disease that gained broader use as an antibiotic.
- Extracts of *Streptomyces* were screened for activity against *T. vaginalis*.
  Azomycin was identified (nitroimidazole).
- Synthetic derivative, Metronidazole, became major drug used for treatment (Flagyl).
- Soon after, was also found effective against *Giardia* and *Entamoeba*.
- 1970's: became useful for treatment against anaerobic bacteria.





Metronidazole Activation

Prodrug requiring a reductive environment



Mode of action:
Reduced in anaerobic organisms
to reactive metabolites which release superoxide anions.

Little toxicity to human - due to aerobic respiration.

Review

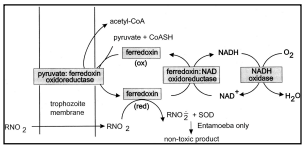
- Glycolysis
  - Compare/contrast mammalian with parasitic
- Mitochondrial respiration
  - Compare/contrast aerobic respiration with anaerobic protist metabolism
  - Future lectures with include variations on the “aerobic” theme.
- KEY TO REMEMBER
  - Adaptations for specific niche
  - Unique biological properties

Carbohydrate Metabolism is Eukaryotes

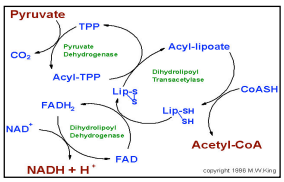
Hexose	Hexose	Hexose
Pyruvate	Pyruvate	Pyruvate + O_2
CO_2 + Acetate	CO_2 + H_2 + Acetate	CO_2 + H_2O
No compartmentalization	Hydrogenosomal compartmentalization	Mitochondrial compartmentalization
Substrate level phosphorylation	Substrate level phosphorylation	Oxidative phosphorylation

Comparison of Pyruvate Decarboxylation

PFO complex?



PDC complex



How to Find New Drugs

1. Random screening
   - no design or biological insight
2. Analogues of known drugs
   - not a new target
3. Rational lead discovery
   - long time & expensive

If you need any of the pathways

enlarged please email me