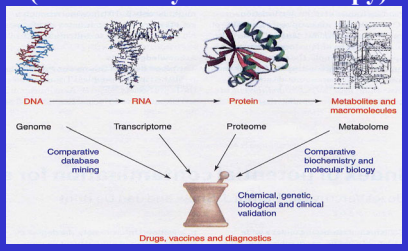
Biomedical Parasitology

Parasite metabolism

(biochemistry & chemotherapy)



Prof Peter O'Donoghue

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Biochemistry

Host biochemistry

- monitoring electrolytes (homeostasis)
- performing organ function tests

Parasite biochemistry

- determining pathogenic mechanisms
- prelude to drug development

Both require understanding of metabolism

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Chemicals of life

Living organisms consist of:

- water
- proteins
  (synthesized from amino acids)
- lipids
  (synthesized from fatty acids)
- carbohydrates/polysaccharides
  (synthesized from simple sugars)
- nucleic acids
  (synthesized from purine/pyrimidine nucleotides)

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Metabolism

Animal metabolism involves:

- catabolize organic substances
  to derive chemical energy
- assemble low MW precursors
  into polymeric components
- form and degrade biomolecules
  for specialized functions

anabolism = synthesis (requires E)
catabolism = breakdown (produces E)
both require enzyme co-factors (metal ions & NAD, nicotinamide adenine dinucleotide)

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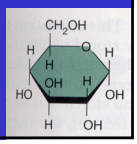
CARBOHYDRATES

Blood glucose

- ingested foods digested by enzymes
- taken up by small intestine
- stored in liver as glycogen
- metabolism under hormonal control (insulin)
- major disorder
  - diabetes (hyper/hypo-glycaemia)



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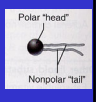
LIPIDS

Comprise:

- long-chain fatty acids
  (stored as triglycerides - lipoproteins)
- phospholipids (constituents of membranes)
- cholesterol
  (precursor of steroid hormones, bile acids)



- insoluble, rely on proteins for transport
- synthesized in most tissues (esp. liver)
- problems - hyperlipidaemia (viscous plasma)

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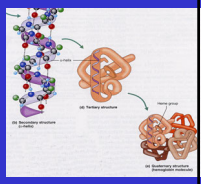
PROTEINS

Polymers composed of up to 20 amino acids



Classified according to structure:
- primary (amino acids)
- secondary (α-helix)
- tertiary (folding)
- quaternary (combination)

or classified according to chemical class
- simple (amino acids)
- conjugated (metalloproteins, nucleoproteins, lipoproteins, phosphoproteins, glycoproteins)

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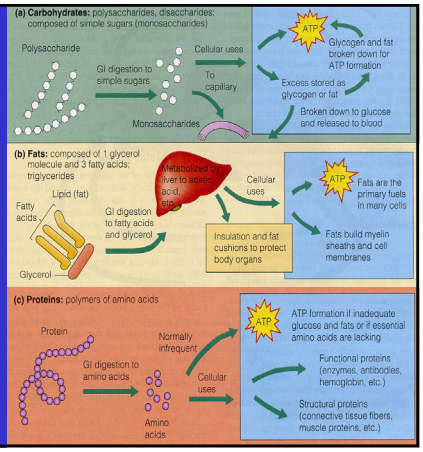
Metabolism

burn:
- sugar
- fat
- protein



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Cellular energy pathways

Five groups of energy-producing reactions

1. glycolysis
2. pyruvate oxidation
3. citric acid cycle
4. respiratory chain
5. fermentation

} cellular respiration (require oxygen)

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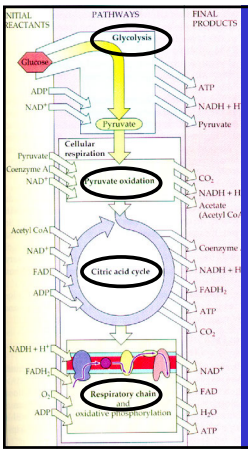
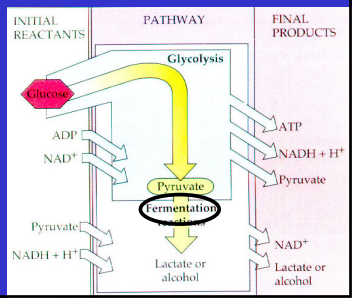


Energy production (5 sources)



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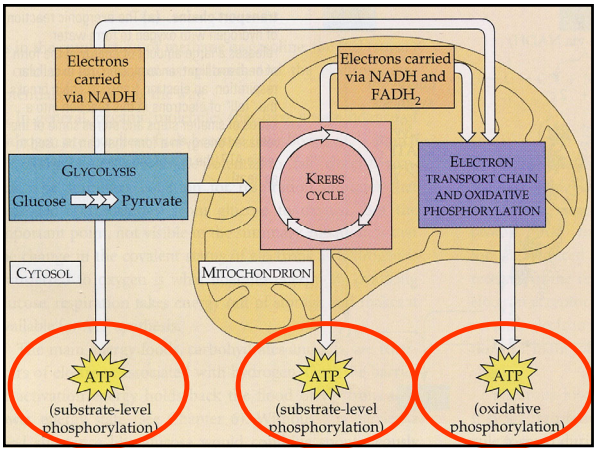

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Metabolic pathways

Blood biochemistry can monitor:

- electrolytes (osmotic balance, pH)
- carbohydrates (energy supply)
- lipids (biosynthesis, stores)
- ketones (excretion)
- serum proteins (buffer, balance)
- enzymology (organ dysfunction)

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Electrolytes

- important for osmotic balance, pH buffering, regulation of membrane permeability
- cations
  - sodium Na, potassium K
  - calcium Ca, magnesium Mg
- anions
  - chloride Cl
  - bicarbonate HCO_3
- elements
  - phosphorus P, copper Cu, zinc Zn, iron Fe

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Carbohydrates

Blood glucose

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Lipids

Comprise:

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- phospholipids (constituents of membranes)
- cholesterol (precursor of steroid hormones, bile acids)

- insoluble, rely on proteins for transport
- synthesized in most tissues (esp. liver)
- problems - hyperlipidaemia (viscous plasma)

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Ketones

Ketone bodies include:

- acetone
- acetoacetic acid

- excreted in body fluids
- metabolic products of breakdown of fatty acids
- liver important organ
- increased levels indicate dysfunction

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Serum proteins

Classified according to structure:

- primary (amino acids)
- secondary (α-helix)
- tertiary (folding) - monomers
- quaternary (combination) - dimers, etc

or classified according to chemical class

- simple (amino acids)
- conjugated (metalloproteins, nucleoproteins, lipoproteins, phosphoproteins, glycoproteins)

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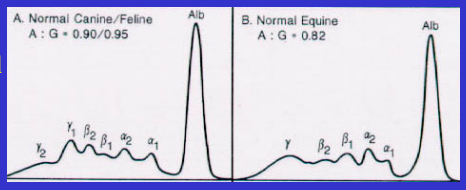
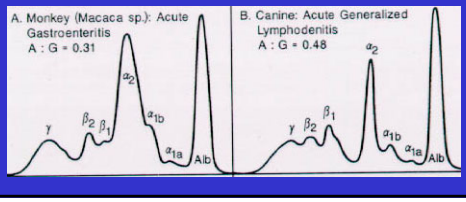
Serum proteins (albumin:globulin)

Normal



Disease



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Dysproteinaemias

Normal A:G ratio
- but both elevated = hyperproteinaemia → dehydration
- but both reduced = hypoproteinaemia → blood loss

Decreased A:G ratio
- decreased albumin → kidney/liver disease, parasites
- increased globulins
  - α-globulin → inflammatory disease, nephritis
  - β-globulin → hepatitis, dermatitis
  - γ-globulin → infectious diseases, tumours

Increased A:G ratio
- increased albumin → dehydration
- decreased globulin → immunodeficiencies

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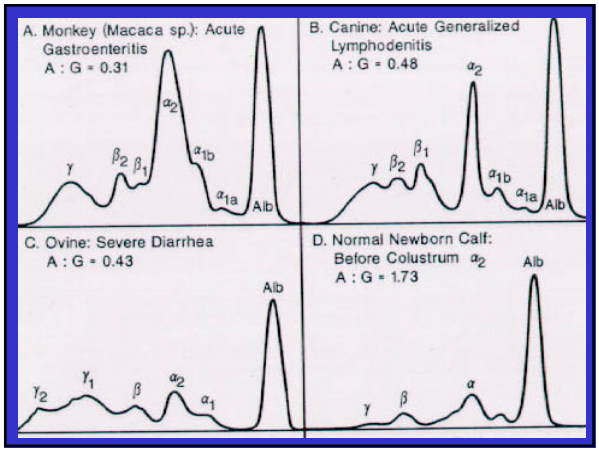

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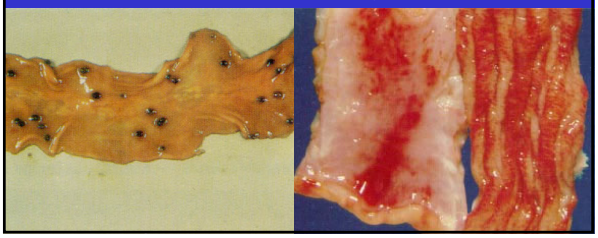
Gut parasites

mucosal damage — *Ostertagia ostertagi*

haemorrhage — *Haemonchus contortus*

↓A:G ratio

[↓ albumin, ↑ immunoglobulins]



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Enzymology

ALP	alkaline phosphatase	- liver (cholestatic)
GGT	γ-glutamyltransferase	- liver (cholestatic)
ALT	alanine aminotransferase	- liver (hepatocyte)
SDH	sorbitol dehydrogenase	- liver (hepatocyte)
LIP	lipase	- pancreas (exocrine)
Amyl	α-amylase	- pancreatitis
-	trypsin	- pancreatitis
PK	pyruvate kinase	- skeletal muscle
CK	creatinine kinase	- cardiac infarction
AST	aspartate aminotransferase	- soft tissue damage
LDH	lactate dehydrogenase	- nonspecific
ChE	cholinesterase	- neurological

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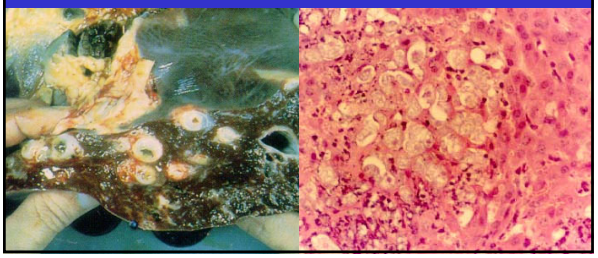
Liver parasites

liver fluke — *Fasciola hepatica*

liver abscess — *Entamoeba histolytica*

[↑ ALP, ↑ GGT, ↑ ALT, ↑ SDH]



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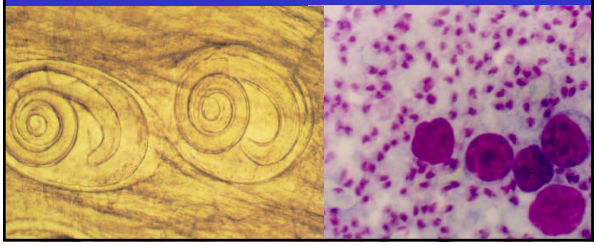
Muscle parasites

trichinosis — *Trichinella spiralis*

Chagas disease — *Trypanosoma cruzi*

[↑ PK, ↑ CK]



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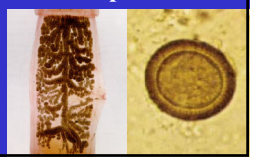
PARASITE METABOLISM

Parasites may utilize/usurp host metabolism

They exhibit many specialized adaptations

- absence of circulatory system in helminths
- absence of digestive tract in cestodes
- absence of mitochondria in some protozoa
- alternation of metabolism between parasitic and free-living stages
- metabolic diapause



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Metabolic variability

Parasites use rich supply of host nutrients

Many do not synthesize their own amino acids, nucleotides or lipids (many lack the genetic capability and use salvage pathways instead)

<u>Stage in nutrient-rich vertebrate host</u>

- substrate level phosphorylation (anaerobic)

<u>Stage in nutrient-poor invertebrate vector</u>

- oxidative phosphorylation (aerobic)

<u>Free-living stages</u>

- use endogenous stores (aerobic)

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DRUG USE

Huge range of chemicals used for parasite:

chemotherapy (curative)

- static drugs (arrest development, reversible)
- cidial drugs (irreversible damage - lethal)

chemoprophylaxis (preventive)

- stop infection
- limit infection

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Pharmaceuticals

Pharmacodynamics - action of drug on body

Pharmacokinetics - action of body on drug

Action based on selective toxicity (parasite first)

Contra-indicated use - side-effects

- synergism/antagonism

With-holding period - 100-1000x ADI (acceptable daily intake)

Maximum residue limits

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Parasite targets

Huge diversity of parasites in terms of:

- organismal biodiversity (multiple phyla)
- developmental cycles (eggs/larvae/adults)
- food requirements (energy sources)
- metabolic pathways (aerobic/anaerobic)
- types of hosts (vertebrate/invertebrate)
- location within host (tissue/organ specificity)

All present challenges to chemical treatment

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CHEMOTHERAPY

Drugs exhibit selective activity on:

- DNA synthesis (alkylation, purine, cofactor)
- protein synthesis (inhibition, translation)
- energy metabolism (electron transport, reduction)
- neurotransmission (blockers, inhibition)
- membrane function (vacuoles, permeability)
- microtubule function (paralysis)
- hem(oglobin) interaction (disruption)

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DNA synthesis affecting drugs

- interference with dihydroorotate dehydrogenase
  - hydroxyquinolines (decoquinate)
- alkyalation reactions
  - nitroimidazoles (metronidazole)
- interference with purine salvage
  - diloxanide (furamide)
- interference with polyamine metabolism
  - melarsoprol (melarsen)
- interference with cofactor synthesis
  - sulfonamides (sulfadoxine)

⇒ STOP REPLICATION

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Protein synthesis-affecting drugs

- emetine (mebadin)
- tetracyclines (oxytetracycline)
- lincosamides (clindamycin)
- macrolide antibiotics (erythromycin)
- aminoglycoside antibiotics (paromomycin)
- glutarimide antibiotics (axenomycin)
- glycopeptide antibiotics (streptothricin)
- diamphenethide (coriban)

⇒ DENY BUILDING BLOCKS

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Membrane function disturbing drugs

- amphotericin B (amphozone)
- polyether antibiotics (monensin)
- mepacrine (atabrine)
- bunamidine (buban)
- praziquantel (droncit)
- diethylcarbamacine (carbam)

⇒ DISRUPT MEMBRANE INTEGRITY/FUNCTION

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Energy metabolism disturbing drugs

- rotenoids
- iodoquinol (ioquin)
- suramin (germanin)
- antimonials (sodium stibogluconate)
- clopidol (clopindol)
- robenidine (robenz)
- amprolium (amprol)
- arsenicals (carbasone)
- clorsulan (curatrem)
- isothiocyanates (bitoscanate)
- halogenated monophenols (disophenol)
- halogenated bisphenols (bithionol)
- salicylanilides (niclosamide)
- cyamine dyes (pyrvinium)

⇒ STARVE or SUFFOCATE PARASITES

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Haem(oglobin) interaction

- artemisinin (artemether)
- amodiaquine (amodiaquine)
- halofantrine (halofantrine)
- chloroquin (chlorochin)
- quinine (various)
- mefloquine (laricur)

⇒ STARVE PARASITES

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Neurotransmission-affecting drugs

Blockers of cholinergic neurotransmission

- organophosphates (dichlorvos)
- ethanolamines (bephenium)
- pyrantel, morantel, oxantel, levamisole

Inhibitory drugs

- piperazine (various)
- macrocyclic lactones (ivermectin)

⇒ PARALYSE PARASITES

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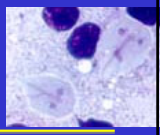
Anti-protozoals (enteric)

	Flagellates blood	Flagellates enteric	Amoebae enteric	Ciliates enteric
1950's diloxanide			+	
chloroquine			+	
1960's iodoquinol			+	
metronidazole	+	+	+	+
furazolidone		+		
1970's emetine	+		+	
erythromycin		+		
tetracyclines		+		
benzimidazoles		+		



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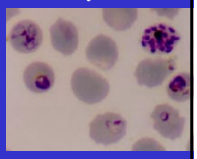
Antiprotozoals (malaria)

1660 - Peruvian Indians use 'fever tree bark' (Cinchona tree)
1820 - quinine isolated from bark
1914-18 - WWI quinine shortage prompted work on synthetics
1928 - pamaquine
1932 - mepacrine
1934 - chloroquine
1939-45 - WWII shortages
1945 - proguanil
1951 - pyrimethamine
1960 - Emergence of chloroquine resistance
1960 - sulphonamides, sulphones
1971 - mefloquine
1974 - series of new compounds from USA
1979 - artemisinin developed in China



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Anthelmintics

1900's N - cupric sulfate, lead arsenate, tin compounds, chenopodium oil, nicotine [toxic]
1920's N - carbon terachloride [toxic]
1930's N - phenothiazine
1960's N - tetrahydropyrimidines (pyrantel, oxantel, morantel)
N - imidazothiazoles (levamisole)
N/C/T - benzimidazoles (thia-, cam-, par-, oxi-, me-, flu-, fen-, al-, cyclo-bendazole)
N - organophosphates (dichlorvos, trichlorfon)
C - chlorinated hydrocarbons (carbon tetrachloride)
T - salicylanilides (rafoxanide)
1970's N - benzimadazole prodrugs (febantel, thiophanate)
N - avermectins/milbemycins (milbemycin, ivermectin)

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Arthropodicidal drugs

1940's	- chlorinated hydrocarbon (DDT)	sodium channel
	- " " (cyclodiens, lindane)	chloride channel
1950's	- organophosphates	AChE
1960's	- carbamates	AChE
1970's	- pyrethroids	sodium channel
	- amidines	biogenic amines
1980's	- avermectines/milbemycins	chloride channel
1990's	- arylpyrazole (fipronil)	chloride channel
	- chloronicotinyles (imidacloprid)	nicotinic AC res

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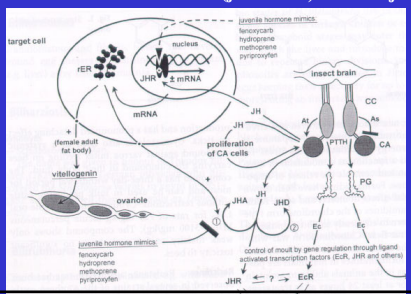
Arthropodicidal drugs

1980's	- insect growth regulator (cyromazine)	disrupt cuticle
	- " " (benzoylphenylureas)	inhibit cuticle
	- " " (juvenoids)	mimic juvenile h



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PROBLEMS

Emergence of drug resistance due to:
- under-dosing (sublethal doses)
- poor compliance (treatment not completed)

Resistance found against:
- antimalarials (chloroquine)
- anticoccidials (ionophores, sulfonamides)
- anthelmintics (white/clear drenches)
- insecticides (DDT, organophosphates)

Need to understand mode of action of drug

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Possible mechanisms of resistance

DRUG LEVEL

- Exclusion
  - decreased drug import
  - increased drug export
- Sequestration
  - drug-binding molecule
  - drug compartmentalization
- Metabolism
  - pro-drug not activated
  - increased drug inactivation

TARGET LEVEL

- Modified
  - decreased affinity
  - protected by substrate
- Amplified
  - increased sequestration
  - increased threshold
- Repaired
  - reduced damage
  - increased damage repair

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Will we get it right in the end!

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The great debate

Chemotherapy versus Vaccination

- broad spectrum (targets whole groups)
- short-acting
- re-infection possible
- drug resistance
- drug residues
- environmental toxins

- narrow activity (species specific)
- long-lasting
- re-infection prevented
- reversion of virulence
- hypersensitivity
- contamination

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