# RC MedReview

## COMLEX Level 1

## Board Review

**2022 - 2023**

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**DEDICATION**

We dedicate this book to Noel, Adrian, Oliver, Owen, and Farida who bring the lightest to our lives.

# Preface

The RC MedReview COMLEX level 1 Board Review is a comprehensive highly compressed Board preparation course. It has been designed specifically for medical students who plan to take the Medical License Examination (COMLEX Level 1). The course material is presented in a case-based format, including subjects that show up frequently on the Boards. The RC MedReview series is not a traditional classroom-style course, instead it focuses on the material in the precise depth needed, covering each of the basic science areas specified by the National Board of Medical Examiners.

My hope is that this book will help you to avoid wasting your time searching over capacious texts, and show you an easy way to excel on the Boards.

Samir Rizk, MD
Founder of RC MedReview

# Acknowledgements

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# Biochemistry

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# General Biochemistry

## Proteins

### *Composition*

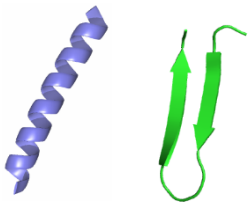
- String of Amino acids (polypeptide chain)
- Made at the ribosome by translation of mRNA

### *Function*

- Structural: maintain cellular structure
- Enzymatic: carry out chemical reactions
- Signaling: hormones, receptors, transcription factors
- Transport: bind to small molecules
- Immune system: antibodies, clotting factors, etc.

## Structure

- Primary structure
  - The sequence of amino acids in a polypeptide chain
- Secondary structure
  - **α-helix:** stabilized by hydrogen bonding (H-bonding) spaced four residues apart
  - **β-Sheets** formed by H-bonds between two regions of a single chain that folds back on it self



In both α-helix and β-sheets, the side chains <u>are not</u> involved in H-bonding.

H bonding is through peptide bond: amide N to carbonyl O

  - Creutzfeldt-Jakob disease
    - Caused by a prion protein
    - Transformation of the α-helical regions into pathogenic β-pleated sheets in the neurons
    - Symptoms include rapidly progressive dementia, personality changes and hallucinations. Speech impairment, jerky movements (myoclonus), balance and coordination dysfunction (ataxia)
- Tertiary structure
  - Three-dimensional conformation of the folded protein chain
  - Usually refers to one polypeptide chain
  - Example: Myoglobin

- Quaternary structure
  - Multiple folded protein molecules in a complex
  - Example: Hemoglobin is an assembly of four globular protein subunits (2 $\alpha$ and 2 $\beta$). Each subunit is associated with a heme group
  - Hb mutant (HbS) leading to Sickle-cell disease
    - Glutamic acid mutated to valine at position 6 of the β-chain
    - Valine (non-polar) fits into a hydrophobic pocket in the neighboring HbS molecule in the deoxy-form
    - Under low oxygen conditions:
      - $\Rightarrow$ Deoxygenated HbS will polymerize sickle shaped RBCs $\rightarrow$ hemolysis and anemia
    - Acute pain crisis, severe hemolytic anemia, functional asplenia
    - Acute chest syndrome:
      - $\Rightarrow$ Most common cause of death in adults patients present with fever, chest pain, and an infiltrate on chest x-ray
    - Kidney:
      - $\Rightarrow$ Isosthenuria - inability to concentrate urine, hematuria - even in sickle trait
    - Increasing likelihood of infections with encapsulated organisms such as *S. pneumoniae*, *Haemophilus* and *Salmonella*
    - Aplastic crises: with parvovirus B19 infection
- Fibrillin
  - An $\alpha$-helical structural protein
  - Major component of the extracellular matrix
  - Marfan syndrome:
    - Mutation in fibrillin results in misfolded protein
    - Long extremities, fingers, and scoliosis
    - ↑ risk of aortic aneurysm
    - Subluxation of the lens and severe myopia
    - Autosomal dominant

## Protein folding

- A process by which a polypeptide (primary structure protein) folds into its functional three-dimensional structure
- Chaperones: Proteins that mediate the folding process of other proteins directly after translation

## Protein misfolding and associated disorders

- Alpha1-antitrypsin (A1AT):
  - Protects tissue from damage by inhibiting elastase, a protein that breaks down elastin
- A1AT deficiency:
  - Results from misfolded alpha 1 antitrypsin protein
  - Misfolded protein accumulates in lungs & liver
  - COPD and liver cirrhosis
  - Increased activity of elastase
    - Over-degradation of elastin
- Elastin:
  - Stretchable protein
  - Found in:
    - Lungs, bladder (allowing for stretching)
    - Elastic ligaments, skin (for elasticity)
    - Large arteries (blood wave propagation)
    - A1AT deficiency results in:
      - ⇒ Overactive elastase = breakdown of elastin
      - ⇒ Emphysema, COPD
- Huntington's disease:
  - Increase in trinucleotide (CAG) repeats in the Huntington gene
  - Leads to Increase in the number of polyglutamine repeats in Huntington protein
  - Result:
    - Aggregation (precipitation) of misfolded Huntington protein
    - Nuclear inclusions and neuronal cell death (most prominent in caudate nucleus)
- Amyloidosis:
  - Misfolded proteins (normally soluble) → insoluble β-pleated fibrils (amyloids)
  - Histologically: amyloid is an extracellular proteinaceous deposit of β-sheets
  - All amyloid proteins: when stained with Congo red produce apple-green birefringence under polarized light

- Types of amyloidoses:
  - Immunoglobulin light chain
    - Associated with multiple myeloma
    - Symptoms: myeloma, kidney failure, Congestive Heart Failure
  - β2- microglobulin
    - Dialysis-associated amyloidosis
    - Symptoms: carpal tunnel syndrome, bone and joint destruction
  - A Cal – chemically related to Calcitonin
    - Associated with medullary carcinoma of the thyroid
    - Look for: amyloid around the C-cells
  - Amyloid-associated (AA) amyloidosis
    - Inflammation-associated amyloidosis
    - Associated with familial Mediterranean fever
    - Symptoms: chronic infections, collagen diseases
  - β-amyloid
    - Associated with Alzheimer's disease
    - Most common cause of dementia
  - Islet amyloid protein (Amylin)
    - Normally secreted along with insulin
    - Associated with Type 2 diabetes mellitus

# Collagen

- Major part of extracellular matrix
  - Most abundant protein in human body
  - Composed of mostly Glycine, Proline and Lysine
    - Synthesis and processing:
      - ⇒ Synthesized as ***Preprocollagen***, in the RER
      - ⇒ Signal peptide is cleaved forming ***Procollagen***
      - ⇒ Hydroxylated at Proline and Lysine
      - ⇒ Glycosylated at Lysine
    - Triple Helix formed, sent to Golgi
    - ***Tropocollagen*** is secreted by exocytosis
    - Covalently cross-linked to form ***Collagen*** fibers
- Types of Collagen
  - Type I: Main component of bone, tendons, skin
  - Type II: Main component of cartilage, nucleus pulposus
  - Type III: Main component of reticular fibers, uterus, fetal tissue
  - Type IV: Forms the basement membrane, basal lamina
- Collagen Defects
  - Osteogenesis imperfecta (Type I)
    - Bone fragility, hearing loss, and blue sclera
  - Collagenopathy (Type II)
    - Hyaline cartilage 50% of all cartilage protein
  - Ehlers-Danlos Syndrome (Type III)
    - Stretchable skin, hypermobility, bowel bleeding
  - Alport syndrome (Type IV)
    - X-linked
    - Renal failure, deafness, and cataract

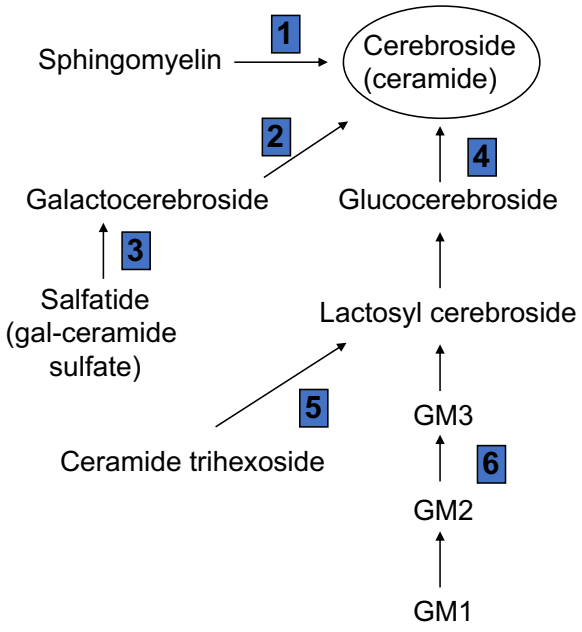
## Post-translational protein modifications

- Post-translation Modification occur after synthesis the protein on the ribosome
- Hydroxylation
  - Addition of -OH group to side-chain of amino acid after protein synthesis e.g. Proline and Lysine of Collagen and Elastin
  - Enzymes: Lysyl hydroxylase and Prolylhdroxylase
    - Both require vitamin C
    - Vitamin C deficiency: ***scurvy*** (anemia, gum and skin bleeding)
  - Reaction takes place in Rough ER
  - Lysyl hydroxylase defect → ***Ehlers Danlos***
- Glycosylation
  - Addition of sugar to side-chain of amino acid after protein synthesis
  - Example: Glycosylation of Collagen at Lysine
    - Converts procollagen to tropocollagen
    - Reaction takes place in Golgi
- Protein processing
  - Cleavage of part of the protein to convert it to an active form
  - Examples:
    - Collagen: Preprocollagen to procollagen by cleavage of signal peptide
    - Insulin:
      - ⇒ Preproinsulin to proinsulin by cleavage of signal sequence
      - ⇒ Proinsulin to insulin by cleavage of C-peptide
- Glutamate Carboxylation
  - At glutamate residues of:
    - Clotting factors II,VII, IX, X, fibrinogen, protein C and S
  - Carried out by the enzyme: ***gamma-glutamyl carboxylase*** (requires vitamin K)
  - Warfarin (anticoagulant) inhibits this process by blocking vitamin K recycling
- Phosphorylation
  - Addition of phosphate group to protein
    - At Serine, Tyrosine or Threonine
  - Carried out by ***kinases***, and requires ATP
  - Function: activation of protein function
    - Usually phosphorylated form of a protein is active
    - De-phosphorylated form of a protein is inactive signaling (phosphorylation cascade)
  - Proteins can be dephosphorylated by ***phosphatases***

## Lysosomal storage diseases

- Sphingoliposis and Mucopolysaccharidoses
- All are enzyme deficiencies
  - Dysfunction in breakdown of a substrate
  - Result: Accumulation of the substrate
- All are autosomal recessive EXCEPT:
  - Fabry's disease (sphingoliposis)
  - Hunter's syndrome (Mucopolysaccharidosis)
    - Both are X-linked Recessive
- All can present in children/infants

## Sphingolyposes




1. *Sphingomyelinase* (Niemann-Pick A&B)
2. *Galactosylceramidase* (Krabbe)
3. *Arylsulfatase A* (Metachromatic Leukodystrophy)
4. *Acid β-Glucosidase* (Gaucher)
5. *α-Galactosidase A* (Fabry)
6. *Hexosaminidase* (Tay-Sachs & Sandhoff)

- Niemann-Pick Types A and B
  - Enzyme: Sphingomyelinase
    - Converts sphingomyelin to ceramide
  - Accumulation: Sphingomyelin
    - In foam cells of the brain, lungs, liver, bone marrow, and spleen
  - Symptoms:
    - Type A: ~6 months, seizure, rapid CNS deterioration, hepatosplenomegaly, cherry-red spots
    - Type B: later onset, progressive pulmonary disease, hepatosplenomegaly, some motor deterioration
    - Reticular infiltrative pattern (chest x-ray)
  - Treatment:
    - Bone Marrow/hepatic transplant, enzyme replacement therapy
- Niemann-Pick Type C
  - NOT a Sphingoliposis
  - Mutation in NPC1 or NPC2 genes responsible for transporting cholesterol OUT OF lysosome
  - Accumulation of cholesterol in lysosomes
  - Diagnosis: Filipin-staining of cholesterol
  - Hepatosplenomegaly, progressive mental/motor deterioration
    - Treatment – manage the symptoms, hydroxypropyl beta-cyclodextrin – compassionate use
- Krabbe Disease
  - Enzyme: Galactosylceramidase
    - a.k.a.: galactosylceramide b-galactosidase
    - Converts Galactoceramide (galactocerebroside) to ceramide
  - Accumulation:
    - Galactoceramide and galactosylsphingosine in Brain
  - Symptoms:
    - Onset: 3-6 months old, mental retardation, seizures, optic atrophy and blindness, spasticity and deafness, usually fatal before age 2
  - Treatment: manage symptoms

- Metachromatic Leukodystrophy
  - Enzyme: Arylsulfatase A
    - Sulfatide (galactosylceramide sulfate) to gal-ceramide
  - Accumulation:
    - Sulfatide (galactosylceramide sulfate) in nervous system (white matter), Kidney and Liver
  - Symptoms:
    - Infantile, juvenile and adult onset forms
    - Mental retardation, ataxia, demyelination of nerves, dementia, infant form fatal in childhood
    - Lab microscopy: Metachromasia in nerve staining
  - Treatment:
    - Adult form may respond to bone marrow transplant
- Gaucher Disease
  - Enzyme: Acid β-glucosidase (β-glucocerebrosidase)
    - Converts glucoceramide to ceramide
  - Accumulation: glucoceramide (glucocerebroside)
    - In brain, spleen, bone marrow and liver
  - Symptoms:
    - Hepatosplenomegaly, "Gaucher Cells": lipid-rich macrophages (in bone marrow biopsy), aseptic necrosis (femur), bone crisis, thrombocytopenia
    - CNS: seizures/ dementia/ mental retardation
  - Treatment:
    - Recombinant enzyme (Cerezyme), joint replacement surgery, manage cytopenia
- Fabry's disease
  - Enzyme: a-galactosidase A
    - Cleaves a-galactoside from trihexosylceramide (GM3) to form lactosyl cerebroside
  - Accumulation: trihexosylceramide (GM3)
    - In the heart, kidney, skin and CNS
  - Symptoms:
    - Child (male) with angiokeratomas (no blanching). Corneal and lenticular lesions (slit-lamp exam), burning of hands/feet upon exercise or fever, high BUN/creatinine
    - Renal/heart failure (long-term)
  - Treatment: phenytoin, carbamazepine, dialysis, renal transplant

- Tay-Sachs Disease
  - Enzyme: Hexosaminidase A
    - Break down of gangliosides
  - Accumulation:
    - GM2 gangliosides (neuronal cells toxicity)
  - Symptoms: (common: Ashkenazi Jews)
    - Infantile form:
      - ⇒ neural degeneration, hyperacusis, retinal cherry-red spot
      - ⇒ ***No*** hepatosplenomegaly
    - Juvenile form:
      - ⇒ ataxia, dementia
    - Adult form:
      - ⇒ progressive motor weakness, psychosis
  - Treatment:
    - Manage symptoms
- Sandhoff Disease
  - Enzyme: Hexosaminidase A and B
    - Break down of GM2 gangliosides
  - Accumulation:
    - GM2 gangliosides (neuronal cells toxicity)
  - Symptoms:
    - Infantile form: neural degeneration
    - ***Hepatosplenomegaly,*** retinal cherry-red spot
  - Treatment:
    - Manage symptoms

## Mucopolysaccharide diseases

- Hunter's Syndrome
  - X-linked recessive
  - Defect in Iduronate sulfatase
- Hurler's Syndrome
  - Autosomal recessive
  - Defect in $\alpha$-L-iduronidase
    - Both result from dysfunction in breakdown of GAGs (glycosaminoglycans)
    - Both lead to accumulation of:

      $\Rightarrow$ Dermatan sulfate and heparan sulfate (urine)
  - Characteristic symptoms:
    - Coarse facial features, vulvar heart disease, joint problems
  - Other Symptoms:
    - Short stature, mental retardation, dwarfism hepatosplenomegaly
  - Distinguishing features:
    - Corneal clouding found *only* in Hurler's
    - Unlike ***I-Cell disease***, *No* elevated plasma levels of lysosomal enzymes

| Disease | Corneal Clouding | Elevated plasma lysosomal enzymes |
|---|---|---|
| Hunter | No | No |
| Hurler | Yes | No |
| I-cell | Yes | Yes |

  - I-Cell Disease: a trafficking disease
    - Coarse facial features (Gargoylism) and corneal clouding,
    - Defect in mannose-6-phosphate synthesis in the Golgi
    - Lysosomal enzymes not transported correctly to the lysosome –> elevated lysosomal enzymes in the plasma

# Metabolism

## General concepts

- Carbon-containing molecules are burned (oxidized) to produce electrons
- Electron carriers FAD and NAD are converted to $FADH_2$ and NADH shuttle electrons to the Electron Transport Chain (ETC)
- Electrons are used to set up gradient of protons
- Proton gradient is used to generate energy

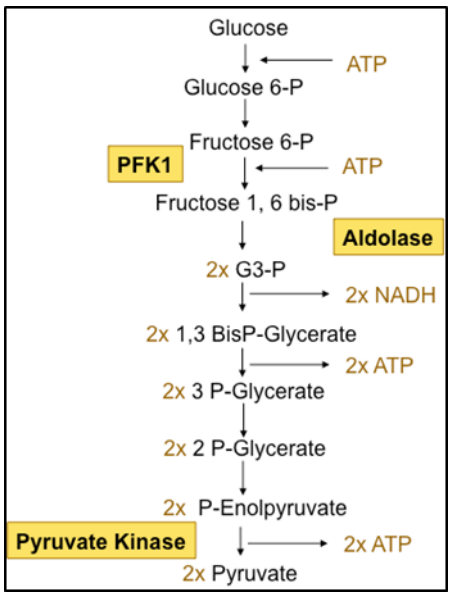
### *Locations of biochemical processes in the cell*

- Cytosol:
  - Glycolysis, pentose phosphate shunt, FA synthesis and glycogen synthesis
- Mitochondrial matrix:
  - β-oxidation of FAs and TCA cycle
- Inner mitochondrial membrane:
  - Oxidative phosphorylation
- Both cytosol and mitochondria:
  - Gluconeogenesis, urea cycle and heme synthesis

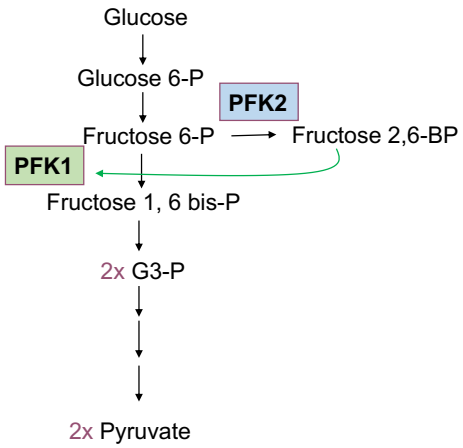
## Glycolysis

- Main pathway for carbohydrate metabolism (glucose, fructose, galactose)
- All the reactions occur in the cytoplasm
- Anaerobic process and can produce ATP in the absence of $O_2$
- Glucose transport into the cell is mediated by glucose transport proteins:
  - GLUT-1: RBCs, brain, testicles and retina
  - GLUT-2: liver, kidney, pancreas, intestine
  - GLUT-3: Brain
  - GLUT-4: Fatty tissue, skeletal and cardiac
    - Insulin stimulates glucose uptake by translocating GLUT- 4 to the plasma membrane
  - GLUT-5: (fructose) intestine and sperm
- ***Glucose is transported by facilitated diffusion:*** NO ENERGY NEEDED

- Overall reaction:
  - 2 moles of ATP are utilized in glycolysis
  - 4 moles of ATP are generated
  - The net production is 2 ATP
  - One mole of glucose → 2 moles of pyruvate + 2 moles of NADH
- First step of glycolysis:
  - phosphorylation of glucose by:
    - Hexokinase:
    - In most tissue
      - ⇒ Activated by insulin
      - ⇒ Inhibited by Glucose 6P (product)
    - Glucokinase:
      - ⇒ In the liver and β-cells of pancreas
      - ⇒ Activated by insulin
      - ⇒ Activated at high intra hepatocyte glucose
      - ⇒ Prevents hyperglycemia after carbohydrate rich meal
      - ⇒ ***Remember: glucokinase mutation → gestational diabetes***
- Glycolysis Key Enzyme
  - Phosphofructokinase 1:
    - Rate-limiting step
    - Activated by: Insulin and Fruc-2,6 bis-P and AMP
    - Inhibited by: Glucagon, citrate, and ATP
  - Pyruvate kinase:
    - Activated by: Insulin, Fruc1,6 bis-P
    - Inhibited by: Glucagon, ATP, Alanine
  - Glucokinase, Phosphofructokinase and Pyruvate kinase activity:
    - ***Increased*** during fed state (by the effect of insulin)
    - ***Decreased*** during starvation or diabetes

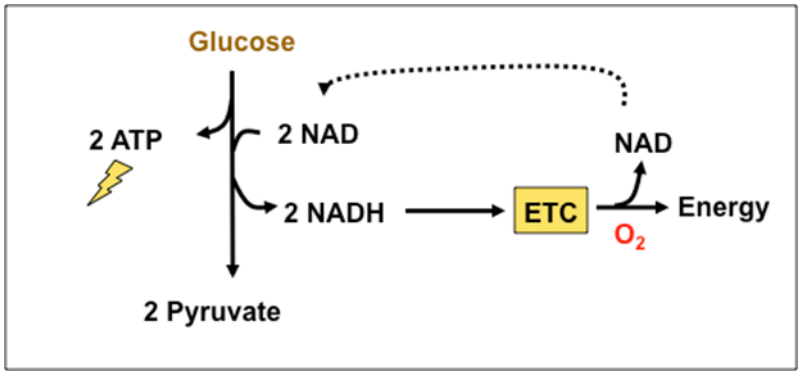


- Phosphofructokinase 1 (PFK1) vs. Phosphofructokinase 2 (PFK2:
  - PFK1 is relatively slow
  - At high [Glucose] Fructose 6P accumulates
  - F6P is converted to Fructose 2,6 bisphosphate (side reaction) by PFK2
  - High levels of F2,6BP activate PFK1 allowing glycolysis to proceed



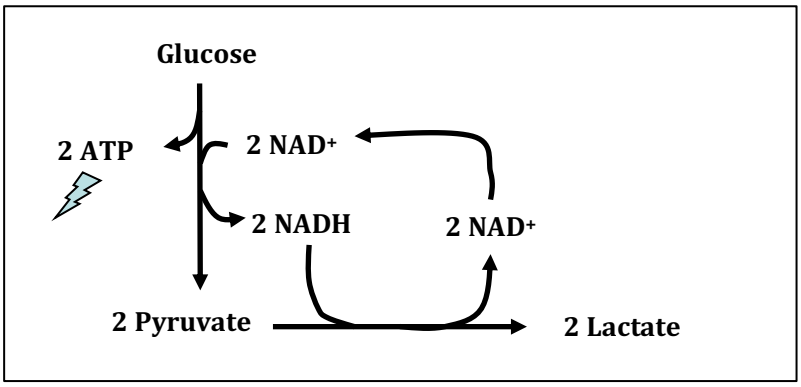

- Aldolase:
  - The only glycolytic enzyme that cleaves a carbon-carbon bond of 6-carbon sugar
    - ⇒ Fructose-1,6-biphosphate → glyceraldehyde 3-phosphate and dihydroxyacetone phosphate
  - Aldolase deficiency: nonspherocytic hemolytic anemia and rhabdomyolysis with fever
- RBCs:
  - contain no mitochondria
  - glycolysis is the only source of energy
- ***Deficiency glycolysis enzymes: Hemolytic anemia, RBC dysfunction***

➢ ***Under normal oxygen conditions,*** NAD is regenerated by the Electron Transport Chain (ETC)



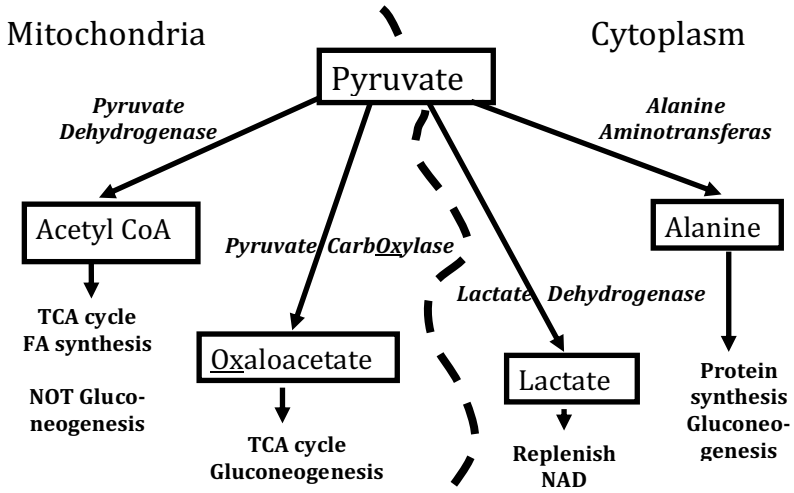

➢ ***In the absence of oxygen:***

- $NAD^+$ becomes limiting (*i.e.* $NAD^+$ levels drop)
- To regenerate $NAD^+$:
  - Pyruvate is converted into lactate by lactate dehydrogenase
  - NADH is converted to $NAD^+$




Regeneration of $NAD^+$ generates lactate under oxygen starvation

## The Fate of Pyruvate

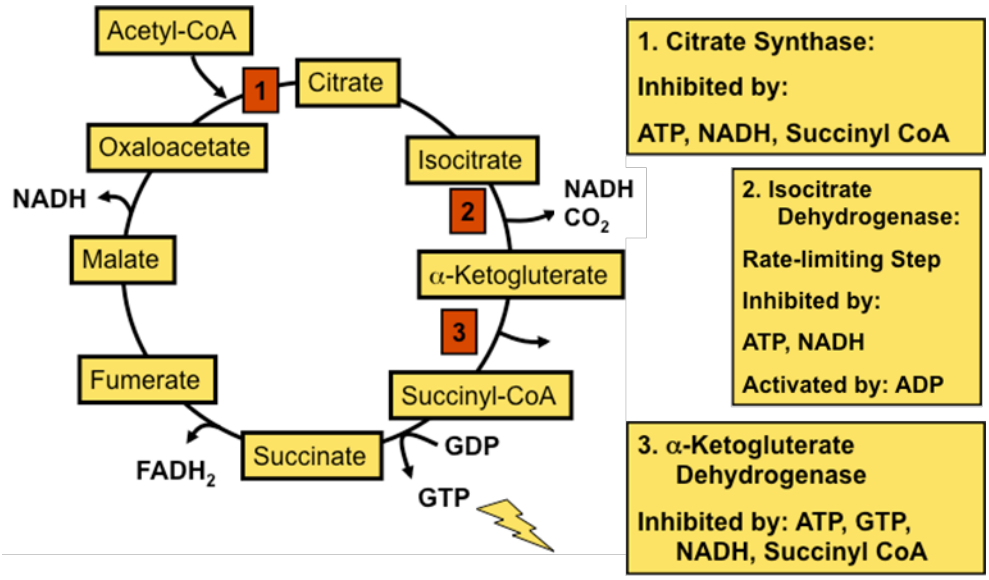



- Lactate:
  - Lactate dehydrogenase (LDH)
  - NADH → $NAD^+$ to keep glycolysis working
  - Cori cycle:
    - Lactate produced by RBCs and active muscles → liver for gluconeogenesis
- Acetyl CoA:
  - Utilized by:
    - TCA cycle: Acetyl-CoA + Oxaloacetate → Citrate
    - Fatty acid synthesis
    - Ketone body synthesis
    - ***Know that Acetyl CoA is NOT a substrate of gluconeogenesis***
  - Made by pyruvate dehydrogenase in the mitochondria
  - Pyruvate DHase Deficiency = lactic acidosis
  - ↓ Pyruvate DHase activity + ↑ Lactate DHase activity = hypoxic lactic acidosis
- Oxaloacetate:
  - Made by pyruvate carboxylase (biotin dependent), stimulated by Acetyl CoA
  - Utilized by brain and liver NOT in the muscles
    - Provides substrates for gluconeogenesis
    - Replenishes intermediate of the TCA cycle
- Alanine
  - Alanine cycle during fasting, major substrate for gluconeogenesis

# TCA cycle

- Overview:
  - Occurs exclusively in the mitochondria
  - For One Acetyl CoA TCA cycle produces:
    - 2 $CO_2$
    - 3 NADH = 3x 2.5 ATP
    - 1 $FADH_2$ = 1.5 ATP
    - 1 GTP = 1 ATP
    - Total ATP = 10 ATP
  - Provides molecules for:
    - Gluconeogenesis
    - FA synthesis
    - Interconversion of amino acids
- First step:
  - Pyruvate to Acetyl-CoA
    - Irreversible, cannot go back to glucose
    - Enzyme: Pyruvate Dehydrogenase
    - Requires lipoic acid
  - Arsenic poisoning:
    - Inhibition of pyruvate dehydrogenase
    - Impaired production of acetyl CoA
- Key enzymes:
  - Citrate Synthase:
    - Inhibited by: ATP, NADH, Succinyl CoA
  - Isocitrate dehydrogenase:
    - Rate-limiting step
    - Inhibited by: ATP, NADH
    - Activated by: ADP
  - $\alpha$-Ketoglutarate Dehydrogenase:
    - Inhibited by: ATP, GTP, NADH, Succinyl CoA
    - Requires thiamine (vitamin B1) as a cofactor
    - Maintains levels of the neurotransmitters:
      $\Rightarrow$ glutamate, $\gamma$–aminobutyric acid (GABA), and aspartate,
    - Maintains levels of protein synthesis

Know that all the enzymes of TCA cycle are in the matrix of the mitochondria except ***succinate dehydrogenase*** is in the inner membrane of the mitochondria




- ***The Malate Shuttle***
  - Shuttles electrons (NADH) from glycolysis (cytoplasm) to the mitochondria
  - In the cytoplasm:
    - Aspartate is converted to malate
    - Malate enters the mitochondria
  - In the mitochondria:
    - Malate is converted into aspartate
    - Aspartate exits the mitochondria
  - Oxaloacetate is the intermediate
- ***Succinate Dehydrogenase***
  - Only Enzyme that participates in both TCA and ETC
  - Only TCA cycle enzyme within the inner mitochondrial membrane
    - TCA: succinate DHase
      - ⇒ Succinate → Fumarate
      - ⇒ $FAD^+$ → $FADH_2$
    - ETC: Complex II
      - ⇒ FADH2 → $FAD^+$
      - ⇒ CoQ → $CoQH_2$

# The Electron Transport Chain

- Overview:
  - Electron carriers NADH and FADH2 give electrons to Protein Complexes: I, II, III, IV
  - Complexes pump protons ($H^+$) to create proton gradient
  - $O_2$: final electron acceptor becomes $H_2O$
  - Remember complex II is succinate DHase DOES NOT PUMP PROTONS
  - ***ATP synthase:*** Complex V, ADP → ATP (Energy)

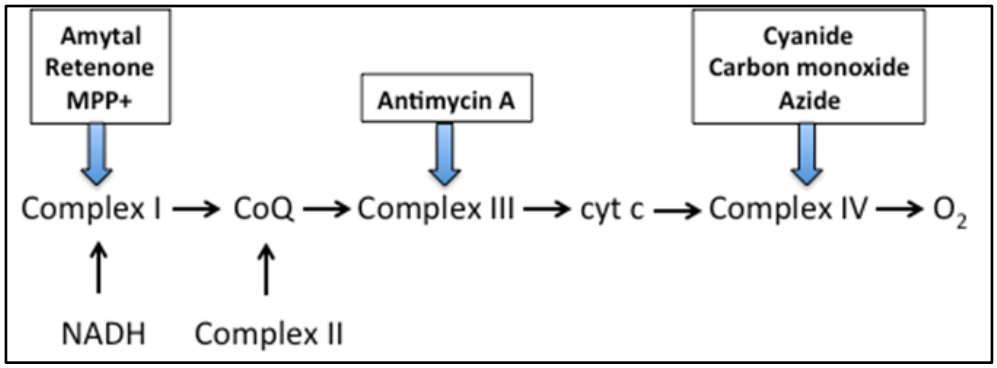
| |
|---|
| Complex I → CoQ → Complex III → cyt c → Complex IV → $O_2$ |
| ↑ ↑ |
| NADH Complex II (From TCA cycle) |

**Flow of electrons in the Electron Transport Chain**

- Complex I:
  - NADH dehydrogenase contains FMN
  - Cofactor: FMN:
    - Riboflavin (vitamin B2) is the central component of the cofactors $FAD^+$ and FMN (Flavin mononucleotide) in Complex I
- CoQ:
  - coenzyme Q, an electron carrier from I and II to III
- Complex III:
  - donates electrons to cyt c
- Cytochrome c:
  - gives electrons to complex IV
  - contains heme
- Complex IV:
  - donates electrons to oxygen to make water
- Complex V:
  - F1-Fo ATP synthase
  - uses proton gradient to make ATP from ADP and Phosphate (oxidative phosphorylation)
  - Inhibited by Oligomycin

- Uncoupling agents:
  - Agents that allow protons to cross the inner mitochondrial membrane without going through ATP synthase leading to a rapid consumption of energy without generation of ATP
  - 2,4-Dinitrophenol and Aspirin:
    - ⇒ Increased rate of $O_2$ consumption
    - ⇒ Increased rate of $CO_2$ production
    - ⇒ Increase in TCA and Electron transport
    - ⇒ Decreased ATP production
    - ⇒ Energy lost as heat
  - Thermogenin:
    - ⇒ Natural uncoupling protein 1 (UCP1) found in the mitochondria of brown adipose tissue
    - ⇒ Bypasses ATP synthase: ↑ heat production, ↓ ATP synthesis
    - ⇒ Generates heat by non-shivering thermogenesis to keep newborns internal temperature higher

➢ Inhibitors of the ETC:
- Cyanide, carbon monoxide and azide
  - Bind to cytochrome oxidase c - at Complex IV
  - Prevent of transport of electron to oxygen at Complex IV
  - Block ATP production, low Oxygen consumption
- Amytal (a barbiturate) and Rotenone (a pesticide):
  - Prevent of transport of electron to from Complex I to CoQ
  - Block ATP production, low Oxygen consumption
- Antimycin A (antibiotic/pesticide)
  - Prevent transport of electrons at Complex III
  - Block ATP production, low Oxygen consumption

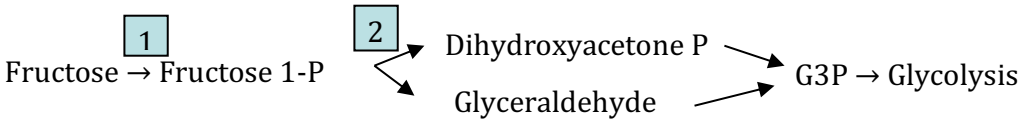



**Inhibitors of the Electron Transport Chain**

## Pentose Phosphate Pathway

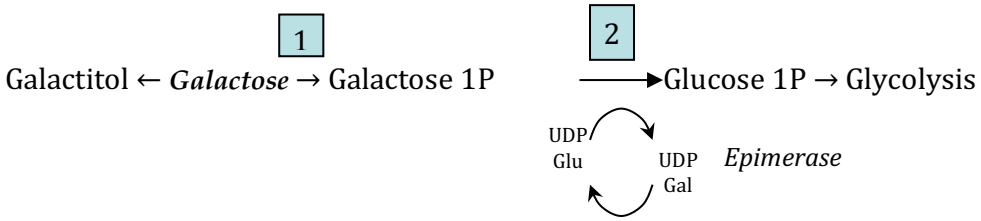
- Also known as the hexose monophosphate (HMP) shunt
- In the cytoplasm
- No ATP required
- Shunt around the first stage of glycolysis
- 2 steps:
  - Oxidative step:
    - Carried out by glucose 6-phosphate dehydrogenase
    - Generates NADPH
      - ⇒ keeps glutathione reduced (RBC)
      - ⇒ FA/steroid synthesis
  - Non-oxidative step:
    - Carried out by ***transketolase***
    - Generates ribose 5-P for DNA and RNA synthesis
- ***Glucose 6-P Dehydrogenase***
  - Rate limiting step in P.P. pathway
  - Produces NADPH from NADP
  - NADPH is used by ***glutathione reductase*** to reduce glutathione
  - Reduced glutathione converts $H_2O_2$ to $H_2O$
  - Oxidizing agents: anti-tuberculosis drugs, sulfonamides, primaquine or fava beans
    - Can lead to decreased levels of NADPH in RBCs results in hemolytic anemia
- ***Glucose 6-P Dehydrogenase*** deficiency
  - X-linked recessive
  - Increases resistance to malaria – P. falciparum
  - Common among African Americans
  - Hemoglobin precipitation in RBCs
    - Heinz bodies

## *Fructose metabolism*




- Fructose-1-P has a fast-metabolic rate
  - Converted to F-1,6-bis P
  - Enters glycolysis, bypasses PFK1 (rate-limiting step in glycolysis)
- Essential Fructosuria
  - Deficiency in ***Fructose Kinase*** 1
  - Asymptomatic (↑ fructose in blood and urine)
  - Fructose can still be metabolized by ***hexokinase***:
    - converted to F6P and enters glycolysis
- Fructose Intolerance:
  - Deficiency in ***Aldolase B*** 2
  - Hypoglycemia, vomiting, jaundice
  - Presentation: infant (~ 6 months) when solid food/juice is introduced
  - Treatment: lower intake of fructose and sucrose

## *Galactose metabolism*




- ***Galactokinase*** Deficiency
  - ↑ Gal in blood and urine 1
  - accumulation of galactitol in lens: infant cataracts
  - Treatment: restrict Gal from diet
- Galactosemia: Autosomal recessive
  - Deficiency in Gal 1-P Uridyl transferase 2
  - More severe: hepatosplenomegaly, cataracts, mental retardation, may result in infant death, *E. coli* sepsis is common
  - Accumulation of Galactitol: produced by ***Aldose reductase***, resulting ***infant cataracts***
  - Treatment: ***No*** Galactose or Lactose in Diet

## *Lactose metabolism*

Lactose → Glucose + Galactose

- Lactose intolerance:
  - ***Lactase*** Deficiency (common among Asian and Black populations)
  - Bloating, flatulence, diarrhea, abdominal pain after consumption of milk (or milk products)
  - Stool: bulky, frothy or watery
  - Treatment: Limit milk or give *lactase* pills

## *Glycogen metabolism*

| | | |
|---|---|---|
| Glycolysis | Breaking down glucose | Anaerobic/cytosol |
| Gluconeogenesis | Making new glucose | Cytosol/mitochondria ***Only*** in Liver, Kidney, intestinal Epithelium |
| Glycogenolysis | Breaking down glycogen to Glucose 1-P | By: ***Glycogen phosphorylase*** and debranching enzyme |
| Glycogenesis | Making glycogen from glucose | By: ***Glycogen synthase*** and branching enzyme |

- Glycogen Structure:
  - Branched Chain of glucose units
    - Main chain: α-1,4 bond:
      - ⇒ Made by ***Glycogen synthase***
      - ⇒ Broken by ***Glycogen phosphorylase*** to Glucose 1-P
    - Branches: a-1,6 bond:
      - ⇒ Made by Branching enzyme
      - ⇒ Broken by Debranching enzyme
- Types of glycogen
  - Liver glycogen:
    - For regulation of blood glucose level during fasting state (4 – 6 hrs after meal)
  - Muscle glycogen
    - Reserve for muscle activity not released into blood (used locally)

## Making or breaking glycogen

| *Process* | *Enzyme* | *Activated by* | *Inhibited by* |
|---|---|---|---|
| Liver glycogenolysis | ***Glycogen phosphorylase*** | Glucagon Epinephrine | Insulin |
| Liver Glycogenesis | ***Glycogen Synthase*** | Insulin Glucose | Glucagon Epinephrine |
| Muscle glycogenolysis | ***Glycogen phosphorylase*** | AMP Epinephrine | ATP Insulin |
| Muscle Glycogenesis | ***Glycogen Synthase*** | ATP Insulin | AMP Epinephrine |

- Fed State:
  - Glucose up, Insulin up, glucagon down, glycolysis
  - ATP up, AMP down
  - Glycogenesis is up-regulated (making glycogen for storage)
- Fasting State:
  - Glucose down, insulin down, glucagon up
  - ATP down, AMP up
  - Gluconeogenesis and glycogenolysis are up-regulated
- Ketone bodies:
  - Acetoacetate and β-hydroxybutyrate
  - Result from prolonged starvation or diabetic keto-acidosis
  - Synthesized from HMG-CoA in liver
  - Used in muscle and brain (under starving conditions)
  - Both excreted in urine: only acetoacetate is detected
  - Breath smells like acetone

# Gluconeogenesis

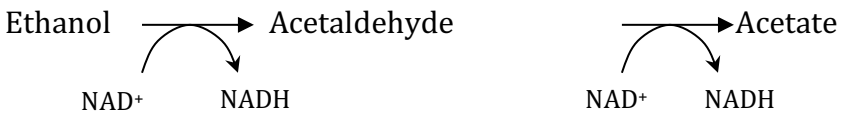
- Reverse of glycolysis
  - Except 3 steps that involve phosphates
    - Pyruvate to PEP
      - ⇒ Intermediate: oxaloacetate
      - ⇒ Energy from GTP produced in TCA can be used here
    - Fructose-1,6 bis-P → Fructose 6-P
    - Glucose 6 P to Glucose
- Substrates for gluconeogenesis:
  - Pyruvate, oxaloacetate, lactate, glycerol, alanine
- Acetyl CoA and Fatty acids are NOT substrates for gluconeogenesis

## *Glycogen Storage diseases*

| ***Disease*** | ***Enzyme*** | ***Clinical picture*** |
|---|---|---|
| I. Von Gierke's<br>***Glycogen in liver*** | ***Glucose 6 phosphatase***<br>(G6P to Glucose) | Baby with hepatomegaly, ↑ lactate hyperuricemia, "doll-face", severe fasting hypoglycemia, short stature |
| II. Pompe's disease<br>***Glycogen in lysosomes*** | ***Lysosomal α-1,4-glucosidase***<br>(acid maltase) | Baby, flaccid, hypotonic, large tongue, myopathy, ***cardiomyopathy***, ↑ serum ***creatine kinase***, early death |
| III. Cori's disease<br>***Incomplete break-down of glycogen*** | Debranching enzyme: ***α-1,6-glucosidase*** | Hepatosplenomegaly, myopathy, hypoglycemia, hyperlipidemia, normal lactate, milder than type I, treat with corn starch |
| V. McArdle's<br>***Glycogen in muscle*** | Muscle ***glycogen phosphorylase*** | Painful muscle cramps, dark urine (myoglobinuria), intolerance to exercise, ↑ ***resting creatine kinase***<br>Ischemic exercise test => no elevation in lactate |

***All*** are autosomal recessive

## Alcohol metabolism

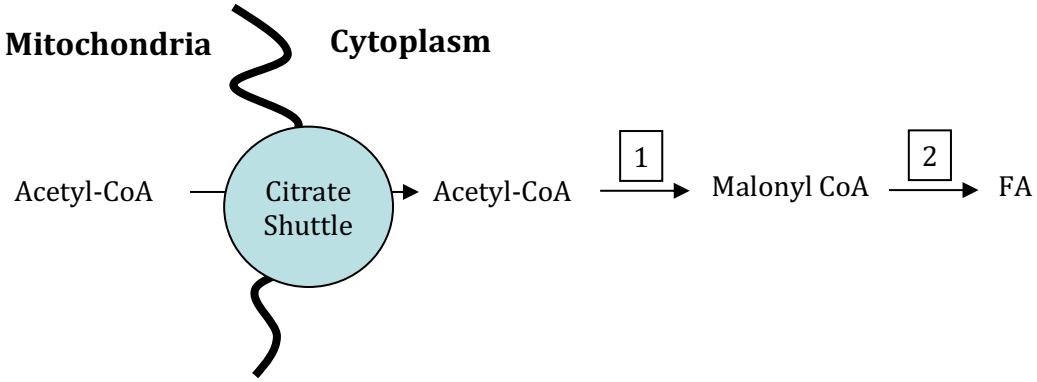



- Reactions deplete $NAD^+$ supply
  - To regenerate NAD:
    - Pyruvate is converted to lactate
    - Oxaloacetate is converted to malate
    - Depletes gluconeogenic molecules
    - ↑ FA synthesis and hepatocellular steatosis (Fatty change in the liver)
- Fetal Alcohol Syndrome:
  - #1 cause of congenital malformations in the US
  - Alcohol consumption by pregnant mother
  - First 3 – 8 weeks is the highest risk
  - Results in:
    - Pre- and postnatal development retardation
    - Microcephaly, abnormalities in facial features
    - Limb dislocation, heart and lung fistulas due to inhibition of cell migration

## Fatty Acid metabolism

- Structure:
  - Long carbon chains with acid group
  - 16 – 20 carbons long
- Types of fatty acids
  - Saturated FA = no double bonds
  - Unsaturated FA = some double bonds
  - Poly unsaturated = many double bonds
  - LCFA = long chain FA
  - MCFA = medium chain FA

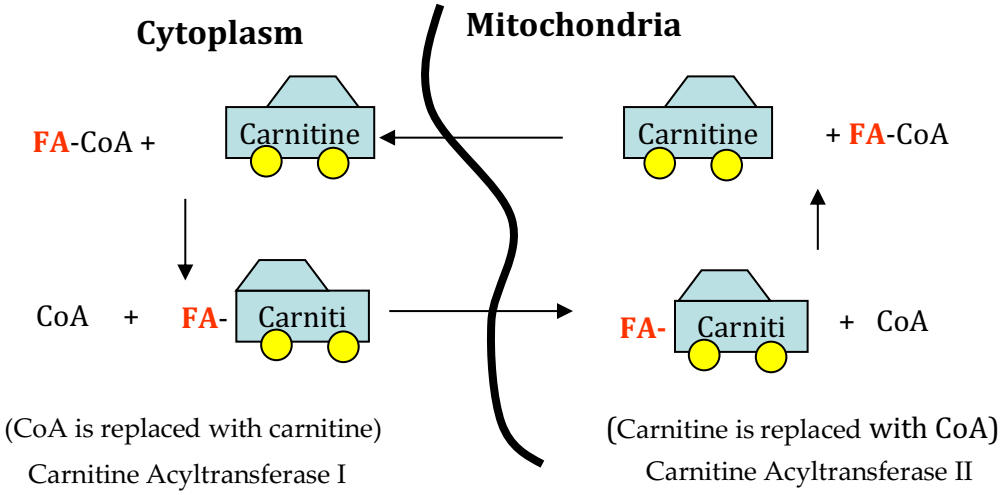
## *Fatty acid biosynthesis*




- Acetyl CoA carboxylase [1]:
  - Adds $CO_2$ to Acetyl CoA to make malonyl CoA
  - Requires Biotin
  - Activated by Insulin
  - Inhibited by glucagon, epinephrine
- ***FA synthase*** [2]:
  - Adds 2 carbons at a time to make FA chain
  - Uses NADPH

➢ FA degradation (β-oxidation)

- First Step: transport of FA into mitochondria by Carnitine Shuttle



- Second Step: break down of FA chain into acetyl CoA in Mitochondria
  - Carnitine Deficiency:
    - ⇒ Inability to break down LCFA (MCFA are OK)
    - ⇒ Accumulation leads to toxicity
    - ⇒ Hypoglycemia, muscle pain/atrophy
    - ⇒ Treatment: Limit diet to MCFA (butter Fat)

- Fatty acid storage:
  - 3 FAs + 1 glycerol = triacyl glycerol (triglyceride)
  - Essential FAs: Linoleic (omega-6) and linolenic acid (omega-3)
- Fatty acid Transport:
  - Liver to Adipose by VLDL
  - FA from diet as chylomicrons: from intestine to blood stream

## Cholesterol metabolism

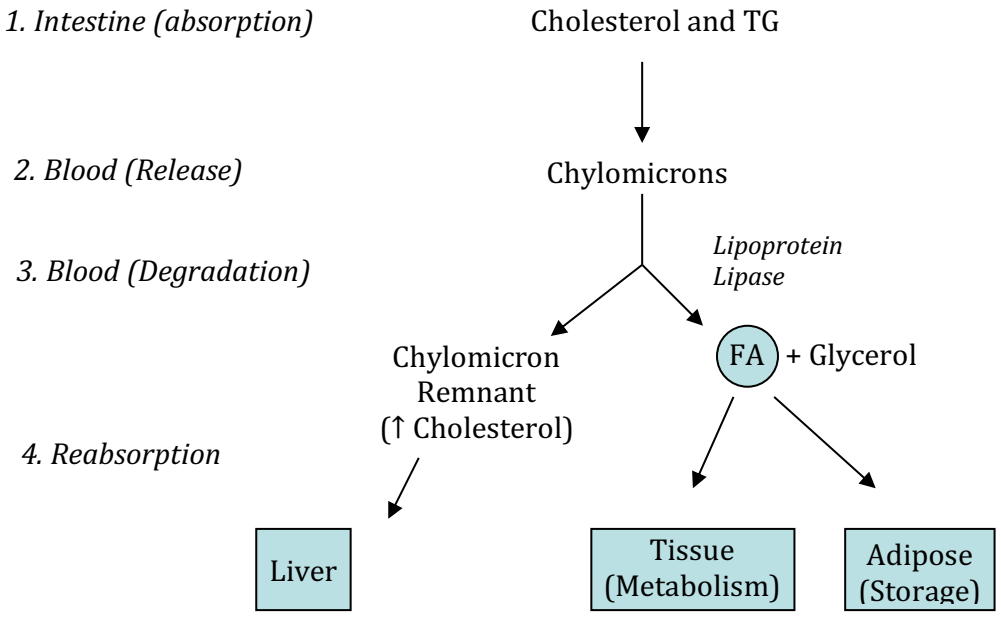
- Cholesterol Biosynthesis

$$\text{Acetyl-CoA} \rightarrow \text{HMG CoA} \xrightarrow{1} \text{Mevalonic acid} \rightarrow\rightarrow \text{Cholesterol}$$

  - Takes place in liver and intestinal mucosa
  - Main enzyme: ***HMG CoA reductase***: 1
    - Rate-limiting step, requires 2 NADPH
    - Inhibited by statins and high cholesterol levels
- Cholesterol modification:
  - Esterified by Lecithin Chol - Acetyltransferase (LCAT)
    - 2/3 of total cholesterol is esterified → trapped as LDL
    - LCAT is activated by Apolipoprotein A-I
  - Cholesterol esters are transported to apolipoprotein particles by CETP (cholesterol ester transfer protein)

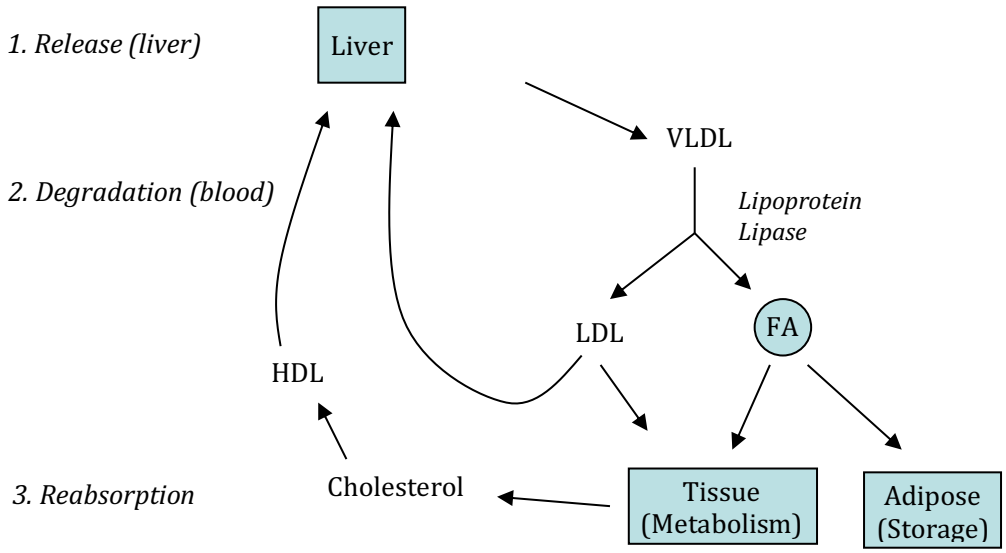
# Lipid transport

## *Exogenous lipid transport*




*Liver can directly absorb Remnants or through HDL*

## *Endogenous lipid transport*



## *TG breakdown enzymes (lipases)*

- ***Pancreatic Lipase:*** TG in Small intestine
- ***Hepatic Lipase:*** TG in LDL
- ***Lipoprotein Lipase:*** TG in VLDL and chylomicrons
- ***Hormone-sensitive Lipase:*** TG in adipose tissue

## *Apolipoproteins*

- ***A-I:***
  - Activates LCAT (cholesterol → LDL)
  - Major component of HDL
  - A-I deficiency:
    - ⇒ Tangiers Disease (Familial alpha apolipoprotein deficiency)
    - ⇒ Low HDL, early onset atherosclerosis
    - ⇒ High levels of intracellular cholesterol
- ***Apolipoprotein B:***
  - Mutations are associated with familial hypercholesterolemia
  - B-100:
    - ⇒ Mediates VLDL secretion by binding to LDL receptor on liver
    - ⇒ Found in VLDL, LDL and IDL
  - B-48:
    - ⇒ Mediates chylomicron secretion
    - ⇒ Found in chylomicron and remnants
- ***C-II:***
  - A "cofactor" (activator) for Lipoprotein lipase
  - Found in VLDL, HDL and Chylomicron
- ***E:***
  - Mediates uptake of remnants by the liver
  - Found in chylomicron and remnants
  - Also found in VLDL, IDL and HDL

## Dyslipidemia

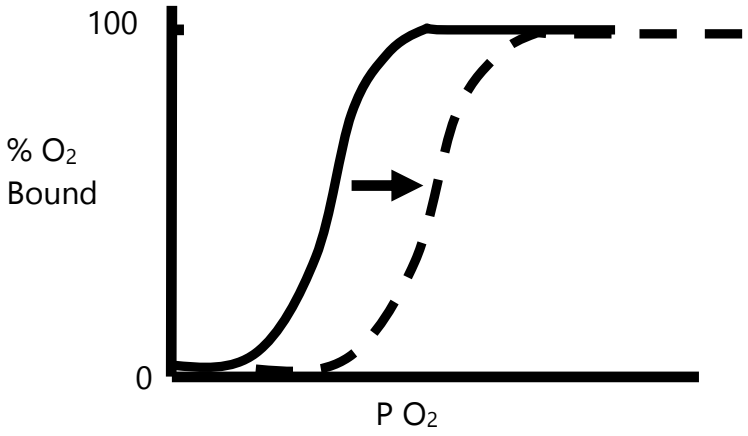
- Type I:
  - Hyperchylomicronemia (autosomal recessive)
  - Deficiency in lipoprotein lipase or C-II mutation
  - Result: high chylomicron, TGs, cholesterol – ***acute pancreatitis***
- Type IIa:
  - Familial hypercholesterolemia (autosomal dominant)
  - LDL receptor mutation
  - High LDL (cholesterol), Xanthelasma, arcus senilis, tendon xanthomas
- Type IIb:
  - Familial combined hypercholesterolemia (autosomal dominant)
  - LDL receptor mutation AND increased ApoB-100
  - High LDL and VLDL (cholesterol)

  ***Both IIa and IIb can lead to early Myocardial Infarction.***
- Type III:
  - Dysbetalipoproteinemia (autosomal recessive)
  - Mutations in the gene coding for Apo E
  - Elevated levels of triglycerides and total cholesterol → ↑ prevalence of early-onset CAD – ***On the Boards*** - look for a patient with palmar and tuberoeruptive xanthomata
- Type IV:
  - Familial hypertriglyceridemia (autosomal dominant)
  - Overproduction of VLDL, high TGs NOT cholesterol – ***acute pancreatitis***

# Hemoglobin

## Basic Properties of hemoglobin (Hb)

- Two states of Hb
  - R form (relaxed): High affinity for Oxygen
  - T form (Taut): Low affinity for Oxygen
- Factors that convert R to T (release $O_2$):
  - ↑ $CO_2$, ↑ $H^+$ (↓ pH), ↑ Temp or ↑ $Cl^-$ (active tissue)
  - High level of 2,3-BPG resulting from:
    - high altitude
    - anemia
    - emphysema
- Hemoglobin curves
  - Binding of $O_2$:
    - Positive co-cooperativity = sigmoidal curve

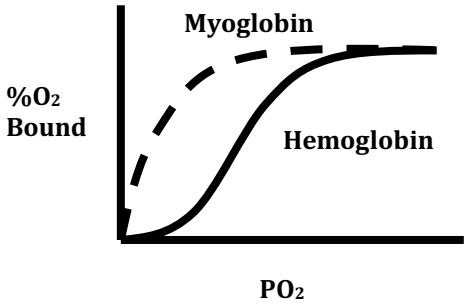



Curve shifts to the ***Right*** (R to T) by $CO_2$, low pH, Cl or BPG

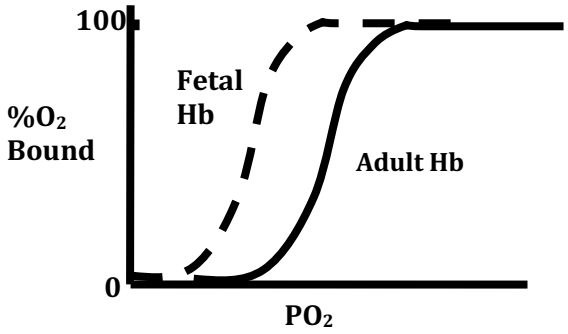
Resulting in ***Lower*** oxygen affinity and release of oxygen

  - In Lungs: High $PO_2$, Hb is saturated with $O_2$
  - In Tissue: Low $PO_2$, Hb releases $O_2$

- Myoglobin
  - One subunit, 1 heme
  - One Oxygen binding site = no cooperativity = no sigmoidal curve

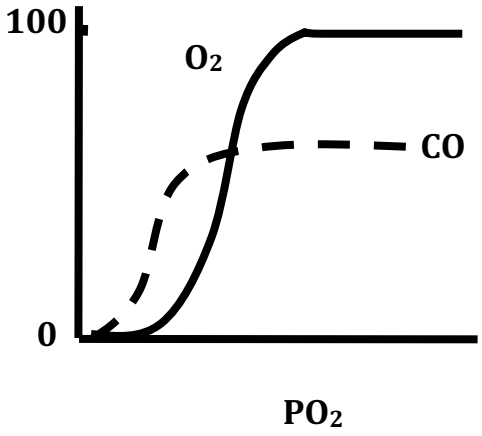



- Fetal Hb
  - four subunits
    - 2 alpha , 2 gamma
  - Lower Affinity for BPG = Higher affinity for $O_2$




Curve shifted to the ***Left*** = ***Higher*** affinity for $O_2$ than Mother's Hb

- Carbon monoxide (CO) poisoning:
  - CO is a non-covalent competitive binder
  - 200 X tighter than $O_2$
  - Prevents full $O_2$ binding
  - Symptoms:
    - Dizziness, chest pain, weakness, loss of consciousness, and death
  - Treatment:
    - High quantities of oxygen




CO also binds to ***porphyrin*** ring of ***Cytochrome oxidase C*** in the ETC preventing ATP production

## Gas Exchange

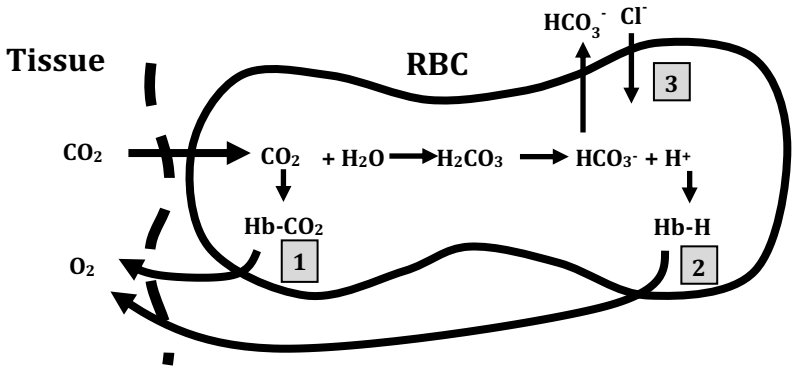
***Dalton's Law*:** Partial pressure = total pressure X fraction of gas concentration

- Under normal conditions
  - $O_2$ is perfusion-limited: $O_2$ equilibrates;
  - $PO_2$ in alveolar air = $PO_2$ in arterial blood
  - $CO_2$ and $N_2O$ are perfusion limited
- Exercise or disease (emphysema or fibrosis)
  - $O_2$ is diffusion-limited: $O_2$ does not equilibrate
  - $PO_2$ in alveolar air ≠ $PO_2$ in arterial blood
  - CO is diffusion-limited

## Partial Pressure Distribution

| | $PO_2$ | $PCO_2$ |
|---|---|---|
| ***Inspired Air (dry)*** | 160 | 0 |
| ***Tracheal Air (humidified)*** | 150<br>(Less because of $H_2O$) | 0 |
| ***Alveolar Air*** | 100<br>(Less because $O_2$ goes into pulmonary capillaries) | 40<br>($CO_2$ comes from pulmonary capillaries) |
| ***Systemic Arterial Blood*** | 100<br>(same as alveolar air) | 40<br>(same as alveolar air) |
| ***Mixed Venous Blood*** | 40<br>(most $O_2$ gone to tissues) | 46<br>($CO_2$ coming from tissue) |

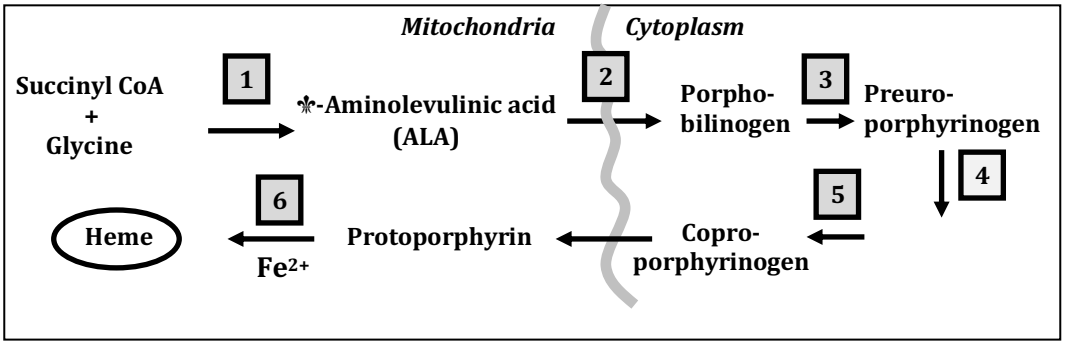
## $CO_2$ transport




- $CO_2$ diffuses from tissue to RBC:
  - Binds directly to Hb [1]
  - Reacts with $H_2O$ to make Carbonic acid ($\uparrow H^+$) [2]
  - Bicarbonates is exchanged for Cl- [3]
  - All result in:
    - Shift from R to T form (Hb curve shifts to the ***right***)
    - ***Lower*** affinity for $O_2$ release of $O_2$ to tissue

## Heme (porphyrin) Synthesis

- Process:
  - Mitochondria → Cytoplasm → Mitochondria
  - Enzyme deficiencies: accumulation of intermediates = porphyrias
  - Low level of heme synthesis = microcytic hypochromic anemia




- ***ALA synthetase:***
  - Main regulatory enzyme
    - Inhibited by ALA (product)
    - Inhibited by Hemin (derivative of Heme)
    - Requires Vitamin B6 (pyridoxal phosphate)
    - Deficiency: sideroblastic anemia (X-linked recessive)

**Uro-**
**Porphyrinogen III**

- Lead Poisoning: 1
  - Inhibited Enzymes:
    - ***ALA dehydratase*** 2
    - ***Ferrochelatase*** 6
  - Lead paint exposure:
    - Children or workers in old building (1970's or before)
  - Symptoms:
    - Abdominal pain, constipation, weakness, neuropathy, "lead-line": Bluish tint on the gum-tooth line, ↑ blood ALA
- Acute Intermittent Porphyria:
  - Defect in Uroporphyrinogen I Synthase 3
    - AKA PBG deaminase
    - Can be caused by barbiturates, valproate, gonadal steroids, poor diet

- Symptoms:
  - Abdominal pain, neuropsychiatric signs, blurred vision, hyponatremia, hyporeflexia, hallucinations
  - ***No Photophobia***
  - Urine: Dark upon exposure to air, ↑ porphobilinogen
- Treatment:
  - Hemin
  - Discontinue precipitating factors

- Congenital Erythropoietic Porphyria:
  - Deficiency in URO III CO synthetase **4**
    - AKA UPG Synthase
  - Presentation:
    - Infant with photosensitivity
    - Disfigurement (face and hands)
    - Skin thickening, friable bullae and vesicles, splenomegaly
  - Treatment:
    - Blood transfusion
    - Beta carotene supplement
- Porphyria Cutanea Tarda:
  - Deficiency in URO III decarboxylase **5**
    - AKA UPG decarboxylase
  - Presentation:
    - Adult onset with photosensitivity
    - Skin thickening, bullae and vesicles, milia (white plaques preceding vesicles)
    - ***No*** neuropathy
  - Treatment:
    - No alcohol
    - Iron supplement or estrogen
    - Repeated phlebotomy
    - Low-dose chloroquine

### *Porphyrias for the Boards*

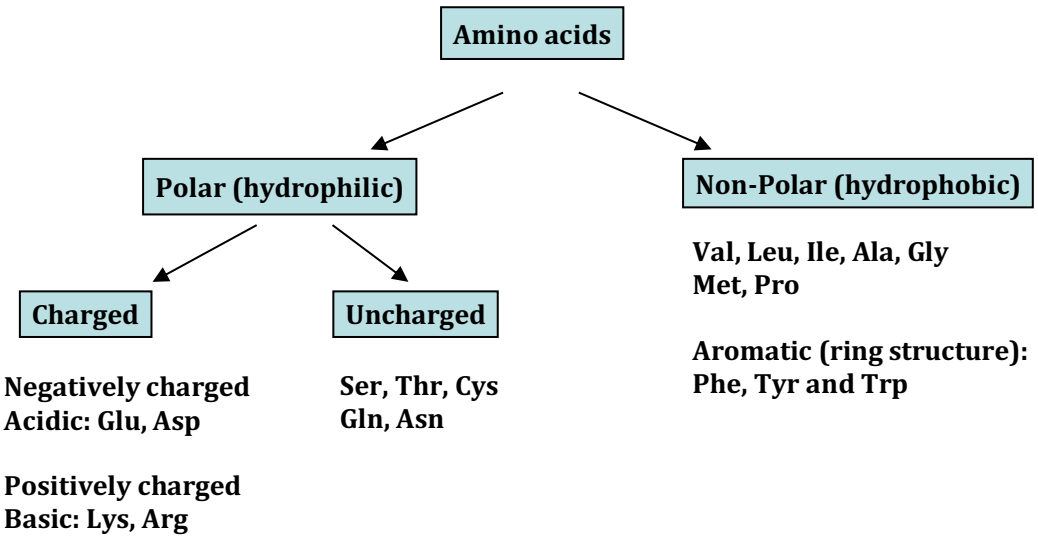
| | *AIP* | *CEP* | *PCT* | *Lead poisoning* |
|---|---|---|---|---|
| ***Photophobia*** | No | Yes | Yes | No |
| ***Urine/blood*** | ↑ ALA, porphobilin-ogen | ↑ Uro-porphyrin I Copro-porphyrin I | ↑ Porphyrins | Lead |
| ***Look for*** | Use of: Barbiturates Valproate | Infant, disfiguring red-brown teeth | Adult, sunburns (vesicles) preceded by milia | Lead paint exposure (old house) |
| ***Mode of Inheritance*** | Autosomal Dominant | Autosomal Recessive | Autosomal Dominant | Acquired |

## Heme Catabolism

- Iron is recycled
- Heme is converted into Bilirubin
- Bilirubin:
  - Toxic to the CNS
  - Transported by albumin in the blood
  - In liver: attached to glucuronic acid and excreted in bile
  - Calcium bilirubinate gall stones can occur in:
    - Sickle cell anemia and hemolytic anemia
    - Liver Fluke or liver cirrhosis

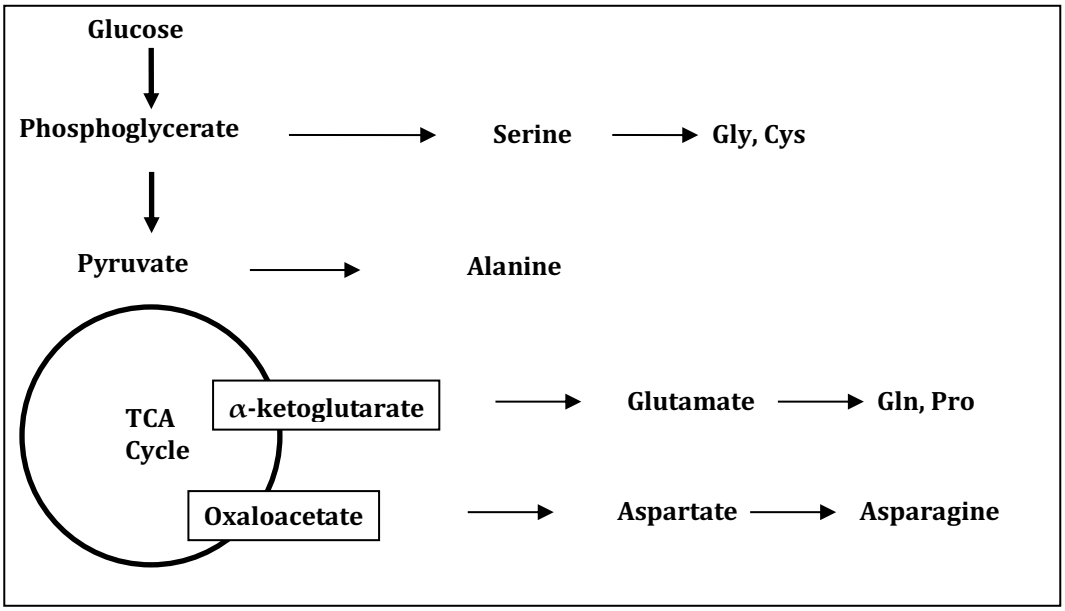
# Amino Acids

## Classification of amino acids




- Cys and Met have sulfur but ***only cysteine*** can form disulfide bonds
- Histidine is basic, aromatic uncharged at physiological pH
- Arg and Lys:
  - Positively charged
  - Major components of histones
  - Histones bind to negatively charged DNA
- Essential amino acids:
  - Leu, Ile, Val, Phe, Trp, Thr, Met, Lys
  - Arg and His: during development
- Glucogenic and ketogenic: Ile, Thr, Phe, Tyr, Trp
- Ketogenic only: Leu, Lys
- Glucogenic only: the rest

## Amino acid Synthesis



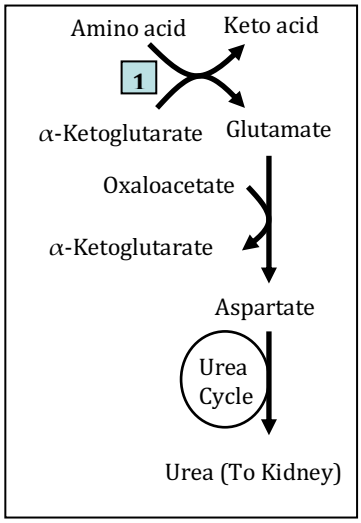

- Serine, Glycine and Cysteine: from phosphoglycerate
- Alanine: from pyruvate
- Glutamate, Glutamine and Proline: from $\alpha$-ketoglutarate
- Aspartate and Asparagine: from oxaloacetate

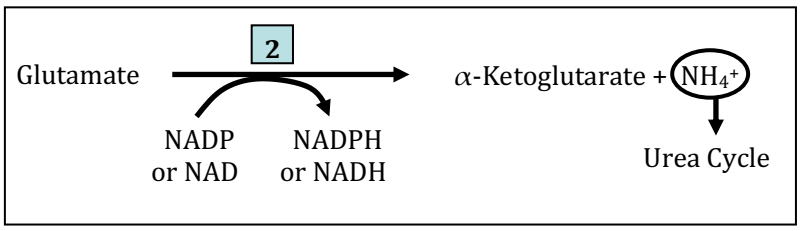
# Amino acid degradation and nitrogen excretion

- The Urea cycle:
  - Degradation of amino acids
  - Excretion of amine group in the form of urea

## *There are two ways for nitrogen to enter the urea cycle*

- 1. Transamination:
  - Enzyme: ***Aminotransferase*** **1**
    - Requires Vitamin $B_6$ (pyridoxal)
    - Amino group is removed to make ***keto-acid***
    - Received by $\alpha$-ketoglutarate to make glutamate
  - Glutamate is converted to Aspartate, which enters ***the Urea cycle***
- 2. Oxidative Deamination:
  - In liver and kidney
  - Ammonia is released and goes to Urea cycle
  - Enzyme: ***Glutamate Dehydrogenase*** **2**
    - Activated by ATP and GTP
    - Inhibited by ADP and GDP

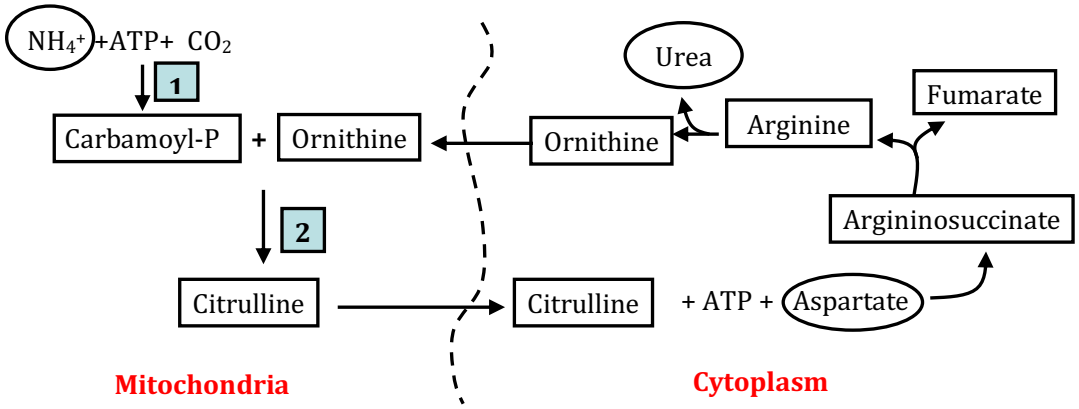


## *The Urea cycle*

- Occurs in mitochondria and cytoplasm
  - Citrulline: from mitochondria to cytoplasm
  - Ornithine: from cytoplasm to mitochondria
- Nitrogen enters the Urea Cycle in the form of:
  - Ammonia (from reductive deamination)
  - Aspartate (from transamination)
- Requires ATP, $CO_2$
- Produces Urea (excreted) and Fumarate (TCA intermediate)

***Deficiency in UC enzymes: Hyperammonemia, Mental Retardation, seizures, coma, death (Ammonia toxicity)***



## Urea Cycle – Pearls

- Key Enzymes of the Urea Cycle:
  - Carbamoyl P synthetase I **1**
    - Activated by *N*-acetylglutamate
    - High protein diet = high glutamate in mitochondria = high *N*-acetylglutamate
  - Ornithine transcarbamoylase **2**:
    - Makes citrulline from carbamoyl-P and ornithine
  - Deficiency in either enzyme results in:
    - ↑ blood glutamine
    - ↑ $NH_4$ (hyperammonemia)
    - ↓ BUN
    - Cerebral edema, convulsions, coma, and death

***Only Ornithine Transcarbamoylase deficiency results in ↑uracil and orotic acid in blood and urine***

### Amino Acid Derivatives

- Glycine: Porphyrin (heme synthesis)
- Histidine: Histamine
- Arginine: Creatine, Urea and Nitric Oxide
- Phenylalanine: Tyrosine
- Tyrosine:
  - Thyroxin, Melanin
  - Dopa, dopamine
  - Epinephrine, norepinephrine
- Glutamate: GABA (by glutamate decarboxylase)
- Tryptophan:
  - Niacin (vitamin $B_3$: $NAD^+$, NADP)
  - Serotonin and melatonin

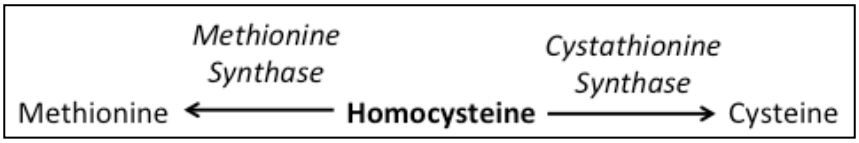
# Disorders of Amino Acid Metabolism

***All amino acid metabolism disorders are Autosomal Recessive***

## *Cystinuria*

- Defect in transporter for cys, lys, arginine, ornithine:
  - In renal tubules and intestinal epithelium
- Cystine (radiopaque) kidney stones, Hematuria
- Labs:
  - ↑ excretion of cys, lys, arg and ornithine (urine a.a. chromatography test)
  - Hexagonal cystine crystals (cooling acidified urine)
- Treatment: Acetazolamide (to alkalize urine)
- Increase fluid intake, decrease Methionine in diet

## *Homocystinuria*




- Can be due to either:
  - 1. ***Cystathionine synthase*** mutation or deficiency
  - 2. ***Methionine synthase*** mutation or deficiency
  - 3. ***Cystathionine synthase*** defect: low affinity for $B_6$
  - 4. Rare: $B_{12}$ deficiency (***Methionine synthase*** requires $B_{12}$)
- Symptoms:
  - Elongated limbs, lens dislocation (like Marfan),
  - Increased serum Met
  - Increased urine homocysteine
  - Mental retardation, ↑ risk for thromboembolism
- Treatment: low Met diet, high folate and cysteine
  - If cause is #1 or #2: low Met diet, high folate and cysteine
  - If cause is #3, High dose of $B_6$

## *Phenylketonuria*

- Caused by either:
  - Deficiency in ***phenylalanine hydroxylase*** (converts Phe → Tyr)
  - Deficiency in tetrahydrobiopterin cofactor ($BH_4$)
- Symptoms:
  - Low pigmentation (blond/blue eyes), microcephaly, mental and growth retardation, and musty body/urine odor
  - Phenyl ketones in Urine
  - Positive Guthrie test (Phe in blood)
  - BH4 also required for serotonin synthesis from Tryptophan, patients may have serotonin deficiency, not responding to Tyrosine supplement
- Treatment:
  - Low Phe in diet (no Aspartame: Neutra sweet)
  - Tyr becomes essential (increase in diet)

## *Alkaptonuria*

- Defect in ***Homogentisic acid oxidase*** (enzyme that degrades tyrosine)
- Results in accumulation of Tyrosine, Phenylalanine and Homogentisic acid
- Symptoms:
  - High pigmentation, degeneration of cartilage, discoloration of connective tissue (ochronosis), arthritis, dark urine (Homogentisic acid polymers)
- Treatment: manage arthritis symptoms

## *Hartnup Disease*

- Caused by defect in sodium-dependent neutral amino acid transport protein in:
  - Proximal tubule in kidney
  - Brush border small intestine
- Result:
  - Low absorption of tryptophan (niacin precursor)
  - Niacin deficiency
- Symptoms:
  - Pellagra, photosensitive dermatitis, cerebellar ataxia, headaches, personality disturbances
  - Indole in urine (Renal aminoaciduria)
- Treatment: Nicotinic acid

### *Maple syrup urine disease*

- Caused by deficiency in ***α-ketoacid dehydrogenase***
- Result:
  - ♦ No degradation of branched a.a. (Leu, Ile, Val)
  - ♦ Accumulation of α-ketoacid of Leu, Ile and Val
  - ♦ High mortality rate
- Symptoms:
  - ♦ Metabolic acidosis (α-ketoacidosis), Brain damage and Maple Syrup urine odor
- Treatment: restrict branched amino acids in diet and dialysis

## Amino acid metabolism disorders: quick guide

| *Disorder* | *Presentation* | *Urine* | *Treatment* |
|---|---|---|---|
| ***Cystinuria*** | Kidney stones<br>Hematuria | Cysteine Hexagonal crystals | Acetazolamide |
| ***Homocystinuria*** | Marfan-like symptoms | Homocysteine | Folate/cysteine or vitamin B6 |
| ***Phenylketonuria*** | Blond/blue eye<br>Musty odor | Phenyl ketones | Decrease Phe, Increase Tyr |
| ***Alkaptonuria (Ochronosis)*** | ↑ Pigmentation arthritis | Dark: Tyr/phe/ Homogentisic acid | Manage arthritis |
| ***Hartnup*** | B3 deficiency symptoms | Indole (aminoaciduria) | Nicotinic acid |
| ***Maple syrup urine*** | $\alpha$-ketoacidosis brain damage | Smells like maple syrup! | Decrease intake of branched a.a., dialysis |

# Vitamins and Nutrients

## Classification of vitamins

- Fat soluble:
  - A, D, E, K
- Water soluble:
  - $B_1$, $B_2$, $B_3$, $B_5$, $B_6$, $B_{12}$, C, Biotin, Folate

Vitamins usually serve as cofactors for enzymes

### *Vitamin A*

- Forms and function:
  - 11-***cis***-retinol: part of rhodopsin (vision pigment)
  - Retinoic acid: growth, reproduction and maintenance of epithelial tissue
  - Beta-carotene (a precursor of vitamin A): Antioxidant
- Deficiency:
  - Night blindness to full blindness, Bitot's spots (conjunctiva)
  - Corneal keratinization
  - Poor wound healing, Infection susceptibility
  - Dry Skin
- ***On the Boards, look for:***
  - Elderly or urban poor, malnutrition
  - Fat absorption disorders:
    - ⇒ Inflammatory bowel syndrome, gastrectomy, pancreatic insufficiency, cholestatic liver disease
  - Laxative/mineral oil abuse
- ***Treatment:*** Vitamin A supplement, early signs can be reversed
- Toxicity:
  - Nausea, vomiting, headache, scaly skin, papilledema
  - Hepatosplenomegaly

## *Vitamin D*

- Forms:
  - $D_2$ : ergocalciferol
    - ⇒ Absorbed by intestine
  - $D_3$ : cholecalciferol (7-dehydrocholesterol)
    - ⇒ Synthesized in skin exposed to UV light
  - 25-hydroxy $D_3$
    - ⇒ hydroxylated in liver (storage form)
  - 1,25-dihydroxy $D_3$
    - ⇒ Hydroxylated in Kidney (active form)
- Function:
  - Stimulates osteoblast activity with help from PTH
  - Bone formation
  - Calcium absorption by kidney (distal tubules) and intestine
- Deficiency:
  - Rickets: Children
  - Skeletal deformation:
    - ⇒ Short stature, sternum protrusion (***pigeon breast***)
    - ⇒ ***Craniotabes***: occipital and parietal bone thinning
  - Osteomalacia: Adults
  - Generalized bone pain
- ***On the Boards, look for:***
  - Malnutrition, decreased sun exposure, fat absorption disorders
  - Liver disease or chronic renal failure
- ***Treatment:*** Vitamin D supplement, exposure to sunlight
- Toxicity:
  - Soft tissue calcification, bone demineralization
  - Hypercalcemia (more calcium absorption)
  - Kidney stones, loss of appetite, stupor
  - Sarcoidosis can lead to vitamin D toxicity
    - ⇒ Due to activation of vitamin D by epithelioid macrophages

## *Vitamin E*

- Function:
  - Anti-oxidant
  - Maintenance of cell membrane integrity (RBCs)
  - Protection from free radicals
- Deficiency:
  - RBC membrane fragility: Hemolytic anemia
  - Vision disturbance
  - Neurologic disfunction and myopathy, ataxia (resembling Friedreich's ataxia)

## *Vitamin K*

- Sources:
  - Diet and synthesis by normal intestinal bacteria
  - Newborns:
    - ⇒ Low absorption from placenta
    - ⇒ Low amount of normal intestinal flora
    - ⇒ ***Always supplement newborns with vitamin K!***
    - ⇒ ***On the boards: home-birth presenting with early cerebral hemorrhage***
- Function:
  - Cofactor for Glutamate Carboxylase
    - ⇒ Enzyme which adds a carboxy group on glutamate residue in clotting factors: II, VII, IX and X
    - ⇒ Leads to activation of coagulation response
- Deficiency:
  - Impaired blood clotting, increased bruising
  - Mucous membrane bleeding
  - Prolonged PT and PTT with normal bleeding time and thrombin time
  - May be asymptomatic
  - ***On the Boards, look for:***
    - ⇒ Elderly or malnutrition
    - ⇒ Recent use of broad-spectrum antibiotics
    - ⇒ Bruising, prolonged PT and PTT
- ***Treatment:*** Vitamin K supplement

## *B vitamins*

| *Name/forms* | *Function* | *Deficiency* |
|---|---|---|
| B1: Thiamine pyrophosphate | Pyruvate DHase, transketolase | Dry and wet Beriberi ***Wernicke-Korsakoff***, amnesia, confabulation, alcoholics |
| B2: Riboflavin | Part of $FAD^+$ and FMN | ***Angular cheilitis***, corneal vascularization |
| B3: Niacin | Part of $NAD^+$ and NADH | No appetite, weakness, ***dermatitis, dementia, diarrhea*** |
| B5: Pantothenic acid | Co-enzyme A (FA synthesis) | Rare (other B deficiencies) Dermatitis, hair loss, gastritis |
| B6: Pyridoxine | Pyridoxal (AA synthesis) | Pregnancy or ***isoniazid***, oral contraceptives, neuropathy, seizures |
| B12: Cobalamin | Homocysteine to methionine | Vegans, pernicious anemia History: Crohn disease, gastrectomy |

- Remember:
  - B1, B2, B3, B5 (and lipoic acid) are cofactors for TCA cycle enzymes:
    - Pyruvate DHase
    - α-Ketoglutarate DHase
  - Deficiency: results in ↑ lactate and alanine
- Vitamin B1 (thiamine):
  - Cofactor for transketolase:
    - Part of HMP shunt (pentose-phosphate pathway)
    - Converts ribulose-5-P to ribose-5-P

> Low erythrocyte transketolase activity = Wernicke's encephalopathy

- B12: Cobalamin is cofactor for:
  - Homocysteine to methionine conversion
  - Methyl malonyl CoA to Succinyl CoA (FA metabolism)

> B12 deficiency - look for a vegan with megaloblastic anemia + neuropathy (damage to dorsal column, spinocerebellar tract)

## *Vitamin C*

- Forms:
  - Ascorbic Acid: from citrus fruits
- Functions:
  - Collagen synthesis:
    - ⇒ Pro and Lys hydroxylation in the Rough Endoplasmic Reticulum
  - Iron Absorption: keeps iron reduced
  - Cofactor for dopamine to norepinephrine conversion
- Deficiency:
  - Scurvy, bleeding gums, easy bruising, poor wound healing, anemia

## *Biotin (Vitamin B7)*

- Function: Cofactor for carboxylation:
  - Pyruvate to oxaloacetate (gluconeogenesis)
  - Acetyl CoA to Malonyl CoA (FA synthesis)
  - Propionyl CoA to methyl malonyl CoA (FA metabolism)
- Sources:
  - From diet or made by normal flora
- Deficiency: Rare
  - Look for Antibiotic use, raw eggs (avidin binds to biotin)
  - Dermatitis, gastroenteritis, elevated cholesterol

## *Folic acid (Vitamin B9)*

- Forms:
  - Reduced to tetrahydrofolate (active form)
- Function:
  - Required for Methyl (1 Carbon) transfers:
    - ⇒ UMP (RNA) to dTMP (DNA) (DNA synthesis)
    - ⇒ Homocysteine to methionine (like B12)
    - ⇒ Serine ↔ glycine
- Deficiency: very common (dietary)
  - Alcoholics, sulfa drugs, methotrexate, phenytoin, sprue
  - Pregnancy: must supplement (neural tube defects)
  - Lab findings: megaloblastic anemia: hypersegmented neutrophils (blood smear)

## *Lipoic acid*

- Function:
  - Cofactor for:
    - ⇒ Pyruvate DHase and $\alpha$-Ketoglutarate DHase
    - ⇒ Remember: Both enzymes also require B1, B2, B3 and B5
  - Inhibited by Arsenic resulting in:
    - ⇒ Vomiting, garlic breath, rice water diarrhea

## *Malnutrition*

- ***Kwashiorkor***: Protein-deficient diet:
  - Skin lesions, hepatomegaly, edema, weight loss
  - Clinical picture: starving child with large belly
- ***Marasmus***: Calorie-deficient diet:
  - Weakness, anemia, stunted growth, muscle wasting, variable edema
  - Clinical Picture: poor child in developing country

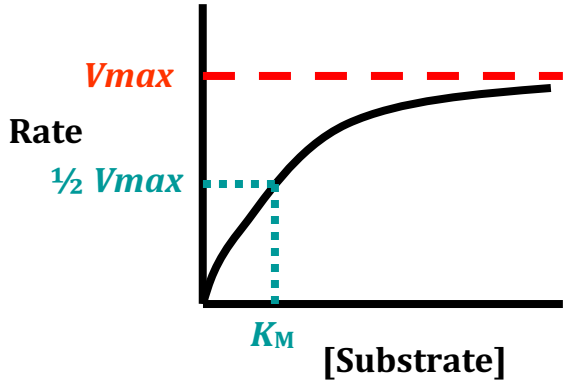
## *Nutrients and trace elements*

- ***Zinc:***
  - Function:
    - ⇒ Cofactor for collagenase (wound remodeling)
    - ⇒ In children: spermatogenesis and growth
  - Deficiency:
    - ⇒ Delay in wound healing
    - ⇒ Decrease in adult hair (facial, pubic and axillary)
    - ⇒ Hypogonadism, loss of taste and smell
  - Look for: Alcohol use predisposes to alcoholic cirrhosis, diabetes mellitus, acrodermatitis enteropathica: autosomal recessive
- ***Copper***
  - Functions as a cofactor for:
    - ⇒ ***Ferroxidase:*** attaches iron to transferrin
    - ⇒ ***Lysyl oxidase:*** cross-links collagen and elastic tissue
    - ⇒ ***Tyrosinase:*** converts tyrosine to melanin
  - Deficiency:
    - ⇒ Microcytic anemia, aortic dissection, poor healing
  - Wilson's Disease
    - ⇒ Autosomal recessive
    - ⇒ Poor ability to eliminate copper into bile
    - ⇒ Look for - chronic liver disease, basal ganglia degeneration, ***Kayser-Fleischer*** ring around cornea
- ***Iodine***
  - Required for thyroxin synthesis
    - ⇒ Tyrosine + Iodine = thyroxine
  - Deficiency:
    - ⇒ Cause: diet (low iodized salt intake)
    - ⇒ Result: Goiter (enlarged thyroid)
- ***Selenium***
  - Function:
    - ⇒ Cofactor for ***glutathione peroxidase*** (antioxidant)
    - ⇒ Converts peroxide to water
  - Deficiency:
    - ⇒ Weakness and muscle pain
    - ⇒ Dilated cardiomyopathy

# Pharmacokinetics

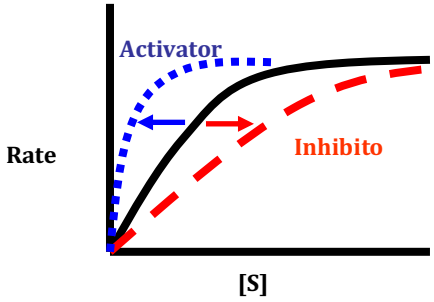
## Enzyme Kinetics

- Michaelis–Menten kinetics:
  - Describes rate of enzymes (reaction speed)
  - Vmax: Maximum rate at very high [S]
  - $K_M$ is the substrate concentration ([S]) that will produce ½ Vmax

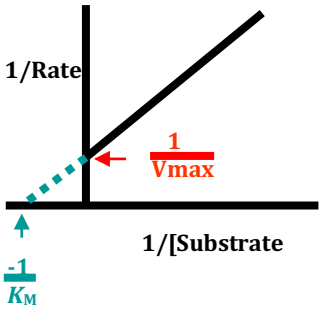



  - When the [S] is equal to the $K_M$ the rate will be equal to ½ $V_{max}$

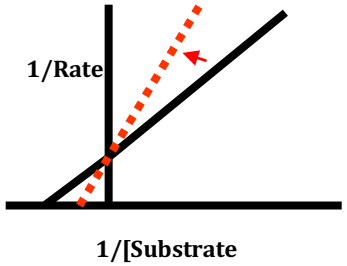
- Inhibition vs. activation
  - Substances influencing the rate of an enzyme: activate or inhibit
    - Activator:
      - ⇒ Moves curve to the LEFT
      - ⇒ Rate higher at lower [S]
    - Inhibitor:
      - ⇒ Moves curve to the RIGHT
      - ⇒ Rate is lower at high [S]



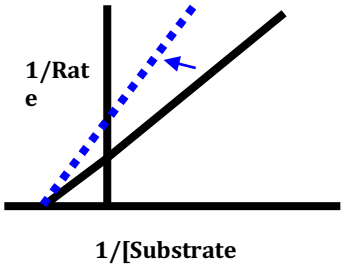

- Lineweaver–Burk plot
  - Double reciprocal plot
  - Plot: 1/rate vs. 1/[S] (instead of rate vs. [S])
    - $-1/K_M$ is the X intercept
    - $1/V_{max}$ is the Y intercept



- Lineweaver–Burk plot: Inhibition
  - Competitive inhibition
    - $K_M$ changes (bigger)
    - $V_{max}$ is the same




  - Non-competitive inhibition
    - $V_{max}$ changes (smaller)
    - $K_M$ is the same




♦ ***Remember: Competitive Changes*** ***Km***

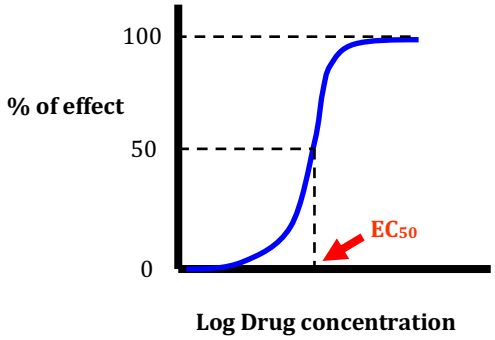
## Pharmacokinetic parameters

- Volume of Distribution ($V_D$):
  - $V_D$ = total amount of drug / plasma concentration
  - High $V_D$ → drug mostly in tissues
  - Medium $V_D$ → drug mostly in extracellular space
  - Low $V_D$ → drug mostly in blood
- Clearance (Cl):
  - $Cl = V_D \times K_e$
  - How much of drug is cleared per hour (L/hr)
  - Rate constant ($K_e$):
    - high = fast, low = slow
- Drug delivery
  - F is the bioavailability
    - F = 1 if given by IV
  - CP is the target plasma concentration
  - Loading Dose = $C_P \times V_D/F$
  - Maintenance Dose = $C_P \times Cl/F$
- Half-life: $t_{1/2}$
  - $t_{1/2} = (0.7 \times V_D) / Cl$
  - The TIME it takes for HALF the drug to be cleared (or infused)
    - After 1 $t_{1/2}$: 50% of drug is cleared (or infused)
    - After 4 $t_{1/2}$: 94% of drug is cleared (or infused)
  - For infusion, after 4 $t_{1/2}$ the drug reaches steady state
- Rate order:
  - Zero order:
    - ***Amount*** of drug eliminated is constant per unit of time
    - Graph shows straight line
  - First order:
    - ***Fraction*** of drug eliminated is constant per unit time
    - Graph shows a curve

# Pharmacodynamics

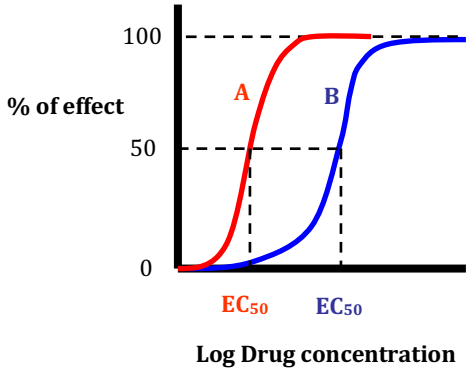
## Half-maximal effective concentration $EC_{50}$

- Median effective concentration of a drug or an agonist
- Drug concentration that will elicit half of the maximal effect




## Potency

- Amount of drug needed to produce a given effect
  - High $EC_{50}$ = Low potency
  - Low $EC_{50}$ = High potency

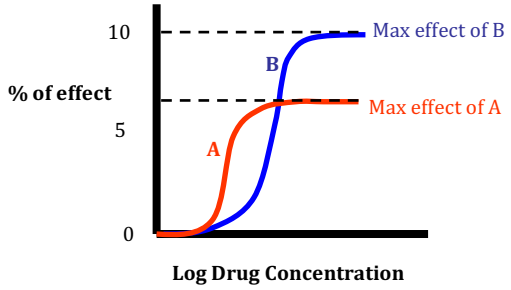



A is more potent than B

- ***More potency: graph shifted to the left, lower $EC_{50}$***
- ***Less potency: graph shifted to the right, higher $EC_{50}$***

## Efficacy

- Maximal effect of a drug



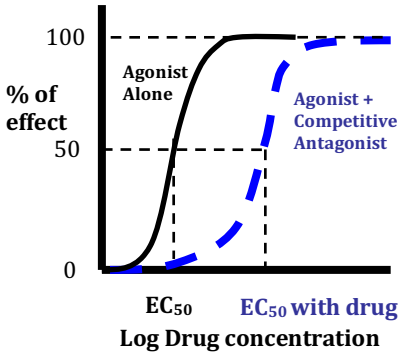

B is more efficacious than A

  - "A" is partial agonist, "B" is a full agonist

## Inhibition by Antagonists

- Competitive Antagonists:
  - ***Increases*** the $EC_{50}$
  - (Lower potency)
  - ***No*** Change in Maximal Effect
  - (no effect on efficacy)
  - Graph shifted to the right

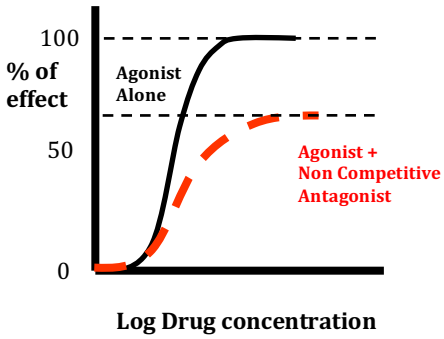
**Competitive antagonist**




## Non-competitive Antagonists

- ***No*** change in the $EC_{50}$
- (no effect on potency)
- ***Decrease*** Maximal Effect
- (lower efficacy)
- Graph shifted down

**Non-competitive antagonist**




- ***Remember:*** Competitive Changes $EC_{50}$

## Therapeutic index

- Measure of effectiveness of drug
- Ratio between toxicity and therapeutic effect
- Therapeutic index = TD50/ED50
  - $ED_{50}$: effective dose
  - $TD_{50}$: toxic dose
  - High therapeutic index (good) = ↓ $ED_{50}$ and ↑ $TD_{50}$

# Neuropharmacology

## Neurotransmitters and Receptors

### *Neurotransmitters*

- Molecules for sending signals:
  - ♦ Between neurons OR from neurons to effector organ

### *Types of Neurotransmitters*

- Acetylcholine (ACh)
- Epinephrine (Epi) and Norepinephrine (Nor)
- Histamine
- Vasopressin
- Dopamine

Most neurotransmitters are quickly degraded or removed by uptake after the desired effect

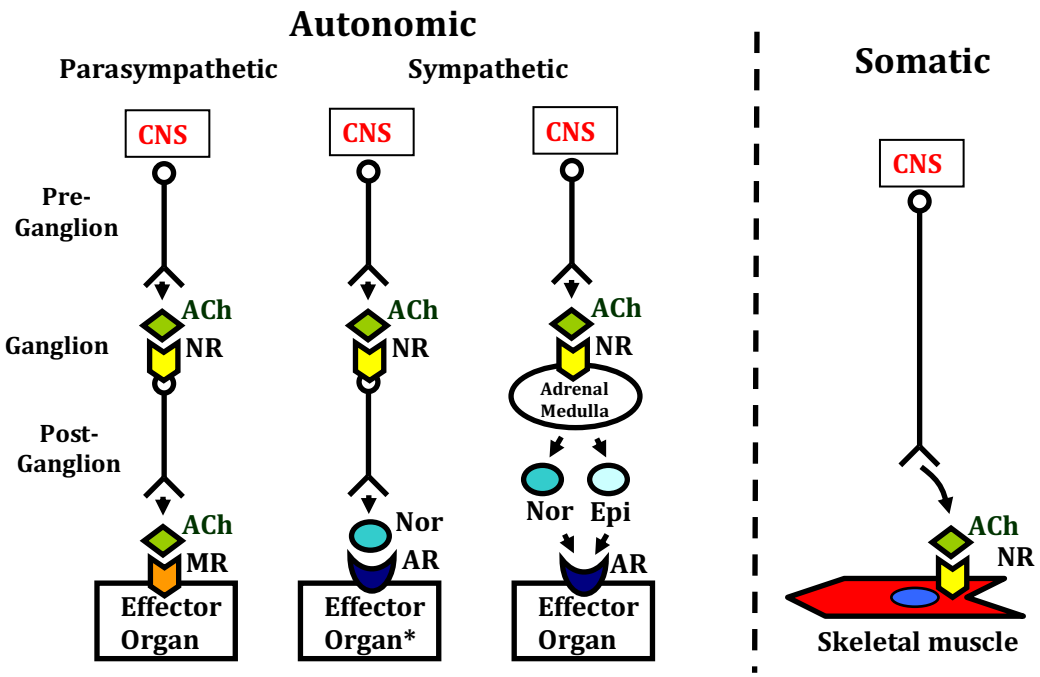
### *Mechanism of receptor activation*

#### Ion Channel-Coupled Receptors

- ♦ Example: cholinergic ***nicotinic*** receptors:
  - ⇒ Binding of ACh → ion influx into cell
  - ⇒ Changes the membrane potential
  - ⇒ Increase in sodium and potassium ion concentration in cell

#### G-Protein Coupled Receptors

- ♦ Targeted by ~40% of drugs
- Binding of neurotransmitter results in activation of a second messenger:
  - ♦ ATP converted to cAMP
    - ⇒ Gs: stimulatory
    - ⇒ Gi: Inhibitory
  - ♦ Release of Inositol triphosphate (IP3), Diacyl-glycerol (DAG), leads to ↑ $Ca^{++}$ in cytoplasm
    - ⇒ Gq




** Except for Sweat glands (MR)*

## Receptors: on surface of neurons and effector organs

- Cholinergic receptors (bind to ACh):
  - Nicotinic
  - Muscarinic
- Adrenergic receptors (bind to epinephrine/norepinephrine)
- Dopamine
- Histamine
- Vasopressin

### *Cholinergic Receptors*

- Nicotinic Receptors:
  - Ion channel coupled receptor
  - Found in:
    - ⇒ All ganglia (sympathetic and parasympathetic)
    - ⇒ Adrenal medulla, Skeletal muscle
    - ⇒ Activation → excitation
  - Agonists:
    - ⇒ ACh, Nicotine, Carbachol

- ♦ Antagonists:
  - ⇒ Curare
  - ⇒ Hexamethonium: ganglionic blocker (only in ganglia, not skeletal muscle)

- Muscarinic Receptors:
  - ♦ Found in *All* parasympathetic effector organ (smooth/cardiac muscles, glands)
  - ♦ Remember that they are found in sympathetic fibers: sweat glands
  - ♦ Activation:
    - ⇒ Heart: M2 receptor (Gi): leads to ↓ rate, ↓ AV node conduction, ↓ atrial contraction
    - ⇒ GI: M3 receptor (Gq): leads to ↑ motility, ↑ acid secretion, relaxed sphincter
    - ⇒ Other: ↑ exocrine (sweat), ↑ contraction of bladder, ciliary muscle, pupillary sphincter
  - ♦ Agonists:
    - ⇒ ACh, Muscarine
    - ⇒ Carbachol: Glaucoma, ↑ pupillary contraction
    - ⇒ Pilocarpine: stimulates sweat/salivary glands
  - ♦ Antagonists:
    - ⇒ Atropine: dilating pupils, resuscitation (inhibit vagus nerve), anti-organophosphate (nerve agents/insecticides), atropine toxicity: ↑ temp, ↓ sweating, ↑ heart rate, flushing, constipation, hyperplasia (prostate)
    - ⇒ Homatropine/tropicamide: pupillary dilators
  - ♦ Antagonists/effect on organs:
    - ⇒ CNS - Benztropine: used in the treatment of Parkinson's disease, Scopolamine for nausea (motion sickness)
    - ⇒ Bladder - Oxybutynin/glycopyrrolate: treatment of bladder spasms
    - ⇒ GI - Propantheline, pirenzepine, methscopolamine: peptic ulcers
    - ⇒ Lungs – Ipratropium: treatment for COPD and asthma
  - ♦ Indirect antagonists (non-competitive):
    - ⇒ Hemicholinium: blocks uptake of choline (ACh precursor)
    - ⇒ Vesamicol: blocks packaging of ACh into secretion vesicles
    - ⇒ Botulinum toxin: clinical use: Botox will block the release of ACh from secretion vesicles → paralysis

- Indirect agonists:
  - Inhibit acetylcholinesterase enzyme that breaks down Ach to acetate + choline
  - Known as anti-cholinesterases
  - Can be clinical drugs or poisons
  - All result in:
    - ⇒ Longer life of ACh at the neuronal junction
    - ⇒ ***Indirect*** stimulation of cholinergic receptors
  - List of Indirect agonists (Drugs):
    - ⇒ Echothiophate: glaucoma
    - ⇒ Physostigmine: glaucoma and atropine toxicity
    - ⇒ Donepezil: Alzheimer's
    - ⇒ Edrophonium: myasthenia gravis (diagnosis)
    - ⇒ Pyridostigmine: myasthenia gravis (treatment)
    - ⇒ Neostigmine: myasthenia gravis (treatment), postoperative reversal of anesthetic effects, treatment of urinary blockade (postoperative)

    Note: Neostigmine and Pyridostigmine do not cross BBB
  - List of indirect agonists (poisons):
    - ⇒ Organophosphates:
    - ⇒ Pesticides:

      ***On the Boards, look for farmers, workers in chemical factories***
    - ⇒ Nerve agents: sarin, Soman, VX, mustard gas
    - ⇒ Symptoms: excitation (somatic and autonomic), salivation, sweating, urination, diarrhea, and possible death
    - ⇒ Treatment: atropine, pralidoxime

## *Adrenergic Receptors*

| *Receptor* | $\alpha_1$ | $\alpha_2$ | $\beta_1$ | $\beta_2$ |
|---|---|---|---|---|
| *Location* | Skin<br>smooth muscle<br>GI/bladder: sphincter<br>Iris: radial muscle<br>Splanchnic regions | Fat cells, platelets<br>GI walls<br>Presynaptic nerve terminals | Heart: SA and AV nodes<br>Ventricular muscle | Vascular smooth muscle: bronchi & skeletal muscle<br>GI/bladder: wall |
| *Effect* | Excitation (contraction) | Relaxation (Dilation) | Excitation (contraction) | Relaxation (Dilation) |
| *Mechanism of action* | IP3 | Gi (↓cAMP) | Gs (↑cAMP) | Gs (↑cAMP) |
| *Agonists* | Epi/NorEpi<br>Dopamine<br>Phenylephrine | Epi/NorEpi<br>Dopamine<br>Clonidine | Epi/NorEpi<br>Dopamine<br>Isoproterenol | Epi, ***not NorEpi***<br>Dopamine<br>Albuterol/ritodrine |

**Adrenergic receptor agonist (sympathomimetics):**

- Phenylephrine ($\alpha_1$):
  - ⇒ Sinus/decongestion
  - ⇒ Result: vasoconstriction, pupil dilation
- Clonidine/Methyldopa ($\alpha_2$):
  - ⇒ Sympathoplegics (↓ sympathetic function)
  - ⇒ Treating hypertension (with kidney disease)
- Isoproterenol ($\beta$1):
  - ⇒ Treatment of AV block, bradycardia
  - ⇒ Also affects $\beta$2 (rarely used for asthma)
- Albuterol/Metaproterenol ($\beta$2):
  - ⇒ Treatment of asthma
- Salmeterol ($\beta$2):
  - ⇒ Long term treatment of asthma
- Ritodrine/Terbutaline ($\beta$2):
  - ⇒ ↓ bladder and uterus contractions

**Non-specific agonists**

- Cocaine:
  - ⇒ Excitatory and vasoconstriction
- Amphetamines:
  - ⇒ ADD, obesity, and narcolepsy
  - ⇒ Result: ↑ metabolism, ↓ sleep
- Ephedrine:
  - ⇒ Obesity, sinus congestion

**Adrenergic receptor antagonists**

- The –zosins ($\alpha_1$):
  - ⇒ Terazosin, prazosin and doxazosin
  - ⇒ Treating: BPH, urinary retention, and hypertension
  - ⇒ Side effects: hypotension and dizziness
- Mirtazapine ($\alpha_2$):
  - ⇒ Antidepressant, antiemetic, and sedative
  - ⇒ Result: increase appetite and hypercholesterolemia

**β-blockers - Heart Disease**

- $\beta_1$: Atenolol, Acebutolol, Betaxolol, Esmolol
- Non-selective β-blockers: Nadolol, Pindolol, Propranolol and Timolol (glaucoma)
  - ⇒ Treatment of hypertension, MI, arrhythmia, angina pectoris, glaucoma
  - ⇒ Avoid in patients with COPD
  - ⇒ ↓ cardiac output, ↓ AV node conduction, ↓ heart failure, ↓$O_2$ consumption, ↓ aqueous humor secretion (eyes)

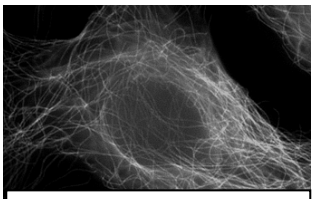
# General Cell Biology

## Plasma Membrane

- A fluid bilayer composed of:
  - Phospholipids: major component
  - Cholesterol:
    - ↑ % cholesterol = ↑ rigidity = ↑ melting temp
  - Minor components:
    - Glycolipids and Sphingolipid
    - Integral membrane proteins
    - Ion channels, transporters, receptors… etc.
- The plasma membrane is ***asymmetric***
  - Outer leaflet contains more phosphatidyl choline

> Phosphatidyl choline is a precursor for dipalmitoylphosphatidylcholine (DPPC), a major lung surfactant

## Cytoskeleton Components

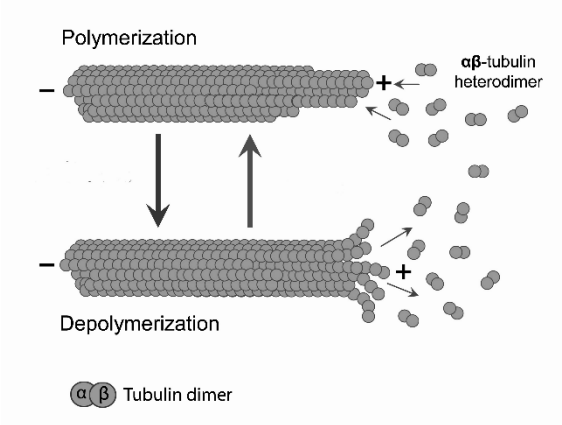


Microtubules

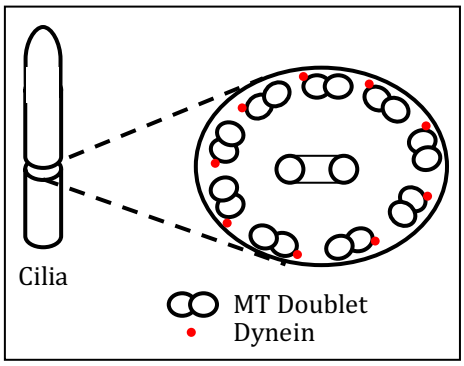
- Make up the cell architecture
  - Microtubules, actin and intermediate filaments

### *Microtubules*

- Microtubules are dynamic polymers
  - Made up of α-β tubulin dimers
  - Polar (+ end, – end)
  - Provide structure for:
    - ⇒ The mitotic spindle
    - ⇒ Cilia and Flagella
    - ⇒ Centrioles
    - ⇒ Neurons



## *Cilia*




- Microtubule-rich organelles
- In trachea:
  - ♦ moving mucus and dirt
- In fallopian tubes:
  - ♦ moving the ovum
    - ⇒ from the ovary to the uterus
- Structure: 9 + 2 arrangement of MT doublets

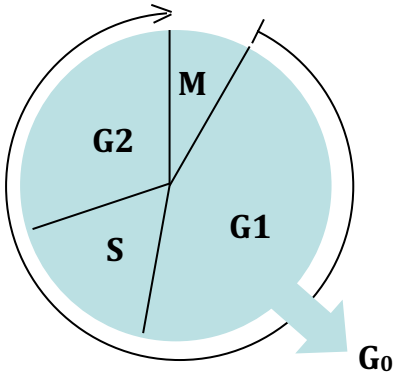
Movement of cilia is caused by the motility of the motor protein ***Dynein*** along the microtubule doublets

- ➢ Molecular Motors that walk along Microtubules
  - Walk in a directional fashion
  - Require ATP
    - ♦ Dynein:
      - ⇒ Retrograde, walks from + to – end
      - ⇒ Function: ciliary motion
    - ♦ Kinesin:
      - ⇒ Anterograde, walks from – to + end
      - ⇒ Cell division and intracellular trafficking
- ➢ Kartagener's syndrome
  - Mutation in the Dynein motor arm
  - Results in immotile cilia and sperm
  - Symptoms:
    - ♦ Male and female infertility
    - ♦ Recurrent sinusitis
    - ♦ Bronchiectasis
    - ♦ May result in situs Inversus: Inverse arrangement of internal organs
- ➢ Drugs that act on microtubules
  - Taxol (anti-breast cancer)
  - Vincristine/vinblastine (anti-cancer)
  - Colchicine – (anti-gout)
  - Mebendazole/thiabendazole (anti-helminthic)

### *Intermediate filament staining for tumor identification*

| Cell type | Type of Tumor | Stain |
|---|---|---|
| Muscle | Rhabdomyosarcoma | Desmin |
| Mesenchymal tissue | Mesenchymal tumors (sarcoma)<br>In addition to endometrial carcinoma, renal cell carcinoma, meningioma | Vimentin |
| Epithelial cells | Epithelial tumors (squamous cell carcinoma) | Cytokeratin |
| Neuroglia (astrocytes, Schwann cells, oligodendrocytes) | Astrcytoma, Glioblastoma | GFAP |
| Neurons | Neuroblastoma | Neurofilaments |

## Cell Cycle




- M: mitosis
  - Short cell division step
  - Prophase, metaphase, anaphase, telophase
- Interphase
  - Gap or growth and DNA synthesis
  - G1, S, G2
  - Can include to $G_0$ (dormant phase)
- G1:
  - Growth and recovery from cell division ***before*** DNA replication
  - Can be long
    - ♦ Know that the normal function of p53 (Tumor suppressor gene) is to arrest DNA damaged cells at G1phase and then undergo apoptosis
- S:
  - DNA synthesis (replication of genome)
- G2:
  - Preparation for cell division ***after*** DNA replication
    - ♦ Know that Bleomycin is G2-phase specific, will form free radicals and cause breaks in DNA strands. Most common side effect is pulmonary fibrosis
      - ⇒ Bleomycin is used in the treatment of testicular cancer and Hodgkin's Disease

## *Checkpoints are critical to ensure proper progression of cell cycle*

- Know the checkpoint proteins
  - Cyclins
  - CDKs (cyclin-dependent kinases)
  - Tumor suppressors

## *Rb Retinoblastoma - tumor suppressor protein*

- Works at the G1 – S transition
- In a healthy individual:
  - The function of Rb is to prevent excessive cell growth by blocking G1-S phase transition to prevent progression of cell cycle until the cell is ready to divide
  - When the cell is ready to divide, Rb is deactivated by phosphorylation to allow for cell cycle progression
- Remember that there are two types of retinoblastoma
  - Familial and sporadic retinoblastoma
  - Know that familial retinoblastoma is associated with ↑ risk for osteosarcoma, sporadic is not

## *Cell types in the human body*

- Labile cells
  - Constantly divide, never in $G_0$
    - ⇒ Skin, hair follicles, intestinal epithelium, and bone marrow
- Stable cells
  - Remain in $G_0$ until stimulation, then go to G1
    - ⇒ Lymphocytes, hepatocytes
- Permanent cells
  - Constantly in $G_0$, never divide
    - ⇒ Skeletal and cardiac muscle, neurons
    - ⇒ Mature RBCs have no nuclei = no cell division

## Subcellular Compartments

### *Rough Endoplasmic Reticulum*

- Rough appearance due to ribosomes
- Synthesis of proteins for secretion
  - Peptide neurotransmitters and hormones
  - Membrane proteins
- Addition of N-linked oligosaccharides to proteins
- Secreting cells have high level of Rough ER:
  - Plasma cells: antibody secretion
  - Small intestine goblet cells: mucous secretion

### *Smooth Endoplasmic Reticulum*

- Smooth appearance: No ribosomes
- Synthesis of lipids and steroids
- Regulation of calcium concentrations
- Detoxification of drugs and poisons
- Regions with high level of smooth ER:
  - Hepatocytes: lipid synthesis and detoxification
  - Adrenal cortex: steroid hormone synthesis

### *Golgi*

- Golgi structures
  - ***cis***-Golgi: close to ER
  - ***trans***-Golgi: close to plasma membrane
- Golgi function
  - Sending proteins and lipids to:

### *Trafficking proteins*

- COPI: from Golgi to ER (retrograde)
- COPII: from RER to Golgi (anterograde)
- Clathrin: formation of vesicles for transport
  - Receptor-mediated endocytosis: plasma membrane to Endosomes
  - Transport within the cell: ***trans***-Golgi to lysosomes
  - Secretion: ***trans***-Golgi to plasma membrane

### *Chédiak–Higashi syndrome*

- Impaired cellular trafficking
- Autosomal Recessive
- Result:
  - Decrease in phagocytosis
  - Decrease in bacteriolysis
  - Impaired lysosomal function
- Symptoms:
  - Frequent bacterial and viral infections

### *Protein modifications in the Golgi:*

- Addition of O-linked sugars: to serine and threonine
- Addition of N-linked sugars: to asparagine
- Assembly and sulfation of proteoglycans
- Addition of mannose-6-phosphate:
  - Targets proteins to lysosome
  - Carried out by mannose-6-phosphotransferase

### *I-cell disease*

- Enzyme: mannose-6-phosphotransferase
- Results:
  - Lysosomal enzymes not targeted to lysosomes
  - Increased level of lysosomal enzymes in plasma
- Symptoms:
  - Infant with mental retardation, coarse facial features, joint problems, corneal clouding, and gum deterioration
- Fatal in childhood

# Molecular Biology

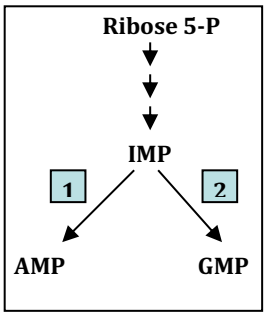
## Purine and Pyrimidine Metabolism

### DNA Bases

- Purines: Adenine (A) and Guanine (G)
  - Structure: 2 rings
  - Synthesized from:
    - Amino acids: Aspartate, Glycine, Glutamine
    - Tetrahydrofolate
- Pyrimidines: Thymine (T) and Cytosine (C)
  - Structure: 1 ring
  - Synthesized from:
    - Aspartate and Tetrahydrofolate
      - ⇒ Deamination of C makes Uracil (RNA)

### Purine Synthesis

- In all tissue
- IMP is intermediate
- Enzymes:



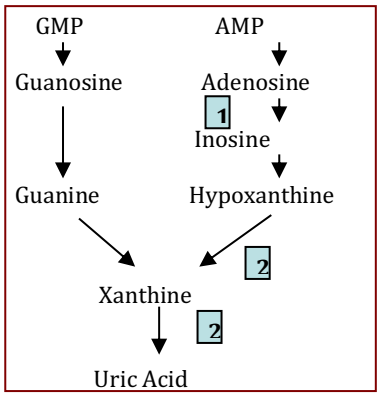

  **1** Adenosyl *succinate Synthase*:
    - Requires GTP
    - Inhibited by AMP

  **2** IMP *dehydrogenase*
    - Inhibited by GMP
  - Both enzymes require tetrahydrofolate (THF)
  - Both enzymes inhibited by methotrexate (THF analog)

## Purine Salvage

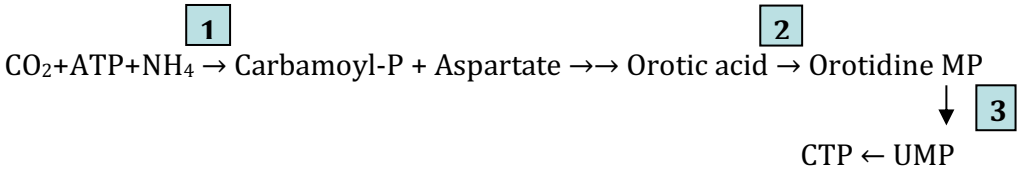
- Synthesis of GMP or AMP from:
  1. Hypoxanthine → IMP → GMP or AMP
     - First step catalyzed by *HGPRT*
  2. Guanine → GMP
     - Catalyzed by *HGPRT*
  3. Adenine → AMP
     - Catalyzed by *Adenine phosphoribosyl transferase*
  - HGPRT (hypoxanthine/guanosine phosphoribosyl transferase)
    - Deficiency leads to Lesch-Nyhan Syndrome
- Lesch-Nyhan Syndrome
  - X-linked recessive
  - Clinical picture:
    - Child, spastic behavior, self-mutilation, gout, mental retardation, orange-red urine, arthritis, kidney stones, hyperreflexia
  - Labs:
    - Hyperuricemia: orange-red uric acid crystals
  - Treatment:
    - Allopurinol: does not improve behavior, lowers uric acid

## Purine Degradation




- Key Enzymes:
  - 1 *Adenosine deaminase Deficiency:*
    - Severe Immune deficiency
  - 2 *Xanthine Oxidase*
    - Inhibited by Allopurinol
    - Treatment of Gout
- Adenosine Deaminase Deficiency:
  - Autosomal recessive
  - Accumulation of adenosine: dysfunctional T and B cells, rarely survive past 1 year
  - Clinical picture:
    - Infant with severe combined immune deficiency (SCID), infections (all types)
  - Treatment:
    - Bone marrow transplant
    - Exogenous modified adenosine deaminase
    - Gene therapy

## De novo Pyrimidine Synthesis




- Key Enzymes
  - 1 Carbamoyl-P synthase II:
    - inhibited by UTP
  - 2 Orotate phosphoribosyltransferase
  - 3 OMP decarboxylase
    - Both 2 and 3 form a complex: *UMP Synthase*
    - Deficiency results in Orotic aciduria
- Orotic aciduria
  - Autosomal recessive
  - Clinical picture:
    - Infant, anemia, growth retardation, lethargic, neurological abnormalities
  - Lab:
    - Blood smear: hypochromic megaloblastic anemia
    - Urine: orotic acid crystals
  - Treatment:
    - Uracil and cytidine supplement

## Pyrimidine Degradation

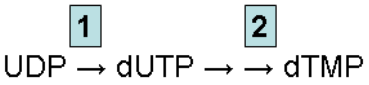
- Unlike Purines which end up as urea, pyrimidines are degraded to useful compounds

  Uracil or Cytosine → → Acetyl CoA → TCA cycle

  Thymine → → Succinyl CoA → TCA cycle

### *RNA/DNA interconversion*

- RNA: ribonucleic acid
- DNA: deoxyribonucleic acid




**1** *Ribonucleotide Reductase*:

- Converts –OH to –H on ribose sugar
- Inhibited by Hydroxyurea: antineoplastic (cancer, sickle cell)

**2** *Thymidylate Synthase:*

- Inhibited by 5-Fluorouracil: Anticancer

# Molecular Cell biology

## DNA

- Double stranded:
  - A binds T (2 H bonds) G binds C (3 H bonds)
  - ↑ % G/C = ↑ melting temperature
- Nucleosome:
  - DNA (-) wrapped twice around histones (+)
  - 2 of each H2A, H2B, H3, H4 = bead
  - H1 links the beads
- Buzz words:
  - Antiparallel: reverse complement
  - → 5′-ATGTCC-3′
  - ← 3′-TACAGG-5′
  - Palindrome: forward = backwards
  - 5′-GAATTC-3′
  - 3′-CTTAAG-5′
    - Restriction enzymes (endonucleases) cut DNA at palindromes

## DNA replication

- Eukaryotes: multiples start sites at AT-rich region
- Prokaryotes: single start site
  - Topoisomerase: releases supercoiling (nick)
  - Helicase: unwinds (separates) strands
  - Single stranded binding proteins: maintain stability of the replication fork
  - DNA Primase: RNA primer (5′→ 3′)
  - DNA pol III: adds to primer (5′→ 3′) proof-reading exonuclease (3′→ 5′)
    - Leading strand (continuous replication)
    - Lagging strand (Okazaki fragments)
  - DNA pol I: replaces RNA primer w/DNA
  - DNA ligase: seals the ends

## Mutations

- A change in the nucleotide sequence of the genome
- The genetic code is: unambiguous and degenerate
  - Unambiguous: 1 codon = only 1 amino acid
  - Degenerate: 1 amino acid > 1 codons

### *Types of mutations*

- Transition: Purine -> Purine or Pyrimidine -> Pyrimidine
- Transversion: Purine -> Pyrimidine or vice versa
- Point mutations: at one base pair
  - Silent: different codon, same a. a.
  - Missense: different codon, different a.a (sickle cell anemia)
  - Nonsense: early stop codon = short protein
- Frame-shift: insertion or deletion of 1 or 2 bases (not 3)
  - Wrong a.a. sequence downstream of (after) mutation
  - Can result in short protein (Nonsense mutation)

### *Mutation Repair*

- Base excision:
  - Damaged base removed by ***glycosylase*** result: base-less sugar (apyrimidinic or apurinic site), later removed
- Nucleotide excision repair:
  - Endonuclease removes a segment from damaged strand (sugars and bases)
  - Result: region of ss DNA
  - Note: Xeroderma pigmentosa
- Mismatch repair:
  - After replication
  - Mutation on new strand is removed (new strand is unmethylated)
  - Result: region of ss DNA
  - Note: Hereditary nonpolyposis colon cancer (Lynch syndrome)
- In all cases above: DNA pol fills the gap, DNA Ligase seals it

## *RNA*

- RNA polymerases
- Unlike DNA pol: no need for primer, no proof reading
- In bacteria: only one multi subunit RNA pol
  - RNA pol I: Makes rRNA

    ***Found in the nucleolus***
  - RNA pol II: Makes mRNA

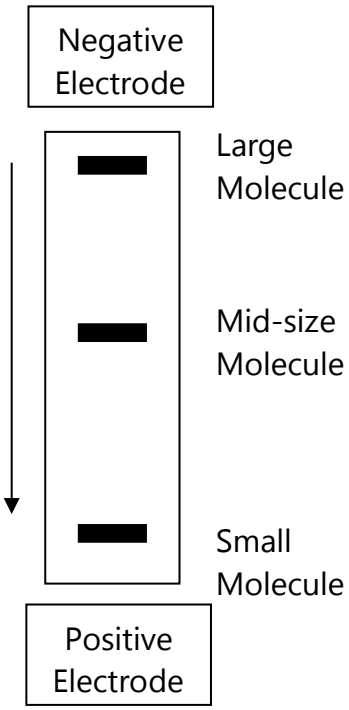
    ⇒ Inhibited by $\alpha$-amanitin from Death Cap mushrooms
  - RNA pol III: Makes tRNA

- mRNA Processing (in nucleus)
  - Polyadenylation:
    - Adds ~ 200 A's to 3′ end
  - Capping:
    - Adds 7-methyguanosine to 5′ end
  - Splicing:
    - Spliceosome cuts out introns and rejoins exons through lariat intermediate
- Transcription regulation
  - Promoters
    - Upstream of the gene (right before the gene)
    - RNA pol and transcription factors binding site
    - A-T rich sequence (TATA box, CAAT box)
  - Enhancers
    - Transcription factors binding site
    - May be close or far from gene
  - Silencers
    - Repressor binding site to down regulate gene expression
    - Negative regulation
- tRNA:
  - Structure: Clover shape
  - Function: deliver amino acids for protein synthesis
  - Charging:
    - Addition of a.a. to 3′ end
    - Carried out by aminoacyl tRNA synthetase
  - Anticodon loop:
    - Complementary sequence to codon
    - Binds to codon in mRNA

- Translation (protein synthesis):
  - In cytoplasm
  - Ribosome: binds to mRNA
    - assembly: 40s and 60s subunits
    - A site: incoming tRNA
    - P site: peptide bond is formed
    - E site: exiting tRNA
  - Termination: stop codon (empty tRNA) – UAA, UAG, UGA

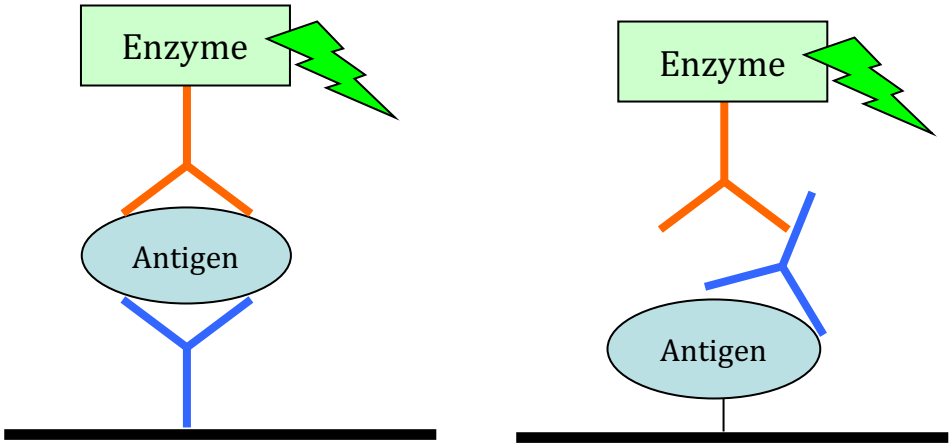
## Molecular Biology Techniques

- Gel electrophoresis – separates DNA, RNA
- or proteins by:
  - Size using an electric current
  - Molecules move to positive electrode (arrow)
  - Larger particles move slower (higher on the gel)
    - Gel electrophoresis is used to identify
    - Monoclonal gammopathy
- Southern blot:
  - Looks for specific piece (sequence) of DNA
  - Using complementary DNA
- Northern Blot:
  - Looks for specific piece (sequence) of RNA
  - Using complementary RNA
- Western Blot:
  - Looks for specific Protein
  - Using antibody




***In all blots, the samples are first separated by gel electrophoresis***

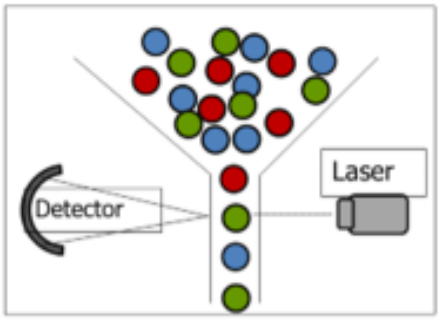
- FISH
  - Fluorescence *in situ* hybridization
    - Used to detect the presence or absence of specific DNA sequence
    - Uses a Fluorescent piece of DNA (probe) with complementary sequence
- ELISA
  - Enzyme-Linked ImmunoSorbent Assay




Detection of Antigen  Detection of Antibody

  - Used to detect the presence of antigen (*e. g.* HIV):
    - ***Antibody*** is immobilized on surface
    - Sample (containing antigen) is added
    - Second antibody attached to an enzyme (enzyme-linked) is added
    - Enzymatic reaction indicates presence of antigen
  - Used to detect the presence of antibody (*e. g.* in serum):
    - ***Antigen*** is immobilized on surface
    - Sample (containing antibody of interest) is added
    - Enzyme-linked secondary antibody is added
    - Enzymatic reaction indicates presence of antibody of interest

- ***Flow cytometry***
  - Cell counting and sorting
  - Cell size
  - Detects cell surface protein expression (immunophenotype)
    - Cells are fluorescently labeled
    - Laser excites fluorophore to emit light at varying wavelengths




  - Used for the diagnosis of HIV by identifying CD4 cells
    - CD4 count in HIV patients will be below 200 cell/ $mm^3$ – know that the normal CD4 count 500 -1500 cell/$mm^3$
  - Immunophenotyping for the diagnosis of hematological malignancies such as lymphoma and acute leukemia

# Genetics

## Chromosomes

- Diploid (Mitosis)
  - 46 (23 pairs)
    - 22 pairs autosomal
    - 1 pair of sex chromosomes (XX or XY)
- Haploid (Meiosis)
  - 23 chromosomes
  - Found in sperm or egg
- Aneuploidy
  - Number of chromosomes is not a multiple of 23, can be caused by:
    - Non-disjunction:
      - ⇒ Chromosomes do not separate during cell division
    - Anaphase lag:
      - ⇒ Loss of chromosome during cell division
      - ⇒ Mosaicism: some cells have the chromosome, some don't
- Trisomy 21: Down Syndrome
  - Mental retardation, low-set ears, broad nasal bridge, short broad hands, epicanthal folds, brushfield spots (small, white or grayish/brown spots on the periphery of the iris), and simian crease
  - Complications:
    - Congenital heart disease
    - Acute Leukemia (most common: lymphoblastic)
    - Brain changes in middle age similar to Alzheimer's disease
- Trisomy 18: Edwards Syndrome
  - Mental retardation, prominent occiput, finger deformities, Micrognathia (small lower jaw), Rocker-bottom feet, congenital heart disease
- Trisomy 13: Patau Syndrome
  - Mental retardation, polydactyly, microphthalmia, congenital heart disease, and Rocker-bottom feet - death ~1 year after birth
- Polyploidy: multiple of 23 greater than 3
  - Normal= 23X2, Polyploidy = 23X3, 23X4. etc.
  - Result is usually spontaneous abortion
- Deletion: whole or part of chromosome
  - Deletion of short arm (p) of #5
    - cri du chat (46,XX,5p-): "Cry of the cat", severe mental retardation, low birth weight, hypertelorism (wide-set eyes), round face, epicanthal folds

- Deletion of small piece known as 11 on #22q
  - DiGeorge/Velocardiofacial disease: (22q11), CATCH 22 Syndrome:
    - ⇒ Cleft palate
    - ⇒ Abnormal facial features
    - ⇒ T-cell deficit
    - ⇒ Cardiac abnormalities
    - ⇒ Hypocalcemia
- Other examples of Deletion:
  - Retinoblastoma –a small percentage of retinoblastomas is due to deletions in the region of chromosome 13 that contains the RB1 gene.
  - Prader-Willi – loss of paternally expressed genes in the human chromosome region 15q
  - Angelman syndrome – deltion of a segment of the maternal chromosome 15

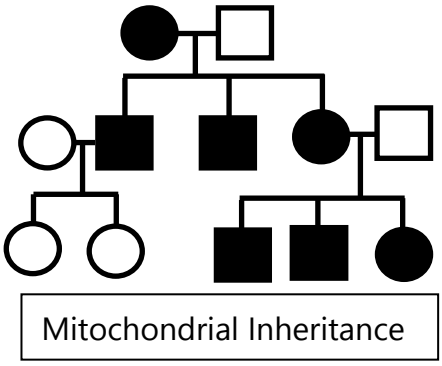
- Translocations:
  - Exchange of DNA from non-homologous chromosomes
    - Balanced (reciprocal) translocation:
      - ⇒ two chromosomes break and exchange
      - ⇒ no information is lost: clinically silent
    - Robertsonian translocation:
      - ⇒ 2 acrocentric chromosomes (q>>>p), usually 14 & 21
      - ⇒ long arms combine, short arms are lost
      - ⇒ designated t(14q;21q)
      - ⇒ Result: form of Down syndrome
- Sex chromosome abnormalities
  - Klinefelter syndrome:
    - 2 or more X + 1 Y (XXY, XXXY, … etc.)
    - Most often caused by maternal meiotic non-disjunction
    - Male phenotype with hypogonadism, tall stature, gynecomastia, ↓testosterone, ↑pituitary gonadotropins, atrophic testes
    - Male infertility, rarely: mental retardation (mild)
  - XYY:
    - ↑ frequency in violent criminals, severe acne,
    - Rarely: mental retardation
  - XXX (or more):
    - Usually asymptomatic (irregular menstrual cycle)
    - Mental retardation increases with number of X chromosomes

- XO: Turner syndrome
  - Complete or partial monosomy of the X chromosome
  - Female phenotype with hypogonadism, short stature, webbed-neck, ↓estrogen, ↑ pituitary gonadotropins, no ovaries (fibrous streaks), infantile genitalia, lymphedema (neck and extremities), coarctation of aorta, and cystic hygroma
  - Important:
    - ⇒ Often: autoimmune hypothyroidism
    - ⇒ Most common cause of amenorrhea
- Fragile X syndrome
  - Caused by ↑ number of trinucleotide repeats (CGG) in the 5′ untranslated regions (UTR) of the familial mental retardation gene (*FMR-1*) → extra-long X chromosome
  - Second most common cause of mental retardation – after Down syndrome
  - Males:
    - ⇒ Mental retardation, enlarged jaws and ears
    - ⇒ Bilateral macroorchidism (enlarged testes)
  - Females:
    - ⇒ Only 50% exhibit mental retardation
  - Both males and females can be asymptomatic carriers
  - Other examples of trinucleotide repeats: Huntington's, myotonic dystrophy and Friedreich's ataxia

| |
|---|
| Huntington's = (CAG) n |
| Myotonic dystrophy = (CTG) n |
| Friedrich' ataxia = (GAA) n |

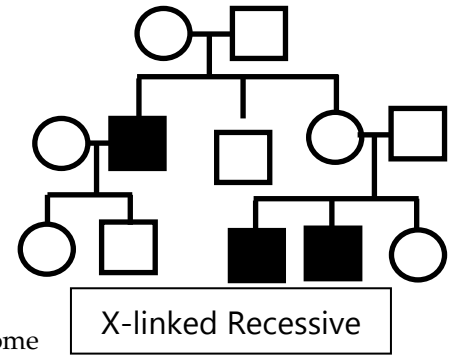
## Modes of inheritance

### *Mitochondrial inheritance*



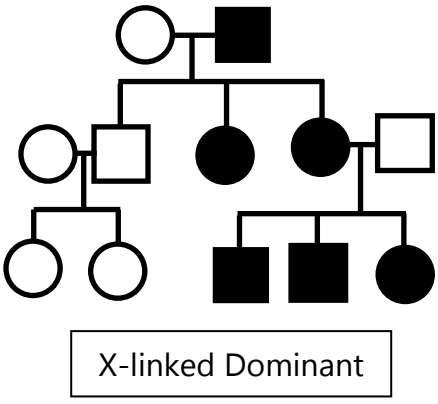

- ALL mitochondrial DNA comes from mother
- ALL offspring of affected mother will have the disease
- Examples:
  - All mitochondrial myopathies
  - Leber's hereditary optic neuropathy

### *X-linked recessive*



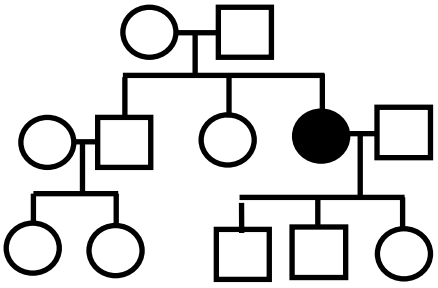

- Affects 50% of MALE offspring of heterozygous Mother
- No male to male transmission
- Examples:
  - Hunter's syndrome, Fabry's disease (lysosomal storage)
  - Hemophilia: Factor VIII gene
  - mutation in long arm of X chromosome
  - Lesch-Nyhan syndrome: purine metabolism (gout, self-mutilation, mental retardation)
  - G6PD deficiency
  - Duchenne muscular dystrophy

### *X-linked Dominant*




- Rare
- Both males and females can be affected
- Heterozygous females may have milder symptoms
- Fragile X syndrome

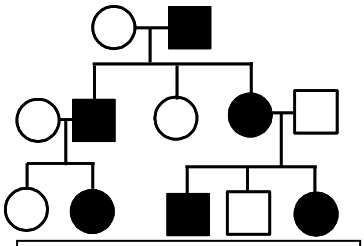
### *Autosomal Recessive*



Autosomal Recessive

- If both parents are carriers (heterozygous), then 25% of offspring will show disease
- Usually manifest in childhood
- Usually enzyme deficiencies
- Affects males or females equally
- May skip generations
- Examples:
  - All glycogen storage diseases
  - All amino acid metabolism diseases
  - All lysosomal storage diseases
    - ⇒ ***except*** Hunter's (X linked recessive)
  - All sphingolipidosis
    - ⇒ ***except*** Fabry's (X linked recessive)
  - Niemann-Pick, Galactosemia
  - Alkaptonuria, Phenylketonuria and albinism
  - Cystic fibrosis, infant polycystic kidney disease
  - Sickle cell anemia, hemochromatosis
  - α1-antitrypsin deficiency

### *Autosomal Dominant*



Autosomal Dominant

- If one parents exhibits the disease:
  - Parent is homozygous (AA): 100% of offspring
  - Parent is heterozygous (Aa): 50% of offspring
    - ⇒ Both AA and Aa are affected (aa is unaffected)
- Family history is crucial for diagnosis
- Males/females affected equally, usually adult onset
- More common than autosomal recessive, but less severe

- Examples:
  - Familial Hypercholesterolemia
  - Familial adenomatous polyposis
  - Achondroplasia (short limbs) and Marfan (long limbs)
  - Neurofibromatosis:
    - ⇒ Type 1: von Recklinghausen disease
    - ⇒ Type 2: central neurofibromatosis
  - Tuberous sclerosis
  - Von Hippel-Lindau
  - Adult polycystic kidney disease

## *Identifying the mode of inheritance in a genetic pedigree*

- Does all offspring of affected mother show disease?
  - If Yes = Mitochondrial
- Does it affect only males, with no male-to-male transmission?
  - If Yes = then X-linked recessive (50% of males)
- Do all daughters of affected father show disease, with no male-to-male transmission?
  - If Yes = X-linked dominant
- Do healthy parents produce offspring with the disease?
  - If Yes = Autosomal Recessive (parents: heterozygous)
  - No = Autosomal Dominant

## Genetic terms

- Hardy-Weinberg genetics:
  - p and q are frequencies of 2 alleles in a locus
  - A population at Hardy-Weinberg equilibrium:
  - Disease prevalence: $p^2 + 2pq + q^2 = 1$
  - Allele prevalence: $p + q = 1$
  - Assumptions of the Hardy-Weinberg law:
    - No mutations at the locus
    - No selection for the genotype at the locus
    - Mating is random
    - No migration into or out of the population
- Codominance:
  - Both alleles are expressed no one dominates
  - Example: blood type AB
- Incomplete penetrance:
  - Mutant genotype does not always = mutant phenotype
- Pleiotropy:
  - Mutation of one gene = multiple phenotypes
- Locus heterogeneity:
  - Mutations at different loci = same phenotype
  - Example: albinism
- Variable expression:
  - Same mutation, variable severity of phenotype
- Anticipation:
  - Severity of phenotype increases or earlier onset in succeeding generations
  - Example: Huntington's disease
- Tumor suppressor gene:
  - Products of these genes protect the cell against cancer
  - Both alleles must be mutated for cancer onset
- Oncogene:
  - Products of these genes cause cell proliferation
  - Mutated or overexpressed in tumor cells
  - Mutation in a single allele is sufficient for the effect to be manifested
  - Example: epidermal growth factor receptor (EGFR)

- Loss of heterozygosity:
  - Individual heterozygous for tumor suppressor gene mutation, the correct copy must be deleted or damaged for cancer to develop
  - Not true for mutations in oncogenes
- Linkage disequilibrium:
  - Alleles at 2 separate loci that occur together more than expected by random chance
  - Measured in populations (not individuals or families)
- Dominant negative mutation:
  - A mutation at one allele that is sufficient to mask (dominate) the effect of the correct allele
- Imprinting:
  - Different phenotype of the same mutation based on paternal or maternal inheritance
  - Example:
    - ***P***rader-Willi syndrome:
      - ⇒ Deletion of ***paternal*** allele
      - ⇒ Mental retardation, obesity, hypotonia, hypogonadism
    - Angel***m***an syndrome:
      - ⇒ Deletion of ***maternal*** allele
      - ⇒ Mental retardation, ataxia, seizures, inappropriate laugh

# Epidemiology and Biostatistics

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# Clinical Biostatistics

## Observational Studies

- Case Study:
  - One patient - usually rare disease, or unique finding with a well-known disease
  - Case series: few cases
- Case-control study:
  - Compares groups with vs. without disease
  - If looking at exposure: can provide exposure odds ratio
- Cross-sectional studies:
  - Large-scale study of patients at a point in time
  - Can provide information about prevalence
- Cohort study:
  - Using individuals of specific group or risk factor over long period of time
    - Retrospective: looking backwards
    - Prospective: following group over time
  - Not suitable for rare diseases
  - Can provide information about incidence
  - Of all observational studies: ***Only cohort studies*** can provide epidemiological data about absolute risk

## Experimental Studies

- Clinical Trial:
  - Phase I:
    - Testing pharmacodynamics/pharmacokinetics
    - Finding limit for safety, Healthy volunteers
  - Phase II:
    - In patients with the disease or condition
    - Efficacy, side effects and dose
  - Phase III:
    - Drug is compared with placebo (control group)
    - Establish safety and efficacy
- Cross-over study:
  - Clinical groups are switched in the middle of the study
  - Each participant serves as his/her own control

## Meta-Analysis

- Pooling data from many studies
- Re-evaluates the results
- Advantages:
  - More statistically sound results and higher confidence in outcome

## Types of Bias

- Sampling Bias:
  - Cause: focusing on subgroup for a study
  - Solution: increase diversity of individuals
- Selection Bias (Allocation Bias):
  - Cause: Bias in dividing individuals within the study into experimental vs. placebo groups
  - Solution: Randomization
    - Patients are randomly assigned to different study groups (treatment vs. placebo)
- Recall bias:
  - Individuals can be biased by current information
  - Result: inaccurate recall of information retroactively
  - To reduce, use blinding
    - *Single blind*: only doctors know who gets treatment vs. placebo
- Observer bias:
  - When researchers know prior information about the patient or sample
  - To reduce, use blinding
    - *Double blind:* both doctors and patients don't know who is receiving treatment vs. placebo
- Confounding Bias:
  - Inability of study (or researcher) to distinguish between coincidence and correlation
  - Solution: re-evaluate results or consider other factors

## Factors influencing studies

- Latent effect:
  - Time needed to show the effect of drug, treatment or exposure to agent
  - Example: vitamin/nutrient consumption, exposure to carcinogen, exercise … etc.
- Effect modification:
  - Additional factor involved in observed effect
  - *Example:* smoking, drug use

Both Latent effect and Effect modification are NOT types of Bias

## Incidence vs. Prevalence

| *Incidence* | *Prevalence* |
|---|---|
| Number of cases in a ***span*** of time | Number of cases at a ***point*** in time |
| *Example*: 1 in 100 per year | *Example*: 1 in 100 |
| Time dependent | Time independent |
| $= \frac{\text{Prevalence}}{\text{Duration}}$ | = Incidence x Duration |

- Incidence reflects the ***RISK*** of acquiring a disease within the next time period
  - Example: exposure to a certain carcinogen results in a 1 in 100 (1%) chance of developing a caner in the next 5 years
- Remember: A drug that increases survival with a disease will:
  - ***Increase*** prevalence
  - Have ***no effect*** on incidence

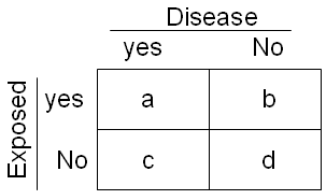
## Relative Risk and Odds Ratio

- Relative risk:
  - Probability of acquiring a disease in an exposed group *divided by* probability in unexposed group
- Attributable risk:
  - Probability of acquiring a disease in an exposed group *minus* probability in unexposed group
- Odds ratio:
  - Odds of acquiring a disease in exposed group *divided by* odds in unexposed group

$$\text{Relative Risk} = \frac{a/(a+b)}{c/(c+d)}$$

Attributable Risk = $a/(a+b) - c/(c+d)$

Odds ratio = $(a/b)/(c/d) = (ad)/(bc)$



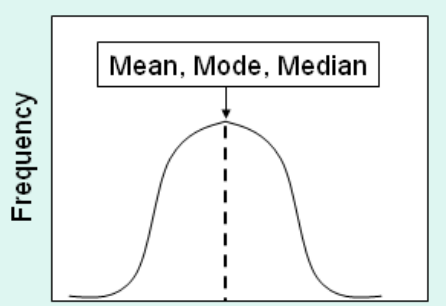
## Statistical Concepts

- Mean:
  - Average = sum of values/ n
  - Where, n is the number of values (*e. g.* # patients in study)
- Median:
  - Middle point of all values
  - Half the points are above the median, half are below
- Mode:
  - Most common value
- Outliers:
  - Extremely high or low values that lie far outside the bulk of the data
  - Can result from:
    - Measurement error
    - Recording error
    - Rare occurrence
  - Affect the Mean and Median
  - Do not affect the Mode
- Accuracy vs. Precision
  - Accuracy: How close the measurement is to the real value
  - Precision: Reproducibility of the measurement

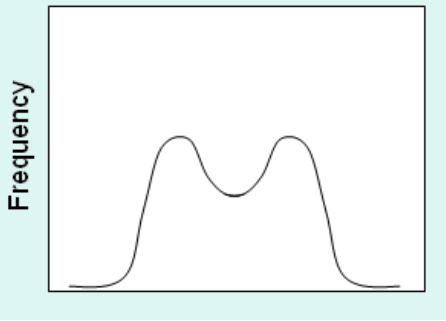
| | | |
|---|---|---|
| ***Systematic error*** | results in | ***High*** precision and ***Low*** accuracy |
| ***Random error*** | results in | ***Low*** precision |

## Statistical analysis

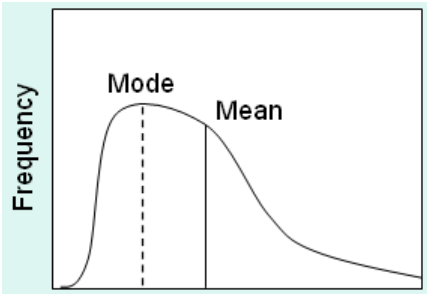
- Bell curve:
  - Also known as Normal or Gaussian distribution
  - Symmetrical distribution
  - Mean = Mode = Median



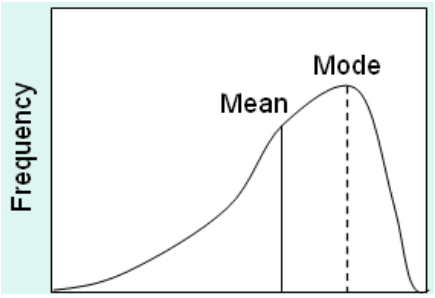

- Bimodal distribution:
  - Two peaks
  - Two tendencies within a population



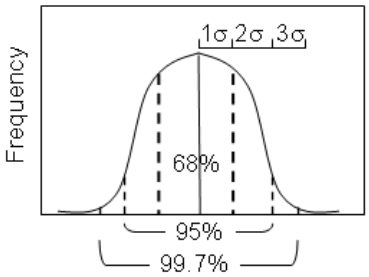

- Positive skew:
  - Tail to the right of the curve
  - Mean and Median > Mode




- Negative skew:
  - Tail to the left of the curve
  - Mean and Median < Mode



Standard Deviation (σ)




- How far away a value is from the mean
- In Gaussian Distribution:
  - 68% of values are ± one standard deviation
    - ⇒ 34% above mean
    - ⇒ 34% below mean
  - 95% ± 2 standard deviation
  - 99.7% ± 3 standard deviation

- Statistical Hypotheses
  - Null Hypothesis ($H_0$)
    - States that there *is no* statistical difference between two groups
    - or
    - States that there *is no* correlation between two factors
  - Alternative Hypothesis ($H_1$)
    - States that there *is* a statistical difference between two groups
    - or
    - States that there *is* a correlation between two factors
- Types of Error and Statistical analyses
  - Type I error ($\alpha$):
    - Probability of test result to be a false positive
    - Null hypothesis is ***true***, but is rejected
    - Stating that there is a difference between two groups, when there is not
  - Type II error ($\beta$):
    - Probability of test result to be a false negative
    - Null hypothesis is ***false***, but is accepted
    - Stating that there is no difference between two groups, when there is
  - Power
    - Probability of rejecting the null hypothesis when it is false
    - How likely is the correlation that we see between two factors is actually true
      ***or***
    - How likely is the difference that we see between two groups is actually true
    - Power = 1-β
    - High power = Low β (low type II error)

- Standard Error of the mean (SEM)
  - SEM = STDEV/√n
  - SEM decreases as n increases:
  - More samples (high n) = less error (low SEM)
- Confidence Interval (CI)
  - How confident we are that the results are within a certain range
  - If CI is 95, then $p = 0.05$
  - $p$ value is the probability of making a Type I error
  - *i.e.* the probability of having a false positive
  - High $p$ value = High chance of Type I error
- Correlation Coefficient (r)
  - Evaluates correlation between two groups or factors
  - Range: -1 to +1
    - $\Rightarrow$ r > 0 positive correlation
    - $\Rightarrow$ r < 0 negative correlation
    - $\Rightarrow$ r close to zero: little to no correlation
- Coefficient of determination: $r^2$
  - Range: Zero to 1, lowest to highest correlation

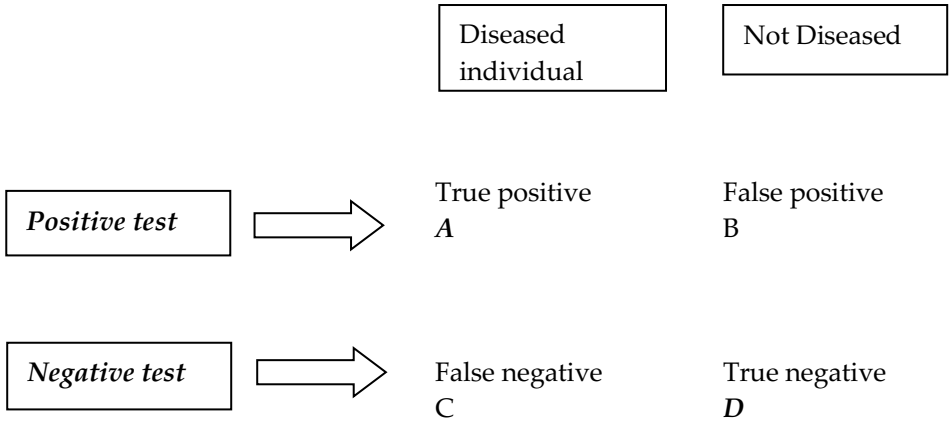
***Remember: $r^2$ does not tell you if the correlation is positive or negative***

- $t$ –test:
  - Compares the averages (mean) between two groups
- $\chi 2$ (chi-squared):
  - Compares the % between two groups
- ANOVA:
  - Analysis of variance
  - Compares 3 or more groups

## Biostatistics for the Boards

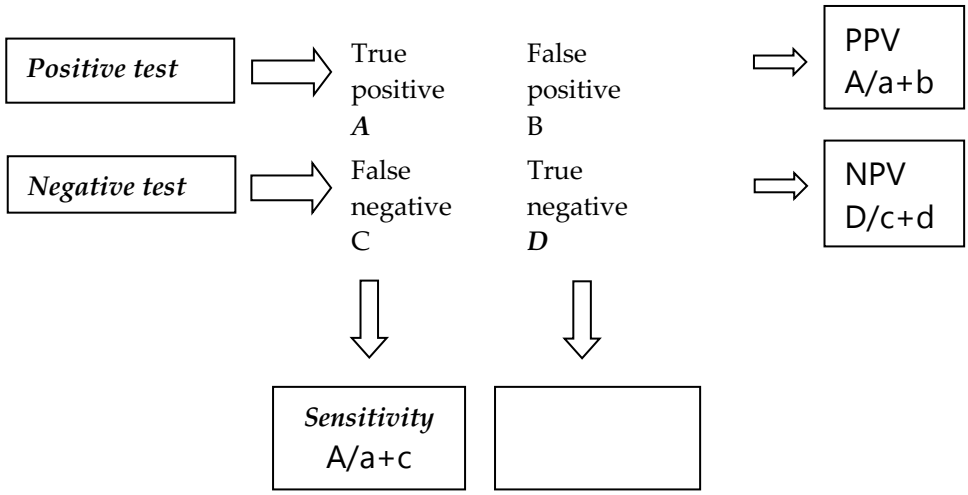
- Sensitivity
- Specificity
- Positive Predictive Value
- Negative Predictive Value
- Prevalence
- Relative Risk Reduction
- Absolute Risk Reduction
- Number Needed to Treat

### *Clinical application of biostatistics*



***From this diagram, we can calculate:***

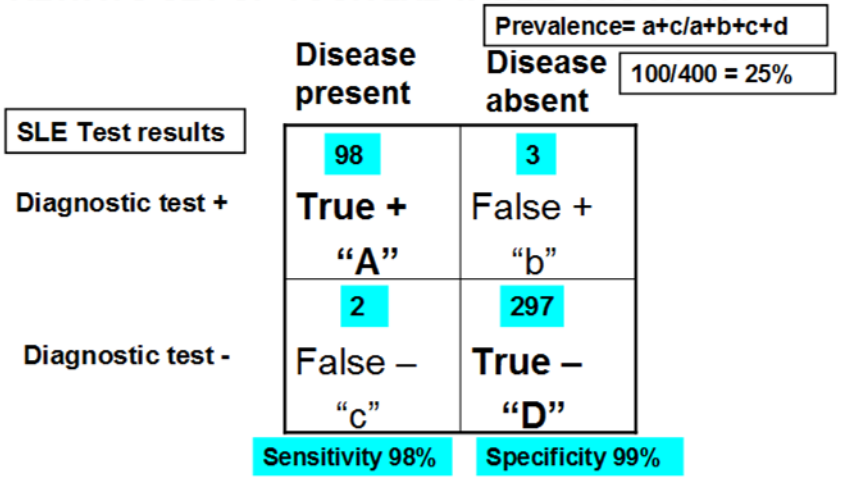
- ***Sensitivity*** from 1st column = A/a+c
- ***Specificity*** from the 2nd column = D/b+d
- ***Positive Predictive Value*** (PPV) is across the 1st row **=** A/a+b
- ***Negative Predictive Value*** (NPV) is across the 2nd row **=** D/c+d
- ***Prevalence*** = a+c/a+b+c+d




- Example # 1
  - Calculate the sensitivity, specificity and positive predictive value of a new test for the diagnosis of SLE. The test is positive in 98 patients who have the disease and three individuals without the disease. The test is negative in 297 patients without the disease and is negative in only 2 patients who have the disease
    - First, put the numbers in the right places in the table then follow the simple equations to calculate each value

## Calculate the Sensitivity, Specificity and Prevalence for a new SLE Test

- **ALWAYS SET UP YOUR 2X2 TABLE THIS WAY:**




- Example # 2
  - For the diagnosis of scleroderma, a new diagnostic serology test was used – the result of the test is positive in 75% of patients who have the disease but gives normal results in 90% of the patients who are truly disease-free. It has been estimated that the prevalence of scleroderma in your population is 10% - What is the positive predictive value of this test?
- From the stem of the question, we can identify the following data
  - Sensitivity = 75%
  - Specificity = 90%
  - Prevalence = 10%
- To solve the problem, follow these steps
  - 1) Consider that the population is 1000; it means that a+b+c+d =1000
    - 10% of that population has scleroderma; means that a+c = 100 (the number of patients who really have scleroderma in the community that has been tested)
  - 2) If a+c =100, and the ***sensitivity*** is 75%, with simple calculation "***a***" must = 75; and therefore "***c***" must = 25
  - 3) 1000 minus 100 (a+c) is 900, which must be = b+d
    - Using the specificity, which was given 90%, so d/b+d, or d/90 = 90%.
    - D = 90X90/100
    - It means that "d" = 810 , so "b" must be = 900-810 = 90
  - 4) Putting these numbers in the table will make it easy to calculate the positive predictive value

| Test result | Disease present | Disease absent | Total |
|---|---|---|---|
| Positive | A | B | |
| | 75 | 90 | |
| Negative | C | D | |
| | 25 | 810 | |
| Total | 100 | 900 | 1000 |

- ***Positive Predictive Value*** (PPV) is across the 1st row **=** A/a+b

  75/90+75 =75/165

  PPV = 50%

### *Biostatistics facts*

- When the prevalence of a disease or condition increases:
  - The positive predictive value increases
  - The negative predictive value decreases
- If you have an effective vaccine that will prevent a disease from occurring - it will decrease:
  - ***Both*** the incidence and the prevalence of this disease

## Interpretation of Therapeutic Results

### *Relative Risk Reduction*

- The usual way used to report the difference between the treated and untreated groups (in research study) is the "***relative risk reduction***" (RRR), which is calculated as ***CER-EER/CER***
- The "***control event rate***" (CER) is the group of patients with no therapy was administered to them
- The "***experimental event rate***" (EER) is the group of patients who received a particular therapy
- However, the RRR often is ***not*** clinically helpful because the number itself does not provide information about the baseline risk rate (i.e., CER)

## *Absolute Risk Reduction (ARR)*

- ARR is clinically more useful to interpret therapeutic results
- The ARR = (CER - EER)
- ***An example:***
- A new medicine has been used in patients with atrial fibrillation and the stroke risk was reduced to 2% per year, from the average 1-year risk for stroke in the placebo group of 5% per year
  - The *"**experimental event rate**"* (EER) is 2% in the group of patients who received the medicine
  - The *"**control event rate**"* (CER) is 5% in the placebo group
- It is very easy to calculate the ARR with the use of this particular medicine
  - ***ARR = CER – EER*** = 5 – 2 = 3%

## *Number Needed to Treat*

- The physician and patient often want to know the number of patients needed to be treated (NNT) with a therapy to prevent one additional bad outcome
- That number can be calculated with the equation: ***1/ARR***
- Let us use the previous example (the medicine used to decrease the incidence of stroke in patients with A fib)
- The ***NNT*** to prevent one stroke by the use of this particular medicine is 1/3% = 1/0.03 = 33
- Therefore, the NNT would be 33 patients; it means that 33 patients should be treated with this particular medicine for 1 year to prevent one additional stroke

## *Answering the questions on "the number needed to treat"*

- All what you need is to know:
  - The "control event rate" (CER)- Patients who have no treatment given
  - The "experimental event rate" (EER)- Patients who received the treatment
  - Absolute risk reduction ARR = CER – EER
  - The number needed to treat = 1/ARR