Contents

ENZYMES : PART 1

----- Active space -----

Basics of Enzymes

00:01:00

Definition : Specialized proteins that act as biological catalyst.

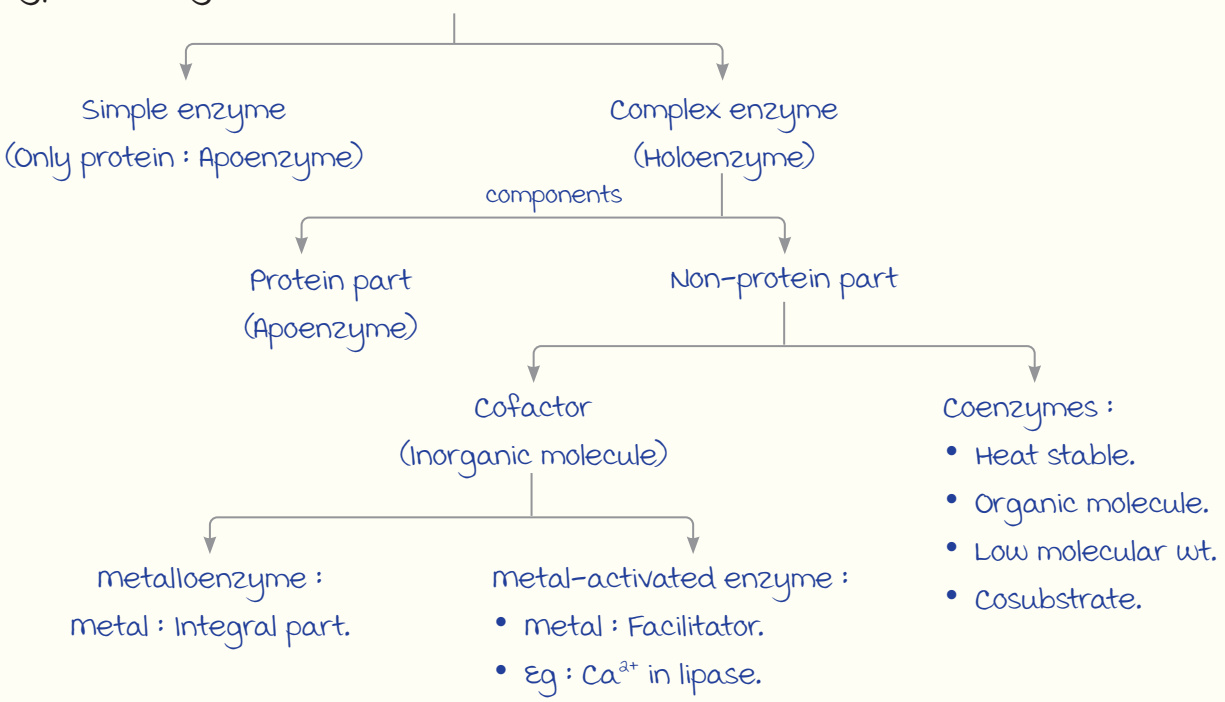
- Exception : Ribozymes (RNA).

Ribozyme	Location	Function
Peptidyl transferase	28 Sr RNA	Translation
Sn RNA	Spliceosome	RNA splicing
Group II introns	–	
Ribonuclease P	–	Post-translational modification of tRNA

Properties of Enzymes :

1. Made of protein.
2. 16% by weight : Nitrogen.
3. Heat labile.
4. Precipitated by protein precipitating agents.

Types of Enzymes :



Prosthetic group : Co-enzyme/Co-factor tightly integrated into apoenzyme.

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Coenzymes :

Vitamin	Active form	Reaction involved
Thiamine	Thiamine di/ pyrophosphate (TDP/TPP)	• Oxidative decarboxylation • Transketolase
Riboflavin	FAD ; FMN	Dehydrogenase (Succinyl, acyl CoA)
Niacin	NAD^+ $NADP^+$	most dehydrogenases
Panthothenic acid	CoA ; Acyl carrier protein	All reactions with acetyl CoA; Succinyl CoA; Fatty acid synthase complex
Pyridoxine	Pyridoxal phosphate	• Transamination • Transulfuration • Decarboxylation } Amino acid metabolism • ALA synthase • Glycogen phosphorylase
Folic acid	THFA	All 1 Carbon reactions
Cobalamin	Methyl B12	Methionine synthase
	Adenosyl B12	Methyl malonyl CoA mutase
Lipoate	Lipoamide	Oxidative decarboxylation
Ascorbic acid	-	Hydroxylation (Prolyl & lysyl)

Cofactors :

Metal	Reaction catalysed
Zinc	• Carbonic anhydrase • Carboxypeptidase A & B • Alcohol dehydrogenase • ALA dehydratase • Cytosolic SOD (Scavenging enzyme)
Magnesium	ATP/PO_4 group involved : • Kinase • Mutase • Phosphatase • Enolase
Iron	Heme iron : • Complex III & IV of ETC (Cytochrome) • No synthase, Peroxidase, Catalase
	Non-heme iron : • Complex I & II of ETC (Fe-S cluster)
Manganese	• Kinase • Mitochondrial SOD • Phosphatase
Molybdenum	Xanthine oxidase

Zn deficiency features

- Acrodermatitis enteropathica
- Visual disturbances (↓Retinol dehydrogenase)
- Alopecia
- Diarrhoea
- Perioral, acral rash

Purine catabolism

Mb deficiency

↓

Hypouricemia

metal	Reaction catalysed
Potassium	• Na^+– K^+ ATPase • Pyruvate kinase
Copper	• Tyrosinase (melanin production) • Complex IV of ETC (Cytochrome C oxidase) • Lysyl oxidase (Covalent cross linking of Collagen)

Cu deficiency
• Depigmentation • Neutropenia • X-ray : Similar to scurvy

----- Active space -----

Note : Vitamin C leads to defective collagen D/t ↓lysyl hydroxylase.

Classification of Enzymes

00:17:14

Class	Class name	Details & examples.
I	Oxidoreductases :	
	a. Dehydrogenase	NAD^+ FAD required as election acceptor (Oxidative decarboxylation)
	b. Oxygenase	• Monooxygenase : Phenylalanine/Tyrosine/Tryptophan hydroxylase, Cytochromes • Dioxygenase : Homogentisate oxidase
	c. Oxidase	Complex IV ETC
	d. Peroxidase	Glutathione peroxidase ($H_2O_2 \rightarrow H_2O$)
	e. Catalase	$H_2O_2 \rightarrow H_2O$; Enzyme marker of peroxisome
II	Transferase	Transfers functional group Eg → Kinases (Hexo/glucokinase) → Phosphorylases (Glycogen phosphorylase)
III	Hydrolase	• Breaks covalent bonds by adding H_2O • Eg : All digestive enzymes, arginase, phosphatase
IV	Lyase	• Breaks covalent bond without H_2O/atom elimination • Eg : Aldolase, fumarase, aconitase, enolase, simple decarboxylase
V	Isomerase	Eg → Isomerase (Produces isomers) → Racemase (D&L isomers) → Mutase (intramolecular PO_4 transfer)
VI	Ligase	• Coupling of molecules with breakdown of ATP • Eg → Synthetase → Carboxylase (Requires biotin)
VII	Translocase	• Transfer of ions/molecules across membrane • Eg: H^+ pump/Ca^{2+} channel

----- Active space -----

Applied biochemistry
Defective synthesis of tetrahydrobiopterin (BH_4) from GTP : • ↓ phenylalanine hydroxylase : Non-classical phenylketonuria • ↓ tyrosine & tryptophan hydroxylase : Neurological symptoms (D/t ↓ catecholamines & serotonin)
• Resistant seizures in neonate : Supplement Vit. B6 (PLP) : – Dopamine, serotonin, epinephrine & norepinephrine require PLP as co-factor (simple decarboxylation)
• Fatigue in chronic alcoholics : D/t Vit. B1 deficiency (↓NADPH ⟶ ↓ATP)
• Polished rice consumption ⟶ Beri-Beri (D/t vit B1 deficiency)
• Raw egg consumption ⟶ Fatigue, hypoglycemia, organic aciduria. – Avidin (Raw egg) inhibits biotin (Vit. B7) & hence, all carboxylase reactions.

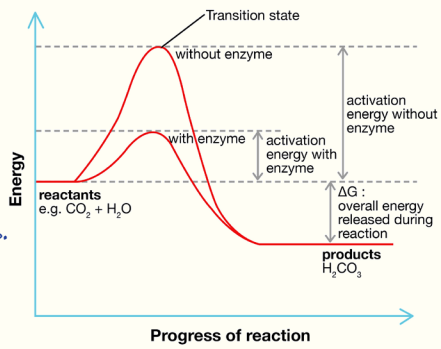
Enzyme Mechanism of Action

00:41:42

- Substrate binding : Active site.
- Site for regulator/modifier Allosteric site.

Free energy change (ΔG) :

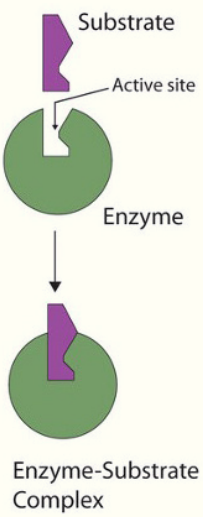
- Free energy change =
  ΔG = Energy of reactants – Energy of products.
- Enzyme ⟶ ↓ activation energy.
  ⟶ No change in ΔG.

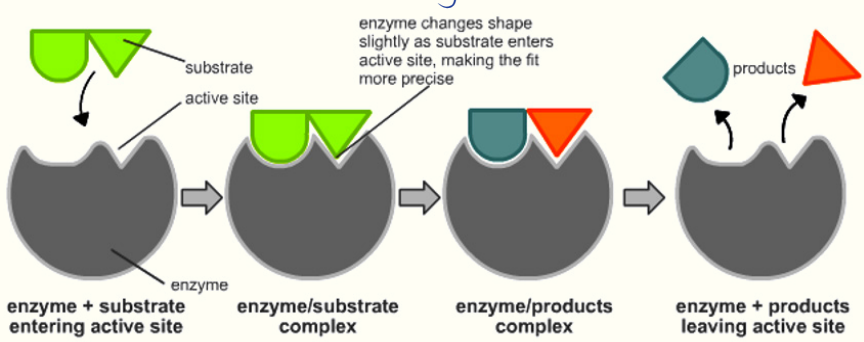


Enzyme-substrate complex :

Emil-Fischer's template theory :
Lock and Key mechanism.

Koshland's induced fit theory :
Conformational change in active site induced by substrate.





ENZYMES : PART 2

----- Active space -----

Enzyme Kinetics

00:00:12

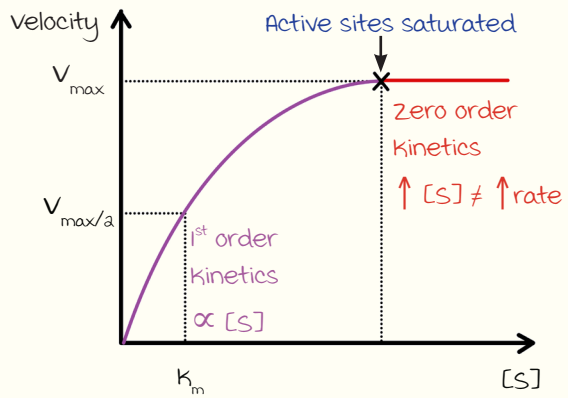
Equilibrium Constant (K_{eq}) :

Independent of enzyme action.

$$K_{eq} = \frac{[Products]}{[Substrates]}$$

Factors Affecting Rate of Reaction :

1. Substrate concentration :

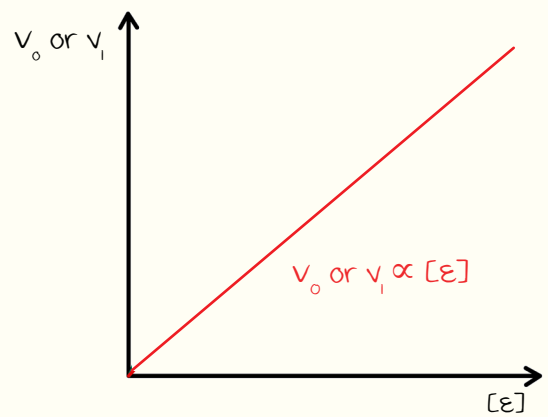


- Hyperbolic curve.
- Michaelis Menten equation :

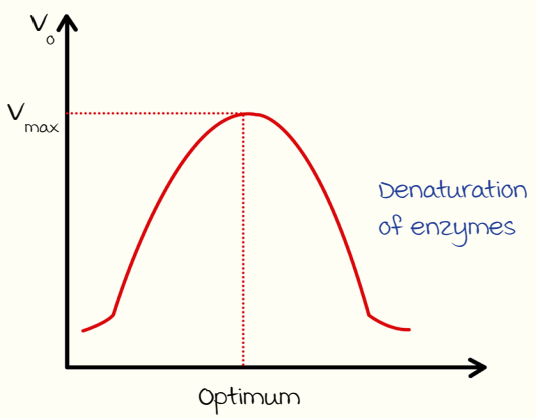
$$V_i = \frac{V_{max} \times [S]}{K_m + [S]}$$

- K_m (Michaelis constant) :
  - [S] at $V_{max}/2$.
  - $\propto \frac{1}{\text{Enzyme affinity to substrate}}$
- Ideal substrate : $\downarrow K_m$.

2. Enzyme concentration :



3. Temperature & pH :



- Temperature (35 to 40 °c).
- pH (5 to 9).

- Q_{10} : 10°c ↑ = 2 x rate of reaction.

----- Active space -----

Catalytic Constant :

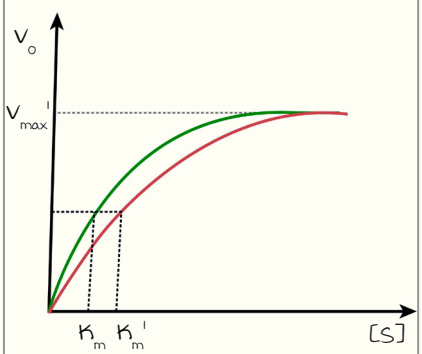
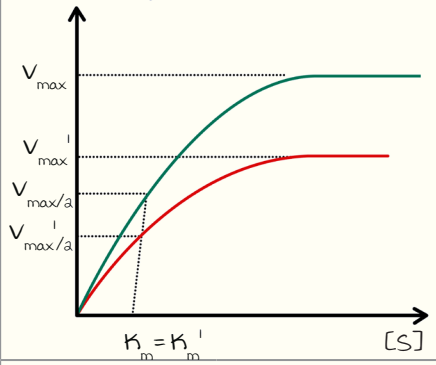
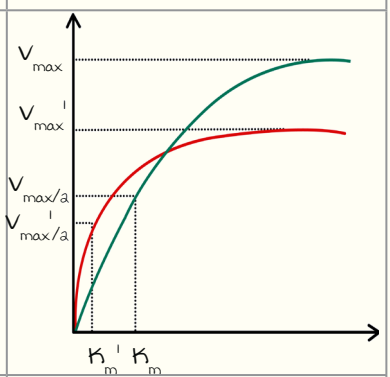
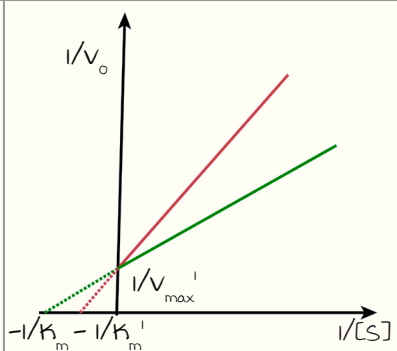
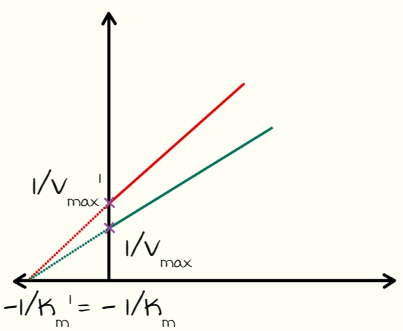
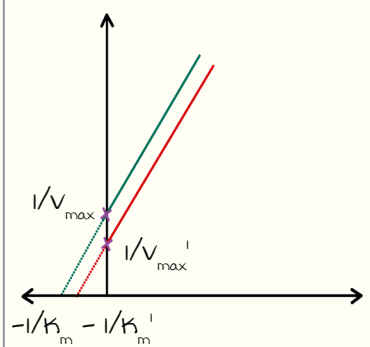
AKA turnover number.

$K_{cat} = \frac{[V_{max}]}{[\varepsilon_t]}$; ε_t = Total enzyme concentration.

Catalytic efficiency = $\frac{K_{cat}}{K_m}$

Enzyme Inhibition

00:12:00

	Competitive inhibition	Non-competitive inhibition	Uncompetitive inhibition
Features	• Inhibitor : Structural analogue of substrate • Compete for same site as substrate	• Inhibitor : Not a structural analogue • Distinct binding site • Usually irreversible	Inhibitor binds to enzyme-substrate complex.
Effect on V_{max} & K_m			
Line weaver Burk plot			

Examples

Inhibitor	Enzyme	Inhibitor	Enzyme	
Methotrexate	Dihydrofolate reductase	Cyanide CO	Cytochrome c oxidase	Phenylalanine inhibits placental alkaline phosphatase
Statins	HMG CoA reductase	Fluoride	Enolase	
Dicumarol	Vit K epoxide	Iodoacetate	Glyceraldehyde 3-P-DH	
Ethanol	Alcohol DH	Fluoroacetate	Aconitase	
Malonate (Poison)	Succinate DH			

----- Active space -----

Applied biochemistry

1. Folic acid supplemented when patient is on methotrexate.
2. Dicumarol : Anticoagulant.
   - Inhibits Vit. K dependent γ carboxylation of clotting factors II, VII, IX & X.
3. Hooch tragedy : Methanol poisoning $\xrightarrow{\text{Antidote}}$ Ethanol (Competitively inhibits formaldehyde).
4. Fluoride oxalate used in estimation of blood glucose (Gray tube).

Suicide Inhibition :

Unreactive inhibitor $\xrightarrow{\text{Binds to enzyme}}$ Reactive inhibitor (Irreversible).

Suicide inhibitor	Enzyme
Allopurinol	Xanthine oxidase
Difluoromethyl ornithine	Ornithine decarboxylase
Aspirin	Cyclooxygenase

Regulation of Enzyme Activity 00:22:53

Enzyme Quantity :

- Heme $\xrightarrow{\text{Represses}}$ ALA synthase gene.
- Dietary cholesterol $\xrightarrow{\text{Represses}}$ HMG CoA reductase gene.

Porphyria : Take detailed drug history

Drugs metabolized by cytochrome

↓

↓Heme levels

↓

↑ALA synthase (Gene induction)

↓ Accumulation of intermediates

Aggravates porphyria.

Covalent Modification :

1. Zymogen activation (Irreversible) :
   - Gastrointestinal enzyme (Eg : Trypsinogen → Trypsin).
   - Clotting factors.

----- Active space -----

a. Phosphorylation – dephosphorylation (Reversible) :

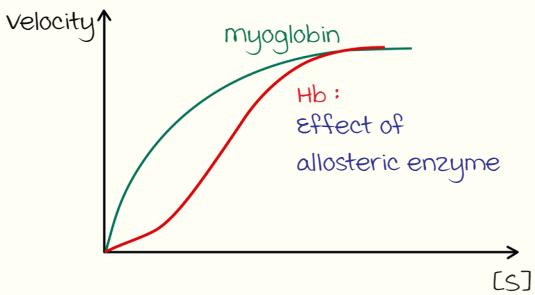
	Phosphorylated state	Dephosphorylated state
Insulin : Glucagon ratio	Low	High
Fed/fasting	Fasting	Fed
Enzymes activated	Inhibited by insulin • Fructose – 1, 6 – bisphosphate • Glycogen phosphorylase • Hormone sensitive lipase	Inhibited by glucagon • Phosphofructokinase • Glycogen synthase • Pyruvate DH • Acetyl CoA carboxylase • HMG CoA reductase

Allosteric Regulation :

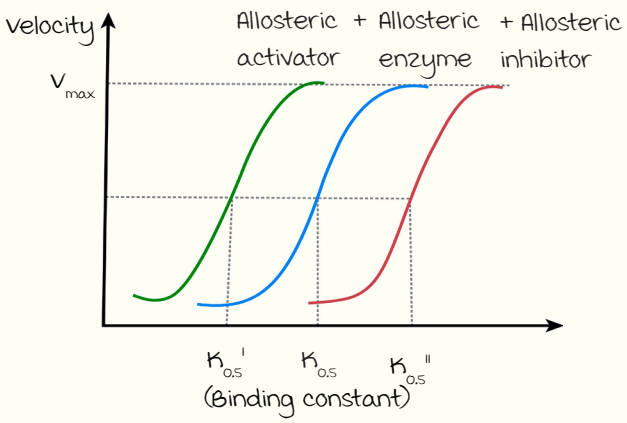
- Substrate : Binds to catalytic site.
- Modifier :
  - Not structural analogue of substrate.
  - Binds to allosteric site.

Allosteric enzymes :

- Multi-subunit.
- Quaternary structure.
- Cooperative binding → Sigmoid curve.
- Usually rate limiting step.



Allosteric activator & inhibitor :



$$K_{0.5} \propto \frac{1}{\text{Affinity of enzyme to substrate}}$$

Examples :

----- Active space -----

Enzyme	Allosteric Inhibitor (Products)	Allosteric Activator (Substrates)
ALA synthase	Heme	-
Aspartate transcarbamoylase	CTP	ATP
HMG CoA reductase	Cholesterol	-
Phosphofructokinase	Citrate, ATP	AMP, F2, 6P
Acetyl CoA carboxylase	Acyl CoA	Citrate
Citrate synthase	ATP	-
CPS-I	-	NAG
CPS-II	ATP	-

Serine Proteases & Markers of Cell Organelles

00:40:30

Serine Proteases :

Serine present in active site.

Enzymes

1. Chymotrypsin : Bulky amino acids.
2. Trypsin : Basic amino acids.
3. Elastase : Small amino acids.

} Breaks protein at this site.

4. Thrombin.
5. Plasmin.
6. Factor X.
7. Factor XI.

Marker Enzymes of Cell Organelles :

Cell organelle	Marker
Plasma membrane	• 5'-nucleotidase • Adenylyl cyclase • Na^+-K^+ ATPase
Smooth endoplasmic reticulum	Glucose-6-phosphate
Golgi apparatus	Galactosyl transferase
Mitochondria	ATP synthase
Lysosome	Cathepsin
Peroxisome	Catalase

Clinical Enzymology

00:42:45

Isoenzymes :

Lactate DH :

Isoenzyme	Subunits	Electrophoretogram	Tissue localization	% in serum
LDH-1	H_4	Fastest	Heart	30
LDH-2	H_3M_1	Faster	RBC	35
LDH-3	H_2M_2	Intermediate	Brain	20
LDH-4	HM_3	Slower	Liver & skeletal muscles	10
LDH-5	M_4	Slowest		5

Myocardial infarction
• Normal : $LDH_2 > LDH_1$ • MI : $LDH_1 > LDH_2$ (Flipped pattern).

----- Active space -----

Creatine Kinase (CK) :

Isoenzyme	Subunits	Electrophoretogram	Tissue localization	% in serum
CK-1	BB	Fastest	Brain	1
CK-2	MB	Intermediate	Heart	5
CK-3	MM	Slowest	Skeletal muscle	80

Clinical significance
CVA
MI
Muscle injury, Myopathies

Alkaline phosphatase :

Isoenzymes	Location
α-1-ALP	Membrane of epithelium of biliary canaliculi
α-2-ALP (Heat labile)	Hepatic sinusoidal cells
α-2-ALP (Heat stable)	Placenta
Pre-β-ALP	Osteoblast
Gamma ALP	Intestinal cells
Leukocyte ALP	Leukocytes

Clinical significance
Marker of cholestasis
Marker of hepatic injury
Most stable
Marker of bone formation : ↑ in Paget's & Vit D deficiency
Ulcerative colitis
Leukemia

Cardiac Biomarkers in MI :

Name	Starts to rise	Peak	Return to baseline
CK-MB (Earliest enzyme marker)	4 – 8 h	24 h	48 – 72 h
Troponin T	4 – 6 h	24 h	7 – 10 d
Troponin I (↑Sensitivity)	4 – 6 h	24 h	7 – 10 d

Note :

NT Pro-BNP (Precursor of brain natriuretic peptide) : Marker of cardiac failure.

Enzyme Markers

00:53:02

Liver :

Markers of hepatic injury :

- S. ALT.
- S. AST.

Markers of cholestasis :

- S. ALP.
- 5' nucleotidase.
- S. GGT.

----- Active space -----

Prostate :

- Acid phosphatase : Tartarate labile.
- Prostate specific antigen : Specific.
  - Cut-off < 4 ng/mL.

Pancreas :

- Serum amylase.
- Serum lipase : Specific.

Bone Disease :

Bone formation (From osteoblast) :

- Pre-β ALP.
- Osteocalcin.
- Propeptide of type I collagen.

Bone resorption (From osteoclast) :

- N-telopeptide of type I collagen.
- C-telopeptide of type I collagen.
- Urine free deoxypyridinoline.

Acute Kidney Injury :

- Kidney Injury Molecule I (KIM I).
- Neutrophil Gelatin Associated Lipocalin (NGAL).
- IL-8.
- ALT.
- Glutathione S-transferase.
- GGT.
- Microalbumin.
- Osteopontin.
- Liver fatty acid binding protein.
- Sodium hydrogen exchange isoform.
- Exosomal fetuin.

----- Active space -----

CARBOHYDRATES : INTRODUCTION

Chemistry of Carbohydrates

00:01:25

Carbohydrates : Aldose or Keto derivatives of polyhydroxy alcohol.

Monosaccharides :

No. of carbon atoms	Aldoses	Ketoses
Triose (Simplest carbohydrates)	Glyceraldehyde	Dihydroxy acetone
Tetrose	Erythrose	Erythrulose
Pentose	Ribose ⟷ (Epimer) Xylose	Ribulose ⟷ (Epimer) Xylulose
Hexose	Glucose, galactose, Mannose	Fructose

Disaccharides :

Reducing disaccharides :

Name	Monomer units	Linkage
Maltose	Glucose + glucose	α 1, 4
Isomaltose	Glucose + glucose	α 1, 6
Lactose	Galactose + glucose	β 1, 4
Lactulose	Galactose + fructose	α 1, β4

Non-reducing disaccharides :

Name	Monomer units	Linkage
Trehalose	Glucose + glucose	α 1, 1
Sucrose	Glucose + fructose	α 1, β2

Polysaccharides :

Homopolysaccharides :

- Made up of one type of monomer units.
- Eg : Starch, glycogen.

Heteropolysaccharides :

- > 1 monomer units.
- Eg : Glycosaminoglycans (GAG).

Dietary Fibres & Glycosaminoglycans

00:12:28 ----- Active space -----

DIETARY FIBRES

Properties :

- Resistant to digestion & absorption by small intestine.
- Undergoes complete/partial fermentation in large intestine.

Classification :

Soluble :

- Gums (Fenugreek)
- Pectin

} ↓Post prandial blood sugar level.

- Mucilage.

Insoluble (Crude fibres) :

- Cellulose, AKA non-starch polysaccharide :
  - Made of beta-D-glucose.
  - Resistant to digestion d/t β-linkage and lack of cellulase in human intestine.
- Hemicellulose.
- Lignin : Neither digested nor fermented.

Note :

Lactose ⟶ Not a dietary fibre as β-linkage in it is digested by lactase (Only digests lactose).

RDA :

40 g/2000 cal.

Energy released per gram : 2 Kcal/g.

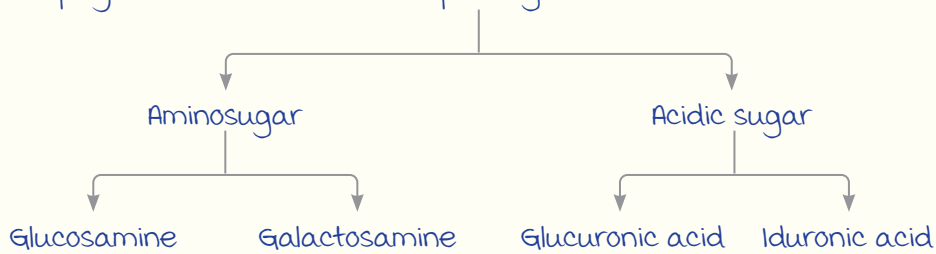
Uses :

- Adds bulk to the stool.
- Regulates bowel movements.
- Improves satiety.
- Prebiotic (Fibre) : Promote colonisation of probiotic bacteria.
- Sequesters bile salts ⟶ ↓Cholesterol.
- Improves glucose tolerance.

----- Active space -----

GLYCOSAMINOGLYCANS (GAG)/MUCOPOLYSACCHARIDES

Unbranched heteropolysaccharides made of repeating disaccharide units



Synthesis Site :

Rough endoplasmic reticulum & golgi apparatus.

Properties :

1. Negatively charged : Confers consistency of mucus & enables mobility at joints.
2. Absorbs water : Provides cushioning effect at weight bearing joints.

Composition :

GAG	Repeating disaccharide unit
Hyaluronic acid	N-acetyl glucosamine + glucuronic acid
Keratan sulphate	N-acetyl glucosamine + galactose (No uronic acid)
Heparin	Glucosamine + iduronic acid

Significance :

1. Chondroitin sulphate :
   - Most abundant.
   - Found in bones & cartilage : Enables compressibility of cartilage.

2. Keratan sulphate (KS) :
   - KS-1 : Cornea → Maintain corneal transparency.
   - KS-2 : Loose connective tissue.

3. Dermatan sulphate :
   - Widely distributed GAG found in the dermis.
   - Maintains structure of sclera.
   - Atherogenic GAG : Attract LDL.

4. Heparan sulphate :
   - Responsible for charge selectiveness of GBM.
   - Present on synaptic vesicle.
   - Anchors lipoprotein lipase to vascular endothelium.
   - Acts as plasma membrane receptors.

----- Active space -----

5. Heparin :
   - Only intracellular GAG.
   - Anticoagulant.
   - Used for estimation of lipoprotein lipase (LPL) in type I hyperlipoproteinemia.

6. Hyaluronic acid :
   - Important role in cell migration : Wound healing, tumor metastases, embryogenesis.
   - Not attached to protein.
   - Sulphate group : Absent.

Mucopolysaccharidoses

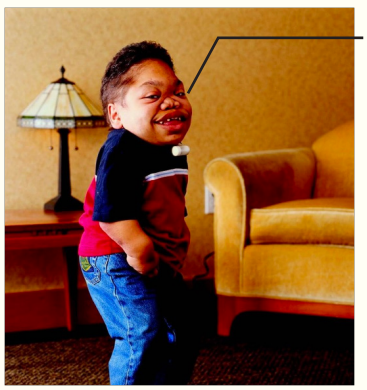
00:37:15

Group of disorders a/w defect in degradation (In lysosomes) of GAG (mucopolysaccharides)

↓

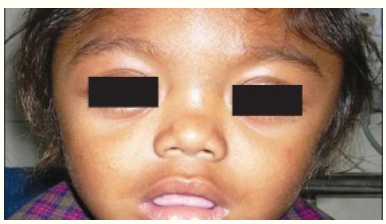
Belongs to lysosomal storage disorder.

General Features :

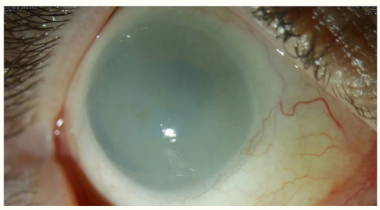


Short stature

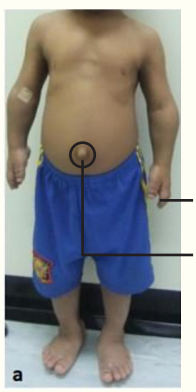
Gargoylic facies : Coarse facial features.
- Frontal bossing.
- Depressed nasal bridge.



Protruding tongue



Corneal clouding



Clawing of hands

Umbilical hernia

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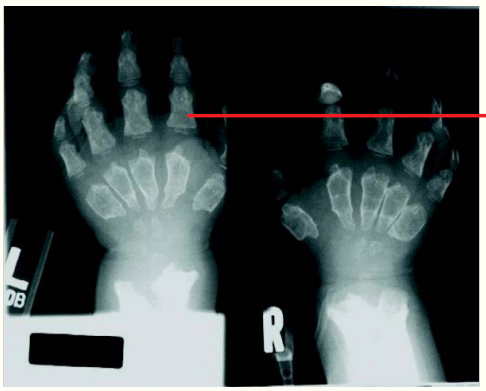
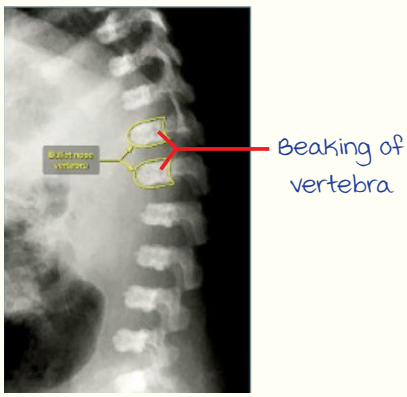
Xray - features :



Dysostosis multiplex



MPS I & II :

All are AR except Hunter's.

	Enzyme defect	Features
Hurler's disease (MPS-I H)	L-iduronidase	• Visual disturbances ⊕ • Mental retardation
Sheie's disease (MPS-I S)	L-iduronidase (Partial defect)	• Hirsutism • Normal intelligence
Hunter's disease (MPS II)	Iduronate sulfatase	• X-linked recessive • Intellectual disability • Clear vision

Other Mucopolysaccharidoses :

	Enzyme defect	Specific feature
Sanfilippo disease (MPS III)	Enzyme that degrades heparan sulfate	• M/c MPS • Corneal clouding : Absent
Morquio disease (MPS IV)	Galactosamine-6-sulphatase, β-galactosidase	• Normal Intelligence • Visceromegaly : Absent • Reilly body (Leucocyte) inclusion : Absent
Maroteaux Lamy disease (MPS VI)	N-acetyl galactosamine-4-sulphatase	Normal intelligence
Natowicz syndrome (MPS IX)	Hyaluronidase	⊖

----- Active space -----

Treatment Modalities :

Treatment modality	MPS
1. Stem cell therapy	MPS-I H
2. Enzyme replacement therapy	
Aldurazyme	MPS-I
Elaprase	MPS-II
Nagalazyme	MPS-VI
3. Substrate reduction therapy : Flavinoids	MPS-I H

I (INCLUSION) CELL DISEASE

- Defect in protein targeting.
- Resembles MPS : Accumulation of GAG in lysosomes.
- Enzyme defect : N-acetyl glucosamine phosphotransferase

  ↓

  ↓mannose-6-phosphate : Signal for proteins tagged with it to get degraded in the lysosome.

Glucose Transporters 00:53:00

SGLT

- Sodium dependent.
- Sodium-glucose symport → ORS contains Na^+ & glucose to facilitate Na^+ absorption.
- Unidirectional.
- Against concentration gradient.
- Secondary active transport.

Types of SGLT :

Type	Location	Function
SGLT-1	Luminal side of intestine	Absorption of glucose
	PCT	Reabsorption of glucose
SGLT-2	PCT	

Applied Biochemistry :

1. Renal glycosuria :
   - Mutation in SLC5A2 → SGLT-2 defect → ↓Renal threshold.

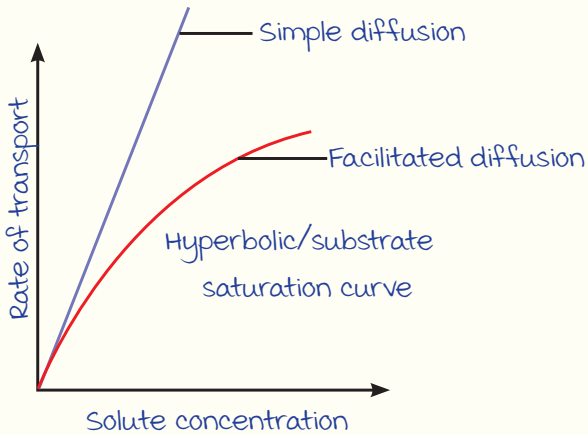
----- Active space -----

- Findings :
  - i. OGTT (Blood glucose) : Normal.
  - ii. Urine Benedict's test : Positive.
  - iii. Urine Glucose oxidase test : Positive.

2. Gliflozines (OHA) : SGLT2 inhibitor → Glycosuria → UTI.

GLUTs

- Sodium independent.
- Bidirectional.
- Along concentration gradient.
- Ping pong mechanism.
- Facilitated carrier mediated process (Passive).



Location :

GLUT	Location	Important points
GLUT-1	Brain, placenta, kidney, RBC, retina, colon	Low Km (High affinity for glucose)
GLUT-2	• β cells of pancreas • Sinusoidal cells of liver • Serosal side of intestine • Proximal renal tubules	• High Km (Low affinity) • Active in the fed state
GLUT-3	Neurons, placenta, kidney	Lowest Km
GLUT-4	• Heart • Skeletal muscle • Adipose tissue	Insulin dependent
GLUT-5	• Luminal side of intestine • Testes & sperm	Fructose transporter
GLUT-6	Spleen, leukocyte	Pseudogene
GLUT-7	Liver SER	Transports glucose-6-PO_4 to SER
GLUT-8	Blastocyst	-
GLUT-9	Intestine & kidney	• Uric acid transporter • Defect → Primary gout

METABOLISM OF CARBOHYDRATES : PART 1

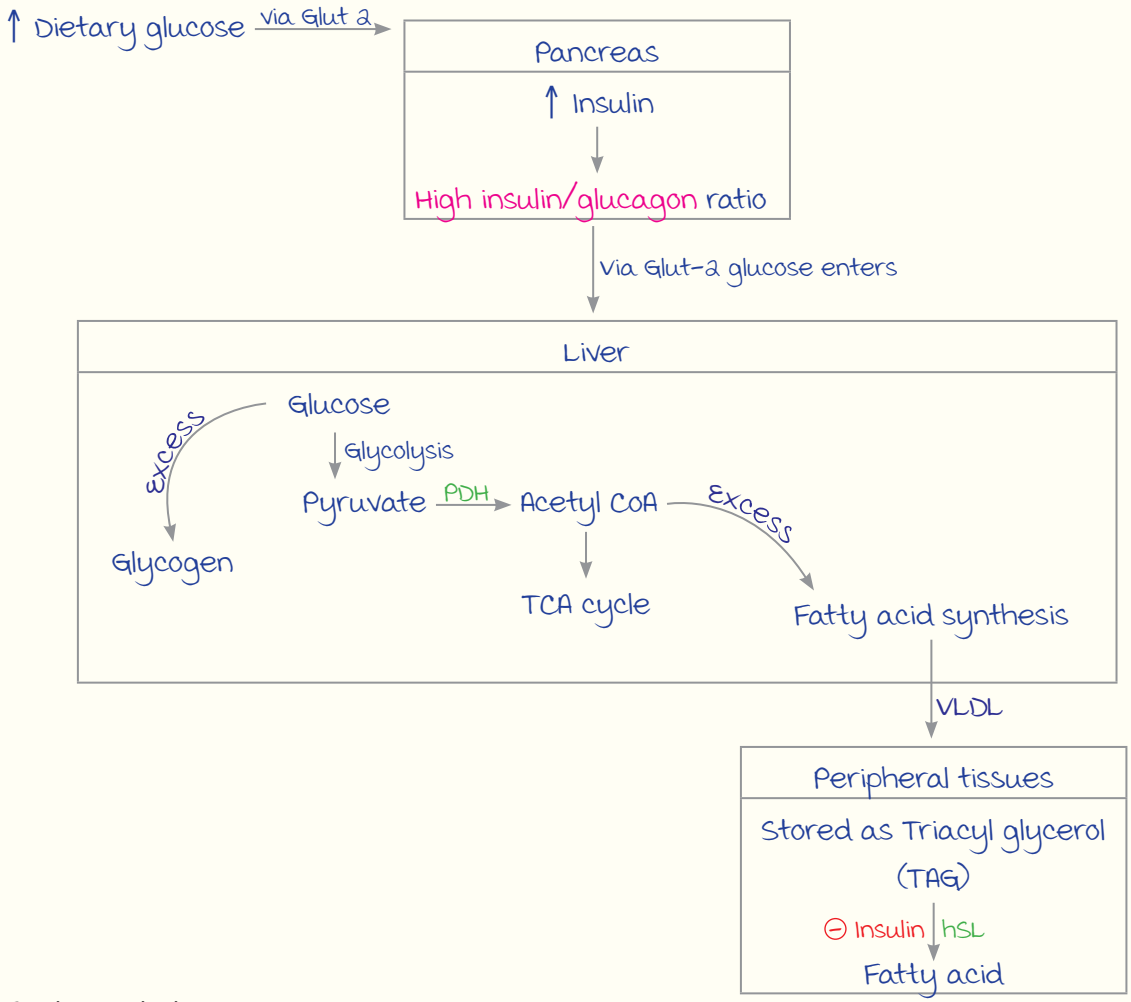
----- Active space -----

Fed & Fasting State Metabolism

00:00:10

Well Fed State :

1-4 hours within food intake.



Fasting State :

Stage	Duration post food intake	Source of energy
Early fasting	4-16 hours	Hepatic glycogenolysis (D/t ↓ Insulin/glucagon ratio)
Fasting	16-48 hours	Gluconeogenesis (ATP from fatty acid oxidation)
Prolonged fasting/starvation	48 hours-5 days	TAG —hSL→ Fatty acid → Acetyl CoA → Ketone body synthesis
Prolonged starvation	>5 days	Muscle proteolysis : Breakdown structural proteins for energy

----- Active space -----

Glycolysis

00:08:30

Features :

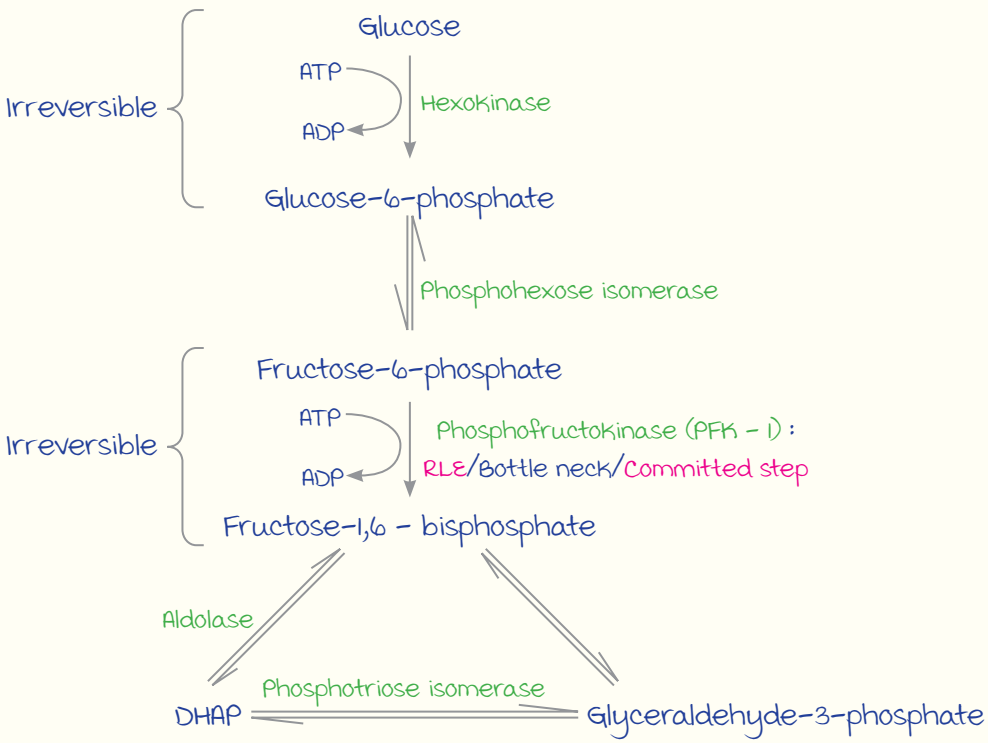
- Site : All organs (In cytoplasm).
- Only pathway that operates both aerobically & anaerobically.

Applied Biochemistry :

Condition		Reason
Defect in glycolytic enzymes → Hemolysis		Mature RBCS → Lack mitochondria → Rely exclusively on anaerobic glycolysis in fed/fasting state
Tolerance to hypoxia	Heart muscles : Low	D/t low glycolytic capacity
	Skeletal muscles : High	D/t enormous glycolytic capacity

Aerobic Glycolysis :

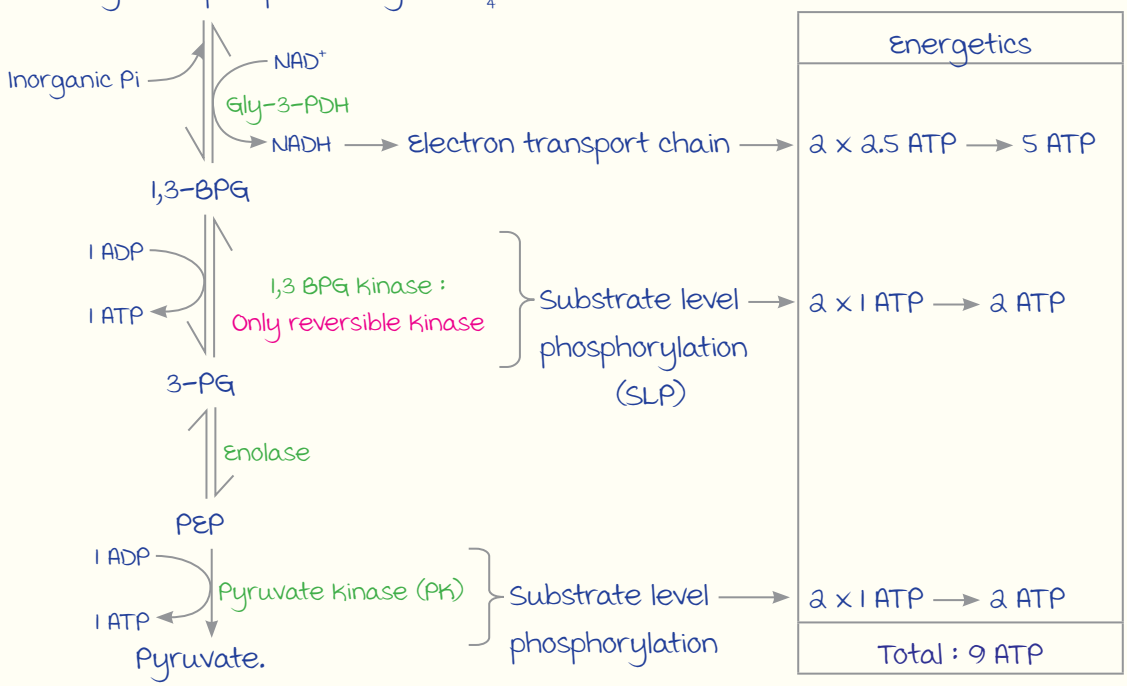
Preparatory phase : Stage of ATP utilization (2 ATPs used).



Pay off phase : Aerobic glycolysis.

----- Active space -----

2 x Glyceraldehyde-3-phosphate (Gly-3-PO_4)

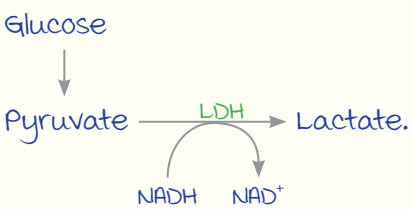


Net ATP : 9 ATP – 2 ATP (utilised in preparatory phase) = 7 ATP.

Applied biochemistry :

S. no	Compound	Enzyme inhibited	Reason/Application
1.	Fluoride	Enolase	Blood glucose estimation : Gray vacutainer containing fluoride oxalate used
2.	Arsenate	Gly-3-PDH	Arsenate & Iodoacetate resembles inorganic phosphate
3.	Iodoacetate		

Anaerobic Glycolysis :



Energetics :

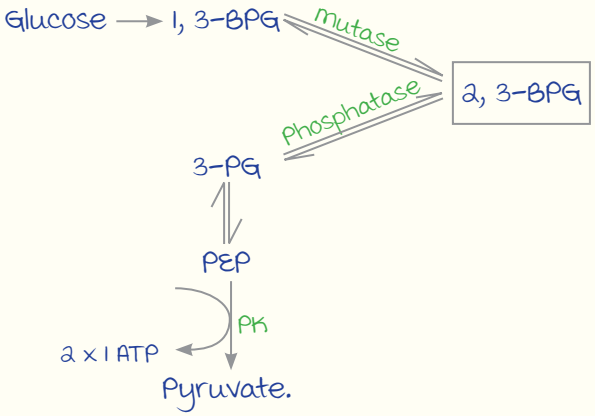
- No net generation of NADH : Utilized by LDH
- 1,3-BPG : 2 x 1 ATP
- Pyruvate Kinase : 2 x 1 ATP } 4 ATP
- Utilization : 2 ATP

Total : 4 ATP–2 ATP = 2 ATP

----- Active space -----

Rapaport Leubering Cycle :

Site : Takes place in 10% of RBCs.



Energetics :
- Pyruvate kinase : +2 ATP
- Hexokinase : -1 ATP
- PFK : -1 ATP

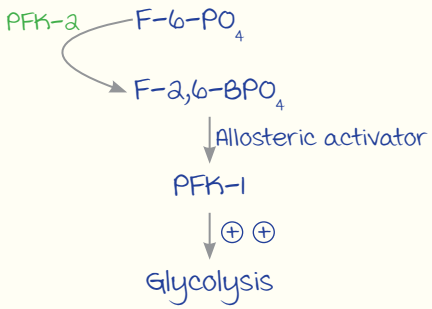
Net ATP : 0

Significance of 2,3-BPG :
- Maintains taut state of hemoglobin.
- Responsible for unloading of O_2 at tissue site → Shifts oxygen dissociation curve to the right.

Regulation of Glycolysis :

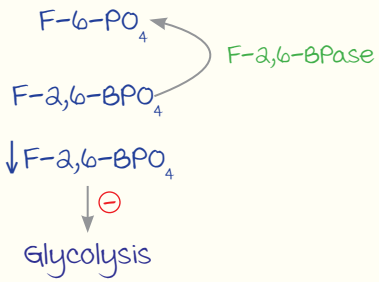
1. Fed state :

High insulin glucagon ratio
↓
Dephosphorylation of enzymes
↓
Activation of PFK-2



2. Fasting state :

Low insulin glucagon ratio
↓
Phosphorylation of enzymes
↓
Activation of F-2,6-BPase



Concept of Allosteric Regulation :

Substrates : Activators.

Products : Inhibitors.

Enzyme	Allosteric activator	Allosteric inhibitor
Hexokinase	-	ATP
PFK-1	• F-6-P • F-2,6-BP • 5' AMP	• ATP • Citrate • Low pH
Pyruvate kinase	-	ATP

----- Active space -----

Metabolism in Cancer Cells :

Cancer cells have ↑ levels of glucose uptake.

Warburg hypothesis :

- Cancer cells undergo aerobic fermentation/aerobic glycolysis :
  Even in the presence of ample O_2, Glucose ⟶ Lactate (used in biosynthetic pathways).

Metabolic reprogramming :

Normal cell :

Glucose
↓
PEP
↓ PKM 1 (Pyruvate kinase) :
- Tetramer
- High catalytic activity

Pyruvate

Cancer cell : Uses glucose via aerobic glycolysis.

Glucose
↓
PEP
↓ PKM2 :
- Dimer
- Low catalytic activity

Pyruvate
↓ O_2
Lactate

Normal cell :

Glucose
↓ 7ATP
Pyruvate
↓ 2 NADH = 5 ATP
Acetyl CoA
↓ 2 x 10 ATP = 20 ATP
TCA cycle

Aerobic oxidation : 1 Glucose ⟶ 32 ATP.

Cancer cell : ↑ Affinity for glucose.

Glucose
↓
Pyruvate
↓
Lactate

Aerobic glycolysis : 2 ATP.

Application :

Usage of Fluorodeoxy glucose in PET scan
↓
↑ Uptake by cancer cells

} Functional screening

----- Active space -----

Pyruvate Dehydrogenase (PDH)

00:41:00

Link Reaction :

- Link glycolysis to TCA cycle.
- Site : Mitochondria.

Enzymes

1. E1 : PDH.
2. E2 : Dihydrolipoyl transacetylase.
3. E3 : Dihydrolipoyl dehydrogenase.

Coenzymes :

1. Thiamine pyrophosphate (B1).
2. Coenzyme A (B5).
3. Lipoamide.
4. FAD (B2).
5. NAD^+ (B3).

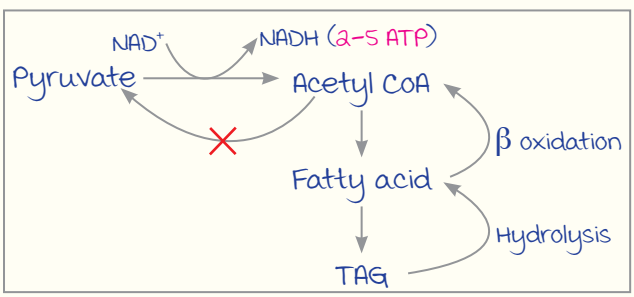
Significance of PDH :

- Irreversible & cannot be circumvented by another enzyme.
- Fat : Never converted to glucose :

  Exceptions :

  a. Glycerol.

  b. Odd chain fatty acid (Propionyl CoA).



Applied Biochemistry :

1. Deficiency of PDH	2. B1 deficiency :
Pyruvate ⟶ Acetyl CoA ↓ Lactate ↓ Lactic acidosis	a) Chronic alcoholics b) Consumption polished rice (↓aleurone layer) } PDH affected ↓ Energy depletion

Note :

- PDH
- α Ketoglutarate dehydrogenase
- Branched chain Keto acid dehydrogenase

} Same coenzymes.

METABOLISM OF CARBOHYDRATES : PART 2

----- Active space -----

Glycogen Metabolism

00:00:10

GLYCOGENESIS

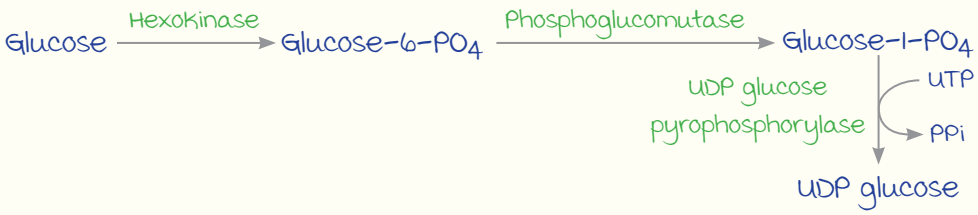
Occurs in the well-fed state, high insulin-glucagon ratio.

Site :

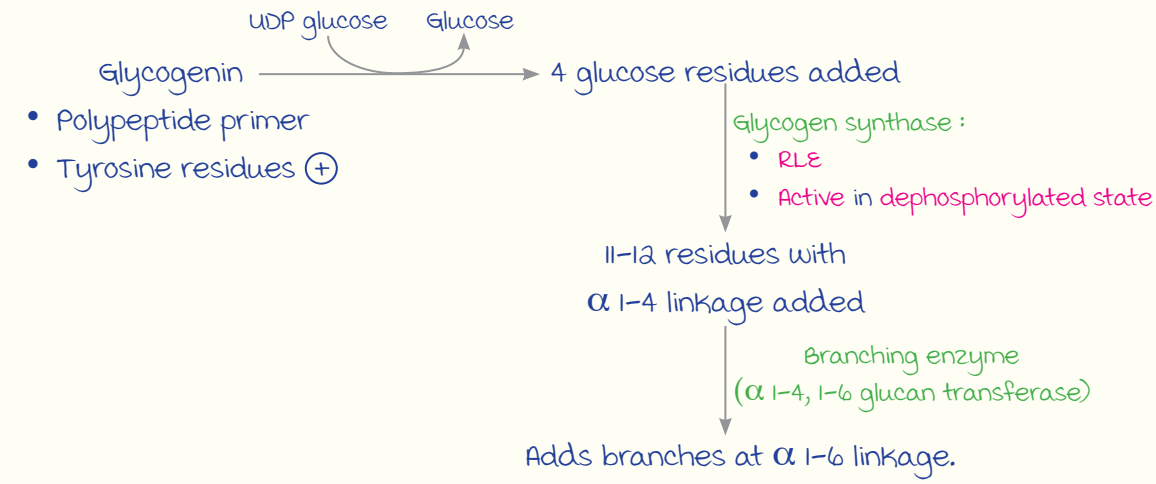
Cytoplasm of liver & skeletal muscles.

Stages :

1. Formation of UDP glucose :



2. Formation of linear polymer :



GLYCOGENOLYSIS

Occurs in the fasting state (4-16 hours).

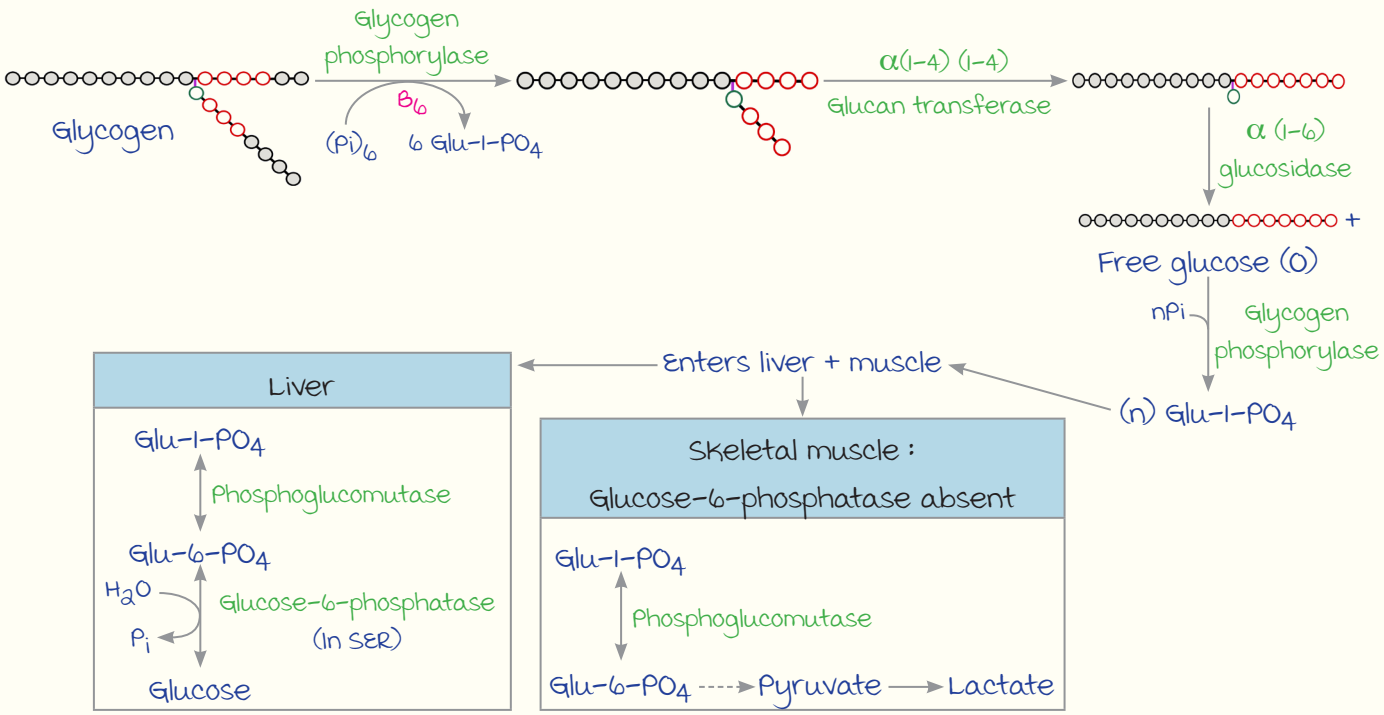
Site :

- Cytoplasm of liver & skeletal muscles.
- Lysosomes → Type II GSD (Pompe's disease) is a lysosomal storage disorder.

Hepatic glycogenolysis : Source of glucose in early fasting states.

----- Active space -----

Stages :



- α (1-4) glycosidic linkage.
- α (1-6) glycosidic linkage.

Debranching enzyme : α (1-4) (1-4) Glucan transferase + α (1-6) glucosidase.

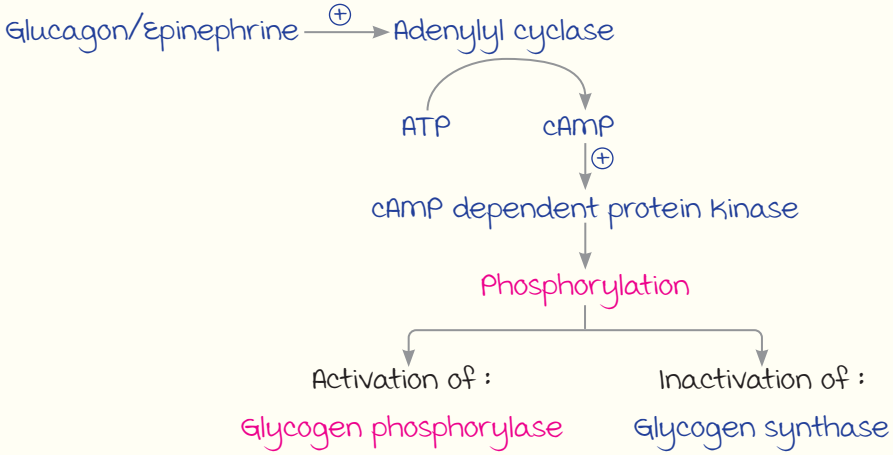
Note :

- Phosphoglucomutase : Common to glycogen synthesis & glycogenolysis.
- Glucose-6-phosphate : Present in SER $\xrightarrow{\text{Hence}}$ Glucose not released immediately.

Energetics in muscle : 1 Glu-6-PO4 → 3 ATP.

HORMONAL REGULATION/COVALENT MODIFICATION

Fasting State :



----- Active space -----

Fed State :

Insulin : cAMP → 5' AMP → ↓↓cAMP

↓

Dephosphorylation

Activation of :	Inactivation of :
Glycogen synthase	Glycogen phosphorylase

In the Muscle :

Regulation by :

1. cAMP dependent pathway.
2. cAMP independent calcium calmodulin dependent pathway.
3. 5' AMP :
   - Allosteric activator of glycogen phosphorylase.
   - Activated in extreme state of anoxia.

ALLOSTERIC REGULATION

Inhibitors of Glycogenolysis :

- Glu-6-PO_4.
- Glucose (Only in liver).
- ATP.

Activator of Glycogenesis :

Glu-6-PO_4.

Glycogen Storage Disorder (GSD) 00:19:20

Liver GSD :

Prominent feature : Fasting hypoglycemia.

GSD type	Enzyme defect
Type Ia GSD : Von Gierke's disease	Glucose-6-phosphatase
Type Ib GSD : Neutropenia ⊕	Glu-6-PO_4 transporter in SER
Type III GSD : Cori's disease/Forbes' disease (Limit dextrinosis)	Debranching enzyme
Type IV GSD : Anderson disease, Amylopectinoses	Branching enzyme
Type VI GSD : Her's disease	Hepatic glycogen phosphorylase

Mnemonic : ABCD.

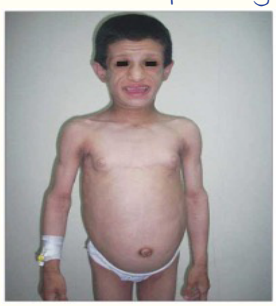
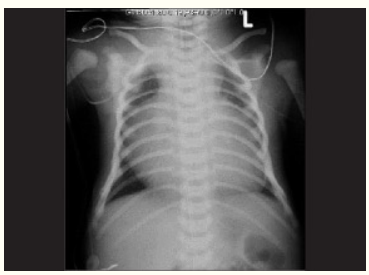
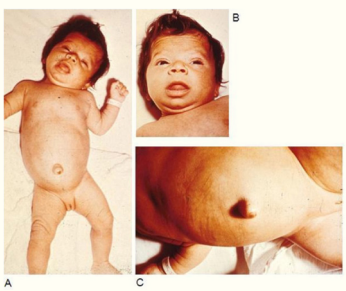
- Anderson disease : Branching enzyme.
- Cori's disease : Debranching enzyme.

----- Active space -----

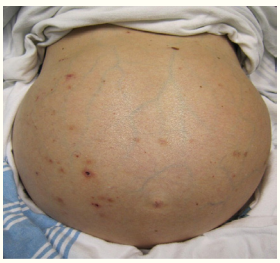
Muscle GSD :

Prominent feature : Exercise intolerance.

Muscle GSD	Enzyme defect
With Hypertrophic cardiomyopathy (HCM) :	
Type II GSD : Pompe's disease	Acid α 1-4 glucosidase/Acid maltase
Without HCM :	
Type V GSD : McArdle's disease (m/c)	Muscle glycogen phosphorylase
Type VII GSD : Tarui's disease	Muscle & erythrocyte PFK

Disease	Features	Investigations
Von Gierke's disease	• Protruding abdomen • Prominent buccal fat • Thin extremities • Convulsions, coma on fasting • O/E : Massive hepatomegaly 	• S. glucose ↓ • Rothera's test : Positive • S. Uric acid ↑↑ • S. Lactate ↑↑ (Lactic acidosis) • AST & ALT : Normal • Liver biopsy : Accumulation of normal glycogen. • Ketosis • Hyperlipidemia
Pompe's disease	• Floppy infant • Generalized hypotonia • Fatal : Death within 2 years d/t cardiac failure.  Chest X-ray : Massive cardiomegaly	-
Cori's disease	• Early morning hypoglycemia • Not fatal  	• S. Glucose ↓ • Rothera's test : Negative • S. Uric acid, S. Lactate } Normal • AST & ALT : ↑ • Liver biopsy : Accumulation of limit dextrin.

----- Active space -----

Disease	Features	Investigations
Anderson's disease	• Fasting hypoglycemia • Portal hypertension • Cirrhosis • Fatal : Death within 5 yrs of age d/t liver failure. 	• S. Glucose ↓ • Rothera's test : Negative • S. Uric acid, S. Lactate } Normal • AST & ALT : ↑↑ • Liver biopsy : Accumulation of amylopectin
McArdle's disease	• Adolescent male • Pain in calf muscle on exercise • No hemolysis • Second wind phenomena : Exercise intolerance Pain in calf during exercise → Rest → Resume activity with more ease • Rhabdomyolysis → Myoglobinuria →	• S. Glucose : ↓ during exercise • S. Lactate : ↓ • AST & ALT : Normal • Creatine Kinase : ↑↑ • Burgundy coloured urine
Tarui's disease	• Exercise intolerance (No 2nd wind phenomena) • Myoglobinuria • Hemolysis	• S. Glucose : ↓ during exercise • S. Lactate : ↓ • Creatine Kinase : ↑
Fanconi Bickel syndrome	GLUT 2 defect	-
Type 0 GSD	Glycogen synthase defect	• No glycogen accumulation • No hepatomegaly

Gluconeogenesis

00:37:28

Synthesis of glucose from non-carbohydrate substrates.

Timeline :

16-48 hours of fasting.

Sites :

1. Liver.
2. Kidney.

Organelle :

Cytoplasm & mitochondria.

Substrate Forming Glucose :

- Glucogenic amino acids : Alanine (Cahill cycle).
- Propionyl CoA (Odd chain fatty acid) → Succinyl CoA.
- Lactate (Cori's cycle).
- Glycerol → DHAP.

Note : Acetyl CoA → Not a substrate for gluconeogenesis.

----- Active space -----

Enzymes :

Irreversible steps of glycolysis	Key enzymes of gluconeogenesis
1. Hexokinase	Glu-6-PO_4 —(Glu-6-phosphatase; H_2O, P_i)→ Glucose
2. PFK	F-1,6-BP —(Fructose-1,6-bisphosphatase; H_2O, P_i)→ F-6-PO_4
3. Pyruvate Kinase (PK)	a. Pyruvate —(Pyruvate carboxylase)→ OAA • Acetyl CoA : Allosteric activator of pyruvate carboxylase • Only mitochondrial step • OAA : Transported to cytoplasm via Malate Aspartate shuttle b. OAA —(PEP carboxykinase; CO_2, GTP, GDP)→ PEP

Conversion of 2 lactate to 1 glucose : 6 ATP required.

- Pyruvate carboxylase : 2 ATP.
- PEP carboxykinase : 2 ATP.
- 1,3-BPG Kinase : 2 ATP.

Galactose & Fructose

00:45:20

Galactose Metabolism :

Types of disorders :

- Classical galactosemia
  - Defect in Galactose-1-PO_4 uridyl transferase
- Non-classical galactosemia

Enzyme defect	Disorders
Galactokinase	Cataract
UDP hexose epimerase	Benign condition with mixed features

Fructose Metabolism :

----- Active space -----

Site : Liver.

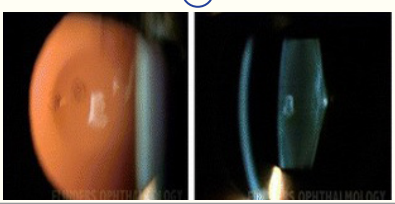
Types of disorders :

Enzyme defect	Disorders
Fructokinase	Essential fructosuria
Aldolase-B	Hereditary fructose intolerance

Applied biochemistry of Fructose

Easily metabolized to Pyruvate → Acetyl CoA → Fatty acid → TAG → Causes dyslipidemia

Galactosemia vs Hereditary Fructose Intolerance (HFI) :

	Classical Galactosemia	HFI
Enzyme defect	Galactose-1-PO_4 uridyl transferase	Aldolase-B
Age of onset	1st week of life	6 months
Trigger	Breastfeeding	Fruit juices
C/f	• Intellectual disability • Convulsions, vomiting, jaundice, failure to thrive • Hepatomegaly, liver failure • Feeding difficulty, poor weight gain	
Cataract		Fructose → Low renal threshold → Fructosuria → No cataract
Accumulated compound	Galactose-1-PO_4 : • Hepatotoxic & neurotoxic • ↓Inorganic PO_4 • ↓Activity of glycogen phosphorylase • Eye : Converted to dulcitol → Cataract	Fructose-1-PO_4 : • Hepatotoxic & neurotoxic • ↓Inorganic PO_4
Lab diagnosis :		
Urine Benedict's test	Positive	Positive
Glucose oxidase test	Negative	Negative
	Enzyme studies & genetic mutation test	
Specific test	Mucic acid test : Positive	Rapid furfural test/ Seliwanoff's test : Positive
Rx	• Stop breastfeeding • Lactose free diet up to 4-5 years of age	Fructose free diet

----- Active space -----

HMP Shunt & Uronic Acid Pathway

00:54:40

HMP SHUNT PATHWAY

AKA Pentose phosphate pathway.

Site :

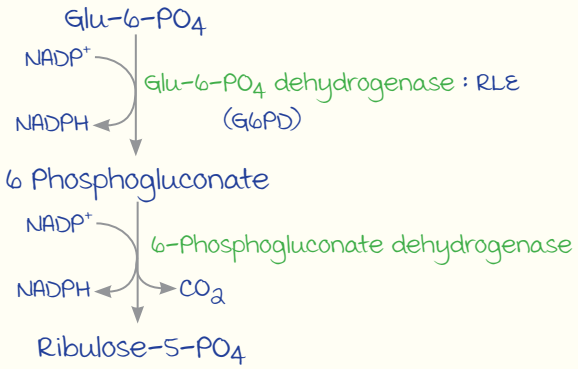
Cytoplasm of liver.

Phases :

Oxidative phase :

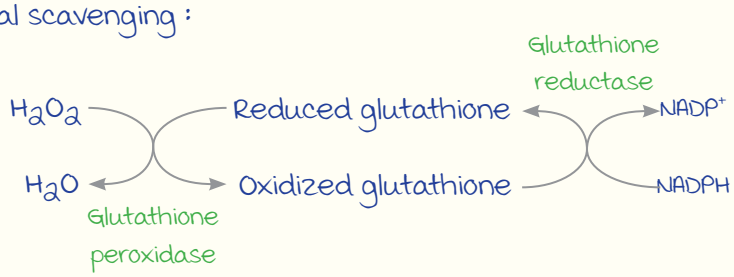
- Irreversible.
- Generate NADPH.

Steps :



Functions of NADPH :

1. Free radical scavenging :



Sites :

- RBC : Prevents RBC lysis.
- Lens : Maintains transparency of lens.

2. Maintains iron in Hb in the reduced state → Prevent formation of methemoglobin.

3. Reductive biosynthesis of fatty acids & steroids :

- Sites :
  - Liver.
  - Adipose tissue.
  - Lactating mammary gland.
  - Gonads.
  - Adrenal cortex.

Non-oxidative phase :

----- Active space -----

- Reversible.
- Generate pentoses required for DNA synthesis.

Enzymes :

- Transketolase : Requires vit. B1
- Transaldolase.

Applied biochemistry
Erythrocyte transketolase : Indicator of Vit B1 status

Site (Rapidly proliferating cells) : Bone marrow, skin, mucosa.

G-6-PD Deficiency :

- m/c enzyme deficiency.
- X-linked recessive.

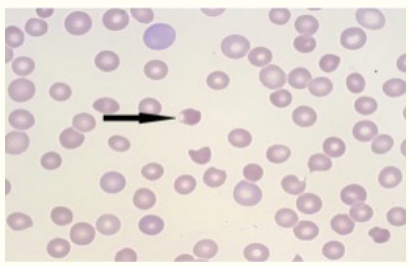
C/f : Defect in G6PD → ↓NADPH.

- Hemolysis → Anemia, jaundice.
- Methemoglobinemia → Cyanosis.

Aggravating factor : Drugs, fava beans, infections.

Protective against : Plasmodium falciparum.

Peripheral smear : Bite cells, Heinz body.



Bite cells

URONIC ACID PATHWAY

Oxidative pathway for glucose.

Site :

Liver.

Organelle :

Cytoplasm.

Functions :

- Produces uronic acid : Glucuronic acid → GAG.
  → Conjugation of bilirubin.
- Minor synthesis of pentoses.
- Synthesis of ascorbic acid : Absent in humans d/t lack of L-gulonolactone oxidase.

Essential Pentosuria :

- Benign condition.
- Benedict test : Positive.
- Bial's test : Positive.
- Defect : Xylitol dehydrogenase/Xylulose reductase.

----- Active space -----

CHEMISTRY OF LIPIDS AND PHOSPHOLIPIDS

Classification of Fatty Acids (FA)

00:00:30

Lipid : Soluble in non-polar solvents only.

	Classification of lipids
Based on no. of carbon atoms	• Short chain (C2 to C6) • Medium chain (C8 to C14) : Coconut oil • Long chain (> C16) • Very long chain (> C20)
Based on double bonds	• Saturated FA (Single bonds only) • Unsaturated FA (Double bonds)
Based on diet	• Essential FA : – Linoleic acid : Safflower oil – α-linolenic acid • Non-essential FA
Unsaturated FA	
Based on no. of double bonds	• Monounsaturated FA : Mustard/Rapeseed oil • Polyunsaturated FA : Safflower oil
Polyunsaturated FA	
Based on position of final double bond	• Omega – 3 FA : – α-linolenic acid : Flax – seed oil – Timnodonic acid – Cervonic acid : Breast milk, Fish & algal oil • Omega – 6 FA : – Gamma linolenic acid – Linoleic acid – Arachidonic acid
Based on isomer	• Cis FA • Trans FA : Vanaspati (Partially hydrogenated vegetable oil) • RDA : 2 to 7 g/d

Applied biochemistry
Essential FA deficiency : • Acanthosis • Follicular hyperkeratosis (aka phrynoderma/Toad skin) • Fatty liver • Mitochondrial membrane damage
Docosahexaenoic acid (DHA)/ Cervonic acid : • Can cross placenta • Deficiency : Retinitis pigmentosa
↑Trans FA : • ↓Fluidity of membrane • Insulin resistance • Dyslipidemia, CV risk • ↑Inflammation
Significance of omega – 3 FA : • Decreases : – Cardiovascular risk (↓TG) – Platelet aggregation – Mental illness, degenerative disease risk – Inflammation • Infant brain development • Benefits in Type 2 DM, ADHD, Non-alcoholic fatty liver disease

■ : Richest source.

Note : Phrynoderma d/d → Vitamin A deficiency.

Phospholipids (PL) & Glycolipids

00:10:00 ----- Active space -----

Glycerophospholipids :

Glycerophospholipid	Constituents	Present in
Phosphatidic acid	Diacyl glycerol (DAG) + PO_4	Cell membrane
Lecithin (most abundant PL in cell membrane)	DAG + PO_4 + choline (Phosphatidyl choline)	Lung surfactant, Cell membrane
Cardiolipin (Diphosphatidyl glycerol)	2 x Phosphatidic acid (PA) + Glycerol	Inner mitochondrial membrane
Phosphatidyl serine	PA + Serine	Apoptosis
Phosphatidyl inositol (2^{nd} messenger in hormonal pathways)	PA + Inositol	Cell membrane

Cardiolipin
• Alw Barth syndrome (Cardioskeletal myopathy) : – Cardiomegaly + myopathy – Mitochondrial disease • Only antigenic PL • Cross reacts with Treponema pallidum : False positive syphilis test

Sphingophospholipids :

Present in :

- Myelin sheath.
- White matter of brain.
- Lung surfactant.

Lecithin : Sphingomyelin ratio
↑Ratio → Lung maturation

Glycolipids :

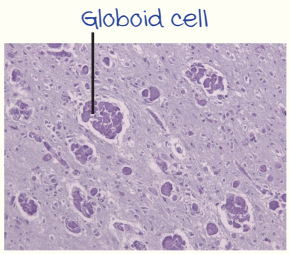
Glycosphingolipids	Constituents	Uses
Cerebroside	Ceramide + monosaccharide	–
	→ Galactocerebroside	Neural tissues
	→ Glucocerebroside	Extra-neural tissues
Globoside	Ceramide + oligosaccharide	–
Ganglioside	Ceramide + oligosaccharide + N-acetyl neuraminic acid (NANA)	–

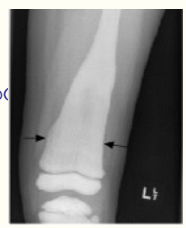
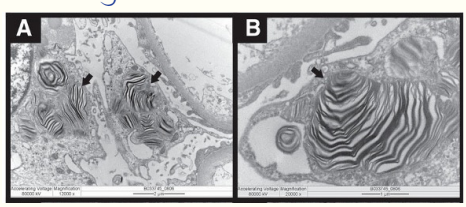
----- Active space -----

SPHINGOLIPIDOSES

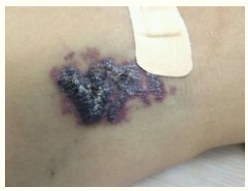
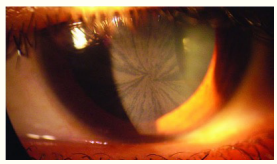
Sphingolipids —(Degraded in)✕→ Lysosomes.

Sphingolipidoses : Type of lysosomal storage disease.

Disorder	Enzyme defect	Features
GM1 gangliosidosis (Autosomal recessive) (AR)	β-galactosidase	• Frontal bossing, depressed nasal bridge, long philtrum, low set ears • Cherry red spot (CRS) on macula • Angiokeratoma
GM2 gangliosidosis (AR)		• Developmental delay • Muscular weakness • Visual disturbances • No hepatosplenomegaly • Hyperacusis • CRS on macula
Tay-Sach's disease	β-hexosaminidase A	
Sandhoff's disease	β-hexosaminidase A & B	
Krabbe's disease (AR)	Galactocerebrosidase/ β-galactosidase (Galactocerebroside accumulation in neural tissue)	• Gross developmental delay • Opisthotonus posture with clenched fists • No hepatosplenomegaly  White matter of brain

Lysosomal storage disease	Enzyme defect	Features
Gaucher's disease (AR)	β-glucocerebrosidase/ β-glucosidase (Glucocerebroside accumulation in bone marrow, liver)	• Protruding abdomen • Crumpled tissue paper appearance • Massive hepatosplenomegaly • Thrombocytopenia • Pain & pathological fractures of long bo • No mental retardation (MR)/CR spot • Erlenmeyer flask deformity 
Neimann-Pick disease (AR)	Sphingomyelinase	• Developmental delay • Zebra body inclusions • CR spot 
Farber's disease	Ceramidase	Resembles rheumatoid arthritis

----- Active space -----

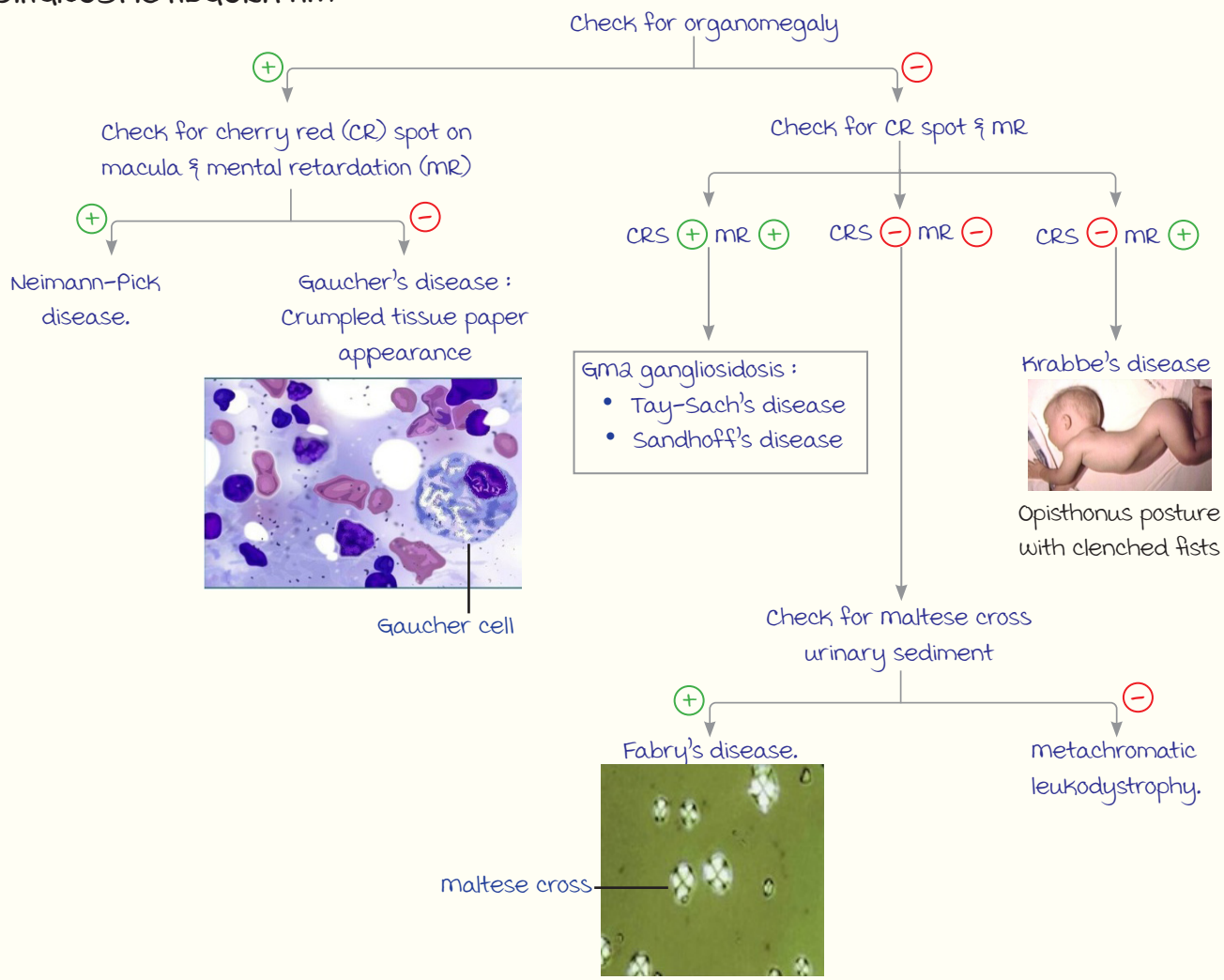
Lysosomal storage disease	Enzyme defect	Features
Fabry's disease (X-linked recessive)	α-galactosidase	• Angiokeratoma  • No CR spot or MR • Lenticular opacities  • Maltese cross : Urinary sediments
Metachromatic leukodystrophy	Aryl sulfatase	No Visceromegaly

Note :

Wolman's disease (Cholesteryl ester storage disease) : Acid lipase defect.
(Lysosomal storage disease)

- Calcification of adrenal gland.
- Watery green diarrhoea.

DIAGNOSTIC ALGORITHM



----- Active space -----

METABOLISM OF LIPIDS

Metabolism in Fasting State

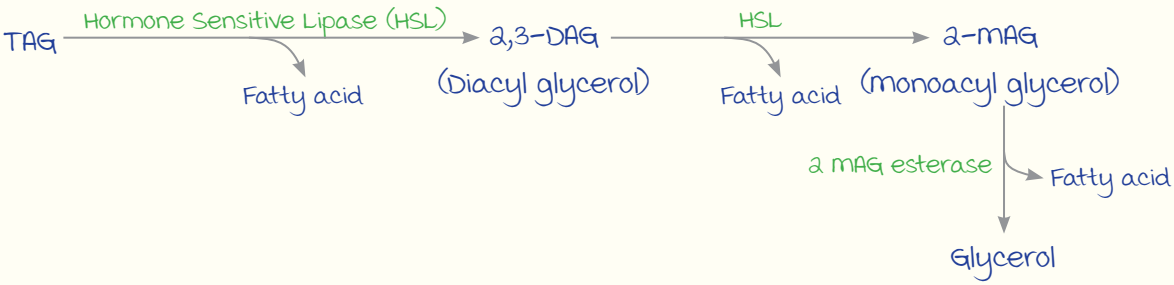
00:00:22

Lipolysis :

Hydrolytic cleavage of TAG.

Site : Adipose tissue.

Steps :



Activators :

- Glucagon
- Epinephrine
- ACTH
- TSH
- Thyroxine
- MSH (melanocyte Stimulating Hormone)

Applied biochemistry :

1. Insulin resistance → ↑Hydrolysis of stored TAG → ↑Free fatty acid level in blood → Fatty liver disease.
2. Niacin : Locks TAG in the adipose tissue → Hence, used to treat hypertriglyceridemia.

Inhibitors :

- Insulin
- PGE_1
- Niacin

metabolic fuel during starvation:

1. Early fasting : Hepatic glycogenolysis
2. 16-48 hrs of starvation : Gluconeogenesis (ATP is given by TAG → FA → (ATP) Acetyl CoA)
3. Prolonged fasting (>2 days of starvation) : Ketone bodies (TAG → FA → Acetyl CoA → KB) → Starvation Ketosis

Beta Oxidation

00:06:49 ----- Active space -----

Sequential removal of 2 carbon acetyl CoA + oxidation of β carbon atoms.

Site :

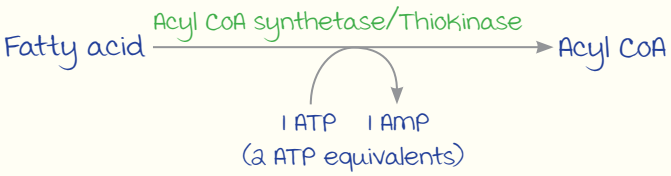
- Liver
- Skeletal muscle
- Adipose tissue

Organelle :

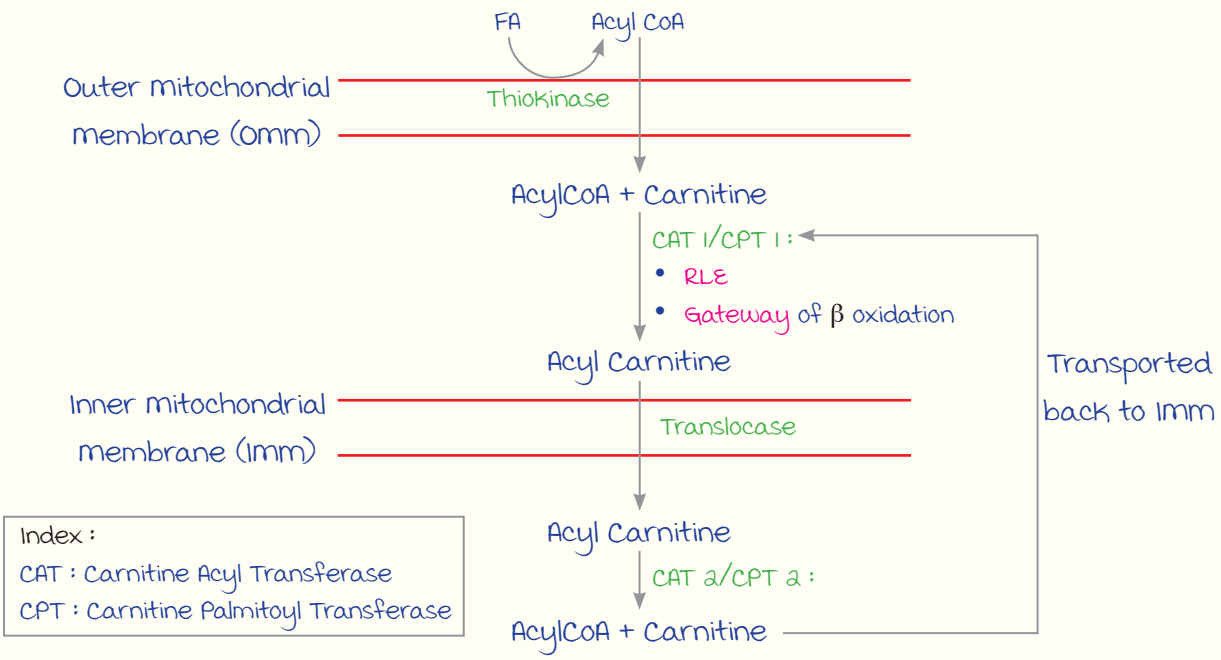
Mitochondria.

Steps :

1. Activation of fatty acid : Occurs in the cytoplasm.



2. Carnitine transport : Transports Acyl CoA into mitochondria.

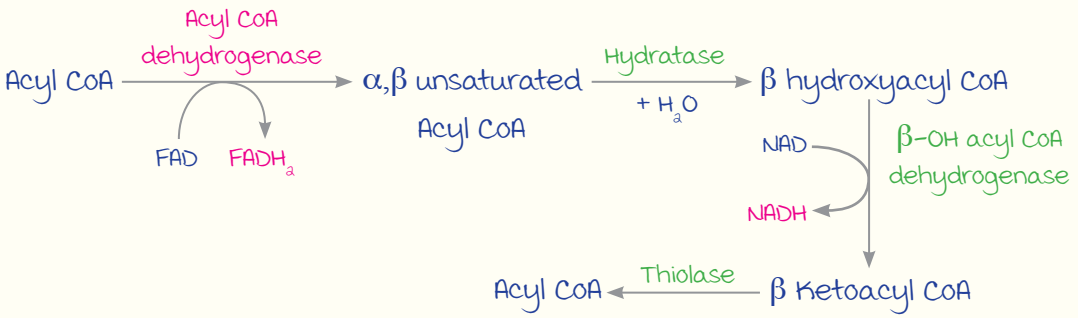


Note :

- FA with <14 carbon atoms ⟶ Do not require carnitine. (Medium chain & short chain FA)
- Carnitine deficiency ⟶ Muscle weakness.

----- Active space -----

3. Fatty acid oxidation :



Energetics :
1 FADH$_a$: 1.5 ATP 1 NADH : 2.5 ATP
Total : 4 ATP

Energetics of palmitic acid (C16) : m/c FA to undergo β oxidation.

C16 → No. of acetyl CoA = $\frac{16}{2}$ = 8 acetyl CoA → 8 × 10 = 80 ATP. C16 → β oxidation = $\frac{C16}{2}$ − 1 = 7 β oxidation → 7 × 4 = 28 ATP. C16 → Activation : Utilizes 2 ATP equivalents → − 2 ATP.
Total : 108 − 2 = 106 ATP

Regulation :

CPT-1 gateway : Inhibited by Malonyl CoA.

- Fasting → Low I/G ratio → (−) Acetyl CoA carboxylase (RLE for FA synthesis) → ↓Malonyl CoA → (+) CPT − 1
- Fed state → High I/G ratio → (+) AcetylCoA carboxylase (RLE for FA synthesis) → ↑Malonyl CoA → (−) CPT − 1

Other Oxidation Pathways & Disorders of Fatty Acid Oxidation 00:19:24

OTHER OXIDATION PATHWAYS

Oxidation pathway	Site	Important feature
β oxidation of unsaturated fatty acid	Mitochondria	Acyl CoA dehydrogenase step is bypassed ↓ 1.5 ATP less for every double bond
β oxidation of odd chain fatty acid	Mitochondria	Biproducts : Propionyl CoA (Gluconeogenic) + Acetyl CoA
Alpha oxidation : Branched chain FA (Phytanic acid)	• Peroxisome : Major • SER : Minor	• No acetyl CoA • No ATP produced
Omega oxidation	SER (Microsome)	• Product : Dicarboxylic acid • No acetyl CoA • No ATP produced

----- Active space -----

DISORDERS OF FATTY ACID OXIDATION

1. MCAD (Medium Chain Acyl CoA Dehydrogenase) Deficiency :

Causes SIDS (Sudden Infant Death Syndrome)/Cradle death.

Medium chain FA
↓ MCAD absent
↓ β oxidation
- ↓ Acetyl CoA → No Ketone bodies synthesis : Rothera's test (−)
- ↓ ATP → Fasting hypoglycemia
- Enters omega oxidation → Dicarboxylic acid

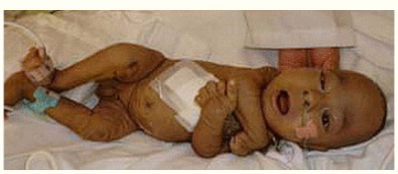
2. Jamaican Vomiting Sickness :

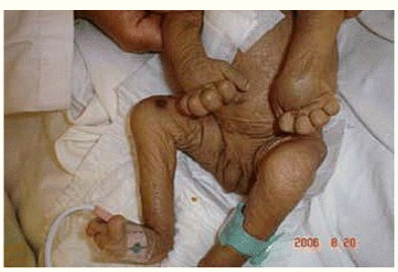
D/t consumption of unripe Ackee fruit → Containe hypoglycin.

Acyl Co A
↓ Acyl CoA dehydrogenase ← (−) Hypoglycin
↓ β oxidation
- ↓ Acetyl CoA → ↓ Gluconeogenesis → Fasting hypoglycemia
- ↓ ATP → Ketone bodies absent : Rothera's test (−)

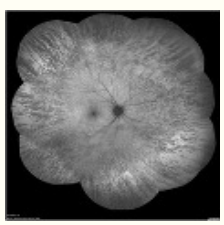
3. Refsum's Disease :

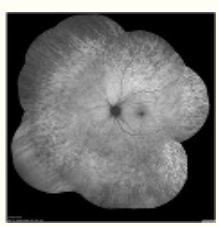
Defect : Phytanoyl CoA oxidase (Hydroxylase) → ✕ Alpha oxidation





Ichthyosis (Scaly skin)





Retinitis pigmentosa

Features :

- Asymptomatic > Symptomatic. (Aggravates on consuming curd/milk)
- Retinitis pigmentosa.
- Ichthyosis
- Peripheral neuropathy.
- Cardiac arrhythmias.

Rx : Restrict dairy products & green leafy vegetables.

----- Active space -----

3. Zellweger Syndrome/Cerebrohepatorenal disease :

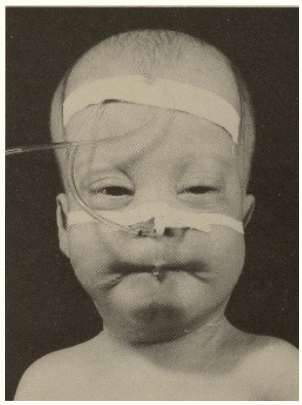
Defect : Peroxisome Targeting Sequence (PTS)

↓

Defective phytanic acid & very long chain FA oxidation.

C/f : Resembles Down's syndrome

- Mongoloid facies.
- Hypertelorism
- Unslanting palpebral fissure.
- Frontal bossing.
- High forehead.
- Brushfield spots.
- Intellectual disability.



Zellweger Syndrome : Resembles Down's syndrome

Diagnosis :

- Peroxisomal (Vacant) ghost.
- Accumulation of VLCFA & phytanic acid.

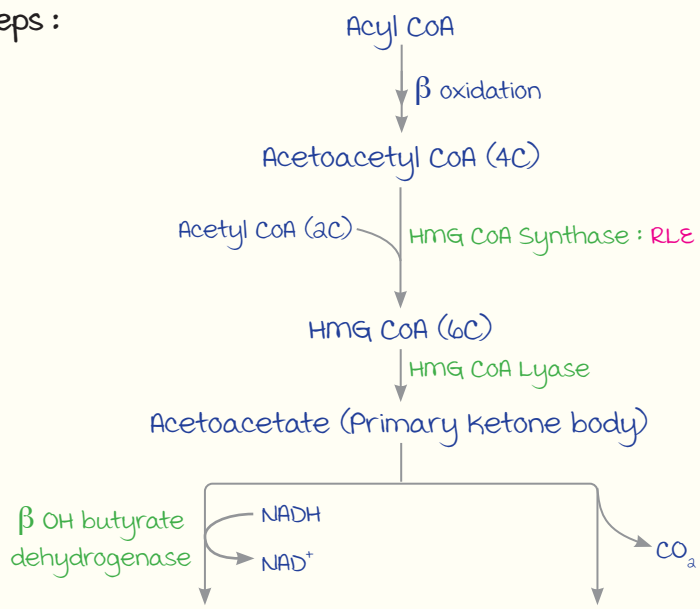
Ketone Body Synthesis

00:33:38

Site :

Exclusively liver mitochondria.

Steps :



----- Active space -----

Ketone Body Utilization :

- Acetoacetate $\xrightarrow[\text{CoA transferase}]{\text{Thiophorase/}}$ Acetoacetyl CoA → Acetyl CoA → TCA.

- Never utilized by :
  a. Liver : D/t lack of thiophorase.
  b. RBC : D/t absence of mitochondria.

Diabetic Ketoacidosis :

Diabetes : ↓Insulin/Insulin resistance → Low I/G ratio (Simulates fasting state).

- ↓Glucose uptake by heart, skeletal muscle, adipocytes (GLUT 4 : Insulin dependent) → ↑↑Blood glucose
- ⊕ HSL → ↑Hydrolysis of TAG → Acetyl CoA → ⊕ KB synthesis
- Liver:
  - ↑Glycogenolysis
  - ↑Gluconeogenesis → Depletion of OAA → ↑↑Blood glucose

Lab Diagnosis :

1. Rothera's test : Purple ring indicative of acetoacetate.
2. Ketostix : Detects urine Ketone bodies.

Rothera's test : Purple ring

Metabolism in Fed State

00:43:20

Fatty Acid Synthesis :

Mnemonic : Car burns fuel, sit & synthesize
- Carnitine : β oxidation.
- Citrate : FA synthesis.

Site : Cytosol (Extramitochondrial).

Substrate : Acetyl CoA.

Transporter of acetyl CoA : Citrate (Tricarboxylic acid transporter).

Release of acetyl CoA : ATP citrate lyase.

Steps :

1. Acetyl CoA $\xrightarrow[\text{(Active in dephosphorylation)}]{\text{Acetyl CoA carboxylase : RLE}}$ Malonyl CoA
   - ATP
   - Biotin
   - CO_2

----- Active space -----

2. Fatty acid synthase complex :
   - Homodimer.
   - Each monomer : 3 subunits
     i. Condensing unit.
     ii. Reduction unit : Requires NADPH.
     iii. Releasing unit : Thioesterase.
   - Acyl Carrier Protein (ACP) : Contains pantothenic acid.

Cholesterol Synthesis :

Exclusive animal steroid, not a metabolic fuel.

Site : Liver, adipose tissue, gonads, adrenal cortex.

Organelle : Cytoplasm + SER

Steps :

2 x Acetyl CoA
↓
Acetoacetyl CoA + Acetyl CoA
↓ HMG CoA synthase
HMG CoA
↓ HMG CoA reductase : RLE
Mevalonate
↓
2 x Isopentenylor (5C) → Geranyl (10C) → 2 x Farnesyl (15C) → Squalene (30C) → Cholesterol (27C)

Applied biochemistry :

Statins
↓ (−)
HMG CoA reductase
↓
↓Coenzyme Q
(Derived from Farnesyl)
↓
myopathy

Compounds Derived from Cholesterol :

1. Bile acids (Excretory product).
2. Steroid hormones.
3. Vitamin D.

Bile Acid Synthesis :

Site :

- Liver (Primary bile acid).
- Intestine (Secondary bile acid).

Steps :

Cholesterol
↓ Vit C | 7 α hydroxylase : RLE
7 OH cholesterol
↓ Conjugation by Taurine + Glycine

1° bile acids → Cholic acid → 2° bile acids → Deoxycholic acid
Chenodeoxycholic acid → Lithocholic acid → Undergoes least Enterohepatic circulation

Deoxycholic acid + Lithocholic acid → Enterohepatic circulation

Lipoproteins

00:52:45 ----- Active space -----

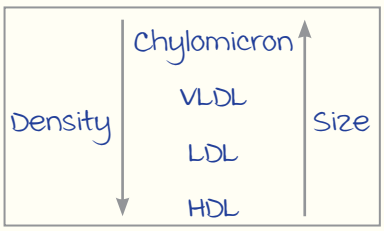
Characteristics :

- Maximum cholesterol : LDL
- Maximum TAG.
- Minimum density.
- Remains at the point of application.
- Maximum size.

Chylomicron

- Maximum density.
- Minimum size.
- Fastest electrophoretic mobility.

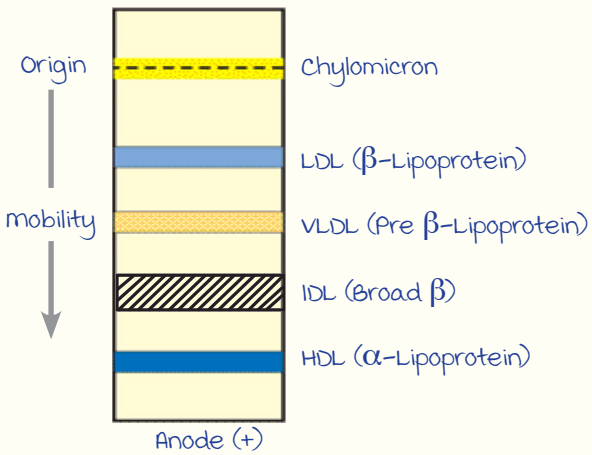
HDL



Functions :

- Carry exogenous TAG to peripheral organs : Chylomicron.
- Carry endogenous TAG to peripheral organs : VLDL.
- Carry cholesterol from peripheral tissue to adrenals : HDL.

Electrophoretic Pattern :



METABOLISM OF LIPOPROTEINS

1. Chylomicron :

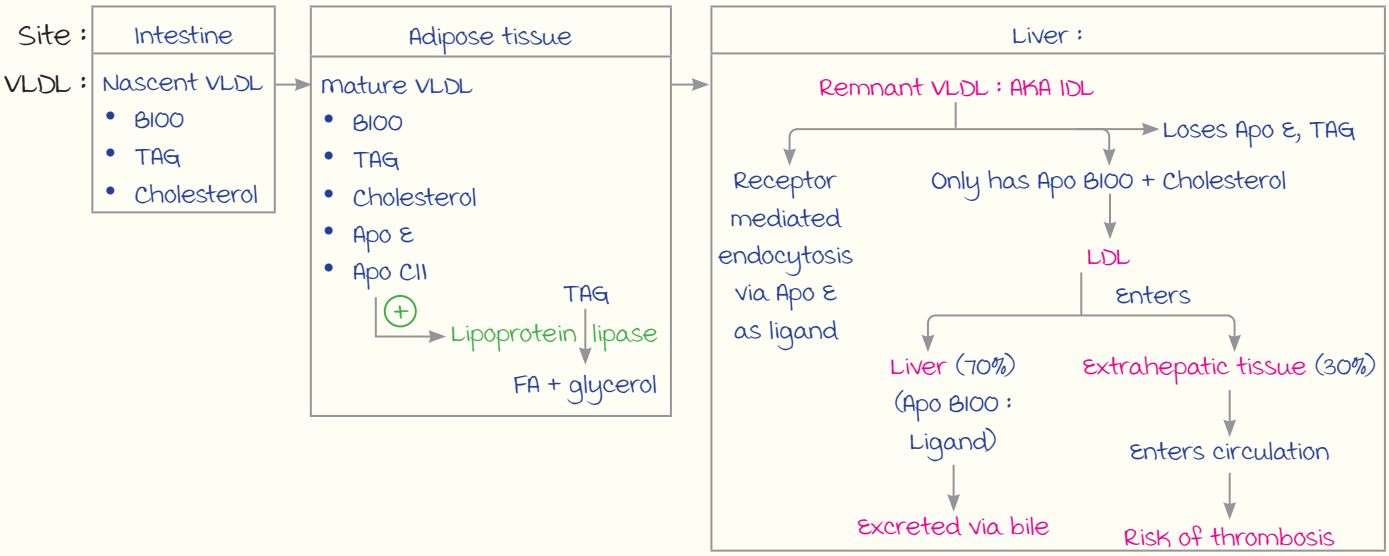
Site :

Chylomicron :

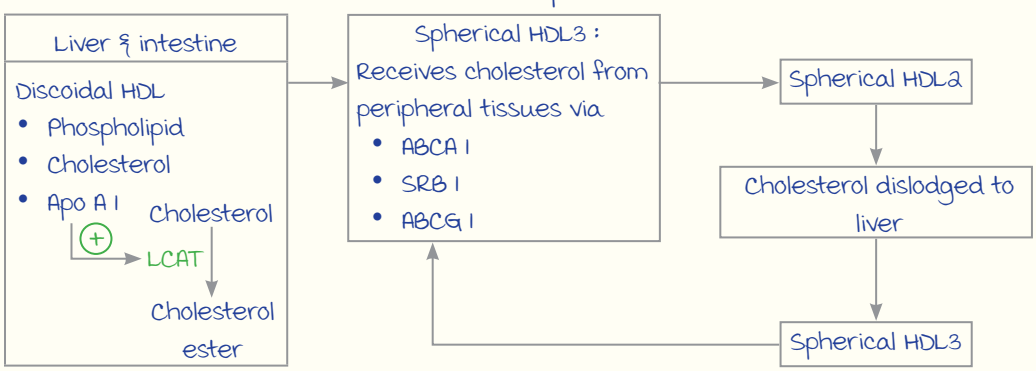
Intestine	Adipose tissue	Liver : Receptor mediated endocytosis
Nascent chylomicron • TAG • B48 • Apo A	Mature chylomicron • TAG • B48 • Apo A • Apo E • Apo CII (+) → Lipoprotein lipase (TAG → FA + glycerol)	Remnant chylomicron • Apo E : Ligand • B48 • Apo C2 • Apo A • ↓↓ TAG

----- Active space -----

a. VLDL/LDL :

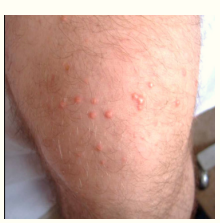
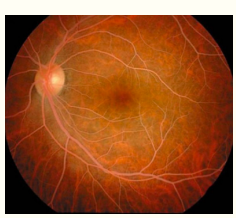


3. HDL : Facilitates reverse cholesterol transport.

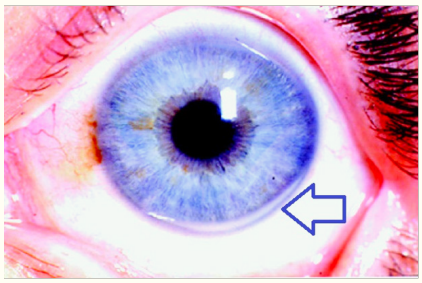
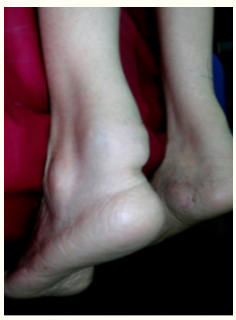
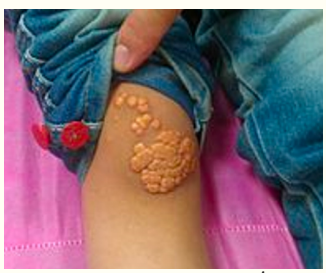
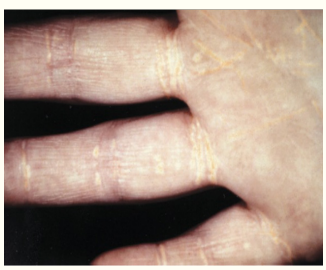


Disorders of Lipoprotein

01:03:43

	Mode of inheritance	Defect	Lipoprotein accumulated	Lipid levels
Familial chylomicronemia (Type I HL) Latest Rx modalities : • Lipogene Tiparvovec. • GOF LpL variant.	AR	LPL or Apo CII	Chylomicron > VLDL	• TAG ↑↑↑ • Cholesterol : Normal
	C/F			
	Pancreatitis (Abdominal pain) can also be seen.  Eruptive xanthoma  Milky white plasma  Lipemia retinalis			

----- Active space -----

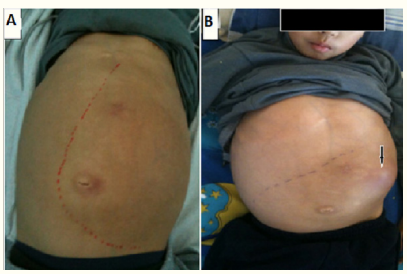
<table>
<tr><th></th><th>Mode of inheritance</th><th>Defect</th><th>Lipoprotein accumulated</th><th>Lipid levels</th></tr>
<tr><td rowspan="3">Familial hypercholesterolemia (Type II HL)
Latest Rx modalities :
• Lomitapide (MTTP).
• Mipomersen
• VERVE 101 : Genome editing on CRISPR cas9.</td><td>AD</td><td>LDL receptor or Apo B100 mutation</td><td>LDL</td><td>• Cholesterol : ↑↑↑ (Risk of CAD)
• TAG : Normal</td></tr>
<tr><td colspan="4">C/F</td></tr>
<tr><td colspan="4">Family h/o CAD (Coronary Artery Disease).
 
Corneal arcus
 
Tendon xanthoma :
m/c achilles tendon</td></tr>
<tr><td rowspan="3">Familial dysbetalipoproteinemia (Type III HL)</td><td>AR</td><td>Apo E</td><td>Remnant chylomicron & VLDL</td><td>• TAG : ↑↑
• Cholesterol : ↑↑</td></tr>
<tr><td colspan="4">C/F</td></tr>
<tr><td colspan="4"> 
Bunch of grapes/
Tubero-eruptive xanthoma
 
Palmar xanthoma</td></tr>
</table>

Tangier's Disease :

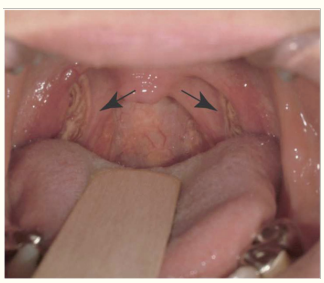
Defect : ABC A1

Findings : Cholesterol ↑↑

Features :



Hepatosplenomegaly



Orange tonsils

----- Active space -----

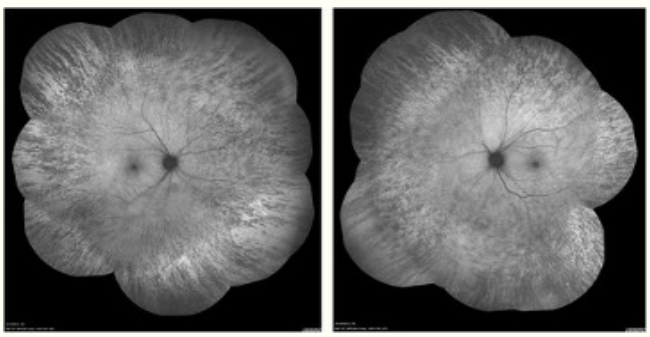
Abetalipoproteinemia :

Defect : MTTP/MTP (microsomal Triglyceride Transfer Protein).

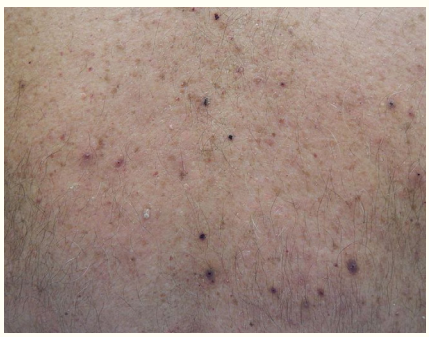
Findings :

- ↓Chylomicron → Bleeding manifestations.
  (Transports fat soluble vitamins such as Vitamin K)
- ↓VLDL
- ↓IDL
- ↓LDL

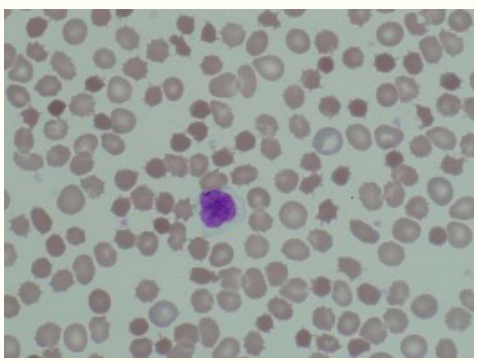
Features :



Pigmentary retinopathy



Petechial rash



Acanthocytes

Sitosterolemia (Type II HL) :

Defect :

- ABC G5
- ABC G8

Finding : ↑Cholesterol.

Fish Eye Disease :

Defect : Partial LCAT deficiency.

AMINO ACIDS : PART 1

----- Active space -----

Classification & Properties of Amino Acids

00:00:20

CLASSIFICATION

Based on Side Chain :

Group	Amino acids
Aliphatic	Simple → Glycine, Alanine
	Branched chain → Leucine, Isoleucine, Valine
OH group containing	• Serine • Threonine • Tyrosine
Sulphur containing	• Cysteine • Methionine
Acidic	• Aspartic acid (Aspartate) • Glutamic acid (Glutamate)
Amides	• Asparagine • Glutamine
Basic	• Histidine • Arginine • Lysine
Aromatic	• Phenylalanine : Benzene ring • Tyrosine : Phenol ring • Tryptophan : Indole ring
Imino acid	Proline : Pyrrolidine ring

Based on Side Chain Characteristics :

- Polar
  - Charged : ABC
    - Acidic : Aspartate, Glutamate.
    - Basic : Histidine, Arginine, Lysine.
  - Uncharged :
    - Amides : Asparagine, Glutamine.
    - OH group : Serine, Threonine.
    - Simple : Glycine.
    - Sulphur containing : Cysteine.
- Non – polar :
  - Branched chain.
  - Aromatic.
  - Simple : Alanine.
  - Methionine.
  - Proline.

----- Active space -----

Based on Metabolic Fate :

- **Ketogenic :**
  5. Leucine.
  6. Lysine.
- **Ketogenic + glucogenic :**
  1. Phenylalanine.
  2. Isoleucine.
  3. Tyrosine.
  4. Tryptophan.
- **Glucogenic :**
  Remaining amino acids.

Based on Nutritional Requirement :

- **Semi-essential :**
  Arginine.
- **Essential :**
  - Methionine.
  - Leucine.
  - Tryptophan.
  - Phenylalanine.
  - Threonine.
  - Lysine.
  - Valine.
  - Histidine.
  - Isoleucine.
- **Non-essential :**
  Remaining amino acids.

DERIVED AMINO ACIDS

Properties :

- No codons.
- Formation :
  - Post translational modification.
  - Intermediate of metabolic pathways.

Classification :

- **Seen in proteins :**
  - Hydroxylysine, Hydroxyproline } Collagen.
  - Gamma carboxy glutamate : Factors 2, 7, 9, 10.
  - Desmosine : Lysine.
  - Methyl lysine : Myosin.
- **Not seen in proteins :**
  - Ornithine, argininosuccinate, citrulline.
  - Homoserine & homocysteine.

STANDARD AMINO ACIDS

	Selenocysteine	Pyrolysine
Reading process	⊕	⊕
Coded by Stop codon	UGA	UAG
Precursor amino acid	Serine	Lysine
Protein formation	21st protein forming amino acid	22nd protein forming amino acid
Cotranslational process	⊕	⊕

Mnemonic : Serina's sister Selena from UGAnda.

----- Active space -----

Enzymes Containing Selenocysteine/Selenium :

- Glutathione peroxidase.
- Thioredoxin reductase.
- Deiodinase.
- Selenoprotein P.

PROPERTIES OF AMINO ACIDS

1. Isomerism :

- D & L isomerism.
- Exception : Glycine (Optically inactive).

2. Absorption of Light :

- Colourless : Do not absorb visible light.
- UV light absorption : Phenylalanine, tyrosine, tryptophan
  Tryptophan : 280 nm (Maximum UV absorption).

3. Buffering :

Maximum with imidazole group of histidine (pH = pKa).

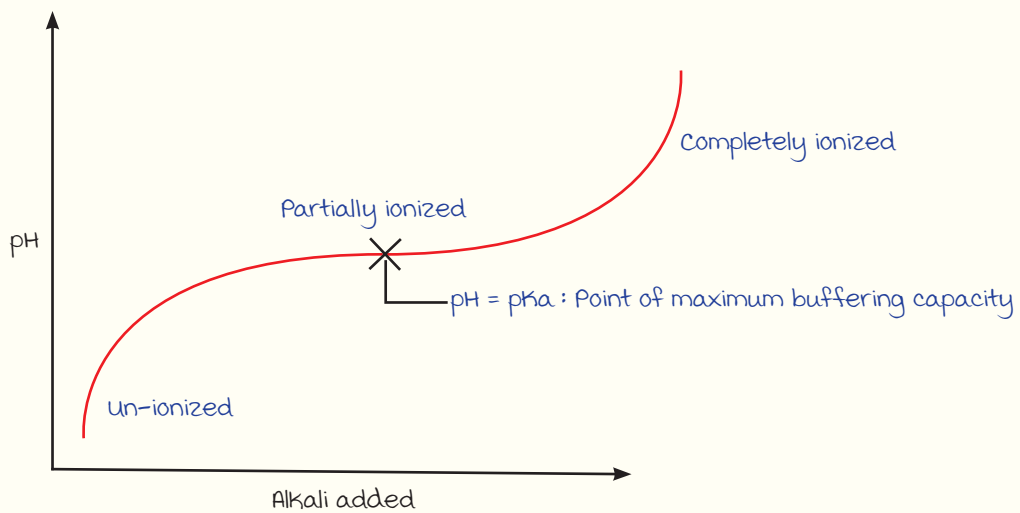
4. Exist in Charged States :

Ampholytes/zwitter ions : Compounds with Isoelectric pH (Net charges = 0).

- Maximum precipitability, minimum solubility.
- Minimum buffering (pH = pI).

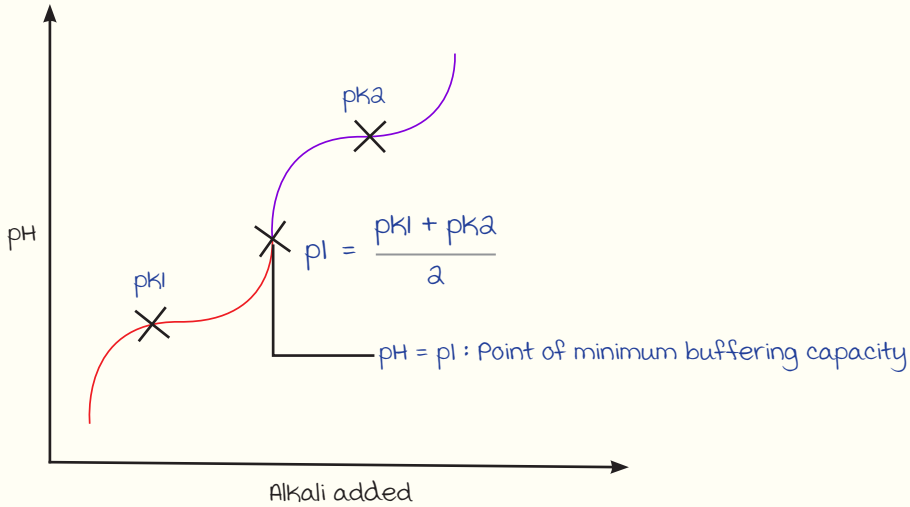
TITRATION CURVE

Compound with Single Ionizable Group :



----- Active space -----

Compound with Multiple Ionizable Groups :



Proteins

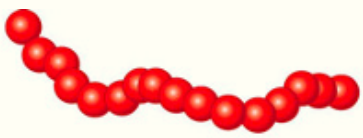
00:25:00

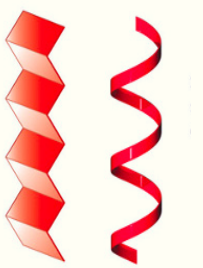
Peptide bond :

- B/w 2 amino acids → Forms proteins.
- Uncharged.
- Partial double bond.
- Trans in nature.

STRUCTURE OF PROTEINS

Primary : Peptide (Covalent) bond.


Primary structure


Secondary structure

Secondary : Coiling of linear structure.

Alpha helix :

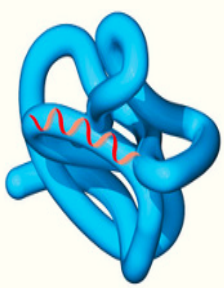
- m/c 2° structure.
- Most stable : Intrachain hydrogen bonds.
- Disrupted by proline.
- Glycine : Induces bend in α-helix.

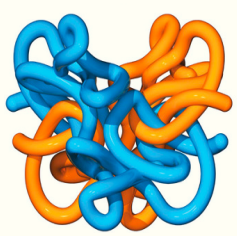
Beta sheets :

- 2nd m/c.
- Zig zag/extended.
- Interchain hydrogen bonds.

Tertiary :

- 3D structure that can perform function.
- Non-covalent forces ⊕.
- Eg : Domain.


Tertiary structure


Quaternary structure

Quaternary :

- > 1 polypeptide interact via subunit.
- Non-covalent forces/subunit interaction.

----- Active space -----

Protein Folding :

Molecular chaperones : Auxiliary proteins.

- BiP (Immunoglobulin heavy chain binding protein).
- GRP-94 (Glucose regulated protein).
- Calreticulin, Calnexin } Bind Ca^{2+}.
- Heat shock proteins.

Enzymes :

- Protein disulfide isomerase.
- Peptide prolyl isomerase.

Protein misfolding diseases :

- Prion diseases.
- Amyloidosis.
- Prion related protein diseases :

7. Alzheimer's disease.
8. Parkinson's disease.
9. β Thalassemia.
10. Cystic fibrosis.
11. Huntington's disease.
12. Pick's Disease (FTD).
13. Amyotrophic Lateral Sclerosis (ALS).
14. Lewy body dementia.

Pathology :

PrP : α helix —mutation→ PrP Sc : β sheet.

- Hydrophobic amino acids exposed.
- Resistant to degradation.

Protein Degradation :

Proteasomal degradation : Ubiquitin mediated (Kiss of death), ATP dependent.

- Proline
- Glutamate
- Serine
- Threonine

} PeST sequence is required for binding with ubiquitin.

Lysosomal degradation : ATP independent.

Collagen 00:37:20

Features :

- Most abundant protein.
- Fibrous protein in ECM.
- Glycine : Most abundant amino acid.

----- Active space -----

Structure :

1. Poly proline α chain :
   - Glycine X-Y repeat.
   - Left handed turn.
   - Composed of 1000 amino acids.

2. Triple helix 3α chain :
   - Vit C → Hydroxylation of proline & lysine.
   - 3 together in Ⓡ direction.

3. Quarter staggered arrangement :
   - Lateral arrangement of triple helix.
   - Cu & lysyl oxidase → Covalent cross-links ⊕.

Synthesis :

	Intracellular	Extracellular
Site	RER of fibroblast	ECM
Product	Procollagen	Tropocollagen
Events	1. Hydroxylation : Prolyl & Lysyl hydroxylase 2. Glycosylation : Hydroxy lysine residue 3. Triple helix	1. Cleavage 2. Quarter staggered 3. Covalent cross-links : Lysyl oxidase

Types :

Type	Diseases associated
Type 1 : • Most abundant type • Present in bone • Ubiquitous in hard & soft tissue	• Osteogenesis imperfecta • Osteoporosis • Ehler-Danlos type VII
Type 2 : Present in cartilage	• Severe chondrodysplasias • Osteoarthritis
Type 3	Ehler-Danlos type IV
Type 4 : Present in GBM	Alport syndrome
Type 6 : Ubiquitous in microfibrils	Bethlem myopathy
Type 7 : Seen in anchoring fibrils	Epidermolysis bullosa (Dystrophic)
Type 10	Schmid metaphyseal dysplasia

Elastin v/s Collagen :

----- Active space -----

	Collagen	Elastin
Types	Many types	Only 1 type
Triple helix	⊕	⊖
Gly – X – Y	⊕	⊖
Hydroxylysine	⊕	⊖
Glycosylation	⊕	⊖
Cross-links	Covalent cross-links	Desmosine cross-link

Disorders a/w Elastin :

1. William Beuren syndrome.
2. Cutis laxa.

Keratin :

- Component of outer layer of skin, nails & hair.
- Rich in cysteine : Confers hardness to nails.
- Associated disorder : Epidermolysis bullosa (Classical type).

Fibrillin :

- Glycoprotein in scaffolding of elastin.
- Fibrillin 1 mutation : Marfan's syndrome.

Amino Acid Metabolism

00:47:40

Reactions :

1. Deamination —(NH_3)→ Ketoacid.
2. Decarboxylation —(CO_2)→ Amines.

Toxic nature of ammonia :

↑GABA (Inhibitory neurotransmitter)

↑ (CO_2)

Glutamate + NH_3 → Glutamine : Causes cerebral edema.

↑

α KG from TCA cycle

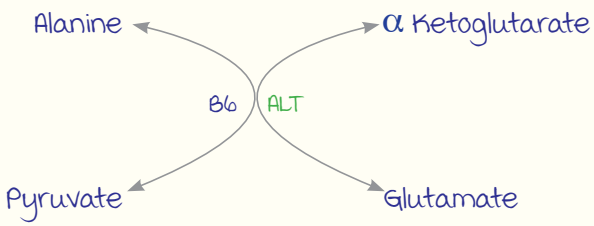
↑NH_3 → ↑Formation of Glutamine → ↓α KG → ↓ATP.

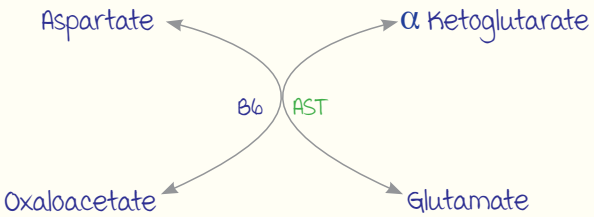
----- Active space -----

1. Transamination :

Site : Cytoplasm of all organs.

Examples :





Properties :

- Toxic amino group → Non-toxic glutamate.
- Reversible reaction.
- Significance : Biosynthesis of non-essential amino acids.
- Ping pong mechanism/Bibi reaction : 2 substrate 2 product reaction.

Transamination of non-alpha amino acid :

Enzyme : δ ornithine aminotransferase.

Applied biochemistry
Gyrate atrophy of retina & choroid :
• Defect in δ ornithine aminotransferase
• Treatment :
– Restrict ornithine & arginine
– Supplement PLP (B6)

Exceptions to transamination :

1. Proline.
2. Hydroxyproline.
3. Lysine.
4. Threonine.

2. Transport of NH_3

Sources of ammonia :

- Amino acids → Glutamate.
- Amino sugars
- Pyrimidine
- Purine
- Porphyrins

NH_3 → Glutamine synthetase (In mitochondria) → Glutamine : Transport form of NH_3

1st line defense of hyperammonemia.

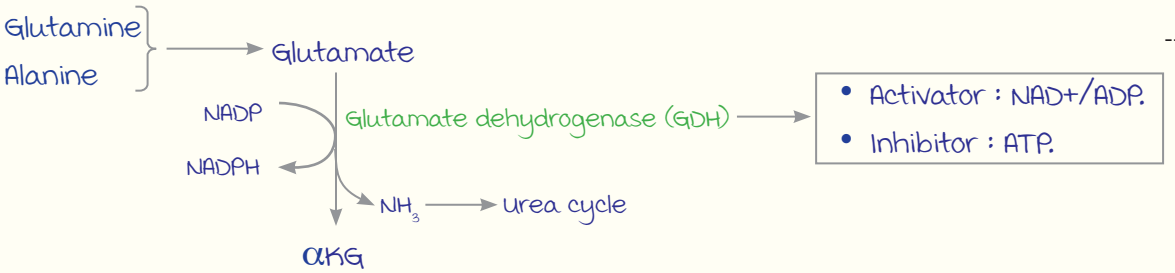
Transport form from skeletal muscles : Alanine.

3. Oxidative Deamination :

Site : Liver & Kidney.

Organelle : Mitochondria.

----- Active space -----



Urea Cycle

01:02:35

AKA Kreb's Henseleit/Ornithine cycle.

Site : Exclusively in liver.

Organelle : Cytoplasm + mitochondria.

Note : Reactions occurring in both cytoplasm & mitochondria

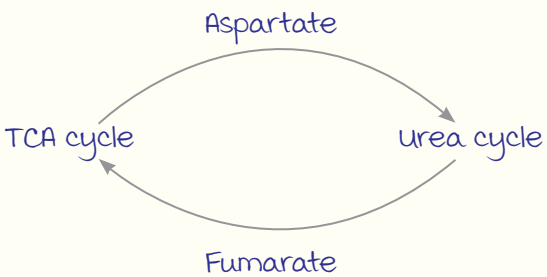
Mnemonic : PUBG

- Pyrimidine synthesis
- Urea cycle
- Blood : Heme synthesis
- Gluconeogenesis

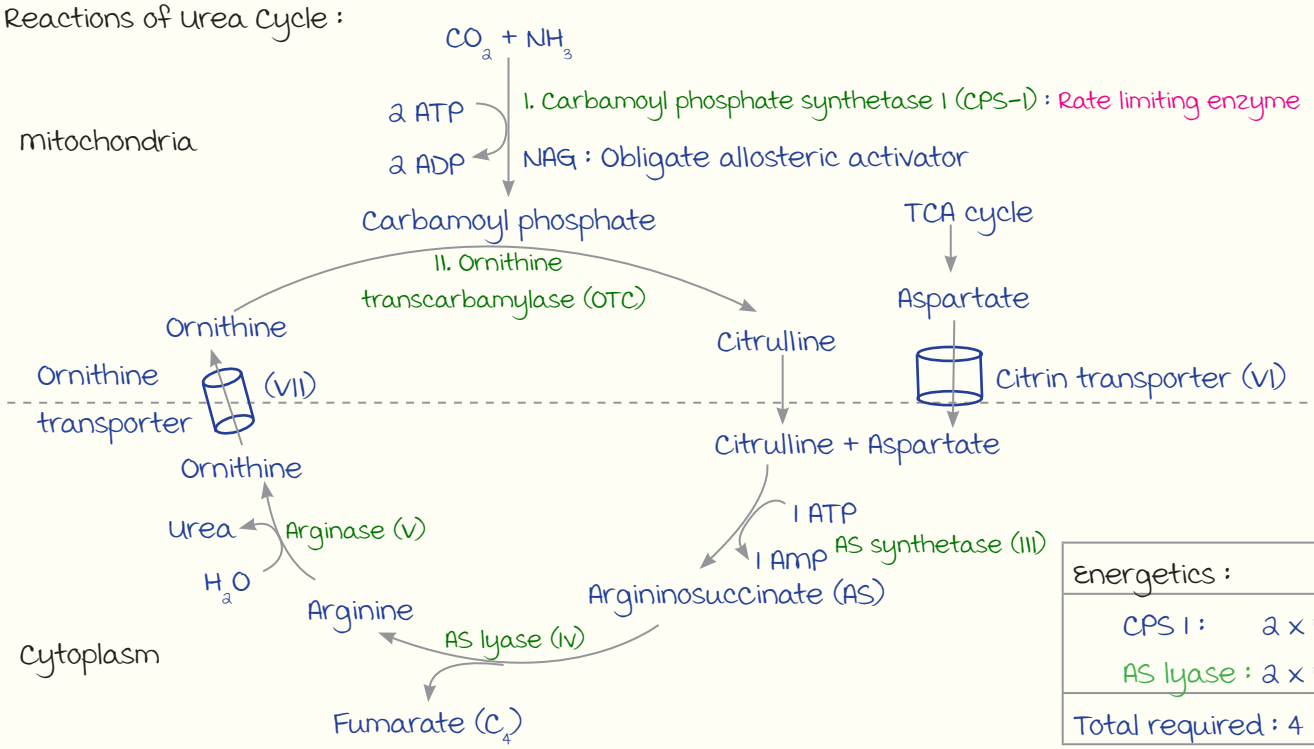
Contributions to Structure :

- 1st Nitrogen : Ammonia.
- 2nd Nitrogen : Aspartate.
- Carbon atom : Respiratory CO_2.

Urea Bicycle :



Reactions of Urea Cycle :



Energetics :
CPS I : 2 × PO_4
AS lyase : 2 × PO_4
Total required : 4 PO_4

----- Active space -----

Urea Cycle Disorders :

Defect	Disorder
CPS I	Hyperammonemia Type I
OTC	Hyperammonemia Type II
AS synthetase	Citrullinemia Type I
AS Lyase	Argininosuccinic aciduria
Arginase	Argininemia
Citrin transporter	Citrullinemia Type 2
Ornithine transporter	HHH syndrome

Hyperammonemia Type II :

- m/c Urea cycle disorder.
- X-linked recessive.
- Defect : OTC.

Carbamoyl phosphate accumulates → (Shunted for) Pyrimidine synthesis

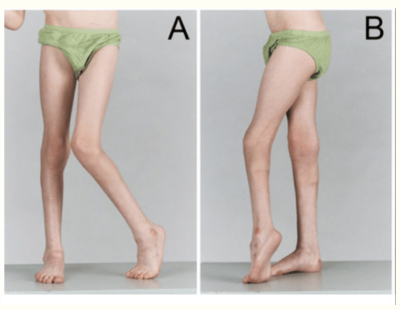
- Excretion of pyrimidines in urine
- Accumulation of orotic acid → Orotic aciduria.

HHH syndrome :

- Defect : Ornithine transporter → Hyperornithinemia.
- Carbamoyl phosphate + lysine → Homocitrulline → Homocitrullinemia.
- Accumulation of NH_3 → Hyperammonemia.

Arginemia :

- Least hyperammonemia.
- Spastic diplegia + scissoring of lower limbs.

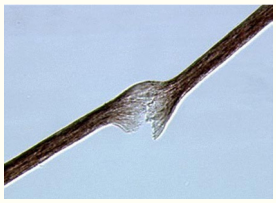


Spastic Diplegia


Scissoring gait

Argininosuccinic aciduria :

Trichorrhexis nodosa : Brittle hair.



Argininosuccinic aciduria

----- Active space -----

General clinical features of urea cycle disorders :

- Encephalopathy.
- Respiratory alkalosis.
- Tachypnoea.
- Hyperammonemia → A/w ↑ Plasma glutamine levels

  Glutamate $\xrightarrow{\uparrow\uparrow NH_3}$ Glutamine

Neonates : Feeding difficulties, failure to thrive, lethargy, convulsions, coma.

Management of Urea Cycle Disorders :

Investigations :

1. pH of blood → ↑/Normal : Urea cycle disorders.
   → ↓Organic aciduria.
2. Tandem mass spectrometry : Gold standard screening technique for all metabolic disorders.

Interpretation :

- Specific amino acids ↑ :
  - ↑Citrulline : Citrullinemia.
  - ↑Ornithine : HHH syndrome.
  - ↑Arginine : Arginemia.
  - ↑Argininosuccinate : Argininosuccinic aciduria.
- Non-specific amino acids ↑
  - Plasma orotic acid
    - ↑ → Hyperammonemia Type II.
    - Normal → Hyperammonemia Type I.

Treatment :

1. Supplement with arginine :
   - Source of ornithine.
   - Activator of NAG.
   - Essential amino acid.
   - C/I in arginase defect.

----- Active space -----

2. Acylation therapy :

Phenyl butyrate & sodium benzoate : NH_3 scavengers.

- Phenyl butyrate (prodrug) → Phenyl acetate + Glutamine → Phenyl acetyl glutamine → Excreted through kidneys.
- Sodium benzoate + glycine —(Glycine synthase)→ Benzoyl glycine → Excreted through kidneys.

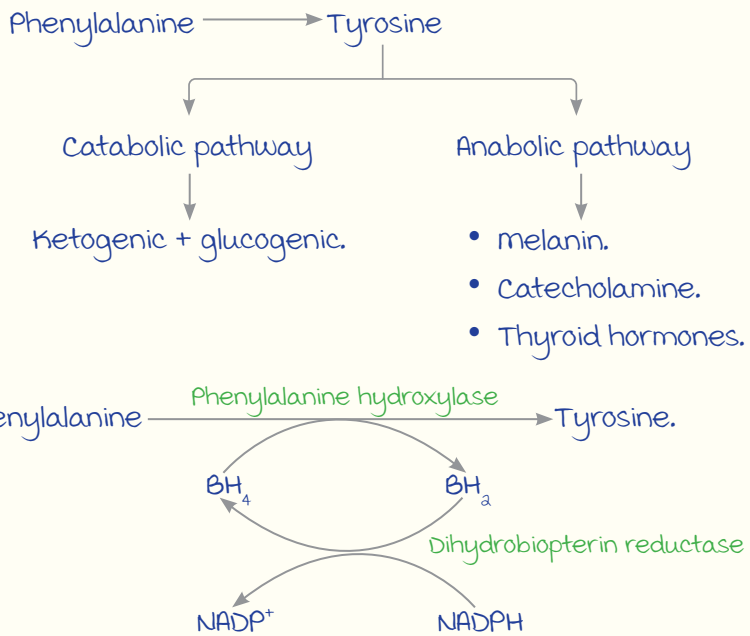
  NH_3 + CO_2 + 1 Carbon group.

AMINO ACIDS : PART 2

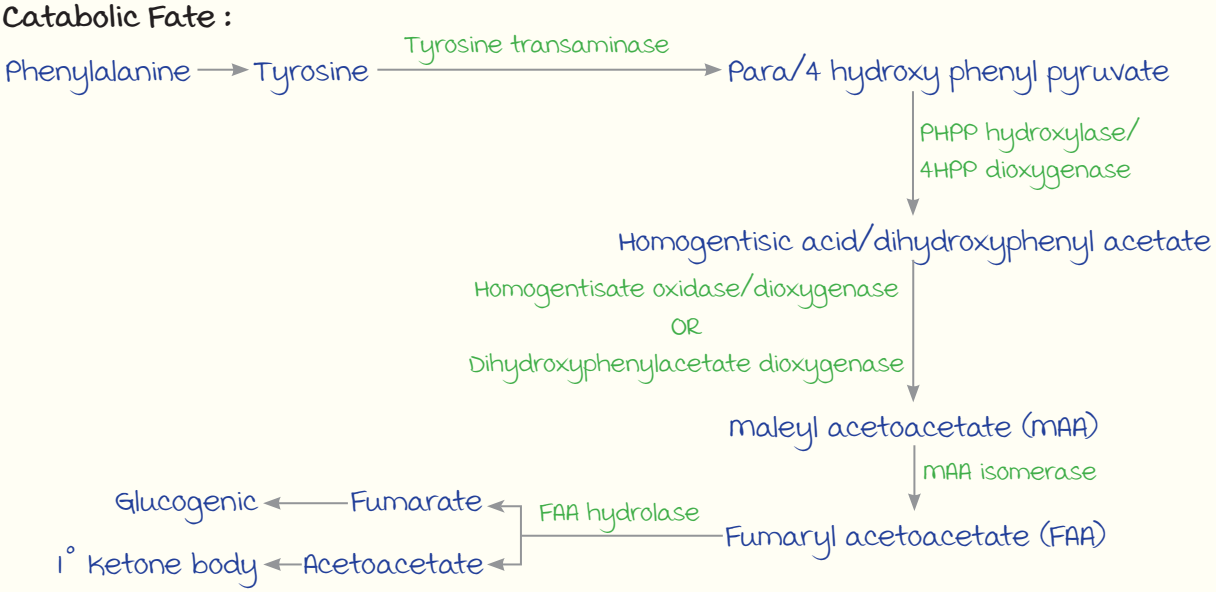
----- Active space -----

Metabolism & Disorders of Phenylalanine & Tyrosine

00:00:10



Catabolic Fate :



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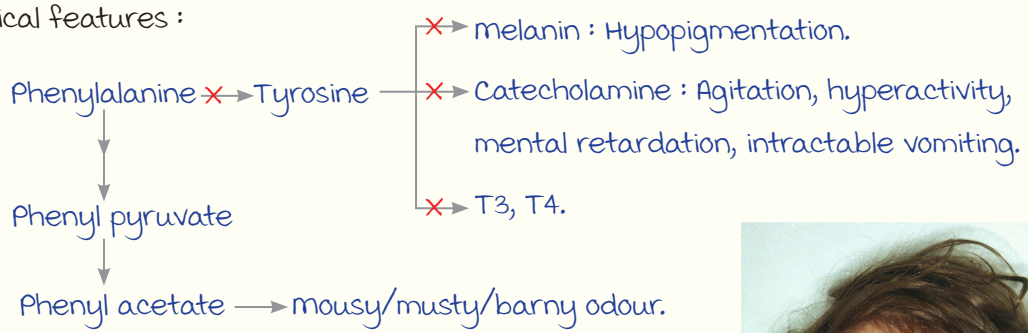
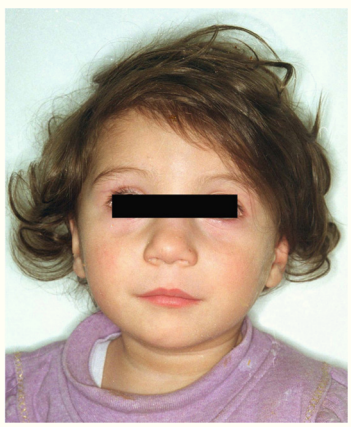
DISORDERS

Enzyme Defect	Disorder
Phenylalanine hydroxylase	Classic phenylketonuria (PKU)
• DHB Reductase • GTP synthesis → BH_4 – GTP cyclo hydrolase – Pyruvoyl THB synthase – Sepiapterin reductase	Non-classic PKU
Homogentisate oxidase	Alkaptonuria
FAA hydrolase	Tyrosinemia type 1
Tyrosine transaminase	Tyrosinemia type 2
PHPP	Tyrosinemia type 3

Classic Phenylketonuria :

Clinical features :





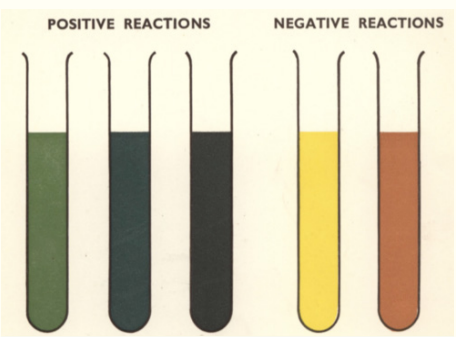
PKU : Hypopigmentation

Note :

Persistence of neurological symptoms after phenylalanine restriction → Non-classic PKU.

Lab diagnosis :

1. Guthrie's test (Bacterial inhibition test) : Growth of Bacillus subtilis.
2. Ferric chloride test : Positive.
3. Blood phenyl alanine levels.
4. Enzyme studies.



Ferric chloride test

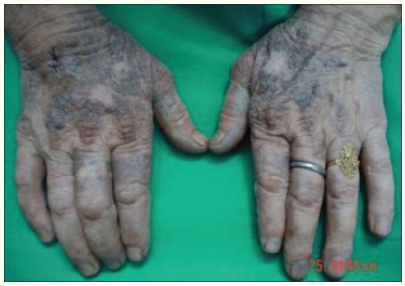
Treatment :

- Phenylalanine restricted diet.
- Synthetic THB (Non-Classic) : Sapropterin dihydrochloride/Kuvan.
- Large neutral amino acid (Tryptophan & tyrosine).

Alkaptonuria :

Part of Garrod's tetrad :

1. Cystinuria.
2. Alkaptonuria.
3. Pentosuria.
4. Albinism.



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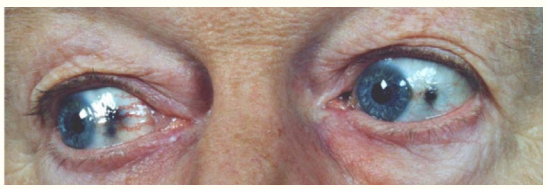


Alkaptonuria : Black pigmentation

Defect :

Phenylalanine → Tyrosine → PHPP → Homogentisic acid —(Homogentisate oxidase ✕)→ MAA.

- Homogentisic acid → Alkaptone bodies
  - → IV disc → Ochronotic arthritis.
  - → Skin.
  - → Cartilage.
- Homogentisic acid → Excreted in urine → Urine turns black on air exposure/ alkalinization.

Clinical features :

- Age of onset : Middle age.
- Present with back pain.
- No intellectual disability.
- Black pigmentation of skin, IV disc, cartilage.
- Blackening of urine on standing.

Lab diagnosis :

- $FeCl_3$ test.
- $AgNO_3$ test.
- Alkalinize urine.
- Benedict's test : Positive.
- X-ray :
  - Bamboo spine.
  - Vacuum phenomenon (Air space in vertebra).

Rx : Nitisinone → Inhibits PHPP hydroxylase.

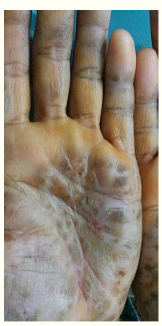
Type 2 Tyrosinemia :

AKA Oculo-cutaneous tyrosinemia/Richner Hanhart syndrome.

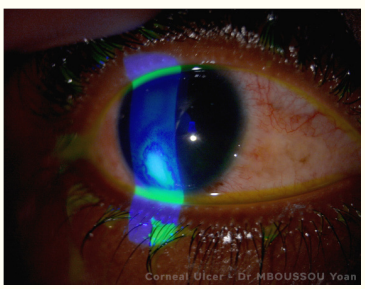
Defect : Tyrosine transaminase.

Features :

- Skin : Non-pruritic hyperkeratotic plaque on soles & palms.



- Corneal ulcers : Poorly stained with fluorescein.



----- Active space -----

Derivatives of Tyrosine

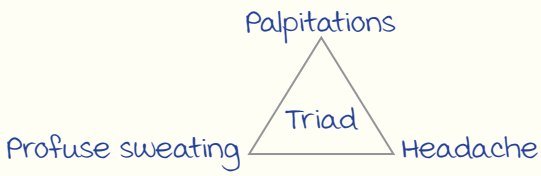
00:18:55

Catecholamines :

Degradation of catecholamines :

- Dopamine → Homovanilic acid (HVA).
- Norepinephrine, Epinephrine } Vanilyl Mandellic Acid (VMA).

Pheochromocytoma :



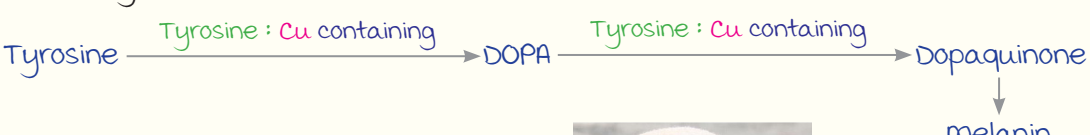
Lab diagnosis :

1. 24 hr urinary tests for :
   - VMA : Highest specificity.
   - Catecholamines.
   - Fractionated metanephrines : Highest sensitivity.
   - Total metanephrines.

2. Plasma tests for :
   - Catecholamines.
   - Free metanephrines : Highest sensitivity.

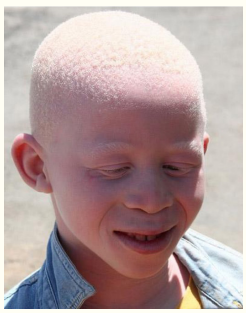
Melanin :

Site of synthesis : Melanosomes (Stratum basale).



Albinism : Defect in tyrosinase.

- Milky white skin & hair.
- Photophobia.
- Lacrimation.



Albinism

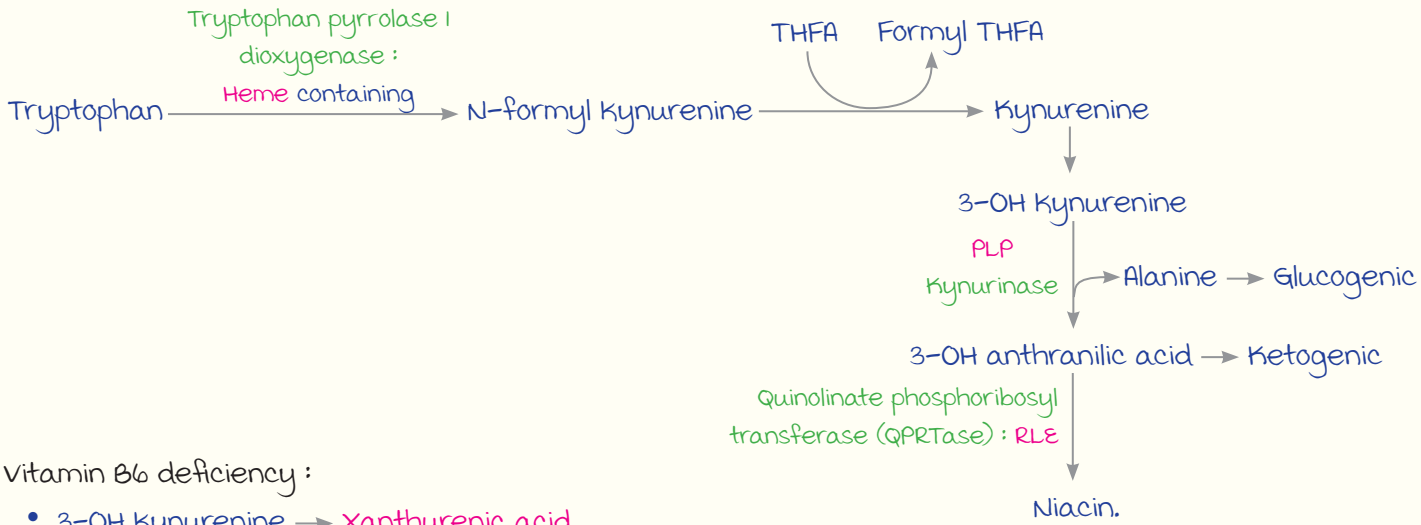
Tryptophan

00:23:00 ----- Active space -----

Properties :

- Aromatic AA.
- Non-polar.
- Essential.
- Ketogenic + glucogenic.

Catabolic Fate :



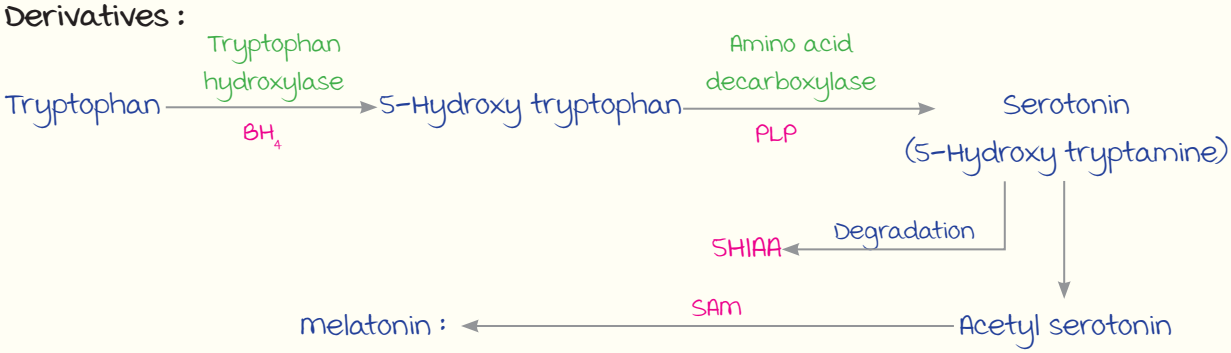
Vitamin B6 deficiency :

- 3-OH Kynurenine → Xanthurenic acid.
- ↓Synthesis of niacin → Pellagra.

Niacin :

- Endogenously synthesized vitamin.
- 60 mg tryptophan → 1 mg niacin.

Derivatives :



- Neurotransmitter
- Antioxidant
- Regulates circadian rhythm.

Site : Argentaffin cells of

1. Intestine.
2. Brain.
3. Platelets.

----- Active space -----

DISORDERS

Carcinoid Syndrome :

Tumor of argentaffin cells → ↑↑Serotonin → ↓Niacin.

C/f :

- Intermittent diarrhea.
- Flushing.
- Sweating.
- Features of pellagra.
- 24-hr 5 HIAA↑.

Hartnup's Disease :

Mutation : SLC 6A 19 Mutation → Defect of tryptophan transporter

↓Tryptophan

↓Serotonin & ↓niacin.

C/f :

- Asymptomatic.
- Accumulation of tryptophan in intestine → (Bacterial decomposition) → Indoxyl compounds → Excreted in urine → Bluish discoloration of diaper.
- Ataxia.
- Wide based gait.
- Cutaneous photosensitivity : m/c symptom.

Test : Obermeyer test.

Rx : Lipid soluble esters of tryptophan.

Derivatives of Tyrosine

00:33:10

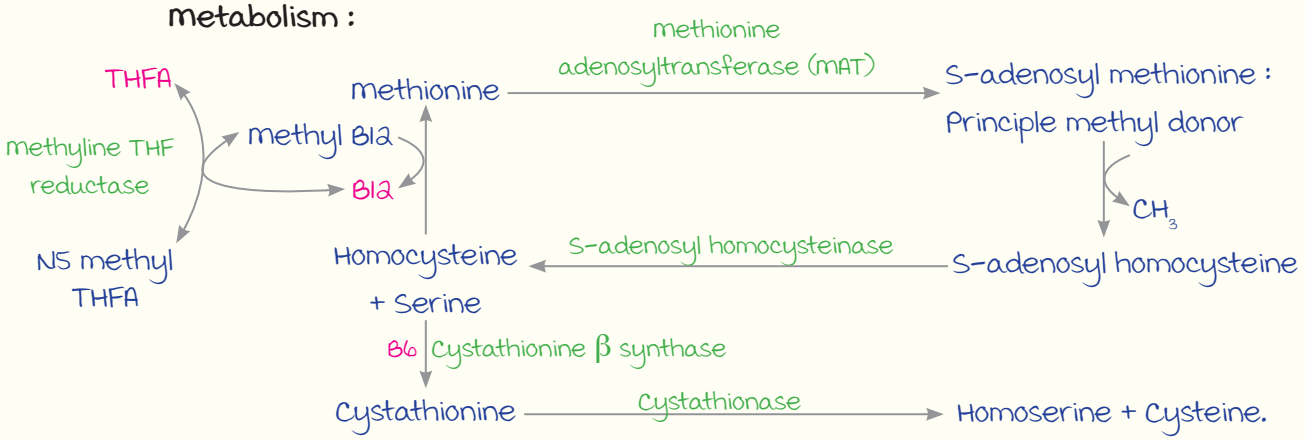
Cysteine :

- Glucogenic
- Polar
- Non-essential.

Methionine :

- Glucogenic
- Non-polar
- Essential.

Metabolism :



DISORDERS

----- Active space -----

Enzyme defect	Disorders
MAT	Primary hypermethioninemia
Cystathionine β synthase	Classic homocystinuria
Cystathionase	Cystathioninuria
• Methyline THF reductase • Defect in methyl B12 formation	Non-classic homocystinuria

Note :

B6, B12, B9 deficiency → ↑Homocysteine in blood → Risk factor for thrombosis.

Folate trap/THFA starvation :

B12 deficiency : N5 methyl THFA → THFA.

↓

Functional deficiency of THFA → ↓DNA synthesis → Nucleocytoplasmic synchrony

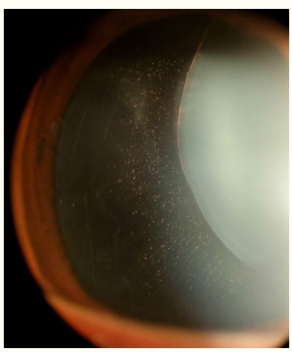
↓

Megaloblastic anemia.

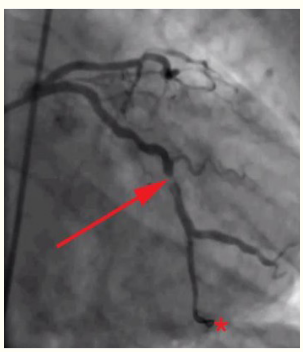
Homocysteinuria :

Clinical features : Resembles marfan's syndrome.

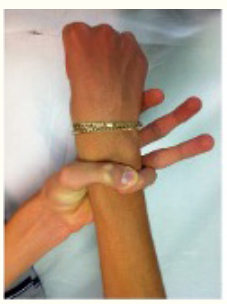
- Developmental delay.
- Mental retardation.
- Tall & thin extremities.
- Skeletal deformities.
- Visual disturbances : Ectopia lentis (m/c : Inferomedial).
- Muscular hypotonia.
- H/o CAD.
- Thromboembolism.



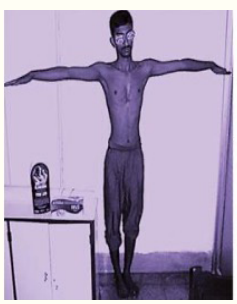
Ectopia lentis



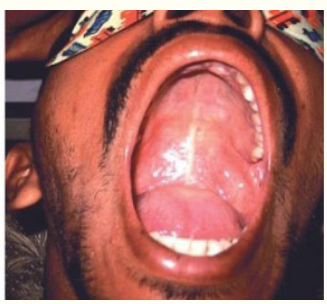
Thromboembolism



Arachnodactyly



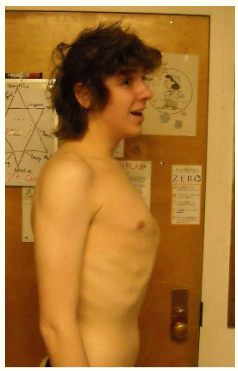
Tall stature



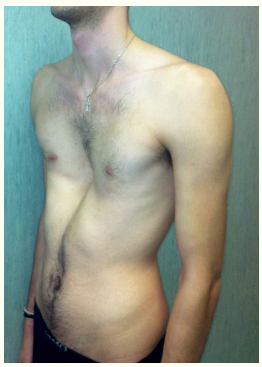
High arched palate

Skeletal deformities in homocysteinuria

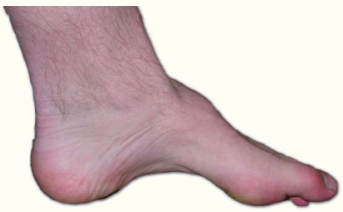
----- Active space -----



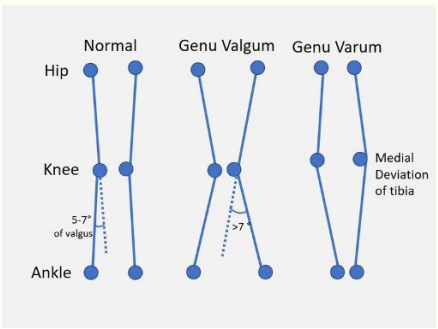
Pectus excavatum



Pectus carinatum



Pes cavus



Genu valgum/varus

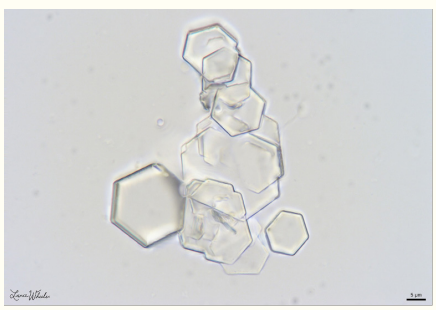
Skeletal deformities in homocysteinuria

Test : Cyanide nitroprusside test ⊕.

	Classic homocysteinuria	Non-classic homocysteinuria
Enzyme	Cystathione β synthase	Methyl B12 formation ; Methylene THF reductase
Defect in	Formation of cysteine	Remethylation of homocysteine to methionine
Homocysteine	↑	↑
Cysteine	↓	Normal
Methionine	Normal	↓
Rx	• Cysteine supplementation • High dose Vit B6 • Betaine (trimethyl glycine) • Vit B12 & folic acid	• Methionine supplementation • Vit B12, B6, folic acid

Cystinuria

- Defect : Dibasic amino acid transporter in Kidney.
- Excretion of : COLA.
  - Cystine.
  - Ornithine.
  - Lysine.
  - Arginine.



Cystine crystals : Colourless, flat, hexagonal ; acidic urine

Cystinosis :

- Defect : Cystine transporter in lysosome.
- Manifestations :
  - Renal failure.
  - Bone marrow suppression.
  - Corneal opacity.
  - Liver failure.

DERIVATIVES OF CYSTEINE

----- Active space -----

Glutathione

Taurine

Glutathione (GSH) :

- Tripeptide : Glutamic acid + cysteine + glycine.
- Active group : SH of cysteine.
- Atypical peptide.

Functions :

1. Transport of ammonia : Meister's cycle/Gamma glutamyl cycle.
2. Free radical scavengers : Glutathione peroxidase.
3. Conjugation.
4. Coenzyme.

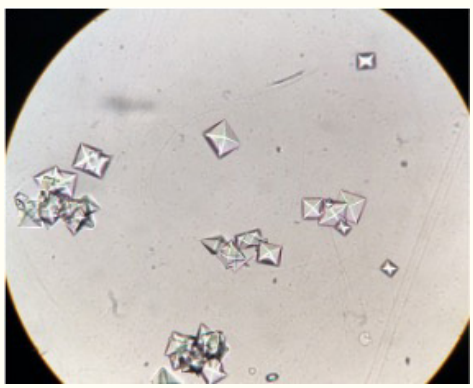
Other Amino Acids

00:49:55

Glycine :

Derivatives :

1. Purine (C4, C5, N7).
2. Creatinine : Glycine + arginine + methionine.
3. Heme.
4. Glutathione.
5. Collagen.



Oxalate stones : Envelope shaped

Hyperoxaluria :

Primary : Defect in glyoxylate amino transferase.

Secondary : D/t

- Vit C excess.
- Vit B6 deficiency.
- Ethylene glycol poisoning.
- Methoxyflurane.

Serine :

Functions :

1. Synthesis of :
   a. Cysteine.
   b. Phosphatidyl serine.
   c. Choline.
   d. Betaine.
2. Produces ethanolamine on decarboxylation.
3. Precursor of selenocysteine.

BASIC AMINO ACIDS

Histidine :

Derivatives :

- FIGLU.
- Histamine.

Histidine

FIGLU (Formimino glutamic acid)

THFA

Formimino THFA

Glutamic acid.

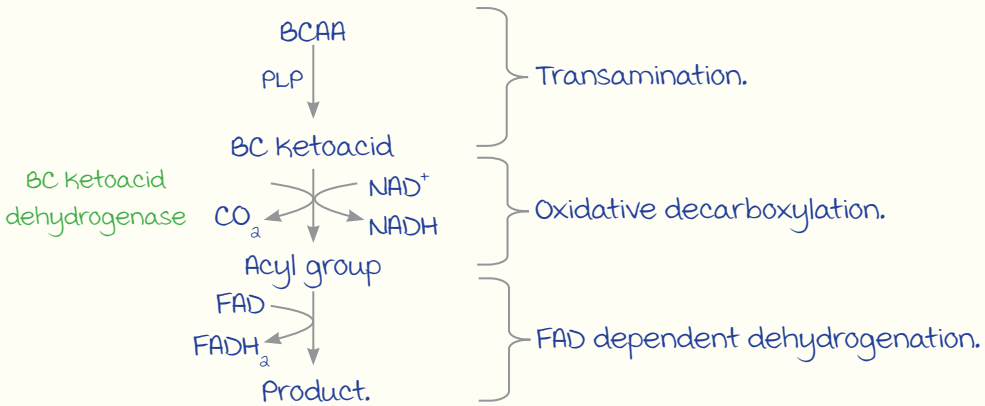
----- Active space -----

Histidine load test : B9 deficiency → FIGLU excreted in urine.

Arginine : Derivative compounds are

1. Nitric oxide.
   - Synthesized by NO synthase.
   - Reactive free radical.
   - Acts via GMP.
   - Endothelium derived relaxing factor.
2. Ornithine & Urea.
3. Creatine

BRANCHED CHAIN AMINO ACIDS (BCAA)



Maple Syrup Urine Disease

Defect : Branched chain Ketoacid dehydrogenase.

Features :

- Age of onset : Neonate.
- Feeding difficulty, convulsion, lethargy, coma.
- Boxing/Kicking movement (Alternating hypo & hypertonia).
- Burnt sugar odour of urine.

Lab diagnosis :

- ↑BCAA & ↑BCKA in blood & urine.
- Dinitrophenyl hydrazine (DNPH) test.
- Rothera's test : Positive.

Rx

- Restrict BCAA.
- Supplement thiamine.

Revision Tables

00:57:35 ----- Active space -----

LABORATORY TESTS

Test	Aminoaciduria
Ferric chloride test	PKU/Alkaptonuria
Dinitro phenyl hydrazine test	MSUD
Guthrie test	PKU
Obermeyer test	Hartnup disease
Cyanide nitroprusside test	Homocystinuria
La Brosse VMA spot test	Pheochromocytoma
5 HIAA	Carcinoid syndrome

ENZYME DEFECTS

Disorder	Defective enzyme
Albinism	Tyrosinase
MSUD	Branched chain keto acid dehydrogenase
Isovaleric acidemia	Isovaleryl CoA dehydrogenase (A/w leucine catabolism)
Homocystinuria	Cystathionine β synthase
Phenylketonuria	Phenylalanine hydroxylase/DHB reductase/Defect in THB synthesis
Alkaptonuria	Homogentisate oxidase
Tyrosinemia type I	Fumaryl acetoacetate hydrolase
Tyrosinemia type II	Tyrosine transaminase
Tyrosinemia type III	PHPP hydroxylase

PECULIAR ODOURS

Disorder	Odour
Glutaric acidemia (Type 2)	Sweaty feet
Hawkinsinuria : Defect in PHPP hydroxylase (Partially active)	Swimming pool
Isovaleric aciduria	Sweaty feet
MSUD	Maple syrup/Burnt sugar
Hypermethioninemia	Boiled cabbage
Multiple carboxylase deficiency	Tomcat urine
Oasthouse urine disease	Hops-like
PKU	Mousy/Musty
Trimethylaminuria : • Defect in trimethylamine oxidase • Rx : Choline restriction	Fish odour
Tyrosinemia	Boiled cabbage/Rancid butter

----- Active space -----

MOLECULAR BIOLOGY : PART 1

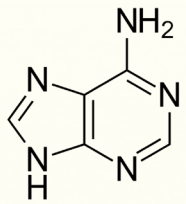
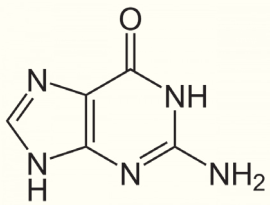
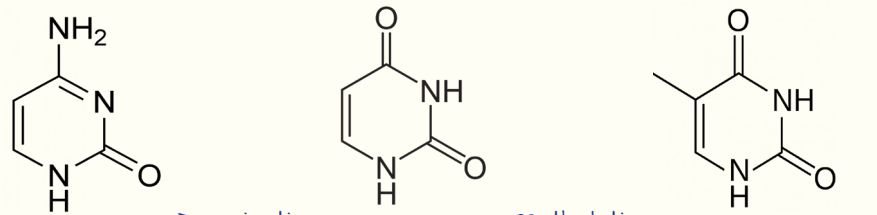
Nucleotides & Nucleic Acids

00:00:10

Steps to Identify Nucleotide :

1. Identify the ribose sugar.
2. Identify the nitrogenous base.
3. Identify if PO_4^{3-} group present.
4. Identify nucleotide → monomer made up of :
   - a. Nitrogenous base
   - b. Pentose sugar
   - } Nucleoside
   - c. PO_4^{3-} group

Types of Nitrogenous Bases :

Double ring : Purine	Single ring : Pyrimidine
Adenine (6-amino purine)  Guanine (2-amino, 6-oxopurine) 	 Cytosine (2-oxo, 4-amino pyrimidine) → Deamination → Uracil (2,4-dioxo pyrimidine) → Methylation → Thymine (2,4-dioxo, 5-methyl pyrimidine)

Important Linkages :

β-N glycosidic bond : B/w N_9 of purine/N_1 of pyrimidine to C_1' of pentose sugar.

Ester bond : B/w nucleoside & 1st phosphate group.

Acid anhydride bond : B/w adjacent PO_4^{3-} groups (Energy rich bonds).

Nucleic Acids :

- Formed by 3'-5' phosphodiester bond b/w nucleotides.
- Exhibit polarity.
- Sequenced from 5' → 3'.

DNA vs. RNA :

----- Active space -----

	DNA	RNA
Hydroxyl group	Only at 3' position	At 2' and 3' positions
Pentose sugar	Deoxyribose sugar	Ribose
Free functional group	⊖	Reactive 2' OH group ⊕
Stability	Stable	Unstable

Nucleotides in RNA vs. DNA :

Nitrogenous base	Nucleoside	Ribose monophosphate	Deoxyribose monophosphate
Adenine	Adenosine	Adenosine monophosphate (AMP)	d AMP
Guanine	Guanosine	Guanosine monophosphate (GMP)	d GMP
Uracil	Uridine	Uridine monophosphate (UMP) (Only in RNA)	-
Hypoxanthine	Inosine	Inosine monophosphate (IMP)	-
Xanthine	Xanthosine	Xanthine monophosphate (XMP)	-
Cytosine	Cytidine	Cytidine monophosphate (CMP)	d CMP
Thymine	Thymidine	-	d Thymidine monophosphate (Only in DNA)

Metabolism of Nucleotides

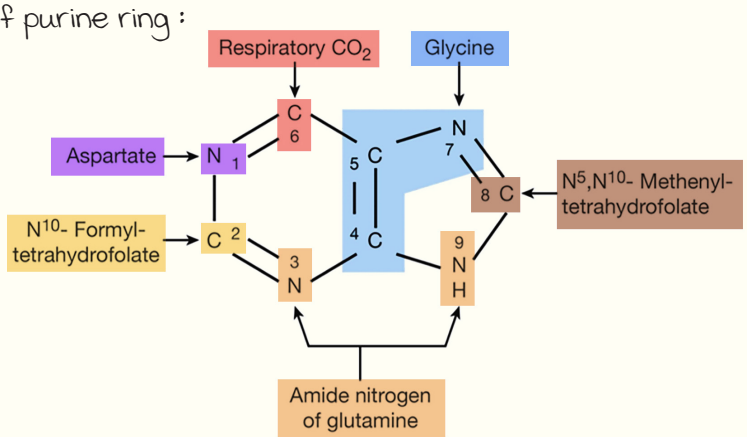
00:13:20

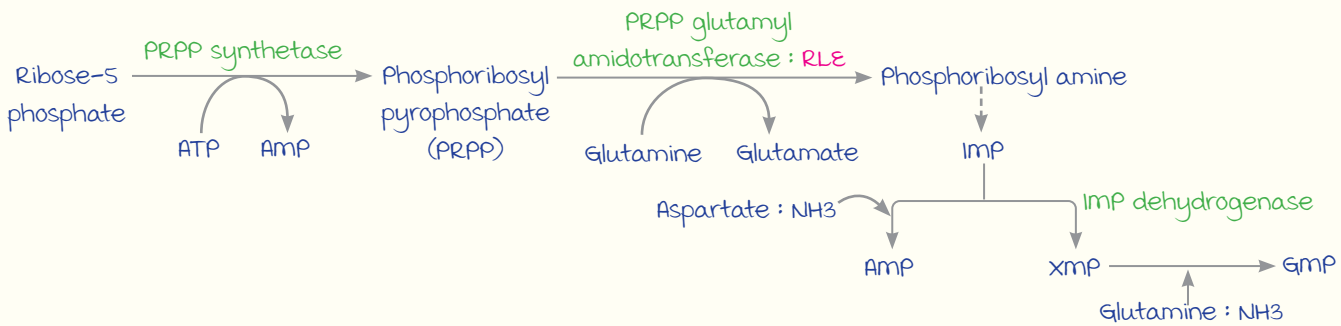
PURINE SYNTHESIS

De-novo Purine Synthesis :

Site : Occurs in all organs except brain, RBC & WBC.

Contributors of purine ring :





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Salvage Pathway :

Significance :

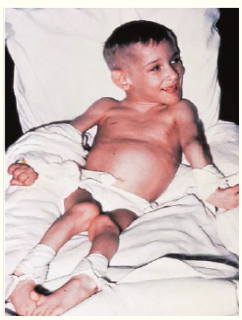
- Recycles purine nucleosides & bases to purine nucleotides.
- Conserves energy in organs without de novo synthesis.

Phosphorylation reactions :

Substrate	Donor	Enzyme	End product
Adenosine	PRPP	Adenine phosphoribosyl transferase (APRTase)	AMP
Hypoxanthine		Hypoxanthine guanine phosphoribosyltransferase (HGPRTase)	IMP
Guanine			GMP
Adenosine	ATP	Kinase	AMP
Guanosine			GMP

Disorders of Purine Synthesis :

Lesch Nyhan Syndrome :



Defect : HGPRTase.

- Hypoxanthine → IMP
- Guanine → GMP

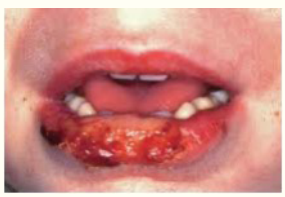
→ ↑Purine catabolism → ↑Uric acid.

C/f :

- Compulsive self-mutilation.
- Hyperuricemia
- Neurological defects.

Rx :

- Allopurinol
- High fluid intake.
- Alkalization of urine.



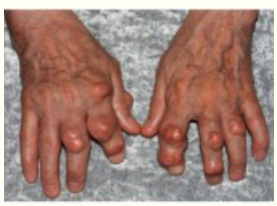
Lesch Nyhan Syndrome :

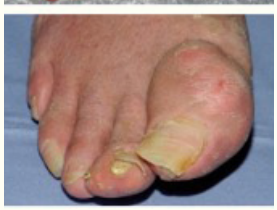
Kelley Seegmiller syndrome :

Partial defect of HGPRTase.

Gout :

C/f :





Tophi

- Acute inflammatory arthritis (m/c : 1st MTP) : Characteristic of acute gout.
- Hyperuricemia.
- Uric acid nephrolithiasis
- Tophi (MSU crystals in s/c tissue) : Characteristic of chronic gout.

MSU (Monosodium Urate) crystals

Definitive diagnosis :

Needle shaped negatively birefringent MSU crystals on polarized light microscopy.

----- Active space -----

Disorders of Purine Catabolism :

End product of purine catabolism : Uric acid.

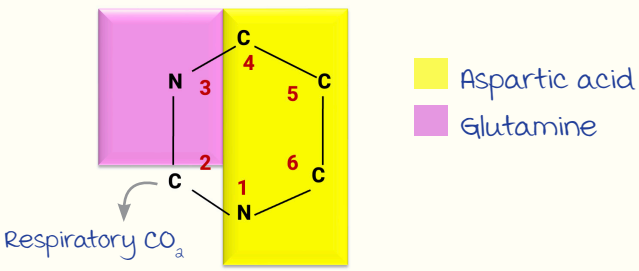
Enzyme deficiency	Features
Adenosine deaminase	SCID : Both T cells & B cells affected
Purine nucleoside ribosyl transferase	Immunodeficiency affecting only T cells
Xanthine oxidase	• Xanthinuria • ↓Uric acid

PYRIMIDINE BIOSYNTHESIS

Site : Liver

Organelle : Cytoplasm & mitochondria.

Sources of Pyrimidine Ring :



Pathway :

CO_2 + Glutamine —CPS II→ Carbamoyl phosphate —Aspartate transcarbamoylase→ Carbamoyl aspartic acid —Dihydroorotase→ Dihydro orotic acid —Dihydroorotate dehydrogenase (NAD⁺ → NADH)→ Orotic acid → OMP → UMP → CMP → TMP

Only step that occurs in mitochondria

End Products of Pyrimidine Catabolism :

- β-alanine (From cytosine & uracil)
- β-amino isobutyrate (From thymine)

} Water soluble

Disorder : Hereditary Orotic Aciduria :

Defect : De novo synthesis of pyrimidines.

Type I orotic aciduria :

Defect in : UMP synthase → Orotate phosphoribosyl transferase.
Orotidine monophosphate decarboxylase.

----- Active space -----

C/f :

- Macrocytic, megaloblastic anemia : Does not respond to Vit B_{12}/Folic acid/ Fe supplementation.
- Intellectual defect ⊕.

DNA

00:30:40

Watson & Crick Model :

- Right handed antiparallel double helical structure.
- Base pairing rule → A = T, G ≡ C
- Chargaff's rule : A + G = C + T (Purines = Pyrimidines)
- Base stacking :
  - Vertical force of interaction b/w base pairs.
  - D/t hydrophobic interactions & Van der Waals forces.

Organization of DNA :

Nucleosome : Histone octamer + ds DNA.

- Basic proteins.
- Rich in arginine & lysine.
- Positively charged.
- Made up of core histones (H2A, H2B, H3, H4).

DNA Replication :

Salient features :

- Both strands act as templates.
- Bidirectional : Always 5'→3'.
- Occurs in S phase
- Semidiscontinuous
- Semi conservative.
- Requires primer.

Steps of replication :

1. Identification of site of origin :

   Ori : Fixed point on DNA where replication begins.
   - E. coli : Ori C.
   - Bacteriophage : Ori λ
   - Yeast : Autonomous Replicating Sequence (ARS).
   - Human : Multiple ori ⊕, similar to ARS.

2. Binding of ori-binding protein to ori → Unwinding of AT rich regions
   ↓
   Binding of SSB (Single Strand Binding) protein :
   Prevents local reannealing of DNA.

----- Active space -----

3. Helicase : Further unwinding of DNA.
   Topoisomerase : Nicking, resealing enzyme that relieves topological constraints.

4. Leading strand synthesis :
   a. Leading strand template (3' → 5').
   b. RNA primase added at 3' end.
   c. DNA polymerase III :
      Adds nucleotides continuously from 5' → 3' of daughter strand.
   d. DNA polymerase I : Removes the RNA primer.

5. Lagging strand synthesis :
   a. Lagging strand template : 5' → 3'.
   b. Multiple RNA primers added & DNA polymerase III adds short segments of nucleotides (Okazaki fragments).
   c. DNA polymerase I : Removes the RNA primer.
   d. Ligase fills the gap on RNA primer removal.

DNA Polymerase (DNAP) :

Enzyme	Function
Prokaryotic DNAP	
DNAP I (AKA Kornberg's enzyme)	• Removal of primers & gap filling. • DNA repair (major). • DNA proof reading.
DNAP II	• DNA repair. • DNA proof reading.
DNAP III	• Leading strand synthesis. • Synthesis of Okazaki fragments. • DNA proofreading.

Enzyme	Function	
Eukaryotic DNAP		
ε	Leading strand synthesis	+ DNA proofreading
δ	Lagging strand synthesis	
γ	Mitochondrial DNA synthesis	
β	DNA repair activity	
α	Primase activity	

Klenow fragment :

- DNAP I is without 5' → 3' exonuclease activity.
- Used in Sanger's sequencing.

Telomere :

- Ends of the chromosomes.
- At 3' end : TTAGGG tandem repeats (+).

----- Active space -----

Hayflick limit:

On removal of primer from 3' end :

The primer nucleotide sequence is not replicated in the daughter strand

↓

End replication error

↓ After multiple cell divisions

Telomere attrition.

(Shortening of ends of chromosomes)

Hayflick limit :
After 50 cell divisions
↓
DNA replication stops.
(Leads to aging)

Telomerase :

Terminal telomere transferase

Function : Adds DNA segments at 3' end

↓

Prevents telomere shortening

(No Hayflick limit.)

Properties :

1. Contains an intrinsic RNA template.
2. Reverse transcriptase activity ⊕.

Absent in : Somatic cells.

Present in : Skin cells, hematopoietic cells, germ line cells, cancer cells, lymphocytes.

Applied Biochemistry

Cancer
↑ ⊕
Telomerase activity
↓ ⊖
Premature aging.

DNA Repair Defects :

Defects in DNA	Repair mechanisms	Associated disorders
Bulky adducts/ Pyrimidine dimers (UV exposure)	Nucleotide Excision Repair (NER)	• Xeroderma pigmentosa – Cutaneous photosensitivity – ↑Risk of skin cancer • Cockayne syndrome • Trichothiodystrophy
Abasic sites	Base Excision Repair (BER)	MUTYH-associated polyposis
Base mismatch	Mismatch Repair (MMR)	Hereditary Non-Polyposis Colorectal Cancer (HNPCC)
• Double strand breaks • Single strand breaks • Intrastrand cross-links	Non-Homologous End Joining (NHEJ)	• SCID • Radiosensitive SCID
	Homologous Recombination (HR)	• Ataxia telangiectasia like disorder • Nijmegen Break Syndrome • Bloom's syndrome • Werner syndrome • Rothmund Thomson syndrome • Breast cancer susceptibility

Transcription

01:00:50 ----- Active space -----

DNA → RNA.

Only one strand transcribed : Template/Minus/Antisense strand.

Other strand : Coding/Plus/Sense strand.

- Strand not involved in transcription.
- Same sequence as that of RNA with T replaced by U.

Enzyme :

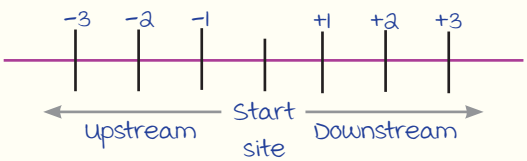
RNA polymerase (RNAP).

- Prokaryotic : Multisubunit
  - β subunit : Catalytic, binds to Mg^{2+}.
  - σ subunit : Binds to promoter.
- Eukaryotic RNAP

	RNAP-I	RNAP-II	RNAP-III
Sensitivity to α-amantin	Least	Highest	Intermediate
Major products	rRNA (most abundant)	• mRNA • miRNA • snRNA • lncRNA	• tRNA • 5S rRNA

Promoters of Transcription :

- Coding region sequences that specify the start site of transcription.
- Gene-specific



E.g :

Prokaryotes

- Pribnow box : -10 bp.
- TGG box : -35 bp.

Eukaryotes :

- TATA box : -25 bp
- CAAT box : -70 bp to -80 bp

Enhancers/Silencers/Repressors :

- ↑ or ↓ transcription of eukaryotic gene.
- Present upstream/downstream.
- Non-specific

ρ Dependent Termination :

ρ factor binds to RUT site (C-rich region of RNA)

↓

Detaches RNA from DNA.

----- Active space -----

POST TRANSCRIPTIONAL MODIFICATION

1. 5' capping :

Step	Enzyme	Site
1. Addition of 7 methyl guanosine cap at 5' end	Guanyl transferase	Nucleus
2. Methylation of N7 of guanine & 2' OH group of ribose	Methyl transferase	Cytoplasm

Functions :

- Stabilizes mRNA : Prevents the attack of 5' → 3' exonuclease.
- Facilitates initiation of translation.
- Binding of mRNA to initiation complex (43s preinitiation complex).

2. 3' Poly A Tailing :

- Addition of 40–200 adenosine residues at 3' end.
- Enzyme : Polyadenylate polymerase.

Functions :

- Stabilizes mRNA : Prevents the attack of 3' → 5' exonuclease.
- Facilitates exit of mRNA from nucleus → Cytoplasm for translation.
- Recruitment of 40s ribosome.

3 Splicing Of Exons & Removal Of Introns :

Carried out by spliceosomes → Sn RNA (Ribozyme) : Rich in uracil + 60 proteins : RR, SR motif + Primary transcript } SnRNP/ Snurps

Steps :

1. Spliceosome cuts at splice sites (Exons–introns junctions) :
   1st exon (Coding) – intron junction : SnRNP binds → SnRNA cuts the junction.
2. The cut end loops back & connects to middle of intron : Lariat formation.
3. Spliceosome makes 2nd cut at end of intron releasing the lariat.
4. Joining of exons via 3' → 5' phosphodiester bond.

Alternate RNA Processing/Differential RNA Processing :

Linking of exons in different sequences

↓

Different proteins formed

Significance : 20,500 genes → >1 lakh proteins synthesized.

RNA Editing :

Exception to central dogma.

Eg :

- Liver : Apo B (CAA) —Fully translated→ Apo B100.
- Intestine : Apo B (CAA) —Cytosine deamination→ UAA (Stop codon)
  - NH_3
  - Partial translation → Apo B48 (Truncated protein)

----- Active space -----

RNA

01:27:28

- Every RNA except mRNA (Coding) is non-coding.
- Histone mRNA : Poly A tail ⊖.
- hnRNA for histone gene : No introns.
- SLE : D/t autoimmune response to snurps.
- Nucleus : M/c site of post-transcriptional processing.
- Nucleolus : Site of post-transcriptional processing of rRNA

tRNA :

Non-coding RNA made of 74–91 nucleotides.

Structure :

- 2° structure : Clover leaf shape.
- 3° structure : Inverted L shape.
- 4 arms :
  a. Acceptor arm : Contains CCA at 3' end → Binds to specific amino acids.
  b. Anticodon arm : Binds to specific codons.
  c. TΨC (Only RNA that contains thymine) arm/Pseudouridine arm : Binds to ribosome.
  d. DHU arm/D-arm : Binds to aminoacyl tRNA synthetase.

Ribosomal RNA :

Present in the ribosomal assembly.

80s ribosome
- 40s : 18S rRNA + 30 proteins
- 60s : 28S rRNA + 5.8S rRNA + 5S rRNA + 50 proteins

↓

Peptidyl transferase activity.

----- Active space -----

miRNA :

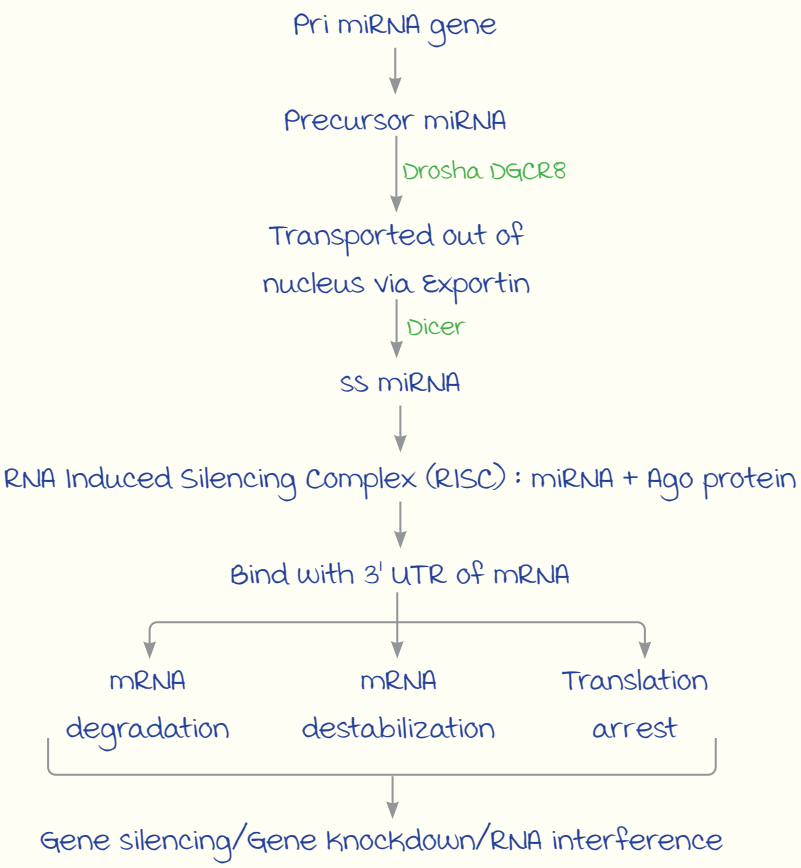
Small non-coding ssRNA of 21-22 nucleotide length.

Function : Post-transcriptional regulation of gene expression.

Sources :

- miRNA : Endogenous (Pri micro RNA gene).
- siRNA : Exogenous.

Formation :



MOLECULAR BIOLOGY : PART 2

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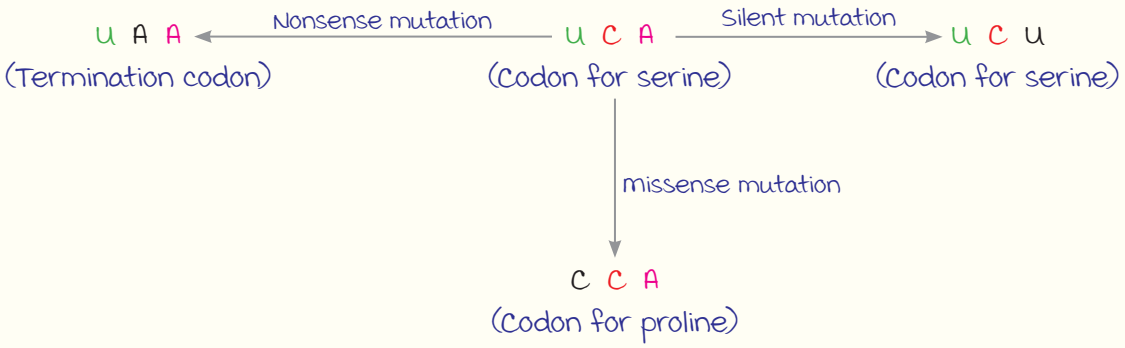
Mutations

00:00:05

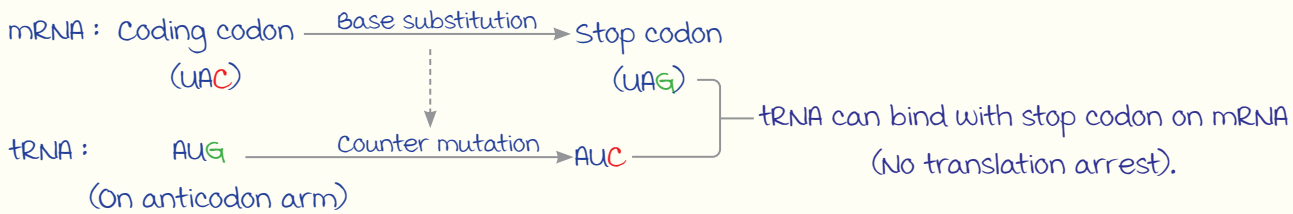
Point mutation (Base substitution) : m/c.



Frame shift mutation :

Insertion/deletion of nucleotide → Distorted reading frame.

Nonsense suppressor tRNA (Suppressor tRNA mutation) :



Epigenetics

00:05:32

Heritable, reversible chemical modification of DNA or chromatin.

No change in DNA sequence.

Functions :

- Regulation gene expression.
- X chromosome inactivation.
- Genomic imprinting.
- Aging process.

Common modifications :

- DNA methylation.
- DNA acetylation.

  Eg :

  - Histone acetylation → Euchromatin formation → Gene activation.
  - Histone deacetylation → Heterochromatin formation → Gene silencing.

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Detection of epigenetic modification :

- Methylation specific PCR.
- DNA chromatin immunoprecipitation (ChIP).
- Bisulphite sequencing.
- Methylation sensitive restriction endonuclease digestion.

Translation

00:09:31

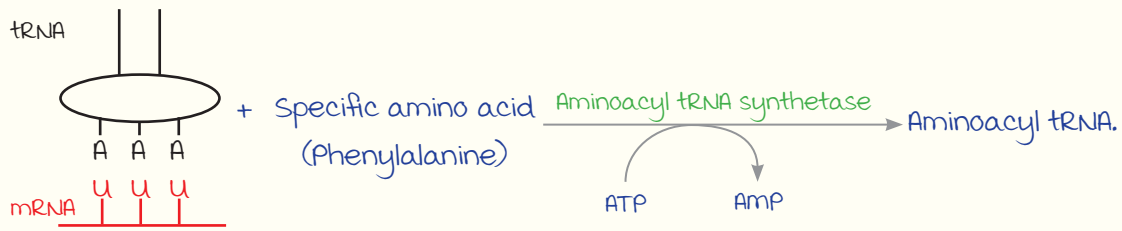
Salient Features of Genetic Code :

- Triplet codon : 3 nucleotides.
- Universal : Each codon codes for same protein in all species.
- Unambiguous : 1 codon codes for only 1 protein.
- Degenerate (Wobble hypothesis) : 1 amino acid can have > 1 codon (In 3rd base).
- Non-overlapping.
- Start codon : AUG.
- Stop codons : UAA, UGA, UAG.

STEPS OF TRANSLATION

Charging of tRNA :

Process of amino acid (AA) attaching on acceptor arm of tRNA.



Initiation :

GTP + eIF-2 (initiation factor) + tRNAi

↓

Ternary complex

↓ + 40s

43s pre initiation complex → (+ mRNA) 48s initiation complex → (+ 60s) 80s initiation complex

Elongation :

Ribosome : 80s (60s + 40s) initiation complex.

1. 3 sites :
   - E site.
   - P site : Initiator tRNA (Codes for methionine).
   - A site : Depending on codon, tRNA charged with AA binds.
2. Peptide bond synthesis (From P site to A site).
3. Translocation of ribosome on mRNA to free A site :
   - E site : Free tRNA.
   - P site : Polypeptide.
   - A site : Free to bind tRNA charged with AA.

----- Active space -----

Termination :

Ribosome reaches stop codon → Binding of releasing factor → (Peptidyl transferase + GTP) → Polypeptide freed.

Hybridisation Techniques

00:17:00

BLOTTING TECHNIQUES

	Southern blot	Northern blot	Western blot/Immuno blot
Target molecule	DNA	RNA	Protein
Transfer medium	Nitrocellulose/Nylon membrane	Nitrocellulose membrane	
Probe used	Labelled DNA probe : Complimentary sequence to target sequence.	Complimentary DNA : Complimentary to RNA (By reverse transcriptase)	Labelled antibody
Application	DNA detection	• RNA detection • Study of gene expression	Detect specific protein/ antigen

South-Western blot : For DNA – protein interaction.

MICROARRAY

DNA identification :

Chip with Known oligonucleotide → (+ Fluorescent labelled Unknown DNA)

RNA identification :

Chip with Known cDNA → (+ Fluorescent labelled Unknown RNA)

Protein identification :

Chip with Known antigen/antibody → (+ Fluorescent labelled Unknown Ag/Ab)

All → Fluorescence detected.

KARYOTYPING

Steps :

1. Collect cells from peripheral vein using heparin syringe.
2. Culture in phytohemagglutinin.
3. Incubation at 37°C for 3 days.
4. Harvest with colchicine.
5. Routine staining with Giemsa.

Banding Techniques :

Banding technique	Use
Giemsa (G)	Light & dark banding
Quinacrine (Q)	Similar to Giemsa, but examination with UV fluorescence microscope
Reverse (R)	Denatured chromosome → Light & dark bands in reverse pattern
Centromeric (C)	Heterochromatin (Centromere) stained preferentially

----- Active space -----

Note :

Human Genome Project : 2003 → Sequencing of human genome complete.

FLUORESCENT IN-SITU HYBRIDISATION (FISH)

23 distinct fluorescent probes used for each chromosome.

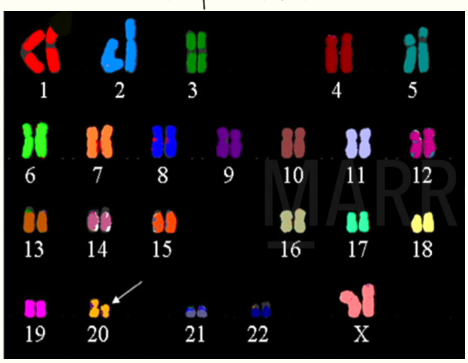
Uses :

To detect :

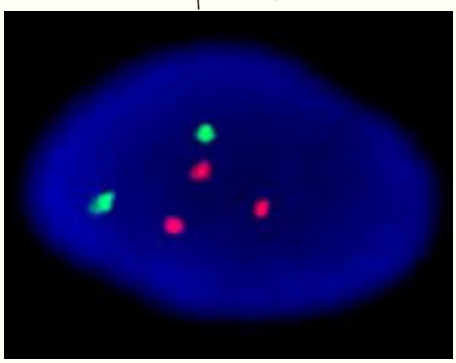
- Microdeletions.
- Amplification.
- Translocations.
- Aneuploidy.

Types :

Metaphase FISH



Interphase FISH



- Non-dividing cells.
- Rapid results & more sensitivity.

Recombinant DNA Technology

00:34:59

Restriction endonuclease (RE) :

- Present in bacteria : Restrict phage entry.
- Molecular scissors : Cut at specific palindromic sequence.

Vector : Carriers of foreign DNA

1. Plasmid : Extrachromosomal bacterial dsDNA → Confer antibiotic resistance.
   Insert size : 0.1 to 10 Kbp.
2. Phage DNA : Linear DNA in phages.
   Insert size : 10 to 20 Kbp.
3. Artificial chromosome (AC).
   - Bacterial (BAC)
   - Phage (PAC)

   } 300 Kbp.
   - Yeast (YAC) : 1000 to 3000 Kbp.

Restriction map :

- Unique band pattern formed when DNA is treated with specific restriction enzyme.
- Same restriction enzyme → Unique pattern in each individual.

Restriction fragment length polymorphism :

----- Active space -----

- Mutation of gene → Abolish restriction site.
  → Create restriction site.
- Based on band pattern → Mutation detected.

DNA Fingerprinting :

- Band pattern of unknown DNA —matched with→ Band pattern of known DNA.
- Used in medico-legal cases (Identifying suspect etc).

DNA Footprinting :

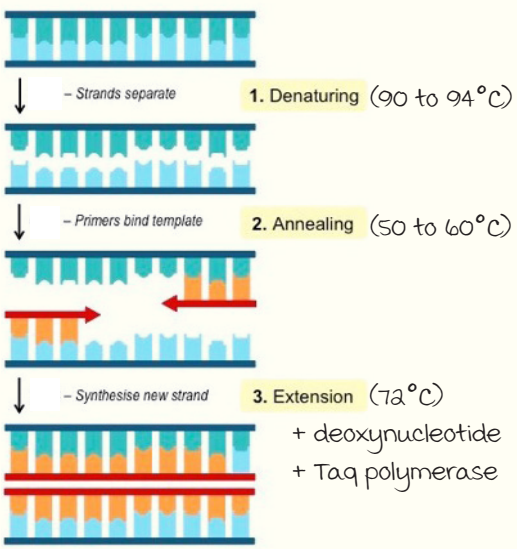
- Study of DNA-protein interaction.
- Method :

DNA is mixed with protein
↓ DNAase
Breakdown of DNA areas not bound to protein.
↓ On gel electrophoresis.

- Only broken fragments seen.
- Fragments bound to proteins not visualized (Footprint regions).

POLYMERASE CHAIN REACTION

- Exponential amplification of target DNA.
- No. of DNA after n cycles = 2^n.



Steps of PCR

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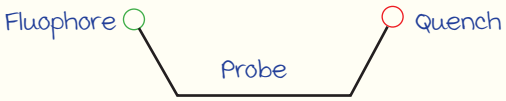
Real Time PCR :

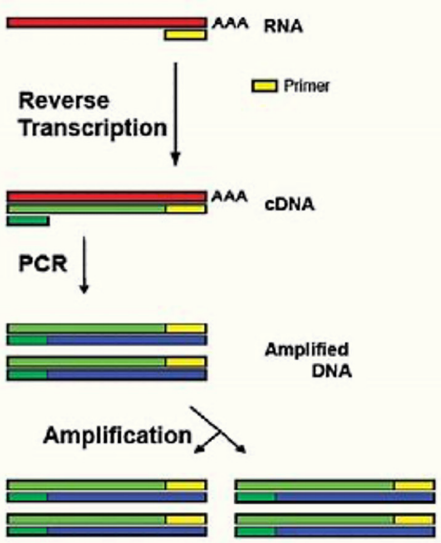
- A fluorescent probe with dye on one end & quencher (nullifies fluorescence when bound to probe) on other end.



- Eg :
  - Taqman probe
  - SYBR green
  - Ethidium bromide
- During elongation probe cleaved & dye displaced emitting fluorescence.
- ↑Fluorescence → Amount of DNA quantified real time.

Reverse Transcriptase PCR (RT-PCR) :

Study of RNA (Gene expression).



DNA SEQUENCING

Sanger's Sequencing :

First sequencing method : Frederick Sanger.

Components :

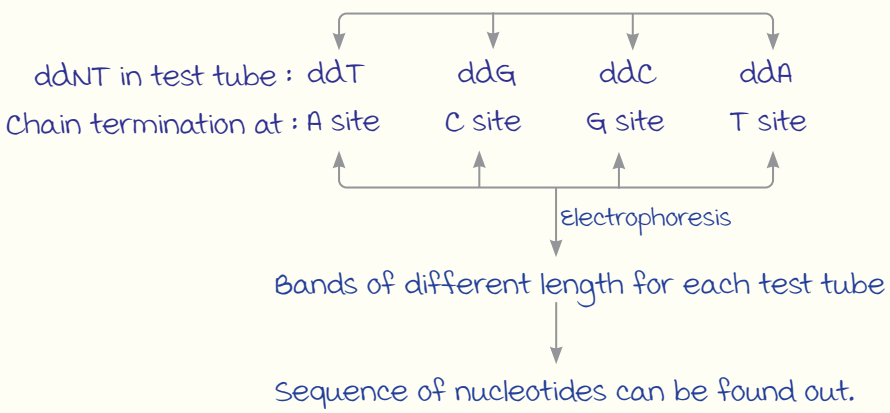
- Dideoxy NTs (ddNT).
- Klenow polymerase.
- dNTs.

Principle :

Dideoxy NT → No functional 3'-OH group → No 3'-5' phospho-diester bond → Controlled chain termination.

Technique :

DNA for sequencing added to 4 test tubes with different ddNT.



MICRONUTRIENTS : VITAMINS AND MINERALS

----- Active space -----

Endogenous vitamins :

- Synthesised by the body :
  - Niacin : From tryptophan.
  - Vitamin D : From cholesterol.
- Produced in the body : By microbiome
  - Vitamin K.
  - Pantothenic acid.
  - Biotin.

Fat soluble vitamins : A, D, E & K.

Water soluble vitamins : B complex & C.

Vitamin A vs Vitamin D

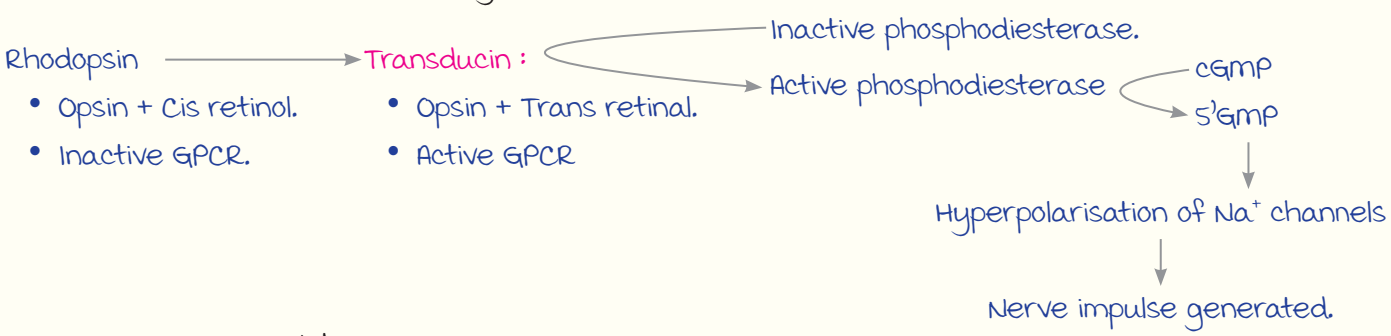
00:01:25

	Vitamin A	Vitamin D
Forms	• Retinal • Retinoic acid • Retinol	• Ergocalciferol (D2) : Plant sources • Cholecalciferol (D3) : Animal sources/Self synthesised
metabolism	Intestine : β-carotene (Diet) → Retinol Liver : Stored in Ito cells ← Carried in chylomicron ← Retinyl ester Plasma : Transported by Retinol Binding Protein (RBP) & transthyretin	Skin : 7-dehydrocholesterol —UVB rays (290–315 nm)→ Cholecalciferol —+ D-binding protein, 25-hydroxylase→ 25-hydroxycholecalciferol Liver : 25-hydroxycholecalciferol —PTH ($\uparrow Ca^{2+}$, $\downarrow PO_4$) ⊖→ 1-α-hydroxylase Kidney : 1,25-dihydroxycholecalciferol (Biologically active form)
Functions	1. Vision : Generation of impulse 2. Regulation of gene expression 3. Normal reproduction 4. Maintainence of normal epithelium of skin & mucosa 5. Anti-oxidant : β-carotene 6. Therapeutic use : – β-carotene cutaneous photosensitivity – All-trans retinoic acid : Promyelocytic leukemia (Differentiation therapy) – 13-cis-retinoic acid (Isotretinoin) : Cystic acne & childhood neuroblastoma	1. Regulation of Ca^{2+} & PO_4^{3-} – Bone – Kidney – Intestine } $\uparrow Ca^{2+}$, $\uparrow PO_4^{3-}$ 2. Immunomodulatory : Prevent TB 3. Anti-proliferative : prevent Ca Colon, Breast, prostate.

----- Active space -----

	Vitamin A	Vitamin D
Deficiency	1. Eye manifestations : – Nyctalopia (Night blindness) : ↑Dark adaptation time – Conjunctival → Corneal xerosis – Bitot's spots – Corneal ulcer → Keratomalacia 2. Skin manifestations : – Follicular hyperkeratosis – Squamous metaplasia	• ↑Unmineralised matrix : – Before closure of epiphysis : Rickets. – After closure : Osteomalacia. • Genu valgum & genu varum • Windswept deformity • Rachitic rosary
Toxicity	1. Acute toxicity : – Pseudotumor cerebri (In arctic explorers) – Exfoliative dermatitis – Hepatomegaly 2. Chronic toxicity (> 50,000 IU/d) : – Bony exostoses – Hepatomegaly (Cirrhosis) 3. Pregnancy : Teratogenic	• Calcinosis : Ca^{3+} deposited in blood vessels
Sources	• Halibut liver oil (Richest) • Plant sources : Carrots (Richest), green leafy vegetables • Animal sources : Egg, milk, fish, meat	• Fish • Fortified food
Assessment	• ↑Dark adaptation time • Carr & Price reaction : Direct assay	• Vit. D assay • S. osteocalcin
RDA (IU/d)	• Children (1 to 6 y) : 400 • Men & women : 600 • Pregnancy : 800 • Lactation : 950	• Children : 400 • Adults : 200 • Pregnancy : 400

Wald's visual cycle :



Note :

- Vit A deficiency : m/c preventable cause of blindness.
- Earliest symptom : Loss of vision to green light.

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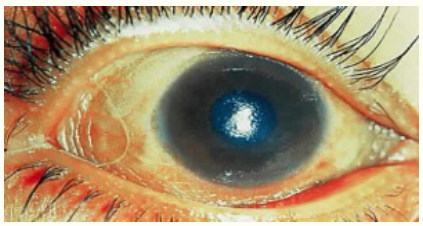
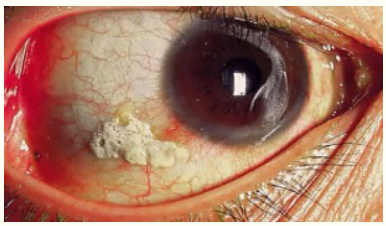
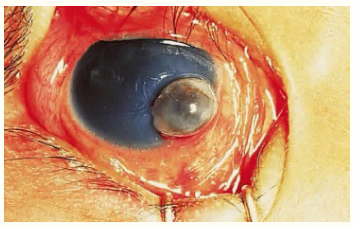
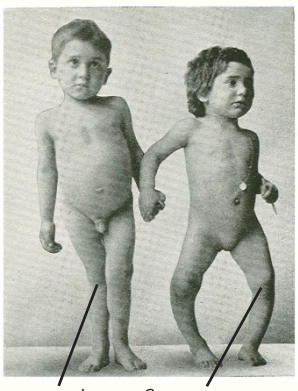
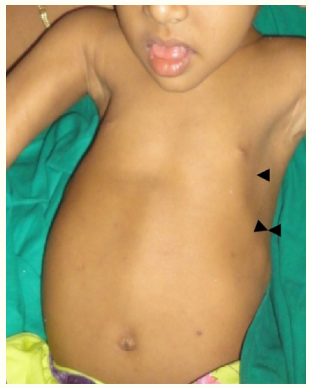

Conjunctival & corneal dryness


Bitot's spot


Corneal ulcer


Genu valgum & Genu varum


Windswept deformity


Rachitic rosary

Vitamin E vs. Vitamin K

00:20:28

	Vitamin E	Vitamin K
Forms	Alpha tocopherol	• K1 : Phylloquinone (Dietary Source) • K2 : menaquinone (Bacterial flora) • K3 : menadione (Synthetic; water soluble)
Functions	1. Most potent anti-oxidant. (Chain-breaking) 2. Prevents LOL Oxidation. 3. Protects PUFA in membranes from lipid peroxidation. 4. Therapeutic uses : – Retrolental fibroplasia – Intermittent claudication – Bronchopulmonary dysplasia – Intraventricular hemorrhage – Slow aging – Prevent fatty liver	1. Post-translational gamma carboxylation (Biotin independent) : – Prothrombin (Factor II) – Factor VII – Factor IX – Product of gene gas-6 – Matrix gla protein – Factor X – Nephrocalcin – Protein C – Protein S – Osteocalcin 2. Pro-coagulant Glutamic acid → Gamma carboxy glutamic acid Reduced Vit. K → Epoxide of Vit. K Vit.K epoxidase Oxidised Vit. K

----- Active space -----

	Vitamin E	Vitamin K
Deficiency	• Axonal degeneration • Peripheral neuropathy • Spinocerebellar ataxia • Hemolytic anemia • Eye manifestations : – Pigmentary retinopathy – Ophthalmoplegia – Nystagmus	• Bleeding manifestations : ↑CT, PT • Common in premature babies (Prevent : Inj Vit. K at birth)
Toxicity	• Interferes with platelet aggregation • Interferes with Vit. K function	Hemolysis → Hyperbilirubinemia ↓ Kernicterus (In babies)
RDA	• Males : 10 mg/d • Females : 8 mg/d • Pregnancy : 10 mg/d • Lactation : 12 mg/d	–

Note :

- Vit. K epoxidase inhibitors : Warfarin & dicumarol (Anticoagulants).
- Breast milk : Poor source of Vit. K.

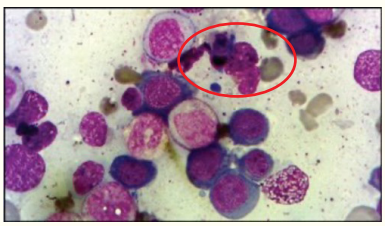
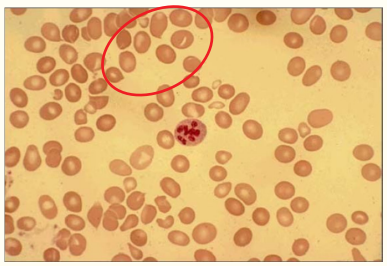
Hematopoetic Vitamins

00:27:02

Folic Acid v/s Vitamin B12 :

	Folic acid (Vit. B9)	Vit B12
Form & function	Tetrahydrofolic acid : Carrier of 1 carbon groups : • Formyl • Methyl • Methylene • Methenyl • Formimino	• Adenosyl B12 : Methyl malonyl CoA mutase Methyl malonyl CoA → Succinyl CoA • Methyl B12 Homocysteine methyl transferase/ methionine synthase Homocysteine $\xrightarrow{B12, B9}$ Methionine
Sources	Green leafy vegetables (Plant sources only)	• Mainly animal sources • Only vegetarian source : curd (D/t lactobacillus)
Deficiency manifestations	1. Megaloblastic anemia 2. Neural tube defects →Spina bifida →Anencephaly 3. Homocysteinemia : D/t defective sulfur-containing amino acid metabolism – ↑ Risk for thrombosis – ↓ Methionine → ↓SAM : Alters epigenetic modifications.	1. Megaloblastic anemia 2. Subacute combined degeneration : – Numbness – Loss of reflex – Peripheral neuropathy

----- Active space -----

	Folic acid (Vit. B9)	Vit B12
Assessment	• Serum folate/Red cell folate • Histidine load test : FIGLU excretion in urine. • Serum homocysteine • Serum Amino Imidazole Carboxamide Ribose-5-phosphate (AICAR)	• Serum cobalamine • Serum homocysteine • Urine homocysteine • Serum methylmalonyl CoA specific • Schilling test • Peripheral smear
Bone marrow & peripheral smear	 megaloblast in bone marrow	 Macrocytes in peripheral sinear

Causes of Vit. B12 deficiency :

1. Nutritional : Strict vegans.
2. Gastric : ↓Intrinsic Factor (IF) from parietal cells
   - Autoimmune pernicious anemia.
   - Gastrectomy.
3. Intestinal :
   - Crohn's disease.
   - Fish tapeworm (Diphyllobothrium latum).
   - Stagnant loop syndrome.

Energy Releasing Vitamins

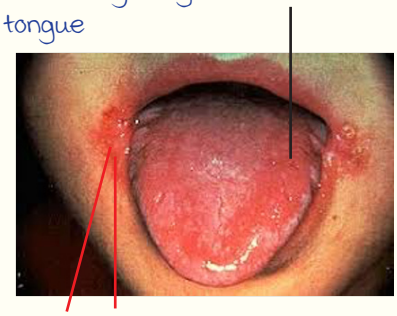
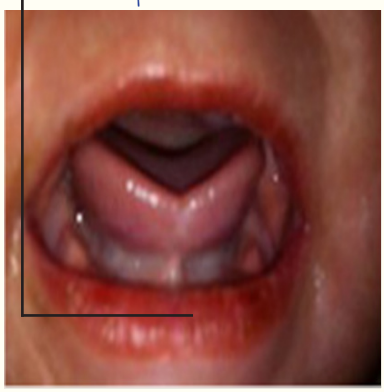
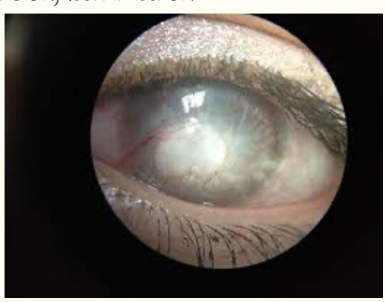
00:40:36

Vitamin B1 vs. Vitamin B2 :

	Vitamin B1 (Thiamine)	Vitamin B2 (Riboflavin)

Features	• Sources :
	  - Aleurone layer of grains
	  - Animal food : Egg, meat
	• Functions : Coenzyme role (TPP)
	  - Oxidative decarboxylation
	  - PDH, BCKDH, AKGDH
	  - Transketolase
		• AKA Warburg yellow enzyme
		• Heat stable
		• Present in flavoproteins.
		• Active form :
		  - FAD : Acyl CoADH, Succinate DH
		  - FMN : Complex I ETC

----- Active space -----

	Vitamin B1 (Thiamine)	Vitamin B2 (Riboflavin)
Deficiency	Causes : - Polishing of rice - Chronic alcoholism Manifestations : 1. Dry beri-beri - Symmetrical motor & sensory neuropathy - Loss of reflexes, muscle cramps - Muscle atrophy (If severe) : PNS affected 2. Wet beri-beri - Peripheral edema - Dyspnea - Cardiomegaly - Pulmonary edema - High output cardiac failure 3. Wernicke's Encephalopathy (WE) : - Horizontal nystagmus - Ophthalmoplegia - Ptosis - Truncal ataxia - Global confusion 4. Wernicke Korsakoff's : Features of WE + dementia + confabulatory psychosis	1. Red beefy/magenta coloured tongue  2. Angular stomatitis & cheilosis 3. Fissures in lips.  4. Corneal vascularisation : Difficulty in vision, lacrimation 
Assessment	• Erythrocyte transketolase level • Urinary thiamine excretion	• Erythrocyte glutathione reductase • Urinary riboflawin

Vitamin B3 (Niacin) :

Functions :

1. Coenzyme :
   - NAD + All OH except Acyl CoA DH & SDH.
   - NADPH generating :
     - HMP oxidative.
     - Cyt isocitrate DH.
     - Malic enzyme.
   - NADPH utilising : All reductases.
2. Therapeutic use : Lipid modifying drug (Hyper triglyceridemia).

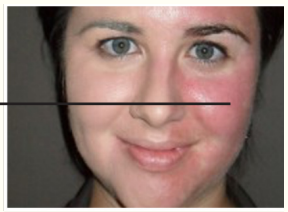
----- Active space -----

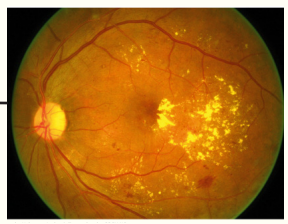
Deficiency of Vit. B3 : Pellagra

1. Dermatitis (Photosensitivity) : Casal's necklace.
2. Diarrhoea
3. Dementia.
4. Death.

Niacin toxicity :

1. PG mediated flushing :
   - Pre treatment : Aspirin.
   - Laropiprant (PG antagonist).
2. Hyperuricemic.
3. Glucose intolerance.
4. Cystoid macular edema.
5. Gastric irritation.
6. Fulminant hepatitis.





Note :

Pellagra-like symptoms.

- Hartnup's disease : ↓Absorption of tryptophan → ↓Niacin.
- Carcinoid syndrome : ↑Tryptophan —Shunted→ Serotonin (↓Niacin production).
- Maize/Corn diet : Niacin in bound form + poor source of tryptophan.
- Jowar/Sorghum staple diet : ↑Leucine —⊖→ QPRTase → ↓Niacin production.

Vitamin B5 (Pantothenic Acid) :

Function : Present in CoA & Acyl carrier protein (FA synthase complex).

Deficiency : Gopalan's burning foot syndrome/nutritional melalgia.

Biotin (Vitamin H/B7) :

Function : Coenzyme role

- Carboxylation reaction
  - Pyruvate carboxylase.
  - Acetyl CoA carboxylase.
  - Propionyl CoA carboxylase.

Deficiency :

- Cause :
  - Raw egg consumption : Avidin —Inhibits→ Biotin.
- Symptoms :
  - Depression, hallucinations.
  - Scaling, seborrheic dermatitis & erythematous rash.

----- Active space -----

Biochemical tests :

- Urinary biotin concentration.
- Serum & urine propionic acid level.
- ↓Activity of biotin dependent enzymes in lymphocyte.

Note :

- Biotin independent carboxylation.
  - Gamma carboxylation.
  - Carbamoyl phosphate synthetase.
  - Malic enzyme.
  - AIR carboxylase.
- Leiner's disease : ↓Biotin linked to complement 5a deficiency.

Vitamin B6 :

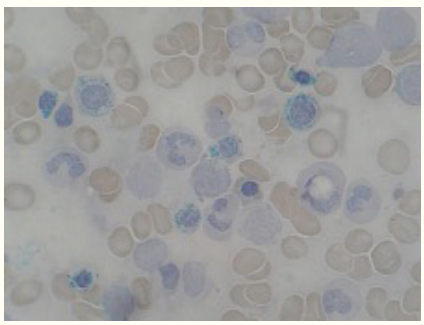
Active form : Pyridoxal phosphate.

Function : Coenzyme role.

- Transamination
- Simple decarboxylation.
- Transsulfuration
- Tryptophan metabolism : Kynureninase.
- Heme synthesis : ALA synthase
- Glycogenolysis : Glycogen phosphorylase.

Deficiency manifestations :

- Pyridoxine dependent seizures.
- Sideroblastic anemia.
- Pellagra-like symptoms : D/t ↓Kynureninase → ↓Niacin.



Ringed sideroblast

Urinary metabolites in B6 deficiency :

- Homocystine.
- Oxalate.
- Xanthurenic acid.

Assessment :

- Enzyme activity : Erythrocyte transaminase.
- Load test : Tryptophan load test → Excretion of xanthurenic acid.
- Direct measurement : Estimation of B6.

Toxicity of B6 : Sensory neuropathy.

Note : RDA of B6 depends on protein intake.

Vitamin C (Ascorbic Acid) :

----- Active space -----

Synthesis : Humans can't synthesise Vit. C → Lack L-gulonolactone (Uronic acid pathway).

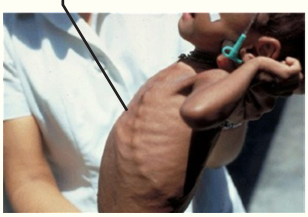
Functions :

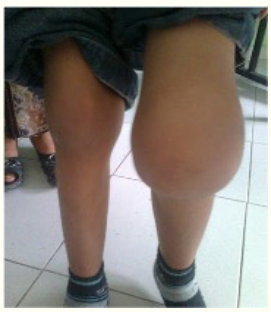
1. Collagen synthesis : Prolyl & lysyl hydroxylase.
2. Iron absorption : Ferrireductase.
3. Tyrosine metabolism : PHPP hydroxylase.
4. Bile acid synthesis : 7-α-hydroxylase.

Sources : Citrus fruits. (Richest source).

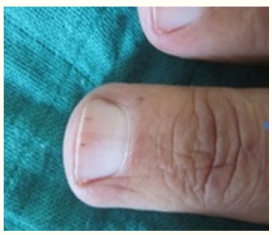
Deficiency :

- Scurvy :
  a. Bleeding manifestation →
  b. Iron deficiency anemia.
  c. Scorbutic rosary.

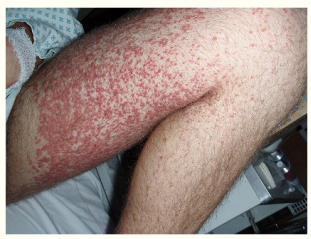




Hemarthrosis



Splinter hemorrhages



Petechial rashes

- Barlow's disease (Infantile scurvy)
- Cause : D/t deficiency during weaning (6 to 12 months)
- Treatment : Supplement Vit. C.

Minerals

01:02:40

Copper :

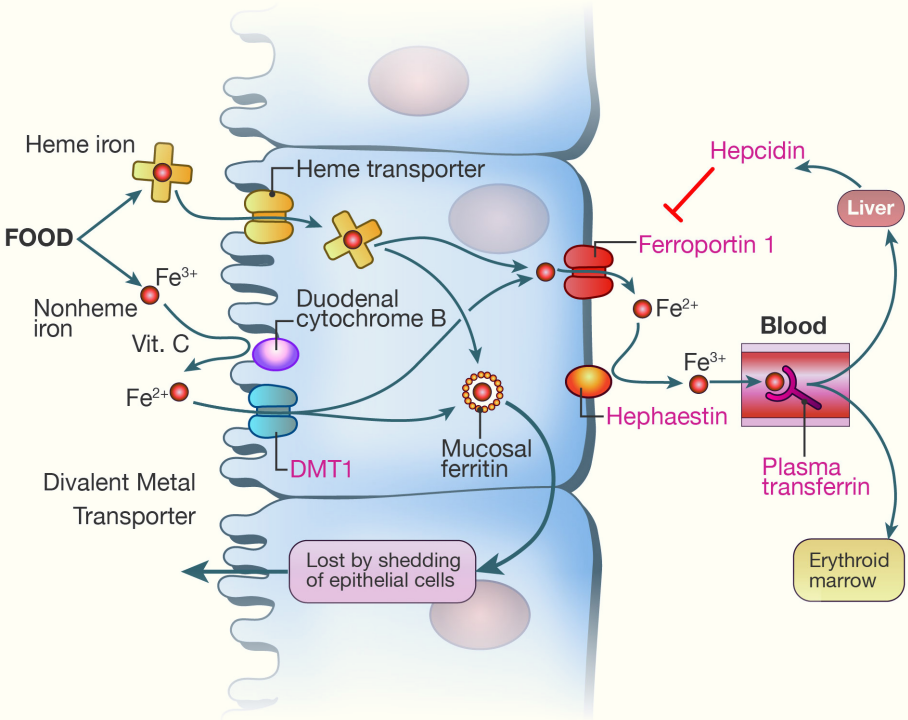
Copper deficiency :

	Wilson's disease	Menke's disease/Kinky or steely hair syndrome
Etiology	Mutation in ATP7B gene : Defective Cu transport (Cu accumulates in tissues)	Mutation in ATP7A : • α-linked recessive • Defect in Cu transporter in intestine
Features	• Kayser Fleischer rings seen • Assessment : – ↓s. ceruloplasmin – Liver copper assay (Gold standard) – ↓3-methyl histidine excretion in urine – 24h urine copper	Enzymes affected : • Xanthine oxidase • Lysyl oxidase (Collagen affected) • Tyrosinase (Depigmentation)

----- Active space -----

Iron

Absorption :



Selenium, Zinc & Chromium :

	Selenium	Zinc	Chromium
Functions	• Anti-oxidant • Selenocysteine containing enzymes : Glutathione peroxidase.	• Metalloenzymes • Stabilise human insulin • Spermatogenesis	Potentiates action of insulin in impaired glucose tolerance.
Deficiency	• Keshan disease (Seen in China) : Endemic cardiomyopathy (D/t dietary deficiency) • Kashinbeck disease : Chronic joint disorder (Also d/t ↓ iodine levels)	• Hypogeusia : ↓ Taste. • Acrodermatitis enteropathica : diarrhoea + perioral & perineal rashes.	-

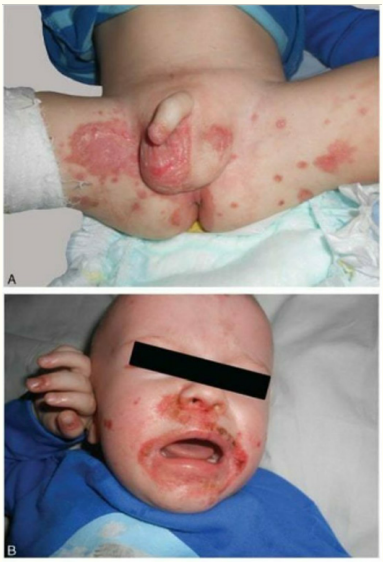
Note :

- Highest concentration of Zn : Hippocampus & prostate.
- Chromium 6 (Hexavalent) ⟶ Pulmonary carcinogen (Stainless steel welding).

----- Active space -----

RDA of minerals :

Mineral	RDA
Calcium	• Adult : 0.5 g • Children : 1 g • Pregnancy : 1.5 g
Iron	• Males : 15–20 mg • Females : 20–25mg • Pregnancy : 40–50 mg
Iodine	• Males : 150–200 µg • Females : 200–250 µg
Zinc	8–10 mg
Selenium	50–200 µg
Copper	1.5–3 mg
Sodium	5–10 g
Potassium	3–4 g



Acrodermatitis enteropathica :
Perioral & perianal rashes

Miscellaneous Topics on Nutrition

01:15:09

Energy available from macronutrients (in KCal/g) :

- Carbohydrate : 4.
- Protein : 4.
- Fat : 9.
- Alcohol : 7.

Specific dynamic action (Thermic effect of food) :

Energy used for digestion of absorption of food :

- Carbohydrate : 5.
- Protein : 30 (max).
- Lipids : 15.
- Mixed food : 10.

Respiratory quotient :

RQ = Co_2 exhaled/O_2 consumed :

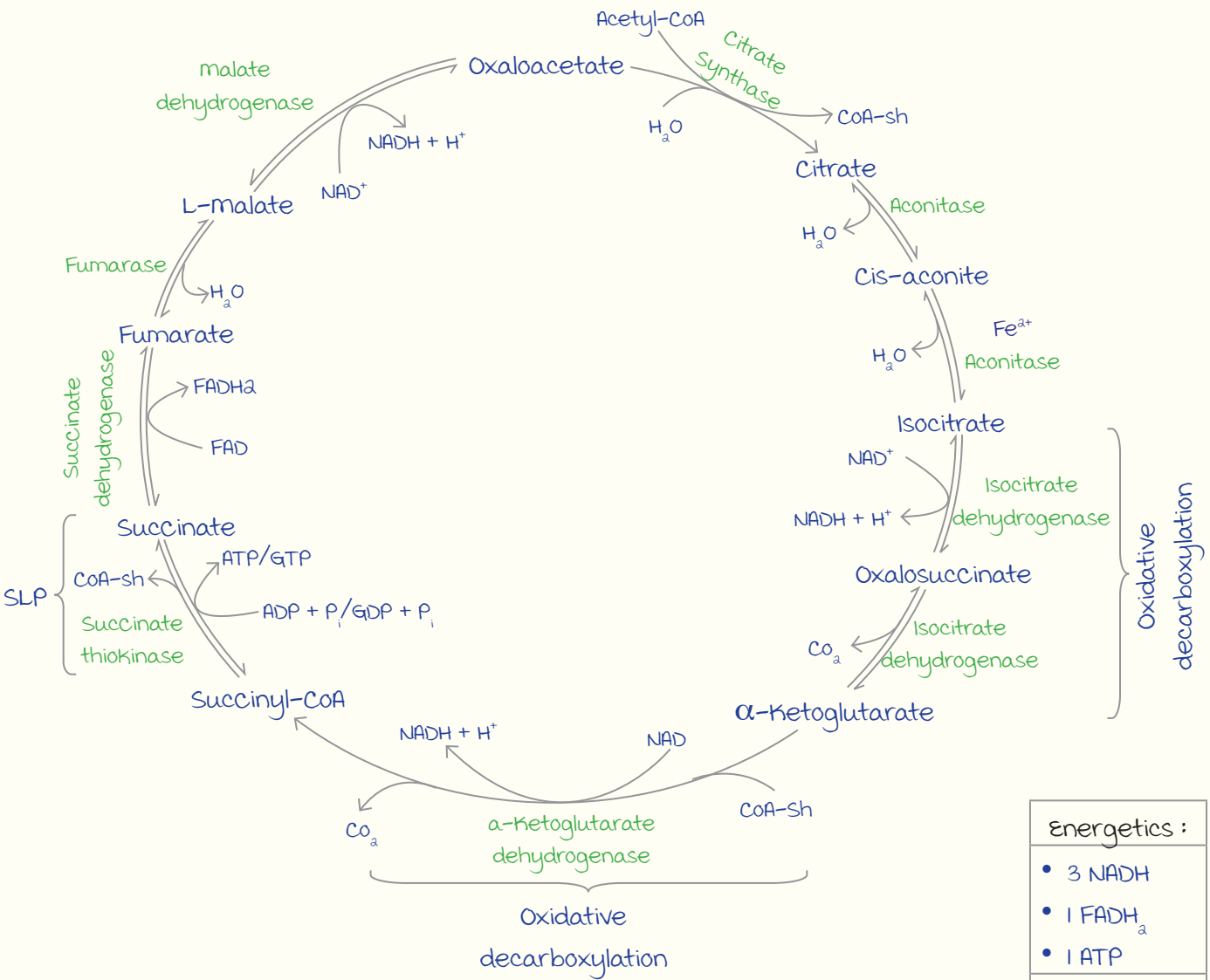
- Carb : 1
- Protein : 0.81
- Lipids : 0.71.
- Alcohol : 0.66.

----- Active space -----

MISCELLANEOUS TOPICS IN BIOCHEMISTRY

TCA Cycle

00:00:10



Energetics :
• 3 NADH
• 1 $FADH_2$
• 1 ATP
Total : 10 ATP

Features :

- Amphibolic pathway.
- Final common oxidative pathway of lipids, carbohydrates & proteins.
- Acetyl CoA : Completely oxidised.
- Unidirectional steps :
  - Citrate synthase.
  - α ketoglutarate dehydrogenase.

- SLP : Succinate thiokinase.
- Anaplerotic reaction : Filling up of intermediates of TCA cycle.
  - Pyruvate carboxylase ← (Allosteric activator) Acetyl CoA.

----- Active space -----

Vitamins Involved :

- Pantothenic acid (CoA).
- Thiamine (α-KGDH).
- Riboflavin (FAD).
- Niacin (NAD^+).

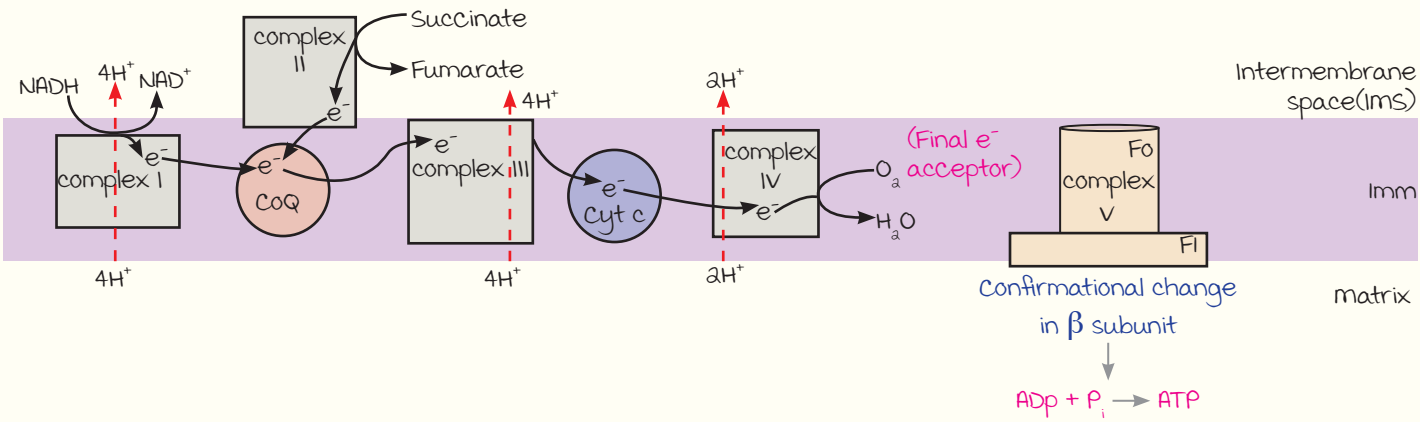
Inhibitors :

Inhibitor	Enzyme
Fluoroacetate	Aconitase
Arsenate	α ketoglutarate dehydrogenase
Malonate	Succinate dehydrogenase

Electron Transport Chain (ETC)

00:06:00

Series of redox couples seen in inner mitochondrial membrane.



Complexes :

Complex	Name	Protons pumped	Inhibitors
Complex I	NADH Q oxido-reductase	$4H^+$	1. Rotenone 2. Amobarbital 3. Piericidin A
Complex II	Succinate Q oxido-reductase	None	1. Malonate 2. Carboxin
Complex III	Q cytochrome c oxido-reductase	$4H^+$	1. Antimycin A 2. BAL (Dimercaprol)
Complex IV	Cytochrome c oxidase	$2H^+$	1. CO 2. Cyanide 3. H_2S
Complex V	ATP synthase	None	Inhibitors of oxidative phosphorylation : 1. Atractyloside : ADP/ATP transporter 2. Oligomycin : F_o complex 3. Venturicidin

----- Active space -----

Uncouplers of Oxidative Phosphorylation :

Chemical :

1. 2, 4 Dinitrophenol.
2. Dinitrocresol.
3. FCCP.
4. High dose aspirin.

Physiological :

1. Thermogenin (Uncoupling protein I).
2. Thyroxine.
3. Long chain fatty acid.
4. Ionophores : Valinomycin, gramicidin.

Note : High energy compounds → ATP, 1-3-Bisphosphoglycerate, Phosphoenol pyruvate, Acetyl CoA, Succinyl CoA, Phospho creatine, Phosphoarginine.

Heme Metabolism

00:16:16

Heme containing proteins :

1. Hemoglobin.
2. Myoglobin.
3. Cytochrome c.
4. Cytochrome P450.
5. Catalase.
6. Tryptophan pyrrolase.
7. Nitric oxide synthase.

HEME SYNTHESIS

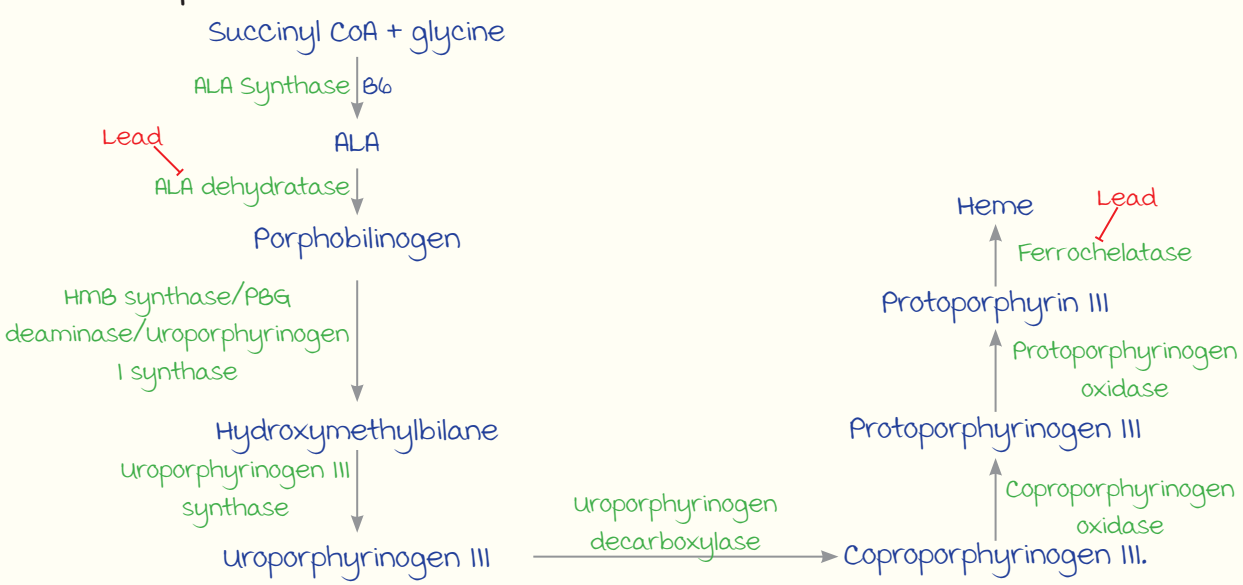
Site :

Liver, erythrocyte precursors.

Organelle :

Partly cytoplasmic & partly mitochondrial.

Steps :



----- Active space -----

Factors Affecting :

Drugs : Drugs utilizing cytochrome → ↓Heme synthesis → Aggravate porphyria.

Lead Poisoning :

- Inhibits : ALA dehydratare, Ferrochelatase.
- H/o : Occupational exposure (Paints), children playing with painted toys.
- C/f : Abdominal pain.
- Biomarkers :
  - Urinary ALA.
  - Coproporphyrin.
  - Protoporphyrin.

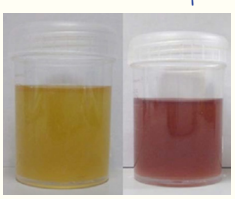
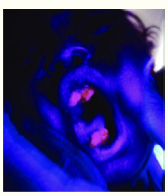
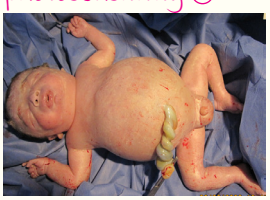
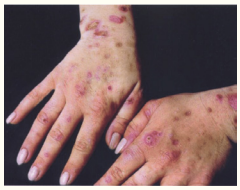
INH : ↓Vitamin B6 → ↓Activity of ALA synthase → ↓Heme.

Porphyrias :

Mode of inheritance : m/c is autosomal dominant except

- Congenital Erythropoietic Porphyria (CEP).
- ALAD enzyme deficiency (ADP).
- Erythropoeitic Protoporphyria (EPP).
- X-Linked Protoporphyria (XLP).

Types :

Porphyria	Defective enzyme	C/F
ADP	ALA dehydratase	-
Acute intermittent porphyria (m/c acutely)	HMB synthase ↓ Accumulation of : • Porphobilinogen • ALA	Neuro visceral manifestations (m/c : Abdominal pain) 
Congenital Erythropoietic porphyria (CEP)	Uroporphyrinogen III synthase	Cutaneous photosensitivity ⊕  Erythrodontia  Non-immune Hydrops fetalis
Porphyria cutanea tarda (m/c)	Uroporphyrinogen decarboxylase	 Cutaneous photosensitivity : Blisters ⊕

----- Active space -----

Porphyria	Defective enzyme	C/F
Erythropoeitic protoporphyria (m/c in children)	Ferrochelatase	Non- blistering photosensitivity
Hereditary coproporphyria	Coproporphyrinogen oxidase	⊖
Variegate porphyria	Protoporphyrinogen oxidase	⊖

Diagnosis :

1. Ehrlich test : Non-specific → Pink : Urobilinogen (UBG).
   → Red : Porphobilinogen (PBG).
2. Hoesch test.
3. Watson Schwartz test : Differentiates b/w UBG & PBG.
4. Soret band at 400 nm.
5. Wood's lamp : Red fluorescence.

Note : Ehrilch's test ⊕ in hemolytic jaundice also.

Differentiation of Jaundice :

Test	Prehepatic	Hepatic	Obstructive
Direct bilirubin (Conjugated)	Normal	Normal/↑	↑
Indirect bilirubin (Unconjugated)	↑	Normal/↑	Normal
Urine bile salt	⊖	+/-	⊕
Urine bile pigment	⊖	+/-	⊕
Urine UBG	⊕	+/-	⊖
ALT	Normal	↑↑	Normal
AST	Normal	↑↑	Normal
ALP	Normal	↑	↑↑↑