B.PHARM SEMESTER II | BP202T

BIOCHEMISTRY (THEORY)

UNIT I

Enzymology and Clinical Chemistry

Short Notes for Quick Revision

What is covered in this unit

UNIT I TOPICS

Part A – Enzymology: introduction, properties, nomenclature, IUB classification, cofactors and coenzymes, enzyme kinetics, inhibitors, regulation and factors affecting activity.

Part B – Clinical enzymology and nutrition: therapeutic and diagnostic enzymes, isoenzymes, digestion and absorption of macronutrients, minerals and vitamins.

Clinical chemistry: liver function tests, renal function tests and ELISA.

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PART A: ENZYMOLOGY

1. Introduction to Enzymes

Enzymes are biological catalysts, almost all of them proteins (the exceptions are catalytic RNAs called **ribozymes**). They speed up chemical reactions by 10^6 to 10^{12} times without being consumed or altering the equilibrium. The term was introduced by **Kuhne (1878)**; **Buchner (1897)** showed that cell-free yeast extract can ferment sugar; **Sumner (1926)** crystallised urease and proved enzymes are proteins.

Important terms

Term	Meaning
Substrate	Molecule on which the enzyme acts; converted to product
Active site	Cleft or pocket formed by folding of the chain, containing the **binding site** and the **catalytic site**
Apoenzyme + cofactor	Protein part plus non-protein part = **holoenzyme** (active)
Zymogen (proenzyme)	Inactive precursor activated by partial proteolysis: pepsinogen, trypsinogen, chymotrypsinogen, procarboxypeptidase, clotting factors
Isoenzymes	Different molecular forms of an enzyme catalysing the same reaction (e.g. LDH1–5, CK-MM/MB/BB)
Ribozyme / abzyme	Catalytic RNA (RNase P, peptidyl transferase) / catalytic antibody
Allosteric enzyme	Has a regulatory site apart from the active site; shows sigmoidal kinetics

Mechanism of enzyme action

- Enzymes **lower the activation energy (Ea)**, the energy barrier between substrate and the transition state. They do not change ΔG or the equilibrium constant.
- **Enzyme–substrate complex:** E + S ⇌ ES → E + P (Michaelis–Menten concept).
- **Fischer's lock-and-key model (1894):** rigid active site that exactly fits the substrate.
- **Koshland's induced-fit model (1958):** substrate binding changes the shape of the active site for a precise fit; the accepted model.
- **Catalytic strategies:** acid–base catalysis, covalent catalysis, proximity and orientation effect, strain/distortion of substrate, metal-ion catalysis.

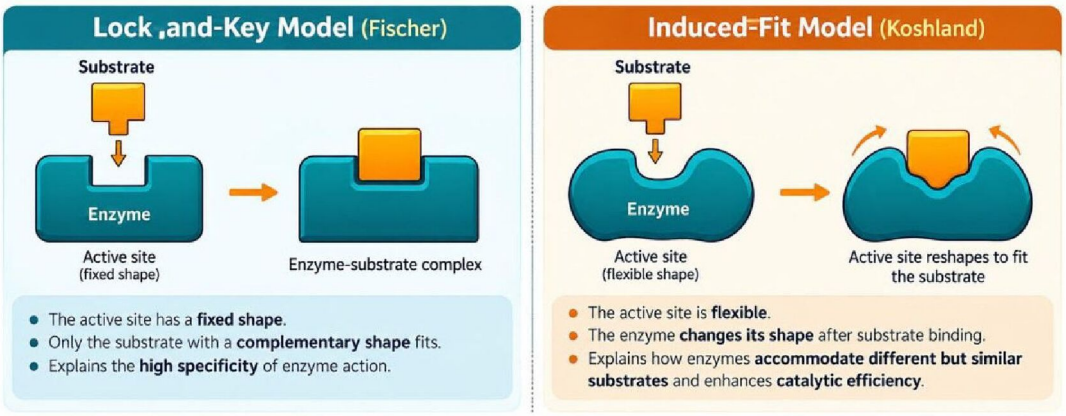

Figure 1: Figure 1: Lock-and-key and induced-fit models of enzyme action

2. Properties of Enzymes

Property	Explanation
Protein nature	Denatured by heat, strong acids/alkalis, heavy metals, alcohol; non-diffusible, precipitated by ammonium sulphate

Property	Explanation
Catalytic efficiency	Catalase converts millions of H_2O_2 molecules per minute; required only in small amounts
Specificity	**Absolute** (urease, glucokinase); **group** (hexokinase, pepsin); **linkage** (lipase, glycosidase); **stereospecific** (L-amino acid oxidase, D-sugar enzymes)
Active site	Small three-dimensional region (about 10–20 amino acids) responsible for binding and catalysis
Reversibility	Catalyse forward and reverse reactions; direction decided by concentrations
Sensitivity	Activity depends on temperature, pH, substrate and enzyme concentration, inhibitors
Regulation	Controlled by feedback, covalent modification, allosteric effectors, gene expression
Not consumed	Remain unchanged at the end; unchanged equilibrium, faster rate

Units of enzyme activity

Term	Definition
International Unit (IU)	Amount of enzyme that converts **1 µmol of substrate per minute** under optimum conditions
Katal (kat)	SI unit: amount converting **1 mol of substrate per second** (1 IU = 16.67 nkat)
Specific activity	Units of enzyme per mg of protein; rises as the enzyme is purified
Molecular activity (turnover number)	Molecules of substrate converted per molecule of enzyme per minute

Enzyme assay and purification (outline)

- **Assay:** measure rate of product formation or substrate disappearance (often absorbance of NADH at 340 nm) under **zero-order** conditions, fixed temperature, pH and excess substrate.
- **Purification steps:** homogenisation → centrifugation → salting out (ammonium sulphate) → dialysis → ion-exchange, gel-filtration and affinity chromatography → electrophoresis; purity checked by specific activity.

3. Nomenclature of Enzymes

- **Trivial names:** older names without a rule (pepsin, trypsin, ptyalin, renin).
- **Recommended names:** substrate name + suffix **-ase** (urease, lipase, lactase) or reaction type (dehydrogenase, transaminase).
- **Systematic (IUB) name:** substrate(s) : reaction type + ase, e.g. **lactate : NAD^+ oxidoreductase**. Each enzyme also has an **EC number** of four digits.
- **Example:** EC 2.7.1.1 = hexokinase (2 = transferase; 7 = phosphate group transfer; 1 = alcohol acceptor; 1 = serial number).

4. IUB Classification of Enzymes

The International Union of Biochemistry (IUB, 1961; Enzyme Commission) divided enzymes into **six classes** on the basis of reaction type.

Class	Reaction type	Examples
1. Oxidoreductases	Oxidation–reduction (electron/hydrogen transfer)	Lactate dehydrogenase, alcohol dehydrogenase, catalase, cytochrome oxidase, glucose oxidase
2. Transferases	Transfer of a group (amino, phosphate, methyl)	AST, ALT, hexokinase, creatine kinase, COMT
3. Hydrolases	Cleavage of bonds by addition of water	Lipase, amylase, trypsin, pepsin, ALP, urease, acetylcholinesterase

Class	Reaction type	Examples
4. Lyases	Non-hydrolytic removal of a group; double bond formation	Aldolase, fumarase, enolase, carbonic anhydrase, decarboxylases
5. Isomerases	Intramolecular rearrangement	Phosphohexose isomerase, racemases, epimerases, mutases
6. Ligases (synthetases)	Joining of two molecules using ATP	DNA ligase, pyruvate carboxylase, glutamine synthetase, acetyl-CoA carboxylase

MEMORY AID

OTHLIL: Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases, Ligases (classes 1 to 6).

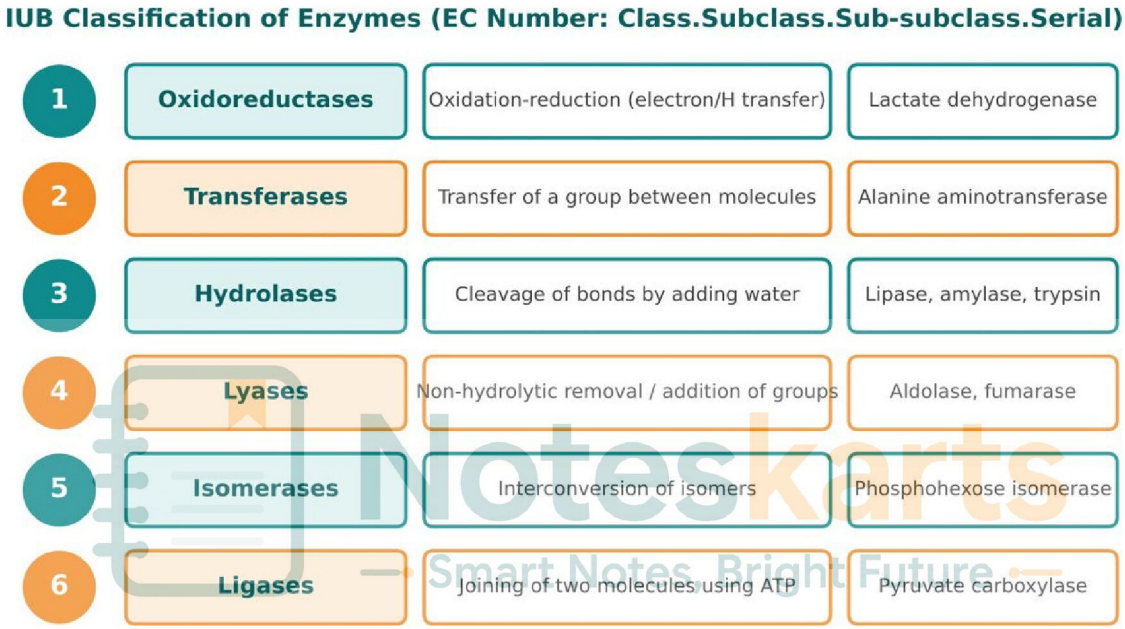

Figure 2: Figure 2: IUB classification of enzymes with examples

5. Cofactors and Coenzymes

Many enzymes need a non-protein partner. **Cofactors** are metal ions or organic molecules; an organic cofactor is a **coenzyme**. A firmly bound coenzyme is a **prosthetic group** (e.g. FAD, heme, biotin); a loosely bound one acts as a **co-substrate** (NAD^+).

Coenzyme	Vitamin source	Group transferred / role	Example enzyme
NAD^+ / $NADP^+$	Niacin (B_3)	Hydrogen and electrons	LDH, G6PD
FAD / FMN	Riboflavin (B_2)	Hydrogen and electrons	Succinate dehydrogenase
Thiamine pyrophosphate	Thiamine (B_1)	Aldehyde (decarboxylation)	Pyruvate dehydrogenase, transketolase
Coenzyme A	Pantothenic acid (B_5)	Acyl groups	Acetyl-CoA formation
Pyridoxal phosphate	Pyridoxine (B_6)	Amino groups	Transaminases, decarboxylases
Biotin	Biotin (B_7)	CO_2 (carboxylation)	Pyruvate carboxylase
Tetrahydrofolate	Folic acid	One-carbon units	Thymidylate synthase
Methylcobalamin	Vitamin B_{12}	Methyl groups, isomerisation	Methionine synthase
Lipoic acid	Not a vitamin (made in body)	Acyl and hydrogen transfer	Pyruvate dehydrogenase

Coenzyme	Vitamin source	Group transferred / role	Example enzyme
Vitamin K	Vitamin K	γ-Carboxylation of glutamate	Clotting factors II, VII, IX, X

- **Metal cofactors (metalloenzymes / metal-activated):** Zn^{2+} (carbonic anhydrase, alcohol dehydrogenase), Mg^{2+} (kinases), Fe^{2+}/Fe^{3+} (catalase, cytochromes), Cu^{2+} (cytochrome oxidase), Mn^{2+}, Mo (xanthine oxidase), Se (glutathione peroxidase), K^{+} (pyruvate kinase).

6. Enzyme Kinetics

Factors affecting reaction velocity

Velocity (v) rises with substrate concentration [S] in a **hyperbolic curve**: first-order at low [S] (v proportional to [S]), mixed order in the middle, and **zero-order** at high [S] when all active sites are saturated and v reaches **Vmax**.

Michaelis–Menten equation

MICHAELIS–MENTEN EQUATION

v = Vmax [S] / (Km + [S])

Km (Michaelis constant) = substrate concentration at which v = ½ Vmax. Units: mol/L.

Derived from $E + S \rightleftharpoons ES \rightarrow E + P$, assuming a **steady state** of ES. **$Km = (k_{-1} + k_2) / k_1$.**

- **Low Km = high affinity** for substrate (glucokinase has high Km ≈ 10 mM; hexokinase low Km ≈ 0.1 mM).
- Km is a characteristic of the enzyme–substrate pair; independent of enzyme concentration. Vmax is proportional to enzyme concentration.
- **Turnover number (kcat)** = substrate molecules converted per enzyme molecule per second; **kcat/Km** measures catalytic efficiency.

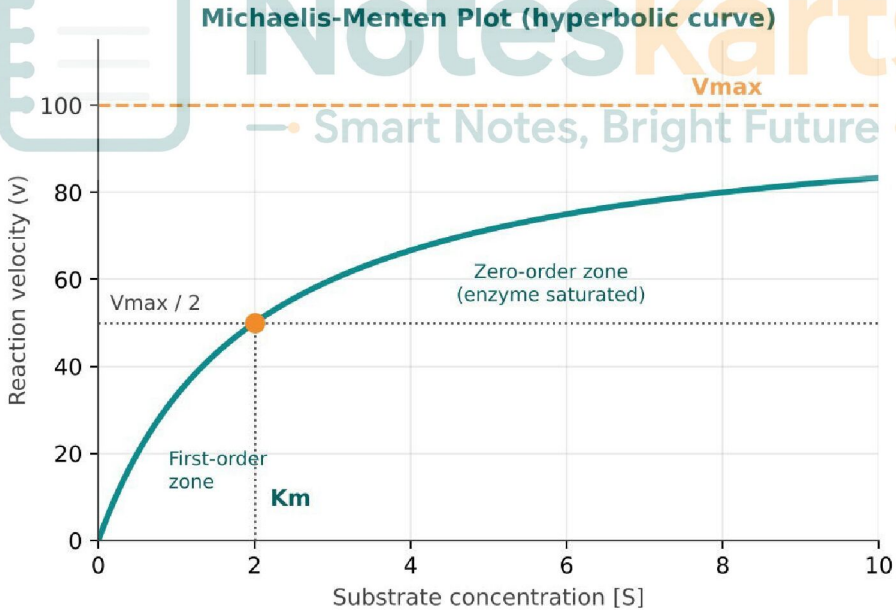

Figure 3: Figure 3: Michaelis–Menten plot (hyperbolic curve)

Lineweaver–Burk (double reciprocal) plot

Taking reciprocals gives a straight line: **1/v = (Km/Vmax)(1/[S]) + 1/Vmax**. Plot 1/v (y-axis) against 1/[S] (x-axis).

Intercept / slope	Value
y-intercept	**1/Vmax**
x-intercept	**−1/Km**
Slope	**Km/Vmax**

The plot gives accurate Km and Vmax and is used to identify the **type of inhibition**.

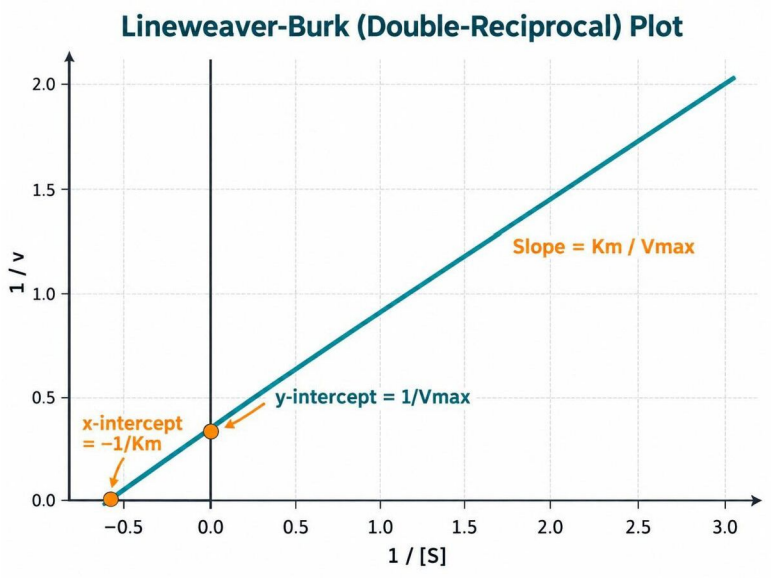

Figure 4: Figure 4: Lineweaver–Burk (double-reciprocal) plot

7. Enzyme Inhibitors

Inhibitors reduce enzyme activity. They may be **reversible** (non-covalent, removable) or **irreversible** (covalent, permanent). Many drugs act as enzyme inhibitors.

Feature	Competitive	Non-competitive	Uncompetitive
Binds to	Free enzyme at the **active site**	Enzyme or ES complex at a **different site**	Only the **ES complex**
Resembles substrate	Yes (structural analogue)	No	No
Vmax	**Unchanged**	**Decreased**	**Decreased**
Km	**Increased**	**Unchanged**	**Decreased**
Reversed by more substrate	Yes	No	No
Lineweaver–Burk	Lines meet on y-axis	Lines meet on x-axis	Parallel lines
Examples	Malonate on succinate dehydrogenase; **statins** (HMG-CoA reductase); methotrexate (DHFR); sulfonamides; captopril (ACE)	Heavy metals (Hg^{2+}, Pb^{2+}) on –SH enzymes; cyanide on cytochrome oxidase	Lithium on inositol phosphatase; phenylalanine on placental ALP

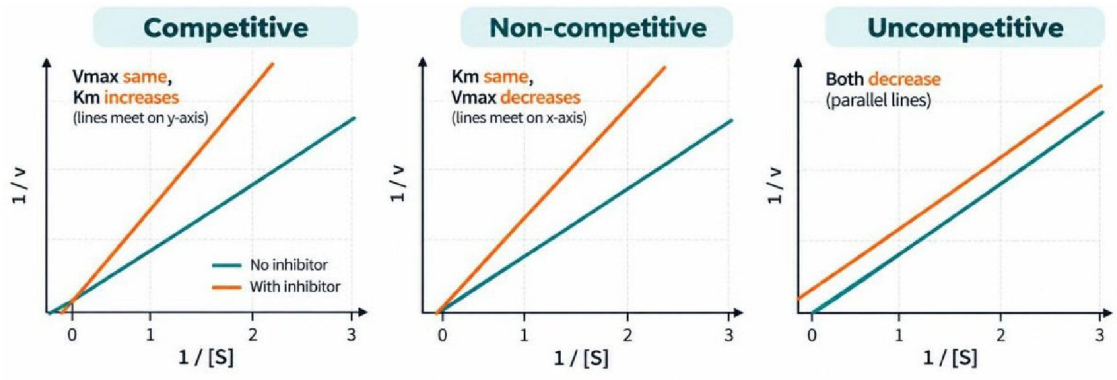

Figure 5: Figure 5: Lineweaver–Burk plots of the three types of reversible inhibition

Irreversible inhibition and suicide inhibitors

- **Irreversible:** organophosphates (DFP, malathion, nerve gases) bind serine of acetylcholinesterase; iodoacetate blocks –SH; **aspirin** acetylates cyclooxygenase; **penicillin** inhibits transpeptidase.
- **Suicide (mechanism-based) inhibitors:** substrate analogue converted by the enzyme into a reactive form that inactivates it, e.g. **allopurinol** → alloxanthine on xanthine oxidase; 5-fluorouracil → thymidylate synthase; disulfiram → aldehyde dehydrogenase.
- **Feedback inhibition:** end product inhibits the first enzyme of its own pathway.

Enzyme inhibitors as drugs

Drug	Enzyme inhibited	Type	Use
Statins (atorvastatin)	HMG-CoA reductase	Competitive	Hypercholesterolaemia
Allopurinol	Xanthine oxidase	Suicide	Gout
Captopril, enalapril	Angiotensin-converting enzyme	Competitive	Hypertension
Methotrexate	Dihydrofolate reductase	Competitive	Cancer, rheumatoid arthritis
Sulfonamides	Dihydropteroate synthase (PABA analogue)	Competitive	Antibacterial
Aspirin	Cyclooxygenase	Irreversible (acetylation)	Analgesic, antiplatelet
Penicillins	Bacterial transpeptidase	Irreversible	Antibacterial
Neostigmine, physostigmine	Acetylcholinesterase	Reversible	Myasthenia gravis
Organophosphates	Acetylcholinesterase	Irreversible	Insecticides, poisons (antidote: pralidoxime)
Acetazolamide	Carbonic anhydrase	Non-competitive	Glaucoma, altitude sickness
Disulfiram	Aldehyde dehydrogenase	Irreversible	Alcohol aversion
Fomepizole	Alcohol dehydrogenase	Competitive	Methanol and ethylene glycol poisoning

8. Regulation of Enzyme Activity

Mechanism	Features	Examples
Enzyme induction and repression	Change in the **amount** of enzyme by gene expression (slow: hours); inducer increases and repressor decreases synthesis	Lac operon (lactose induces β-galactosidase); insulin induces glucokinase; glucose represses lactose enzymes
Allosteric regulation	Effector binds a regulatory site; changes conformation (T ⇌ R). **Sigmoidal** v–[S] curve; positive/negative modulators; usually multi-subunit; rapid	PFK-1 (ATP, citrate inhibit; AMP, F-2,6-BP activate); ACC; aspartate transcarbamoylase
Covalent modification	Reversible phosphorylation/dephosphorylation (kinases, phosphatases); adenylation, methylation, ADP-ribosylation	Glycogen phosphorylase (active when phosphorylated); glycogen synthase (inactive when phosphorylated)
Proteolytic activation	Irreversible cleavage of zymogen	Pepsinogen → pepsin; trypsinogen → trypsin by enteropeptidase; clotting cascade
Feedback control	Product inhibits early enzyme	CTP on aspartate transcarbamoylase; cholesterol on HMG-CoA reductase
Compartmentation / isoenzymes	Enzymes confined to organelles; tissue-specific forms	Fatty acid synthesis (cytosol) vs β-oxidation (mitochondria)

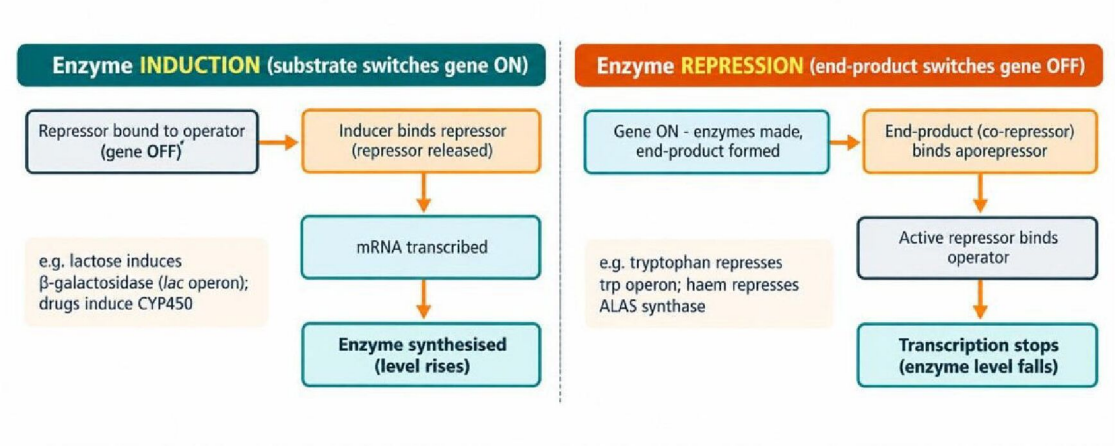

Figure 6: Figure 6: Enzyme induction and repression

Allosteric models

- **Concerted (Monod–Wyman–Changeux) model:** all subunits switch together between tense (T, low affinity) and relaxed (R, high affinity) states.
- **Sequential (Koshland–Némethy–Filmer) model:** subunits change shape one at a time after substrate binding.
- **Cooperativity:** binding of one substrate molecule increases (positive) the affinity of other sites, giving the sigmoidal curve; the **Hill coefficient** (n > 1) measures it. Haemoglobin is the classic example.
- **K-type** allosteric enzymes change Km; **V-type** change Vmax.

Key regulatory (rate-limiting) enzymes

Pathway	Regulatory enzyme	Main control
Glycolysis	**Phosphofructokinase-1**	Allosteric: ATP and citrate inhibit; AMP and fructose-2,6-bisphosphate activate
Gluconeogenesis	Fructose-1,6-bisphosphatase	Inhibited by AMP and F-2,6-BP; induced by glucagon
Glycogenolysis	Glycogen phosphorylase	Covalent (phosphorylation by cAMP-dependent cascade), allosteric (AMP, Ca^{2+})
Cholesterol synthesis	HMG-CoA reductase	Feedback, covalent (AMPK), induced by insulin, inhibited by statins
Fatty acid synthesis	Acetyl-CoA carboxylase	Citrate activates; palmitoyl-CoA and phosphorylation inhibit
Urea cycle	CPS-I	Activated allosterically by N-acetylglutamate
Heme synthesis	ALA synthase	Heme represses; induced by drugs

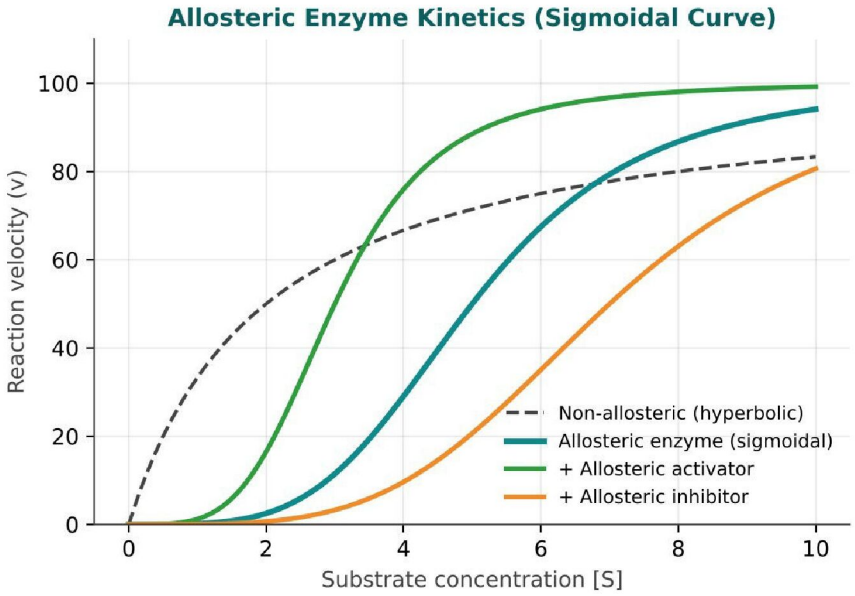

Figure 7: Figure 7: Allosteric enzyme kinetics (sigmoidal curve)

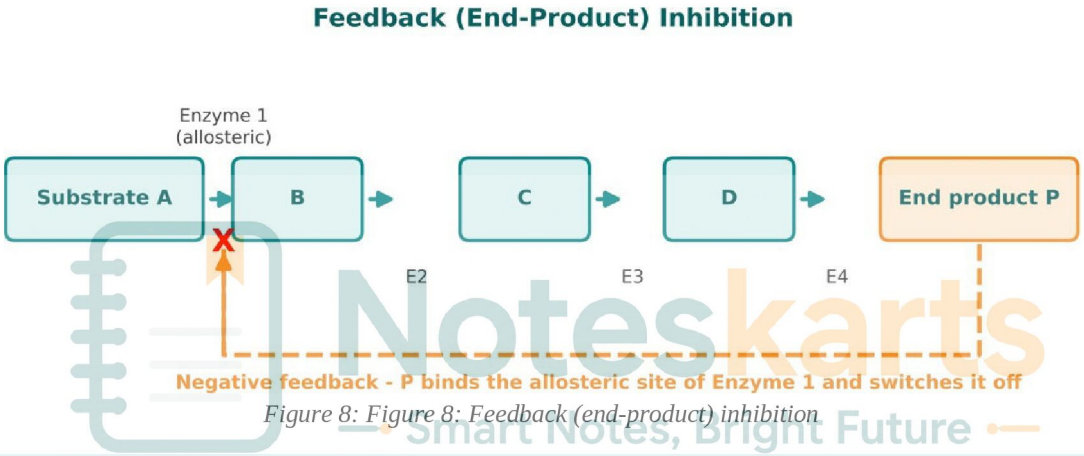

Figure 8: Figure 8: Feedback (end-product) inhibition

9. Factors Affecting Enzyme Activity

Factor	Effect
Enzyme concentration	Velocity is directly proportional to enzyme concentration when substrate is in excess
Substrate concentration	Hyperbolic curve to Vmax (Michaelis–Menten)
Temperature	Rate roughly doubles for every 10 °C rise (Q_{10} = 2) up to the **optimum** (about 37 °C for human enzymes); above 50–60 °C denaturation destroys activity. Bell-shaped curve; Taq polymerase optimum is 72 °C
pH	Each enzyme has an optimum: pepsin 1.5–2, salivary amylase 6.8, trypsin 8, alkaline phosphatase 9–10; extremes denature and alter ionisation of the active site (bell-shaped curve)
Product concentration	Accumulation slows or reverses the reaction
Activators	Cl^- for salivary amylase; Mg^{2+} for kinases; Ca^{2+} for lipase; bile salts for pancreatic lipase
Inhibitors	Competitive, non-competitive, uncompetitive, irreversible (see section 7)
Time and radiation	Prolonged incubation and UV/X-rays inactivate enzymes

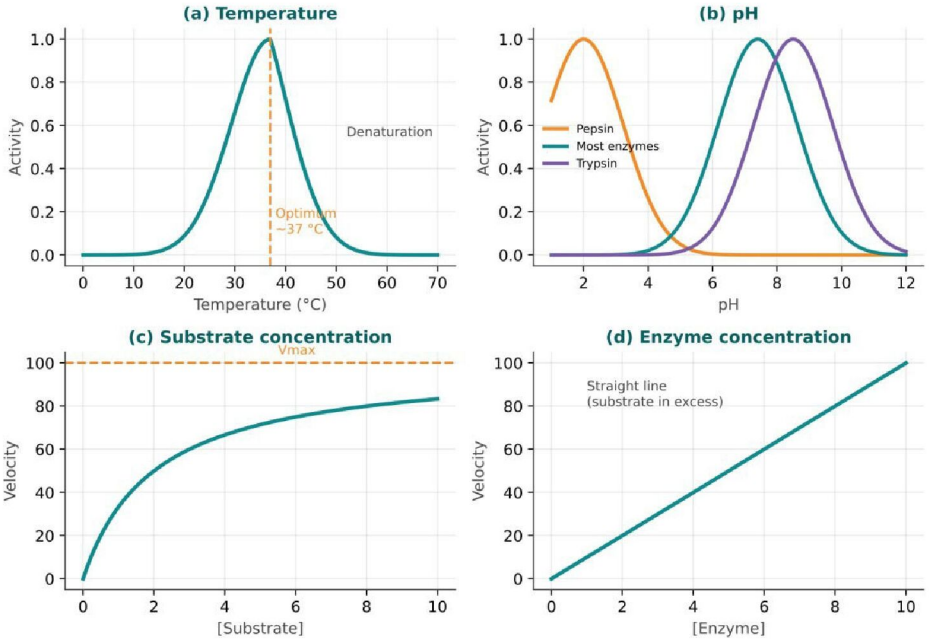

Figure 9: Figure 9: Effect of temperature, pH, substrate and enzyme concentration on velocity

PART B: CLINICAL ENZYMOLOGY AND NUTRITION

10. Therapeutic and Diagnostic Enzymes; Isoenzymes

Diagnostic enzymes

Enzymes normally act inside cells. Cell damage releases them into blood, and their rise in serum helps diagnose disease (**functional plasma enzymes** like clotting factors are made for the plasma; **non-functional** ones leak from tissues).

Enzyme	Raised in	Remarks
AST (SGOT)	Myocardial infarction, liver disease, muscle injury	Rises 6–8 h after MI, peaks 24 h, normal by 4–5 days
ALT (SGPT)	Viral hepatitis, hepatocellular damage	More specific for liver; ALT > AST in viral hepatitis, AST > ALT in alcoholic hepatitis (ratio > 2)
ALP	Obstructive jaundice, bone disease (rickets, Paget's, osteomalacia), hyperparathyroidism	Normal 3–13 KA units; 40–120 U/L
Acid phosphatase (prostatic)	Carcinoma prostate with metastasis	Replaced by PSA
γ-Glutamyl transferase (GGT)	Alcoholism, obstructive liver disease	Sensitive marker of alcohol abuse
Amylase and lipase	Acute pancreatitis	Amylase rises in 2–12 h; lipase more specific
Creatine kinase (CK-MB)	Myocardial infarction	Rises 4–8 h, peaks 24 h, falls in 48–72 h
LDH	MI, haemolysis, liver and muscle disease	LDH1 > LDH2 (flipped pattern) in MI
Cholinesterase (serum)	Decreased in organophosphate poisoning and liver disease	Used to monitor pesticide exposure
5′-Nucleotidase	Hepatobiliary disease	Not raised in bone disease, so differentiates source of raised ALP
Troponin T / I (not enzyme)	MI	Rises 3–6 h, stays 7–14 days; the gold-standard marker

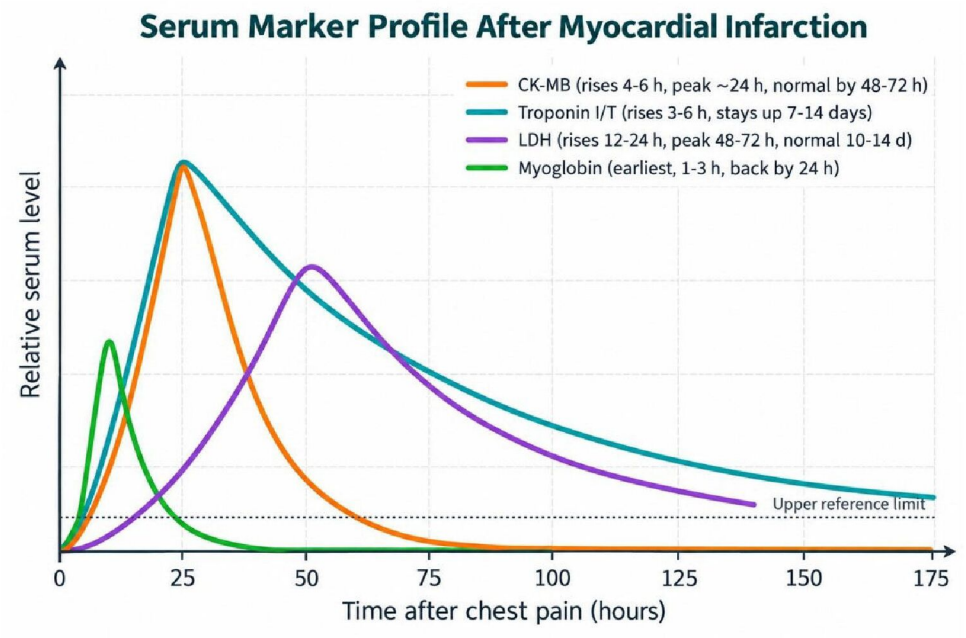

Figure 10: Figure 10: Serum marker profile after myocardial infarction

Cardiac markers in myocardial infarction

Marker	Starts rising	Peak	Returns to normal	Comment
Myoglobin	1–3 h	6–9 h	24 h	Earliest but non-specific
Troponin I / T	3–6 h	12–24 h	7–14 days	Most sensitive and specific; detects late presenters
CK-MB	4–8 h	24 h	48–72 h	Useful for detecting **re-infarction**
AST	6–8 h	24–48 h	4–5 days	Non-specific, now obsolete
LDH	12–24 h	48–72 h	10–14 days	LDH1:LDH2 ratio > 1 (flipped)

Enzyme patterns in other diseases

- **Acute pancreatitis:** serum amylase and lipase up 3–10 times; urinary amylase raised for longer.
- **Skeletal muscle disease (Duchenne dystrophy):** CK-MM, aldolase and AST rise; CK normal in neurogenic atrophy.
- **Bone disease:** ALP raised with active osteoblasts (rickets, osteomalacia, Paget's disease, bone metastasis).
- **Cancer:** PSA/acid phosphatase (prostate), LDH (lymphoma, germ-cell tumours), ALP (bone metastasis), CEA and AFP as tumour markers.
- **Inborn errors:** deficiency of one enzyme causes disease, e.g. G6PD (haemolysis), phenylalanine hydroxylase (PKU), glucose-6-phosphatase (von Gierke), hexosaminidase A (Tay–Sachs), glucocerebrosidase (Gaucher).
- **Precautions for sampling:** avoid haemolysis (raises AST, LDH, K^+); fasting sample for lipids and glucose; prompt separation of serum.

Isoenzymes

Isoenzyme	Composition and tissue	Clinical use
LDH (tetramer of H and M)	LDH1 (H_4) heart, RBC; LDH2 (H_3M) RBC; LDH3 lungs; LDH4 kidney; LDH5 (M_4) liver, skeletal muscle	LDH1 > LDH2 in MI; LDH5 raised in liver disease
CK (dimer of M and B)	CK-MM skeletal muscle; **CK-MB heart**; CK-BB brain	CK-MB for MI; CK-MM in muscular dystrophy

Isoenzyme	Composition and tissue	Clinical use
ALP	Liver, bone, intestine, placenta isoforms	Heat-stable placental form; Regan isoenzyme in cancer
Amylase	Pancreatic (P-type) and salivary (S-type)	Differentiates pancreatitis from parotitis

Therapeutic enzymes

Enzyme	Use
Streptokinase, urokinase, alteplase (tPA)	Thrombolysis in MI, stroke, pulmonary embolism
Asparaginase	Acute lymphoblastic leukaemia (depletes asparagine)
Hyaluronidase	Spreading agent that aids drug absorption
Pancreatin, papain, pepsin, diastase	Digestive aids in pancreatic insufficiency
Trypsin, chymotrypsin, serratiopeptidase	Anti-inflammatory, wound debridement
Collagenase, DNase (dornase alfa)	Burns and ulcers; cystic fibrosis mucus
Glucocerebrosidase, α-galactosidase	Enzyme replacement in Gaucher and Fabry disease
Lactase, uricase (rasburicase)	Lactose intolerance; tumour lysis hyperuricaemia

11. Digestion and Absorption of Macronutrients

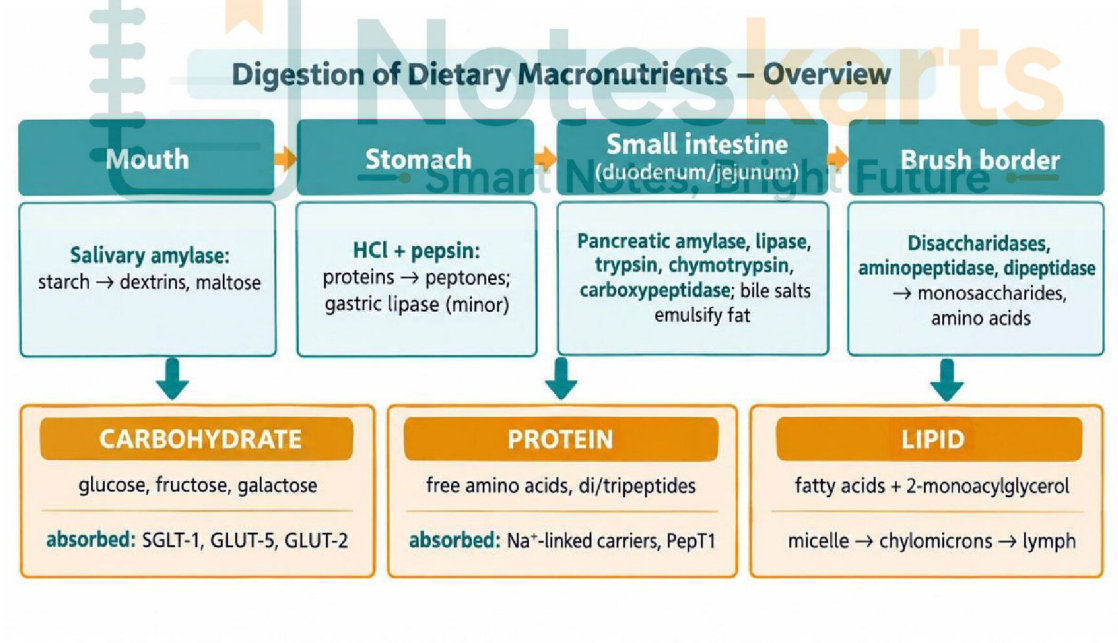

Figure 11: Figure 11: Digestion of dietary macronutrients: overview

Carbohydrates

- **Mouth:** salivary α-amylase (needs Cl^-, optimum pH 6.8) splits starch into dextrins and maltose; inactivated in stomach acid.
- **Stomach:** no carbohydrate digestion. **Small intestine:** pancreatic α-amylase gives maltose, isomaltose and limit dextrins.
- **Brush border enzymes:** maltase, sucrase, isomaltase, lactase (β-galactosidase), trehalase convert disaccharides to **glucose, galactose, fructose**. Lactase deficiency causes lactose intolerance (diarrhoea, gas). Cellulose (fibre) is not digested.

- **Absorption:** glucose and galactose by **SGLT-1** (secondary active transport with Na^+, driven by the Na^+–K^+ ATPase); fructose by **GLUT-5** (facilitated diffusion); exit to blood via **GLUT-2**. Pentoses by simple diffusion.

Proteins

Site	Enzyme (precursor)	Action
Stomach	**Pepsin** (pepsinogen, activated by HCl, pH 1.5–2)	Endopeptidase; splits bonds of aromatic and hydrophobic amino acids; **rennin** (infants) clots milk
Small intestine (pancreatic)	**Trypsin** (trypsinogen, activated by enteropeptidase), **chymotrypsin**, **elastase**	Endopeptidases: trypsin cleaves at Lys/Arg; chymotrypsin at Phe/Tyr/Trp
Small intestine (pancreatic)	**Carboxypeptidase A and B**	Exopeptidases from carboxyl end
Brush border	**Aminopeptidases, dipeptidases**	Release free amino acids and di/tri-peptides

- **Absorption:** free amino acids by Na^+-dependent carriers (at least 6 groups: neutral, basic, acidic, imino, β, glycine); di- and tripeptides by **PepT1** (H^+-coupled). **γ-Glutamyl (Meister) cycle** helps transport. Defects: **Hartnup disease** (neutral amino acids) and **cystinuria** (cystine, ornithine, lysine, arginine – kidney stones).

Lipids

- **Emulsification** by bile salts in the duodenum. **Pancreatic lipase** (with **colipase**) hydrolyses triacylglycerol at positions 1 and 3 to give **2-monoacylglycerol + 2 free fatty acids**. **Cholesterol esterase** and **phospholipase A_2** act on cholesterol esters and phospholipids.
- **Micelles** (bile salts + monoglycerides + fatty acids + fat-soluble vitamins) carry products to the enterocyte. Short and medium chain fatty acids pass directly to portal blood.
- Long chain fatty acids are re-esterified in the enterocyte and packed into **chylomicrons**, which enter lymph (lacteals) then blood via the thoracic duct. Bile salts are reabsorbed in the ileum (**enterohepatic circulation**).
- **Malabsorption:** steatorrhoea in pancreatic insufficiency, obstructive jaundice, coeliac disease.

Summary of digestive enzymes

Source	Enzyme	Substrate	Product
Saliva	α-Amylase	Starch	Dextrins, maltose
Stomach	Pepsin, gastric lipase	Proteins, fats	Peptides, fatty acids
Pancreas	Amylase, lipase, trypsin, chymotrypsin, elastase, carboxypeptidase, nucleases	Starch, fats, proteins, nucleic acids	Maltose, MAG + FFA, small peptides, nucleotides
Intestinal mucosa	Sucrase, maltase, lactase, isomaltase, aminopeptidase, dipeptidase, enteropeptidase	Disaccharides, peptides	Monosaccharides, amino acids

- **Dietary fibre** (cellulose, pectin, lignin) is not digested; it adds bulk, delays glucose absorption, lowers cholesterol and is partly fermented by colon bacteria to short-chain fatty acids.
- **Disorders:** lactose intolerance (lactase deficiency), coeliac disease (gluten damages villi), cystic fibrosis (pancreatic enzyme deficiency), glucose–galactose malabsorption (SGLT-1 defect).

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12. Micronutrients: Minerals and Vitamins

Minerals

Mineral	Daily need / source	Functions	Deficiency / excess
Calcium	800–1200 mg; milk, ragi, leafy vegetables	Bone and teeth, clotting (factor IV), muscle contraction, nerve impulse, second messenger	Rickets, osteomalacia, osteoporosis, tetany
Phosphorus	800 mg; milk, cereals	Bone, ATP, nucleic acids, buffer	Rare; hypophosphataemia in rickets
Iron	10–18 mg; liver, green leaves, jaggery	Haemoglobin, myoglobin, cytochromes, catalase	Iron-deficiency anaemia; haemochromatosis (excess)
Iodine	150 µg; iodised salt, seafood	Thyroid hormones T_3, T_4	Goitre, cretinism, hypothyroidism
Zinc	10–15 mg; meat, nuts	Carbonic anhydrase, insulin storage, wound healing	Acrodermatitis enteropathica, delayed healing
Copper	2 mg; liver, nuts	Cytochrome oxidase, ceruloplasmin, lysyl oxidase	Menkes disease; **Wilson disease** (excess)
Fluoride	1 mg; water	Dental enamel (fluorapatite)	Dental caries; fluorosis (excess)
Selenium	55 µg	Glutathione peroxidase	Keshan disease (cardiomyopathy)
Magnesium, Na, K, Cl	–	Kinases; fluid and electrolyte balance, nerve conduction	Hypo/hyper-natraemia, hypo/hyper-kalaemia
Chromium, molybdenum, manganese, cobalt	Trace	Glucose tolerance factor, xanthine oxidase, B_{12} component	Impaired glucose tolerance (Cr)

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Vitamins

Vitamins are organic micronutrients needed in small amounts. **Fat-soluble: A, D, E, K** (stored in the body, can cause toxicity). **Water-soluble: B-complex and C** (not stored, excess excreted).

Vitamin	Active form and function	Deficiency disease	Sources
A (retinol)	Retinal in rhodopsin (vision), epithelial differentiation, immunity	Night blindness, xerophthalmia, keratomalacia, Bitot spots; excess: hypervitaminosis A	Liver, carrot, papaya, milk
D (cholecalciferol)	Calcitriol; raises Ca^{2+} and phosphate absorption	Rickets (children), osteomalacia (adults)	Sunlight, fish oil, egg yolk
E (tocopherol)	Antioxidant protecting membranes	Haemolytic anaemia in premature infants, neuropathy	Vegetable oils, nuts
K	γ-Carboxylation of clotting factors II, VII, IX, X	Bleeding, haemorrhagic disease of newborn	Green leaves, gut bacteria
B_1 (thiamine)	TPP: PDH, transketolase	**Beriberi**, Wernicke–Korsakoff	Whole grain, pulses
B_2 (riboflavin)	FAD, FMN	Cheilosis, glossitis, angular stomatitis	Milk, eggs, liver
B_3 (niacin)	NAD^+, $NADP^+$	**Pellagra** (dermatitis, diarrhoea, dementia)	Meat, groundnut, cereals
B_5 (pantothenic acid)	Coenzyme A	Burning feet syndrome	Widespread
B_6 (pyridoxine)	PLP: transamination	Convulsions, sideroblastic anaemia, neuropathy (isoniazid)	Meat, cereals

Vitamin	Active form and function	Deficiency disease	Sources
B_7 (biotin)	Carboxylase coenzyme	Dermatitis, hair loss (raw egg white – avidin)	Liver, gut bacteria
Folic acid	THF: one-carbon transfer, DNA synthesis	Megaloblastic anaemia, neural tube defects	Green leaves, liver
B_{12} (cobalamin)	Methylmalonyl-CoA mutase, methionine synthase	Pernicious anaemia, neuropathy	Animal foods only
C (ascorbic acid)	Collagen hydroxylation, antioxidant, iron absorption	**Scurvy** (bleeding gums, poor healing)	Citrus, amla, guava

Fat-soluble vitamins: excess and special points

- **Vitamin A:** daily need 600–900 µg RE; **excess** (> 25,000 IU/day) causes headache, bone pain, teratogenicity, hepatotoxicity; β-carotene is the provitamin (safe antioxidant).
- **Vitamin D:** made in skin by UV-B from 7-dehydrocholesterol; **activated** by 25-hydroxylation (liver) then 1α-hydroxylation (kidney, stimulated by PTH); excess causes hypercalcaemia and calcification.
- **Vitamin E:** α-tocopherol is the most active; protects PUFA from lipid peroxidation; works with selenium and vitamin C.
- **Vitamin K:** K_1 (phylloquinone, plants), K_2 (menaquinone, gut bacteria), K_3 (menadione, synthetic); **warfarin** blocks vitamin K epoxide reductase; newborns receive prophylactic vitamin K.

Water-soluble vitamins: points to remember

- **Thiamine deficiency:** wet beriberi (cardiac) and dry beriberi (neuropathy); raised blood pyruvate and decreased erythrocyte **transketolase** activity are the diagnostic tests.
- **Niacin:** can be made from tryptophan (60 mg tryptophan = 1 mg niacin); **Hartnup disease** and carcinoid syndrome cause pellagra-like illness.
- **Vitamin B_{12}** needs **intrinsic factor** from gastric parietal cells for absorption in the terminal ileum; deficiency raises methylmalonic acid and homocysteine; **folate trap** explains megaloblastic anaemia.
- **Vitamin C** is needed for hydroxylation of proline and lysine in collagen, synthesis of catecholamines and bile acids, and absorption of non-haem iron; RDA 40–75 mg.
- **Antagonists:** isoniazid (B_6), methotrexate (folate), sulfonamides (PABA), dicoumarol (K), avidin (biotin).

13. Liver Function Tests (LFT)

The liver has a large reserve, so a battery of tests is used to detect damage, assess synthetic and excretory function, and differentiate types of jaundice.

Group	Test	Normal value	Significance
Excretory	**Serum bilirubin** (total / direct)	0.2–1.0 mg/dL / 0–0.3 mg/dL	Direct raised in obstruction; indirect raised in haemolysis
Excretory	Urine bilirubin, urobilinogen, bile salts	Bilirubin absent	Present in obstruction; urobilinogen raised in haemolysis and absent in complete obstruction
Enzymes (damage)	**ALT, AST**	ALT 7–56 U/L; AST 10–40 U/L	Raised markedly in hepatitis (up to 10–100×)
Enzymes (cholestasis)	**ALP, GGT, 5′-nucleotidase**	ALP 40–120 U/L; GGT 10–50 U/L	Raised in obstructive jaundice
Synthetic	**Serum albumin**	3.5–5.0 g/dL	Low in chronic liver disease (albumin:globulin ratio reversed)

Group	Test	Normal value	Significance
Synthetic	**Prothrombin time (PT)**	11–15 s	Prolonged in severe disease; not corrected by vitamin K in hepatocellular failure
Metabolic	Serum ammonia, blood glucose, cholesterol	Ammonia 15–45 µg/dL	Ammonia rises in hepatic encephalopathy
Special	Serum α-fetoprotein, hepatitis markers (HBsAg), BSP excretion	AFP < 10 ng/mL	AFP raised in hepatocellular carcinoma

Pattern in jaundice

Parameter	Haemolytic (pre-hepatic)	Hepatic	Obstructive (post-hepatic)
Serum bilirubin	Unconjugated ↑	Both ↑	Conjugated ↑
Urine bilirubin	Absent	Present	Present
Urine urobilinogen	↑↑	Variable	Absent
Stool colour	Dark	Pale or normal	Clay-coloured
ALT/AST	Normal	↑↑↑	Mild ↑
ALP	Normal	Mild ↑	↑↑↑

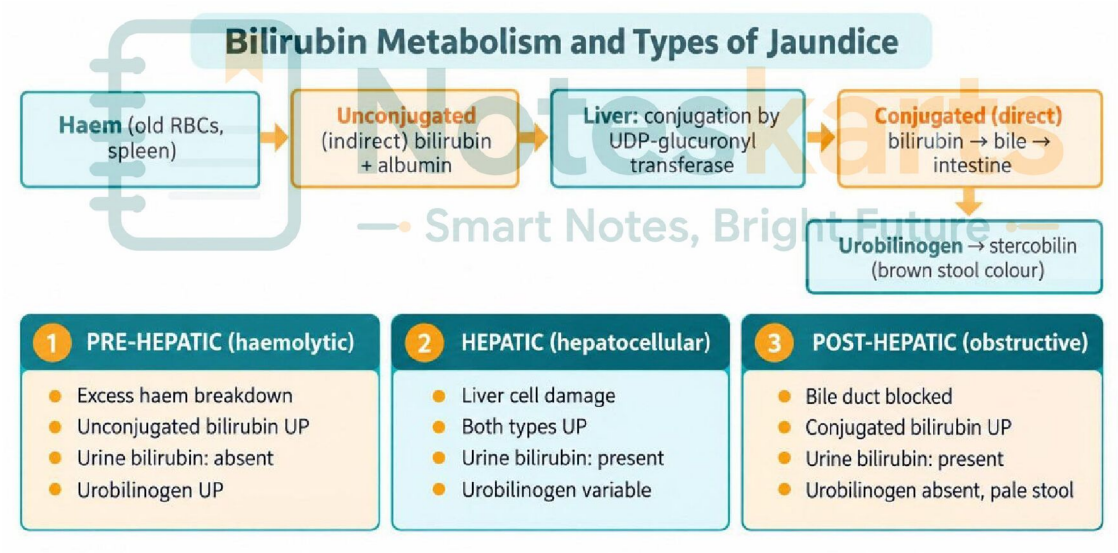

Figure 12: Figure 12: Bilirubin metabolism and the three types of jaundice

14. Renal Function Tests (RFT)

RFTs assess glomerular filtration, tubular function and detect kidney disease. About 20% of the cardiac output goes to the kidneys; GFR is about 125 mL/min.

Test	Normal value	Significance
Blood urea	15–40 mg/dL (BUN 7–20 mg/dL)	Raised in renal failure, dehydration, high protein diet; affected by non-renal factors
Serum creatinine	0.6–1.3 mg/dL	More reliable than urea; rises when GFR falls by about 50%; depends on muscle mass
Creatinine clearance (C = U × V / P)	Males 95–140; females 85–125 mL/min	Best routine estimate of GFR; 24 h urine needed. Also eGFR by Cockcroft–Gault, MDRD, CKD-EPI

Test	Normal value	Significance
Inulin clearance	125 mL/min	Gold standard for GFR (freely filtered, not reabsorbed)
PAH clearance	About 600 mL/min	Measures renal plasma flow
Serum uric acid	Males 3.5–7.2; females 2.6–6.0 mg/dL	Raised in gout and renal failure
Electrolytes	Na^+ 135–145; K^+ 3.5–5.0 mEq/L	Hyperkalaemia is a danger in renal failure
Urine analysis	Specific gravity 1.003–1.030	Proteinuria, haematuria, casts; fixed gravity ~1.010 in chronic disease
Concentration test (water deprivation)	Urine SG > 1.025	Tests tubular function
Microalbuminuria	< 30 mg/day	Early marker of diabetic nephropathy
BUN : creatinine ratio	10 : 1 to 20 : 1	> 20 in pre-renal azotaemia; low in liver disease

- **Azotaemia:** raised urea and creatinine; **uraemia:** clinical syndrome with nausea, drowsiness, pruritus, acidosis. **Nephrotic syndrome:** proteinuria > 3.5 g/day, hypoalbuminaemia, oedema, hyperlipidaemia. **Nephritic syndrome:** haematuria, hypertension, oliguria.

Tubular function and clearance concept

- **Clearance (C) = U × V / P:** the volume of plasma completely cleared of a substance per minute (U = urine concentration, V = urine flow in mL/min, P = plasma concentration).
- **Glomerular tests:** inulin, creatinine and urea clearance, serum cystatin C. **Tubular tests:** concentration and dilution tests, urine acidification (ammonium chloride load), PAH tubular maximum, glucose Tm (renal threshold for glucose is about 180 mg/dL).
- **Stages of chronic kidney disease (by GFR, mL/min/1.73 m²):** G1 ≥ 90, G2 60–89, G3 30–59, G4 15–29, G5 < 15 (kidney failure).
- **Urine examination:** colour, pH (4.5–8), protein (dipstick, sulphosalicylic acid), glucose (Benedict's), ketones (Rothera's), microscopy for casts, cells and crystals.

15. ELISA (Enzyme-Linked Immunosorbent Assay)

ELISA is an immunoassay in which an antigen–antibody reaction is detected by an **enzyme-labelled** antibody that converts a colourless substrate into a coloured product. Intensity of colour (read at 405–450 nm in a plate reader) is proportional to the amount of analyte. It is sensitive, specific and needs no radioisotopes.

Principle and steps (sandwich ELISA)

- **Coating:** capture antibody is adsorbed on a 96-well plate. **Blocking** with BSA or milk protein stops non-specific binding.
- **Sample** added; the antigen binds the capture antibody. **Wash.**
- **Detection antibody** (enzyme-linked: HRP or alkaline phosphatase) added; binds another epitope. **Wash.**
- **Substrate** (TMB for HRP, pNPP for ALP) added; colour develops; **stop solution** (H_2SO_4); absorbance read.

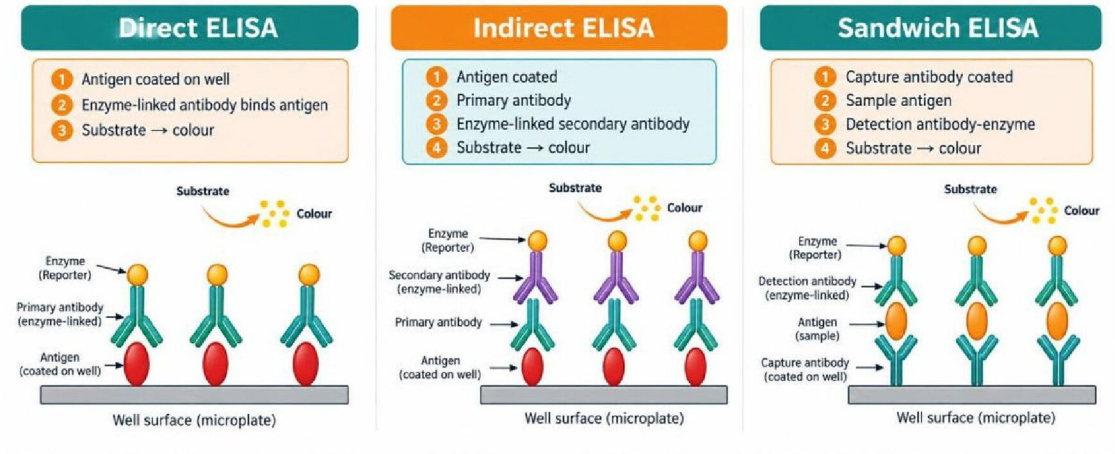

Figure 13: Figure 13: Direct, indirect and sandwich ELISA

Types of ELISA

Type	Design	Use
Direct	Antigen coated; enzyme-labelled primary antibody	Simple antigen detection
Indirect	Antigen coated; unlabelled primary then enzyme-labelled secondary antibody	Detect **antibodies** in serum (HIV, hepatitis)
Sandwich	Capture antibody + antigen + labelled detection antibody	Most specific; hormones, cytokines, tumour markers
Competitive	Sample antigen competes with labelled antigen; colour is **inversely** proportional	Small molecules (drugs, hormones)

Applications

- Diagnosis of **HIV, hepatitis B/C, dengue, TB, malaria**; **pregnancy tests (hCG)**; hormone assays (TSH, insulin); tumour markers (PSA, AFP); allergy testing; drug and toxin screening; quality control in vaccine and biopharmaceutical production.
- **Advantages:** high sensitivity, specificity, safe, suited to bulk screening and automation. **Limitations:** false positives from cross-reactivity, cost of antibodies, window period in infections.

Related techniques and quality points

- **Related immunoassays:** RIA (radioisotope label), CLIA (chemiluminescence), ELFA (fluorescence), lateral-flow rapid tests (pregnancy, COVID antigen), **Western blot** (confirmatory for HIV).
- **Controls:** positive and negative controls and a **standard curve** (absorbance vs known concentrations) must run on every plate; results reported as concentration or as positive/negative against a **cut-off**.
- **Washing steps** are critical: poor washing gives high background and false positives; incubation time and temperature must be uniform.

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