Helminth Parasite Biochemistry

- Parasitic life style = ADAPTATIONS
- All parasites still require a supply of energy for biosynthesis of macromolecules, growth, mechanical activity, reproduction etc.
- Major nutritional requirements are supplied by the host, - abundant, not limiting.
- Limited range of biosynthetic pathways - evolution of salvage pathways - no purine (A, G) biosynthetic pathway in any parasites - only salvage pathways.
- Specific niche = diversity in adaptations
  - Aerobic vs. Anaerobic - important transitions
  - Free-living forms, nutrients are limiting
  - Full range of biochemical reactions.

Helminth Biochemistry

- Lumen or tissue dwelling adults
  - Generally a lower O₂ environment
- Variations of fermentative metabolism
  - In the presence or absence of O₂
- Different end products
- Range of metabolic oddities
  - Anaerobic mitochondrial
- Anaerobic or microaerophilic organisms
  - Do not require O₂ for survival and multiplication
  - O₂ is not the terminal electron acceptor
  - Can tolerate low O₂ conc
  - Growth is inhibited under higher O₂ conc.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Environment</th>
<th>Metabolic rate</th>
<th>Temperature</th>
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</thead>
<tbody>
<tr>
<td>UE</td>
<td>Egg, microaerobic → aerobic</td>
<td>Low</td>
<td>Ambient</td>
</tr>
<tr>
<td>L1</td>
<td>Egg, aerobic</td>
<td>High</td>
<td>Ambient</td>
</tr>
<tr>
<td>L2</td>
<td>Egg, aerobic</td>
<td>Quiescent, low</td>
<td>Ambient</td>
</tr>
<tr>
<td>L3 (hooklet)</td>
<td>Gut, microaerobic</td>
<td>High</td>
<td>36-47°C</td>
</tr>
<tr>
<td>L4</td>
<td>Gut, microaerobic</td>
<td>High</td>
<td>36-47°C</td>
</tr>
<tr>
<td>L4-8</td>
<td>Gut, microaerobic → anaerobic</td>
<td>High</td>
<td>36-47°C</td>
</tr>
</tbody>
</table>

Oxygen environment - lumen

- Gradient of O₂ availability - microenvironments
- Diversity depending on size of the worm
- Marked aerobic to anaerobic transition
**Synthesis of ATP**

- Oxidative phosphorylation - coupling of ATP formation to the respiratory chain (electron transport, membrane associated, O2 as final e- acceptor).
- Substrate level phosphorylation - direct phosphorylation of ADP via the transfer from a high-energy intermediate.
- Linked to branched-chain fatty acid production

**Helminth Glycolysis**

- Early steps are identical to mammals
- Large glycogen stores
- Utilization of glycogenolysis
  - Tightly regulated
- Two main routes from PEP
  - Lactate production
    - Pyruvate kinase
    - Excreted
  - Malate production
    - PEP carboxykinase
      - Not excreted, transported tomitochondria

**Comparative Mitochondrial Metabolism**

1. Phosphoenolpyruvate carboxykinase
2. Transporter
3. Malate
4. No TCA
5. Production of branched-chain fatty acids
Mitochondrial Metabolism

- Malate dismutation
- Branched pathway
  - Oxidation
  - Dismutation
- Fumarate reduction is linked to electron transport

Branched Electron Transport

Larval aerobic

Adult anaerobic

Modifications of Classic Aerobic Mitochondria for Anaerobic Energy Generation in Body Wall Muscle of the Adult Parasitic Nematode Ascaris suum

- Deletion or reduction of nonessential activities
  - Ubiquinone
  - Complex III (ubiquinone:cytochrome c oxidoreductase)
  - Reduced cytochrome c oxidase
  - Citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase
- Overexpression of key enzyme
  - Pyruvate dehydrogenase complex (PDC)
  - ETF:Rhodoquinone oxidoreductase (ETF:RO)
  - 2-methyl branched chain enoyl CoA reductase
- Altered kinetics
  - Apparent Kms for pyruvate and CoA of the PDC
  - Fumarate stimulation of Malic enzyme
- Expression of novel components
  - Rhodoquinone
  - Protein Y of the PDC
- Expression of anaerobic-specific isoenzymes
  - E1α subunit of the PDC
  - 2-methyl branched chain enoyl CoA reductase
  - Others???
**Pyruvate Decarboxylation**

- **PDC complex**

  - E1
  - E2
  - E3
  - Acetyl-CoA
  - Pyruvate

**SDS-PAGE of Purified PDC:**

1 - bovine kidney PDC
2 - Ascaris suum adult muscle PDC

**Developmental regulation**

- Figure 5: Immunoblot of A. suum larval homogenates with antisera against the adult A. suum E2, p45, E1alpha, and E1beta. Larval homogenates and the affinity-purified antisera were prepared as described under Experimental Procedures. E2 and p45 antisera were used at dilutions of 1:5000 and 1:2000, respectively. E1alpha and E1beta antisera were used at dilutions of 1:5000. UE, unembryonated egg (150 μg); L1 (150 μg); L2 (150 μg); L3 (80 μg); and M, adult muscle PDC (1 μg). *, rabbit IgG; L3 are recovered from rabbit lungs and homogenates often contain rabbit IgG.
Regulation by Phosphorylation

Ascaris suum E1α inactivation

Review

- Glycolysis
  - Compare/contrast mammalian - comparative but with lactate production

- Mitochondrial respiration
  - Compare/contrast aerobic respiration with anaerobic helminth metabolism

KEY TO REMEMBER

- Adaptations for specific niche
- PDC is a big regulatory point is mammals
- PDC is adapted for function under anaerobiosis

Comparison

Aerobic

- Triglyceride
- Fatty acids

Pyruvate

Acetyl CoA

Enolpyruvyl CoA

α-Ketoglutarate

Phosphoenolpyruvate

CO₂

NADH

NAD⁺

Anaerobic

- Malate

Malate dismutation

Malate

Fumarate

Succinate

Acetyl CoA

Propionyl CoA

2-Methyl branched-chain enoyl CoA

3-Methyl branched-chain enoyl CoA

3-Methyl branched-chain acyl CoA

Branched-chain fatty acids

NADH

NAD⁺
Specialized Electron Transport

Parasite Chemotherapy

- Why so important?
  - Absence of vaccines
  - Vector control is difficult
- What is the basis of selectivity?
  - Uptake of drug
  - Activation of drug
  - Detoxification of drug
  - Importance of drug target
  - Binding to drug target
  - Unique drug target

Paul Ehrlich (1854-1915)
- “chemotherapy”
- Magic bullet
- Idea of selectivity
- Selective dyes
  - Chemotherapeutic index: maximum dose tolerated by host / minimum curative dose

Idea of a Magic Bullet

“If we picture an organism as infected by a certain species of bacterium, it will be easy to effect a cure if substances have been discovered which have a specific affinity for these bacteria and act on these alone while they possess no affinity for the normal constituents of the body... such substances would then be... magic bullets.”

Paul Ehrlich

Observation: Chemical dyes
- Methylene blue (1901)
- Trypan red (1904)
- Compound 606 (1910)

Interacted with particular cells or tissues in specific ways
- Idea of specific affinity (selectivity)
Parasite Chemotherapy

- Is chemotherapy the perfect solution?
  - Re-infection in endemic areas
  - Few drugs 100% effective
  - Drug Active against only a few stages
  - Parasite resistant to Drug
  - Drug cannot reach migrating parasite
  - Some (most) drugs expensive
  - Serious side effects
  - Many cannot be given orally

Properties of an Ideal Anti-parasitic

- Information based on the huge interest of veterinary market - driving force especially for anthelmintics!
  - Should possess a wide margin of SAFETY (men, women, children, fetus).
  - MINIMAL TOXICITY (tolerable side effects)
  - FEW CONTRAINDICATIONS (drug-drug interactions)
  - BROAD SPECTRUM of activity (all disease species including resistant lines)
  - EFFICACIOUS (relatively short treatment, <14 days)
  - RESISTANCE (low potential for drug resistance)
  - EASY Administration (orally active, avoid needles or hospitalization)
  - AFFORDABLE (diseases of the poor; ~$1 per patient)
  - STABLE (2 years shelf life at 40 °C, 75 % humidity)

Current State of Parasite Drug Development

- Protozoa
  - Military Interest
    - Malaria
    - Leishmaniasis
  - Immunocompromised
    - Cryptosporidiosis
    - Toxoplasmosis
  - Bioterrorism
    - Select agents
      - Cryptosporidium
      - Cyclospora
      - Giardia
      - Entamoeba
      - Toxoplasma

- Helminths
  - Veterinary Market - Huge
    - Cattle Industry
    - Companion animals
    - Sheep Industry
    - Equine Industry
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

Discovery of Ivermectin

- Top 40 Pharmaceuticals
  - Drugs that changed our world - Aspirin - Viagra

- Ivermectin - A Wonder Drug
  - 25 years and still going strong
  - High potency (as low as 1 nM)
  - Extremely safe
  - Broad spectrum use
  - Various formulations
  - Single annual dose (slow release)
  - Few reports of resistance
  - ~$1 billion USA industry - the most highly effective antiparasitic drug ever introduced

Wonder Drug Highlights

- 1972 - Dr. Satoshi Omura, proposed important chemicals exist in fermentation products of microbes.
  - 1972 - Formed partnership with Merck, Sharp and Dohme (MSD)
  - 1974 - Second year of collaboration - isolated organism that produced compound with high antihelminth activity.
  - 1988 - first mass drug donation program
Professor Satoshi Omura

- President of Kitasato Institute
- More than 40 years of studying bioactive compounds from microbes
- Successful Philosophy
  - Unlimited supply of novel compounds
  - Produce gold-standard screening
  - Screening is not just an exercise
  - Contribution of basic research
  - Keep the human connection
- Huge success
  - ~1 in 3 soil isolates have produced antimicrobial substances!

"Success by being able to stand on the shoulders of giants" - Sir Isaac Newton

Discovery of Ivermectin

- Screened 40,000 samples - basic curiosity are there compounds that microbes make that can inhibit the worms?
- Found one that worked! (in vitro studies)
  - Japanese golf course - Dr. Satoshi Omura
  - Surprisingly powerful against the worms
  - Streptomyces avermectis produced compounds they called avermectins
- Division of Merck (MSD) - drugs to treat parasitic worms in animals (in vivo studies)
  - Simple screen
    - Infected mice with worms
    - Fed worms cultures of microbes from soil
    - Soil samples from around the world

Dr. William Campbell
Dr. Mohammed Aziz

Ivermectin - Broad Spectrum

- Class of compounds called 
  Avermectins - macrocyclic lactones
- Lacked antibacterial and antifungal properties
- Ivermectin is the most widely used drug in veterinary medicine
  - Control nematodes
  - Control arthropod infestations
- Led to the most important contribution of the pharmaceutical industry
- Mectizan Donation Program
  - Mectizan would be provided free of charge for as long as needed.

"Medicine is for the people. It is not for profits" - George W. Merck
Ivermectin - Mechanism of Action

- Ivermectin works by acting as a potent agonist at Glutamate-gated chloride ion channels.
- In nematodes and arthropods, γ-aminobutyric acid (GABA) sends inhibitory signals to motor neurons.
- Ivermectin potentiates these inhibitory signals and this results in the paralysis of the parasite.
- In mammals, GABA receptors are confined to the CNS. Ivermectin does not cross the blood brain barrier, so it does not cause paralysis.

Simplified schematic representation of the invertebrate and vertebrate chloride ion channels under avermectin control

Parasite

<table>
<thead>
<tr>
<th>Glutamate (C. elegans)</th>
<th>Ivermectin (High Affinity)</th>
</tr>
</thead>
</table>

Host

<table>
<thead>
<tr>
<th>Mammalian (Pit Brain)</th>
<th>GABA</th>
<th>Ivermectin (Low Affinity)</th>
</tr>
</thead>
</table>

Prevents closure of ion channel
Hyperpolarization of neurons

Ivermectin and Oncocerciasis

- Mid 1970's - avermectins did not work against hookworms or tapeworms
- 1980 Dr. Mohammed Aziz - observation that ivermectin killed a horse parasite
- Simple clinical study
  - Skin snips prior to treatment - high numbers of microfilaria
  - Single dose of Mectizan - skin snip in 1 month - NO microfilaria
  - Skin snips 3 months later - still NO microfilaria!
  - First evidence for a single annual dose of Mectizan
- 1987 French government approved use in humans
- Eradication was a possibility - Mectizan donation program was initiated.
Onchocerciasis Control Program

- Good News - Bad News
- OCP ended in 2002
- Conclusion - Onchocerciasis cannot be eradicated in most endemic areas with the current tools
- What is needed:
  - New drug regimens
  - Macrofilicicides
  - Better diagnostics
- Overlap with another program African Program for Onchocerciasis Control (1995)
  - Aim: by 2007 create a sustainable community-directed distribution system for ivermectin in over 17 African nations

Analogues of Known Drugs

Diethylcarbamazine

- Synthetic organic compound with no toxic metallic components
- Tissue and blood nematodes (filarial worms)
- Hyperpolarizing neuromuscular blockade
  - Paralysis of worm
- Headache, malaise, nausea, inflammation
- Most useful in a combined treatment regimen
Adult worms do not multiply in the mammalian host.

The most effective chemotherapeutic targets have been:

**Motility**

**Energy Metabolism**

### 1. Drugs affecting motility
- Worms have complex nervous systems
- Active motility is essential for the worms to resist expulsion by bowel peristalsis

### 2. Drugs affecting energy generation
- Enteric helminths exist in an anaerobic environment, and have developed special mechanisms for generating energy.

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<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Nucleic acid synthesis</th>
<th>Protein synthesis</th>
<th>Metabolism</th>
<th>Energy metabolism</th>
<th>Neutrophilic function</th>
</tr>
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<th>Different animal species</th>
<th>Drugs affecting motility</th>
<th>Drugs affecting energy generation</th>
<th>Drug resistance in host and parasite</th>
<th>Anthelminthic effect</th>
<th>Mechanism of action</th>
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13
Scheme for Rationale Drug Design

Year 1
- Identify key biochemical feature
- Show feature important to parasite survival
- Purify/clone & resolve 3D structure of target
- Design & synthesis inhibitors
- Screening of target in vitro
- Validate target in vivo: animal models & trials

Year ~6
- Approval & launch of new drug

Year 20

Compound Success Rate by Stages

- Preclinical
- Clinical
- Approval
- Withdrawal

Success 1 in 5,000
6 years
2 years
5-10 years

Cost >$800 Million

Source: Pharmaceutical Industry Profile 2001

New Antihelminth Drugs

Potency of Compounds

Dosage (mg/kg)

Phenothiazine
Organophosphates
Thiabendazole
Pyrimethamine
Granulam
Ivermectin
Moxidectin
Abamectin
Boronectin

Year of Introduction

New helminth drugs:
- What target?
- Any better potency?
- Any incentives?