B.PHARM SEMESTER II | BP202T

BIOCHEMISTRY (THEORY)

UNIT IV

Amino Acids and Protein Metabolism

Short Notes for Quick Revision

What is covered in this unit

UNIT IV TOPICS

Part A – Classification and functions: classification and physiological roles of amino acids; structure and functions of proteins; plasma proteins.

Part B – General metabolism of amino acids: protein digestion, transamination, oxidative and non-oxidative deamination, decarboxylation, urea cycle, fate of carbon skeletons (glucogenic and ketogenic).

Part C – Specific amino acids and disorders: catabolism of phenylalanine and tyrosine, PKU, albinism, alkaptonuria, tyrosinaemia, inborn errors of branched-chain and aromatic amino acids.

Part D – Neurotransmitters and hormones: serotonin, melatonin, dopamine, noradrenaline and adrenaline.

Part E – Heme and jaundice: heme synthesis (background), heme catabolism, bilirubin metabolism and jaundice.

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PART A: CLASSIFICATION AND BIOLOGICAL FUNCTIONS

1. Amino Acids: Classification and Physiological Roles

Amino acids are the building blocks of proteins. About 300 amino acids occur in nature, but only **20 standard (proteinogenic) amino acids** are coded by DNA and incorporated into proteins. All 20 are **α-amino acids**: an amino group ($–NH_2$) and a carboxyl group (–COOH) are attached to the same carbon (the α-carbon), which also carries a hydrogen atom and a variable side chain (R group). The R group decides the size, charge, polarity and chemical behaviour of each amino acid. **Proline** is an exception, because it is an imino acid (the nitrogen is part of a ring).

Classification 1: based on the chemical nature of the side chain

Group	Amino acids	Features of the side chain
Aliphatic (non-polar)	Glycine, Alanine, Valine, Leucine, Isoleucine	Hydrocarbon chains; Val, Leu, Ile are the **branched-chain amino acids (BCAA)**
Hydroxyl-containing	Serine, Threonine	–OH group; sites of phosphorylation and O-glycosylation
Sulphur-containing	Cysteine, Methionine	Cysteine has a –SH group and forms disulphide bonds (cystine); methionine carries the methyl donor SAM
Acidic (negatively charged)	Aspartic acid, Glutamic acid	Extra –COOH group, ionised at pH 7
Amides	Asparagine, Glutamine	Amide derivatives of Asp and Glu; transport ammonia
Basic (positively charged)	Lysine, Arginine, Histidine	Extra amino, guanidinium or imidazole group
Aromatic	Phenylalanine, Tyrosine, Tryptophan	Ring structures; absorb UV light at 280 nm (Tyr, Trp)
Imino acid	Proline	Secondary amine in a ring; breaks α-helices; abundant in collagen

Classification 2: based on polarity of the side chain

Class	Amino acids	Behaviour in water
Non-polar (hydrophobic)	Gly, Ala, Val, Leu, Ile, Pro, Met, Phe, Trp	Cluster in the interior of globular proteins and in membrane-spanning segments
Polar, uncharged	Ser, Thr, Cys, Tyr, Asn, Gln	Form hydrogen bonds; lie on the protein surface
Polar, negatively charged (acidic)	Asp, Glu	Form ionic bonds and salt bridges
Polar, positively charged (basic)	Lys, Arg, His	Bind to nucleic acids (histones), form ionic bonds

Physiological roles of amino acids

Role	Details and examples
Protein synthesis	Basic monomers of all enzymes, hormones, structural, transport and defence proteins
Energy source	Carbon skeletons are oxidised for ATP, especially in starvation (about 10–15% of body energy needs)
Gluconeogenesis	Alanine, glutamine and other glucogenic amino acids supply the carbon for blood glucose in fasting
Neurotransmitters	Glutamate (excitatory), glycine and GABA (inhibitory); serotonin from Trp; dopamine, noradrenaline and adrenaline from Tyr; histamine from His
Hormones	Thyroxine (from Tyr), insulin and other peptide hormones, melatonin (from Trp)
Nitrogenous compounds	Purines and pyrimidines (Gly, Asp, Gln), heme (Gly), creatine (Arg, Gly, Met), choline and phosphatidylserine (Ser), glutathione (Glu, Cys, Gly), nitric oxide (Arg)
Vitamins and coenzymes	Niacin (NAD^+, $NADP^+$) from Trp; β-alanine for coenzyme A; PLP is the coenzyme for amino acid metabolism
Methyl donors	Methionine forms S-adenosylmethionine (SAM), the universal methyl donor
Detoxification	Glycine conjugates benzoate (hippurate) and bile acids; glutamine and aspartate carry ammonia; cysteine in glutathione
Buffering and osmotic role	Histidine buffers pH; free amino acids and taurine help in cell volume regulation
Ammonia transport	Glutamine (from tissues to liver and kidney) and alanine (from muscle to liver)

2. Structure and Functions of Proteins

Proteins are linear polymers of L-α-amino acids joined by **peptide bonds**. They are the most abundant organic molecules in cells (about 15–20% of body weight; 50% of the dry weight of a cell) and carry out nearly every biological function. Each protein has a unique amino acid sequence, which fixes its three-dimensional shape and, through the shape, its function.

The peptide bond

- A peptide bond is an **amide (–CO–NH–) linkage** between the α-carboxyl group of one amino acid and the α-amino group of the next, formed with loss of one water molecule (condensation).
- It has **partial double-bond character** (resonance), so it is **rigid, planar and usually in the* trans *configuration**. Rotation is possible only around the bonds on either side of the α-carbon (φ and ψ angles), which is plotted in the Ramachandran diagram.
- Peptides with 2 to 10 residues are oligopeptides; longer chains are polypeptides. A protein usually has more than about 50 residues. Examples: **glutathione** (tripeptide), **oxytocin and vasopressin** (nonapeptides), **insulin** (51 amino acids in two chains joined by disulphide bonds; first protein sequenced, by Sanger).

Levels of protein structure

Level	Description	Stabilised by
Primary	The linear **sequence of amino acids** in the chain, read from the N-terminus to the C-terminus. Genetically determined; a single change can cause disease (HbS: Glu → Val at position 6 of the β-chain)	**Peptide (covalent) bonds**; disulphide bonds
Secondary	Local, regular folding of the backbone: **α-helix** (right-handed, 3.6 residues per turn, 0.54 nm pitch), **β-pleated sheet** (parallel or antiparallel strands), **β-turns** and loops, random coil	**Hydrogen bonds** between the C=O and N–H groups of the backbone
Tertiary	Complete three-dimensional folding of a single polypeptide, bringing distant residues together. Forms domains, motifs and the active site	Hydrophobic interactions (main force), hydrogen bonds, ionic (salt) bridges, **disulphide bonds**, van der Waals forces
Quaternary	Arrangement of two or more polypeptide subunits into a functional protein, e.g. **haemoglobin ($\alpha_2\beta_2$)**, lactate dehydrogenase (4 subunits), immunoglobulins	Same non-covalent forces as tertiary structure; sometimes disulphide bonds

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POINTS TO REMEMBER

Proline and **glycine** disrupt the α-helix (proline has no free N–H and a fixed ring; glycine is too flexible). The α-helix is stable in the presence of Ala, Leu, Glu and Met.

Supersecondary structures (motifs) include the helix-turn-helix and zinc finger (DNA-binding proteins) and the β-α-β unit. **Domains** are independently folded compact regions of a protein.

Haemoglobin and myoglobin

Myoglobin (Mb) is a single polypeptide of 153 amino acids with one heme group; it stores oxygen in muscle. **Haemoglobin** (Hb) is a tetramer of two α and two β chains ($\alpha_2\beta_2$, 64.5 kDa) with four heme groups, and transports oxygen in blood. Oxygen binds to the **Fe^{2+}** of heme. Myoglobin has a **hyperbolic** oxygen dissociation curve and high affinity (P50 about 2.8 mmHg). Haemoglobin has a **sigmoid curve** (P50 about 26 mmHg) because of **cooperative binding**: each O_2 bound increases the affinity of the remaining sites, shifting the molecule from the tense (T) to the relaxed (R) state.

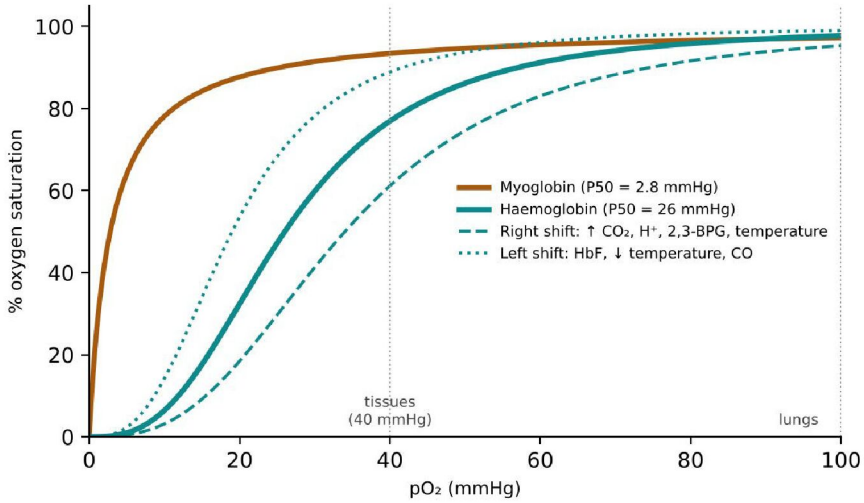

Figure 1: Figure 1: Oxygen dissociation curves of myoglobin and haemoglobin, and factors that shift the haemoglobin curve

Factor	Effect on Hb curve	Reason / significance
↑ H^+ (↓ pH) and ↑ CO_2 (Bohr effect)	Right shift	Active tissues unload more oxygen; H^+ binds Hb and stabilises the T state
↑ 2,3-Bisphosphoglycerate (2,3-BPG)	Right shift	Binds in the central cavity of deoxy-Hb. Raised at altitude, in anaemia, chronic hypoxia. Stored blood loses 2,3-BPG, so its O_2 delivery is poor
↑ Temperature	Right shift	Working muscle, fever
Fetal haemoglobin (HbF, $\alpha_2\gamma_2$)	Left shift	Binds 2,3-BPG poorly, so the fetus takes oxygen from maternal blood
Carbon monoxide (CO)	Left shift + lowers capacity	CO binds Hb about 250 times more strongly than O_2 (carboxyhaemoglobin); treat with 100% oxygen

Haemoglobin type	Chains	Remarks
HbA	$\alpha_2\beta_2$	About 97% of adult Hb
HbA_2	$\alpha_2\delta_2$	2–3%; raised in β-thalassaemia trait
HbF	$\alpha_2\gamma_2$	Fetal Hb; below 1% in adults; raised in thalassaemia, treated with hydroxyurea in sickle cell disease
HbS	$\alpha_2\beta_2$ (β6 Glu → Val)	**Sickle cell anaemia**: deoxy-HbS polymerises, red cells sickle and block capillaries
HbC	$\alpha_2\beta_2$ (β6 Glu → Lys)	Milder haemolytic anaemia
Methaemoglobin	Fe^{3+} form	Cannot carry O_2; caused by nitrites, aniline, drugs; treated with methylene blue and vitamin C

3. Plasma Proteins

Plasma proteins are the proteins dissolved in blood plasma. The **total plasma protein concentration is 6.0–8.0 g/dL** (albumin 3.5–5.0 g/dL; globulins 2.5–3.5 g/dL; fibrinogen 0.2–0.4 g/dL). Serum is plasma without fibrinogen and other clotting factors. Nearly all plasma proteins, except the immunoglobulins (made by plasma cells) and some hormones, are **synthesised in the liver** by ribosomes of the rough endoplasmic reticulum. Most are **glycoproteins**; albumin is the main exception.

Important plasma proteins and their functions

Protein	Function	Clinical significance
Prealbumin (transthyretin)	Carries thyroxine and retinol-binding protein; half-life about 2 days	Sensitive marker of nutritional status; falls in malnutrition (negative acute-phase protein)
α_1-Antitrypsin	Major inhibitor of serine proteases, especially **neutrophil elastase**	Deficiency: **emphysema** (panacinar, lower lobes) and neonatal hepatitis or cirrhosis. Absent α_1 band on electrophoresis

Protein	Function	Clinical significance
α_1-Fetoprotein (AFP)	Fetal counterpart of albumin	Tumour marker for hepatocellular carcinoma and germ-cell tumours; raised in neural tube defects
Haptoglobin	Binds free haemoglobin and prevents renal loss of iron	Low in **intravascular haemolysis**; raised in inflammation
Ceruloplasmin	Carries about 90% of plasma copper; has ferroxidase activity (Fe^{2+} to Fe^{3+})	**Wilson's disease:** low ceruloplasmin and raised urinary copper. **Menkes disease:** low copper and ceruloplasmin
α_2-Macroglobulin	Broad protease inhibitor; carries cytokines and growth factors	Raised in nephrotic syndrome because of its large size
Transferrin	Carries Fe^{3+} in plasma, 2 iron atoms per molecule	Raised in iron deficiency; low in chronic disease and inflammation. Transferrin saturation is a test for haemochromatosis
Ferritin	Iron storage (mainly tissue; a little in plasma)	Plasma ferritin reflects body iron stores; also an acute-phase reactant
C-reactive protein (CRP)	Binds phosphocholine on bacteria and activates complement	Rapid acute-phase marker of infection and inflammation; high-sensitivity CRP predicts cardiovascular risk
Fibrinogen and clotting factors	Factor I and the coagulation cascade; formed in the liver (II, VII, IX, X need vitamin K)	Prolonged PT in liver disease and warfarin therapy; fibrinogen is an acute-phase protein (high ESR)
Complement (C3, C4)	Innate immune defence, opsonisation, lysis	Low in immune complex disease (SLE, glomerulonephritis)
β_2-Microglobulin	Light chain of MHC class I	Raised in myeloma, lymphoma and renal failure
Immunoglobulins	Antibodies from plasma cells	See below

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PART B: GENERAL METABOLISM OF AMINO ACIDS

4. Protein Digestion, Amino Acid Pool and Nitrogen Balance

Dietary proteins are large molecules that cannot be absorbed. They are hydrolysed to free amino acids and small peptides by **peptidases (proteases)** in the stomach, pancreas and small intestine. Endopeptidases cut internal bonds; exopeptidases remove amino acids from the ends. The proteolytic enzymes are secreted as inactive **zymogens (proenzymes)** to protect the secreting cells from self-digestion.

Digestion of proteins

Site / enzyme	Source and activation	Specificity (cuts at)
Stomach: HCl	Parietal cells; pH 1–2	Denatures proteins, kills bacteria, activates pepsinogen
Pepsin	Chief cells secrete pepsinogen; activated by HCl and autocatalytically; **optimum pH 1.5–2**	Endopeptidase; bonds of aromatic amino acids (Phe, Tyr, Trp) and Leu
Rennin (chymosin)	Gastric juice of infants	Curdles milk by converting caseinogen to casein (with Ca^{2+})
Trypsin	Pancreas secretes trypsinogen; activated by **enteropeptidase (enterokinase)** of the duodenum, then autocatalytic	Endopeptidase; carboxyl side of **Lys and Arg**. Activates all other pancreatic zymogens
Chymotrypsin	Chymotrypsinogen activated by trypsin	Carboxyl side of **aromatic** amino acids (Phe, Tyr, Trp) and Leu
Elastase	Proelastase activated by trypsin	Small neutral amino acids (Ala, Gly, Ser)
Carboxypeptidases A and B	Procarboxypeptidases activated by trypsin	Exopeptidases removing C-terminal residues (A: aromatic; B: basic)
Aminopeptidases, dipeptidases, tripeptidases	Brush border and cytosol of enterocytes	Remove N-terminal amino acids; split di- and tripeptides

CLINICAL CORRELATION

Acute pancreatitis: premature activation of trypsinogen inside the pancreas leads to autodigestion (raised serum amylase and lipase). **Cystic fibrosis** and chronic pancreatitis cause protein and fat malabsorption. **Coeliac disease** (gluten-sensitive enteropathy) damages the brush border. **Zollinger–Ellison syndrome** gives excess acid and pepsin and ulcers.

Nitrogen balance

Nitrogen balance is the difference between nitrogen taken in (as dietary protein; 1 g N = 6.25 g protein) and nitrogen lost in urine (urea, creatinine, ammonia, uric acid), faeces and sweat.

State	Nitrogen intake vs output	Conditions
Nitrogen equilibrium	Intake = output	Healthy adult on adequate diet
Positive balance	Intake > output	Growth, pregnancy, lactation, convalescence, recovery from illness, anabolic steroid or growth hormone
Negative balance	Intake < output	Starvation, protein deficiency, lack of an essential amino acid, fever, burns, trauma, major surgery, uncontrolled diabetes, cancer cachexia, immobilisation

- **Recommended dietary allowance (RDA):** about **0.8–1.0 g protein per kg body weight per day** (about 55–60 g for adults); 1.5 g/kg in children, and more in pregnancy and lactation (ICMR suggests 1 g/kg).
- **Biological value (BV)** is the percentage of absorbed nitrogen retained for use. Egg protein has the highest BV (about 100), milk 85–90, meat 75, soya about 70, wheat about 50–60, pulses 50–60. **Net protein utilisation (NPU)** = BV × digestibility. **Protein efficiency ratio (PER)** is the weight gain per gram of protein consumed.

- **Complementary proteins:** cereals lack lysine and pulses lack methionine; eaten together (dal and rice) they give a protein of high BV. **Limiting amino acid:** the essential amino acid present in the lowest amount relative to need (lysine in wheat; methionine in pulses; tryptophan in maize).

5. Transamination

Transamination is the transfer of an α-amino group from an α-amino acid to an α-keto acid, forming a new amino acid and a new keto acid. It is **freely reversible** and needs no free ammonia. It is catalysed by **aminotransferases (transaminases)**, which use **pyridoxal phosphate (PLP)**, the active form of **vitamin B_6**, as the coenzyme. Aminotransferases are named after the amino acid donor, and they all use **α-ketoglutarate** (or less often oxaloacetate and pyruvate) as the acceptor of the amino group, so that **glutamate** is the funnel through which nitrogen is collected.

6. Deamination and Ammonia Metabolism

Deamination is the removal of the α-amino group from an amino acid as **free ammonia (NH_3)**. It takes place mainly in the **liver and kidney**. It prepares the carbon skeleton for oxidation and the nitrogen for excretion as urea. It is of two types: oxidative and non-oxidative.

A. Oxidative deamination

Here the amino group is removed by oxidation (dehydrogenation) to give an imino acid, which is then hydrolysed to the keto acid and ammonia.

Enzyme	Coenzyme / location	Details
L-Glutamate dehydrogenase (GDH)	NAD^+ or $NADP^+$; **mitochondrial matrix** of liver and kidney	Glutamate + $NAD(P)^+$ + H_2O ⇌ **α-Ketoglutarate + NH_3 + NAD(P)H**. The most important and active enzyme of oxidative deamination; reversible. **Allosteric:** inhibited by **ATP and GTP** (high energy), activated by **ADP and GDP**. Gain-of-function mutations cause hyperinsulinism-hyperammonaemia syndrome
L-Amino acid oxidase	FMN; microsomes of liver and kidney	Acts at alkaline pH on some L-amino acids; low activity, so a minor pathway. The $FMNH_2$ is reoxidised by O_2 giving H_2O_2, which catalase destroys
D-Amino acid oxidase	FAD; peroxisomes of liver and kidney	Acts on D-amino acids from bacteria and diet, converting them to keto acids that can be used
Glycine oxidase / glycine cleavage system	FAD; PLP, THF, NAD^+ (cleavage system)	Glycine ⇌ CO_2 + NH_3 + one-carbon unit (N^5,N^{10}-methylene-THF)

Transdeamination: the major pathway

Most amino acids are not directly deaminated, since GDH is specific for glutamate. Their amino groups are first transferred to α-ketoglutarate by **transamination** to form glutamate, and glutamate is then **oxidatively deaminated** by GDH to release ammonia. This combined process is called **transdeamination** (coupled or indirect deamination). It can be reversed to make non-essential amino acids from ammonia (reductive amination of α-ketoglutarate).

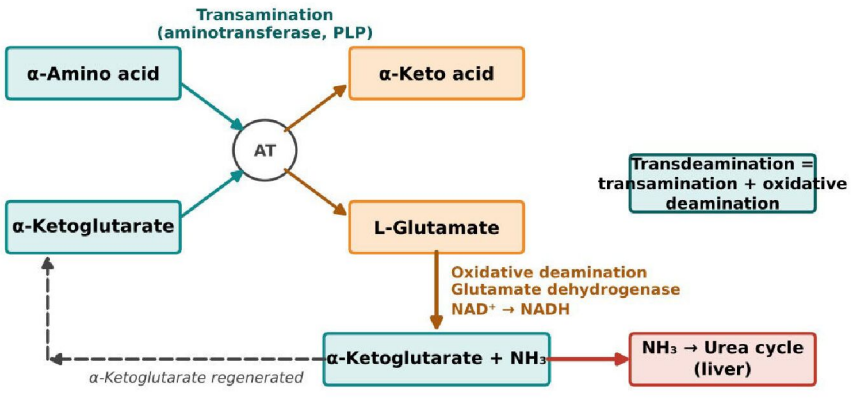

Figure 3: Figure 3: Transdeamination: transamination coupled with oxidative deamination by glutamate dehydrogenase

Ammonia toxicity and hyperammonaemia

- **Normal blood ammonia:** about **20–50 µg/dL (12–30 µmol/L)**. Even a rise to 100–200 µg/dL is serious.
- **Mechanism of brain toxicity:** (1) excess NH_3 is trapped by glutamine synthetase, which depletes **glutamate** (a neurotransmitter) and **α-ketoglutarate**, so TCA cycle activity and **ATP production in the brain fall**. (2) **Glutamine accumulates in astrocytes**, causing osmotic swelling and **cerebral oedema** and raised intracranial pressure. (3) Altered GABA and glutamate neurotransmission.
- **Symptoms:** tremors (asterixis), slurred speech, vomiting, blurred vision, irritability, lethargy, convulsions, coma and death.
- **Acquired causes: liver cirrhosis** and portosystemic shunts (hepatic encephalopathy), fulminant hepatic failure, Reye syndrome, drugs (valproate). **Inherited causes:** urea cycle enzyme defects and organic acidaemias.

TREATMENT OF HEPATIC ENCEPHALOPATHY

Restrict dietary protein temporarily; give **lactulose** (acidifies the colon so NH_3 becomes non-absorbable NH_4^+, and acts as a laxative); **rifaximin** or neomycin (reduce ammonia-producing gut bacteria); **L-ornithine L-aspartate** (stimulates urea and glutamine synthesis); treat the cause such as GI bleeding, infection and electrolyte problems.

7. Decarboxylation of Amino Acids

Decarboxylation is the removal of the α-carboxyl group of an amino acid as CO_2, forming a **biogenic amine**. Most amino acid **decarboxylases** need **PLP** as coenzyme and are found in the liver, kidney, brain, gut and bacteria. Many of the resulting amines are physiologically very active, and they are cleared by **monoamine oxidase (MAO)** and diamine oxidase.

Amino acid	Amine formed	Enzyme	Function
Histidine	**Histamine**	Histidine decarboxylase	Mast cell mediator: vasodilation, increased vascular permeability, bronchoconstriction, gastric acid secretion (H_2 receptor), neurotransmitter, itching
Tyrosine	Tyramine	Aromatic L-amino acid decarboxylase	Releases noradrenaline; in cheese (see below)
DOPA (from Tyr)	**Dopamine**	DOPA decarboxylase	Precursor of noradrenaline; neurotransmitter
5-Hydroxytryptophan	**Serotonin (5-HT)**	Aromatic L-amino acid decarboxylase	Neurotransmitter, vasoconstrictor, gut motility
Tryptophan	Tryptamine	Aromatic L-amino acid decarboxylase	Trace amine
Glutamate	**γ-Aminobutyric acid (GABA)**	Glutamate decarboxylase (PLP)	Main inhibitory neurotransmitter of the CNS. Low GABA in epilepsy; vitamin B_6 deficiency in infants causes seizures
Cysteine (via cysteine sulphinate)	Taurine, cysteamine	Cysteine sulphinate decarboxylase	Taurine conjugates bile acids; cysteamine is part of coenzyme A
Serine	Ethanolamine	Serine decarboxylase	Phospholipids (cephalin); choline after methylation (SAM)
Aspartate	β-Alanine	Aspartate decarboxylase	Part of coenzyme A and carnosine
Ornithine	**Putrescine** → spermidine → spermine	Ornithine decarboxylase	Polyamines for cell growth and DNA stabilisation
Lysine	Cadaverine	Lysine decarboxylase (bacteria)	Putrefaction product
Arginine	Agmatine	Arginine decarboxylase	Minor

8. Urea Cycle: Nitrogen Disposal and Detoxification

Humans are **ureotelic**: the toxic ammonia produced by amino acid breakdown is converted into the non-toxic, highly soluble **urea**, which is excreted in urine. Birds and reptiles are uricotelic (uric acid) and fishes ammonotelic (ammonia). The **urea cycle** (Krebs–Henseleit ornithine cycle, 1932, the first cyclic pathway discovered) takes place **only in the liver**, partly in the mitochondria and partly in the cytosol. About **20–30 g of urea** are formed daily, which carries about 80–90% of excreted nitrogen.

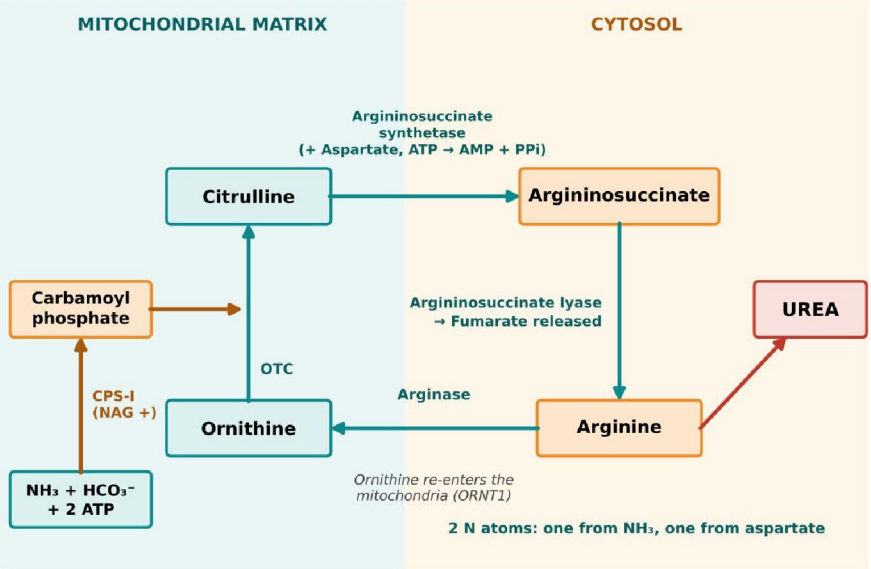

Figure 4: Figure 4: The urea cycle, showing mitochondrial and cytosolic reactions

Reactions of the urea cycle

Step	Reaction and enzyme	Site	Remarks
1	$NH_3 + CO_2$ (HCO_3^-) + **2 ATP** → **Carbamoyl phosphate** (+ 2 ADP + Pi) *Carbamoyl phosphate synthetase I (CPS-I)*	Mitochondria	**Rate-limiting and regulatory step.** Allosteric activator: **N-acetylglutamate (NAG)**. Two ATP are used (one to activate bicarbonate, one to phosphorylate carbamate)
2	Carbamoyl phosphate + **Ornithine** → **Citrulline** + Pi *Ornithine transcarbamoylase (OTC)*	Mitochondria	Citrulline leaves the mitochondria through a transporter
3	Citrulline + **Aspartate** + ATP → **Argininosuccinate** + AMP + PPi *Argininosuccinate synthetase*	Cytosol	Second nitrogen enters (from aspartate). ATP is cleaved to AMP + PPi, which uses **2 high-energy bonds**
4	Argininosuccinate → **Arginine** + **Fumarate** *Argininosuccinate lyase (argininosuccinase)*	Cytosol	Fumarate enters the TCA cycle, linking the two cycles
5	Arginine + H_2O → **Urea** + **Ornithine** *Arginase (Mn^{2+})*	Cytosol (liver)	Urea is released; ornithine returns to the mitochondria through the ORNT1 transporter

OVERALL REACTION AND ENERGETICS

$CO_2 + NH_3$ + Aspartate + 3 ATP + 2 H_2O → Urea + Fumarate + 2 ADP + AMP + 2 Pi + PPi.

Three ATP molecules are used, but **four high-energy phosphate bonds** are consumed (ATP to AMP + PPi). The fumarate formed is converted to malate and then to oxaloacetate, producing NADH (about 2.5 ATP), which partly **recovers the energy cost**. One nitrogen of urea comes from free ammonia and the other from aspartate; the carbon comes from CO_2. Aspartate is formed from oxaloacetate by transamination with glutamate, so all the amino groups funnel into the cycle.

Regulation of the urea cycle

- **Short-term regulation: N-acetylglutamate (NAG)**, formed from acetyl-CoA and glutamate by **NAG synthase**, is the obligatory allosteric activator of CPS-I. **Arginine** activates NAG synthase. After a protein meal, glutamate rises, NAG rises, and the cycle speeds up.
- **Long-term regulation:** the five enzymes are **induced** (transcription raised) by high-protein diets, starvation (muscle protein breakdown) and glucagon or glucocorticoids, and reduced by low-protein diet.
- **Substrate availability:** the supply of ammonia, ornithine (a catalyst) and aspartate drives the rate.
- **Link with the TCA cycle:** the **aspartate–argininosuccinate shunt** connects the urea cycle to the TCA cycle through fumarate.

Disorders of the urea cycle

Each enzyme defect causes **hyperammonaemia**, and all are **autosomal recessive** except **OTC deficiency, which is X-linked**. The more proximal the block, the more severe the hyperammonaemia. Newborns become well after birth for 24–48 hours, then develop

poor feeding, vomiting, lethargy, hypothermia, seizures and coma. Blood ammonia and plasma glutamine are raised, with respiratory alkalosis.

Disorder	Enzyme defect	Key features and biochemical findings
CPS-I deficiency	Carbamoyl phosphate synthetase I	Severe hyperammonaemia; **low citrulline and arginine; no orotic aciduria**
NAGS deficiency	N-Acetylglutamate synthase	Same as CPS-I deficiency; responds to **carglumic acid** (NAG analogue)
OTC deficiency (most common)	Ornithine transcarbamoylase (X-linked)	Carbamoyl phosphate accumulates, enters the pyrimidine pathway: **high orotic acid in urine**, low citrulline. Males severely affected; female carriers variable. Protein aversion
Citrullinaemia type I	Argininosuccinate synthetase	**Very high citrulline** in blood and urine
Argininosuccinic aciduria	Argininosuccinate lyase	High argininosuccinate; **trichorrhexis nodosa** (brittle hair); hepatomegaly
Argininaemia	Arginase	High arginine; **progressive spastic diplegia**; hyperammonaemia mild or intermittent
HHH syndrome	Ornithine transporter ORNT1	**H**yperammonaemia, **h**yperornithinaemia, **h**omocitrullinuria

MANAGEMENT OF UREA CYCLE DEFECTS

Acute: stop protein intake, give intravenous glucose and lipids to prevent catabolism, **nitrogen scavengers (sodium benzoate and sodium phenylacetate/phenylbutyrate)**, arginine (except in arginase deficiency) and haemodialysis for very high ammonia (over 400–500 µmol/L). **Long-term:** low-protein diet with essential amino acid supplement, oral scavengers, citrulline or arginine, carglumic acid in NAGS deficiency, and liver transplantation.

How scavengers work: benzoate is conjugated with glycine to **hippurate**, and phenylacetate with glutamine to **phenylacetylglutamine**; both are excreted in urine, which removes nitrogen without the urea cycle.

9. Fate of Carbon Skeletons: Glucogenic and Ketogenic Amino Acids

After removal of the amino group, the **carbon skeletons** (α-keto acids) of the 20 amino acids are converted to only **seven metabolic intermediates**: **pyruvate, oxaloacetate, α-ketoglutarate, succinyl-CoA, fumarate, acetyl-CoA and acetoacetate**. The first five can be used for gluconeogenesis (**glucogenic**) and the last two give acetyl-CoA and ketone bodies (**ketogenic**).

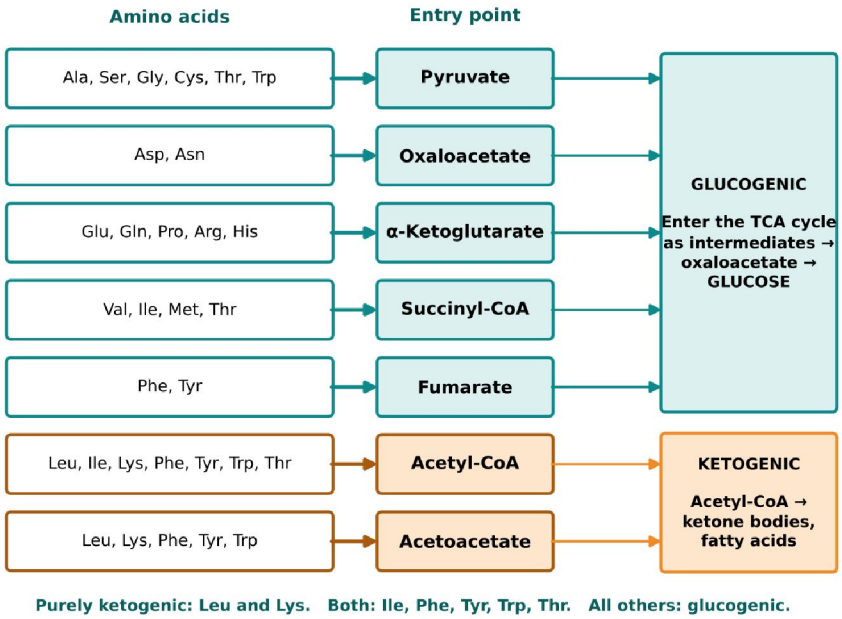

Figure 5: Figure 5: Fate of the carbon skeletons of amino acids: entry points and classification

Entry points of the carbon skeletons

Entry point	Amino acids that give it	Remarks
Pyruvate	Alanine, Serine, Glycine, Cysteine, Threonine (one path), Tryptophan (via alanine)	Pyruvate → oxaloacetate → glucose (gluconeogenesis) or acetyl-CoA
Oxaloacetate	Aspartate, Asparagine	Direct entry into gluconeogenesis and the TCA cycle
α-Ketoglutarate	Glutamate, Glutamine, **Proline, Arginine, Histidine**	Through glutamate (glutamate γ-semialdehyde for Pro and Arg; formiminoglutamate for His, which needs **folate**)
Succinyl-CoA	**Valine, Isoleucine, Methionine, Threonine**	Through **propionyl-CoA → methylmalonyl-CoA** (needs **biotin** and **vitamin B_{12}**)
Fumarate	**Phenylalanine, Tyrosine**	From fumarylacetoacetate (Section 10)
Acetyl-CoA	Leucine, Isoleucine, Lysine, Tryptophan, Threonine, Phenylalanine, Tyrosine	Can form ketone bodies, fatty acids and cholesterol; cannot form net glucose
Acetoacetate	Leucine, Lysine, Phenylalanine, Tyrosine, Tryptophan	Ketone body (directly or from acetoacetyl-CoA)

Group	Amino acids	Clinical relevance
Purely glucogenic	Ala, Arg, Asp, Asn, Cys, Glu, Gln, Gly, His, Met, Pro, Ser, Val	Supply glucose during fasting; in diabetes they are the main source of excess glucose (gluconeogenesis)
Purely ketogenic	**Leucine and Lysine**	Cannot give glucose. A ketogenic diet with leucine can reduce seizures. Leucine stimulates the mTOR pathway of protein synthesis
Both	**Isoleucine, Phenylalanine, Tyrosine, Tryptophan** (Thr in some)	Part of the skeleton goes to glucose and part to ketones

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PART C: SPECIFIC AMINO ACIDS AND RELATED DISORDERS

10. Catabolism of Phenylalanine and Tyrosine

Phenylalanine is an essential amino acid and **tyrosine** is non-essential because it is made from phenylalanine. Both are **glucogenic and ketogenic**: complete oxidation gives **fumarate** (glucogenic) and **acetoacetate** (ketogenic). Their catabolism takes place mainly in the **liver**. Tyrosine is also the precursor of catecholamines, thyroid hormones and melanin, and the pathway is the best-known site of inherited metabolic disorders.

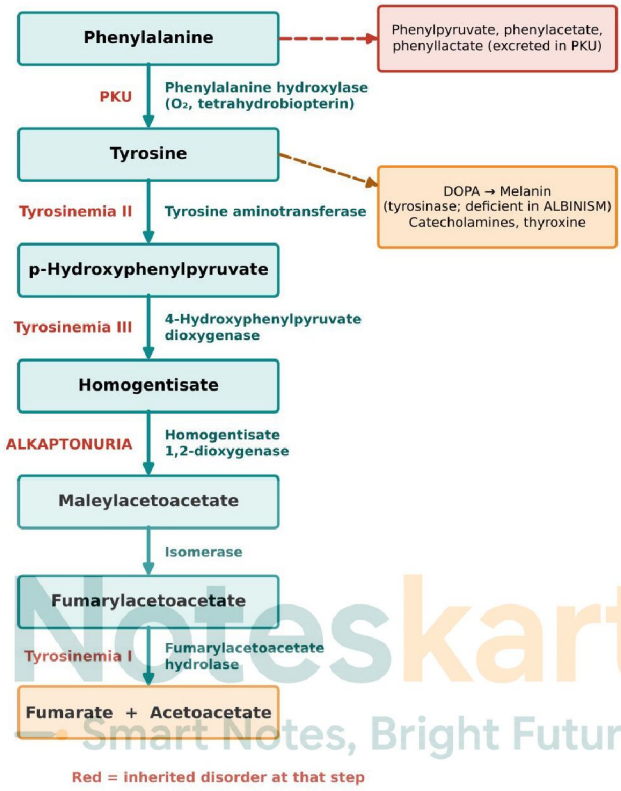

Figure 6: Figure 6: Catabolism of phenylalanine and tyrosine and the sites of inherited metabolic blocks

Steps of the pathway

Step	Reaction	Enzyme (cofactor)	Defect
1	Phenylalanine → **Tyrosine**	**Phenylalanine hydroxylase** (a mixed-function oxygenase; needs O_2 and **tetrahydrobiopterin, BH_4**; liver only)	**Phenylketonuria (PKU)**
2	Tyrosine → **p-Hydroxyphenylpyruvate**	**Tyrosine aminotransferase** (PLP; transamination with α-ketoglutarate)	**Tyrosinaemia type II** (Richner–Hanhart)
3	p-Hydroxyphenylpyruvate → **Homogentisate**	**p-Hydroxyphenylpyruvate dioxygenase** (hydroxylase; needs O_2, Fe^{2+} and **vitamin C**)	**Tyrosinaemia type III**; transient neonatal tyrosinaemia (immature)
4	Homogentisate → **Maleylacetoacetate**	**Homogentisate 1,2-dioxygenase** (oxidase; ring opening, Fe^{2+})	**Alkaptonuria**
5	Maleylacetoacetate → **Fumarylacetoacetate**	Maleylacetoacetate isomerase (glutathione)	Rare
6	Fumarylacetoacetate → **Fumarate + Acetoacetate**	**Fumarylacetoacetate hydrolase**	**Tyrosinaemia type I** (hepatorenal)

11. Metabolic Disorders of Phenylalanine and Tyrosine

Garrod (1902) used **alkaptonuria** to propose the concept of the **inborn error of metabolism**: an inherited deficiency of an enzyme causes accumulation of the substrate or lack of the product. Most are **autosomal recessive**. The disorders of phenylalanine and tyrosine metabolism are among the commonest and best studied.

A. Phenylketonuria (PKU)

- **Cause:** deficiency of **phenylalanine hydroxylase** (gene on chromosome 12q), autosomal recessive. Incidence about 1 in 10,000–15,000 births (lower in some populations). Phenylalanine accumulates in blood (classical PKU: **above 20 mg/dL**; normal 0.8–1.8 mg/dL) and tyrosine becomes an essential amino acid.
- **Pathogenesis:** high Phe competes with other large neutral amino acids at the blood-brain barrier (LAT1 carrier), reducing brain amino acid and neurotransmitter synthesis, impairing myelination and protein synthesis, and is toxic to developing neurons.
- **Clinical features** (untreated; babies are normal at birth): **severe intellectual disability** (IQ under 50), delayed milestones, seizures, hyperactivity, microcephaly, tremors; **fair skin, blond hair and blue eyes** (high Phe inhibits tyrosinase, so less melanin); **eczema**; **musty or mousy odour** of urine and sweat (phenylacetate).
- **Diagnosis:** newborn screening after 48 hours of protein feeding on a dried blood spot: **Guthrie bacterial inhibition test** (older method; *Bacillus subtilis* growth is supported by Phe), fluorometric assay or **tandem mass spectrometry** (now preferred). Urine **ferric chloride test** gives a **green colour** (phenylpyruvic acid); also **2,4-DNPH** (yellow precipitate). Confirmed by plasma amino acids and by mutation analysis; BH_4 deficiency is excluded by pterin analysis and DHPR assay.
- **Treatment: low-phenylalanine diet** started in the first weeks of life and continued lifelong (phenylalanine-free amino acid formulae, restriction of meat, milk, eggs, fish, nuts; use of low-protein foods); **tyrosine supplementation**; target blood Phe about **2–6 mg/dL** (120–360 µmol/L). **Sapropterin** (synthetic BH_4) helps patients with residual enzyme activity; **pegvaliase** (PEGylated phenylalanine ammonia lyase) is an enzyme therapy for adults; large neutral amino acids are an alternative. Avoid **aspartame** (contains Phe; labelled on sugar-free products).

Variant	Defect	Notes
Classical PKU	PAH nearly absent (activity below 1%)	Phe above 20 mg/dL; needs strict diet
Mild PKU / hyperphenylalaninaemia	Residual PAH activity	Phe 2–20 mg/dL; milder diet restriction, may respond to BH_4
Malignant PKU (BH_4 deficiency)	Defect in BH_4 synthesis (GTP cyclohydrolase, PTPS) or regeneration (DHPR), about 1–2% of cases	Hyperphenylalaninaemia **plus progressive neurological deterioration** (hypotonia, seizures, dystonia) even if the diet is good, because dopamine and serotonin synthesis fails. Treated with **BH_4 + L-DOPA/carbidopa + 5-hydroxytryptophan** (and folinic acid in DHPR defect)
Maternal PKU	Untreated mother with high Phe	Phe crosses placenta: **microcephaly, intellectual disability, congenital heart disease, low birth weight** in the baby (who is not genetically PKU). Strict diet before conception and through pregnancy prevents it

B. Albinism

- **Cause:** inherited absence or reduced activity of **tyrosinase** (oculocutaneous albinism type 1, **OCA1**; autosomal recessive) or of other pigmentation proteins (OCA2: P protein, the most common type in Africa and in India; OCA3, OCA4). **X-linked ocular albinism** affects only the eyes.
- **Features: lack of melanin** in skin, hair and eyes: white hair, pink-white skin, pale blue or red-looking iris, **photophobia, nystagmus, reduced visual acuity and strabismus** (abnormal optic nerve routing), **marked sunburn and high risk of skin cancers** (squamous cell and basal cell carcinoma).
- **Diagnosis and management:** clinical; hair bulb incubation (tyrosinase) test historically; gene testing. **Sun protection** (clothing, broad-spectrum sunscreen), ophthalmic care with refractive correction and tinted glasses, regular skin examination. Intelligence is normal. Note that melanin is made from tyrosine, so albinism is **not due to tyrosine lack** but to the enzyme.
- **Difference from vitiligo:** vitiligo is acquired, patchy loss of melanocytes due to autoimmunity; in albinism melanocytes are present but cannot make pigment.

C. Alkaptonuria (alcaptonuria)

- **Cause:** deficiency of **homogentisate 1,2-dioxygenase** (HGD gene; autosomal recessive; rare, about 1 in 250,000, higher in Slovakia and the Dominican Republic). **Homogentisic acid (homogentisate)** accumulates and is excreted in urine.

- **Dark urine:** homogentisate oxidises and polymerises in air, especially in **alkaline urine**, so the urine **turns brown to black on standing**. Often the first sign is dark stains on diapers. Urine gives a positive **reducing test (Benedict's)** but a negative glucose-oxidase strip, and is blackened by **ferric chloride** and alkali.
- **Ochronosis:** pigment (oxidised homogentisate) deposits in connective tissue, giving **blue-black discolouration of cartilage of the ear, nose, sclera and tendons**, and **ochronotic arthropathy** (early degenerative arthritis of the spine and large joints after age 30–40), also heart valve calcification and kidney and prostate stones.
- **Treatment:** low-protein (low Phe and Tyr) diet, vitamin C (limited effect), joint care and replacement; **nitisinone** (inhibits p-hydroxyphenylpyruvate dioxygenase and so lowers homogentisate) is an approved treatment. Life span is normal; the course is benign apart from arthritis.

12. Inborn Errors of Branched-Chain and Aromatic Amino Acid Metabolism

Catabolism of branched-chain amino acids (BCAA)

Valine, leucine and isoleucine are essential amino acids with branched side chains. Unlike most amino acids, they are catabolised mainly in **skeletal muscle, heart, kidney and brain** (and not in the liver, which lacks the first enzyme). Their metabolism has three common steps, similar to the oxidation of fatty acids and pyruvate.

- **Step 1: Transamination** with α-ketoglutarate by **branched-chain amino acid aminotransferase (BCAT)** to the corresponding **branched-chain α-keto acids** (α-ketoisovalerate, α-ketoisocaproate, α-keto-β-methylvalerate).
- **Step 2: Oxidative decarboxylation** by the **branched-chain α-keto acid dehydrogenase (BCKD) complex** (mitochondrial; E1 decarboxylase with **thiamine pyrophosphate**, E2 transacylase with **lipoic acid and CoA**, E3 dihydrolipoyl dehydrogenase with FAD and NAD^+). It is regulated by phosphorylation (BCKD kinase); it is like the pyruvate and α-ketoglutarate dehydrogenases and shares the E3 subunit.
- **Step 3: Dehydrogenation** by acyl-CoA dehydrogenases (isovaleryl-CoA dehydrogenase for leucine), then reactions as in β-oxidation to the final products.
- **Products: valine** → propionyl-CoA → methylmalonyl-CoA → **succinyl-CoA** (glucogenic); **isoleucine** → acetyl-CoA + propionyl-CoA (both glucogenic and ketogenic); **leucine** → acetyl-CoA + **acetoacetate** (purely ketogenic).
- **Propionyl-CoA to succinyl-CoA:** propionyl-CoA carboxylase (**biotin**) → methylmalonyl-CoA epimerase → **methylmalonyl-CoA mutase (vitamin B_{12}, adenosylcobalamin)**.

Inborn errors of BCAA metabolism

Disorder	Enzyme defect	Clinical and biochemical features	Treatment
Maple syrup urine disease (MSUD)	**BCKD complex** (E1α, E1β or E2 subunit); AR	**Sweet maple syrup or burnt-sugar odour** of urine, sweat and ear wax (sotolone from isoleucine). Poor feeding, vomiting, lethargy, hypotonia then hypertonia, seizures, metabolic **ketoacidosis**, coma and brain damage in the first week of life. **High leucine, isoleucine, valine and alloisoleucine** and their keto acids. **Urine 2,4-DNPH test** gives yellow precipitate; ferric chloride gives blue-green colour	**Diet restricted in BCAA** (special formula) with careful monitoring; **thiamine** in thiamine-responsive variants; dialysis or exchange transfusion in crisis; liver transplantation. Avoid catabolic states (fever, fasting)
Isovaleric acidaemia	**Isovaleryl-CoA dehydrogenase** (leucine pathway)	**Odour of sweaty feet**, vomiting, acidosis, hyperammonaemia; isovalerylglycine in urine	Low-leucine diet; **glycine and carnitine** to conjugate isovaleryl-CoA
Propionic acidaemia	**Propionyl-CoA carboxylase** (biotin)	Ketoacidosis, hyperammonaemia, lethargy; propionate, methylcitrate in urine	Protein restriction, carnitine, biotin trial, metronidazole (reduces gut propionate), avoid fasting
Methylmalonic acidaemia	**Methylmalonyl-CoA mutase** or defect of B_{12} metabolism	Severe acidosis, vomiting, hypotonia, developmental delay, **kidney disease**; high methylmalonic acid in blood and urine	Low-protein diet, **hydroxocobalamin (B_{12})** in responsive types, carnitine
Hypervalinaemia / others	Valine transaminase	Rare	Low-valine diet

KEY DIAGNOSTIC CLUE

Odour of urine often points to the disease: **musty or mousy = PKU**; **maple syrup = MSUD**; **sweaty feet = isovaleric acidaemia**; **cabbage = tyrosinaemia I or methionine malabsorption**; **rotten fish = trimethylaminuria**; **boiled cabbage = hypermethioninaemia**.

PART D: NEUROTRANSMITTERS AND HORMONES DERIVED FROM AMINO ACIDS

13. Serotonin (5-Hydroxytryptamine, 5-HT)

Serotonin is a biogenic amine derived from the essential amino acid **tryptophan**. It acts as a neurotransmitter in the brain and as a local hormone and vasoactive substance in the gut and blood. Only 1–2% of tryptophan is used for serotonin, but it has wide actions on mood, sleep, appetite, temperature, pain, vascular tone and gut motility.

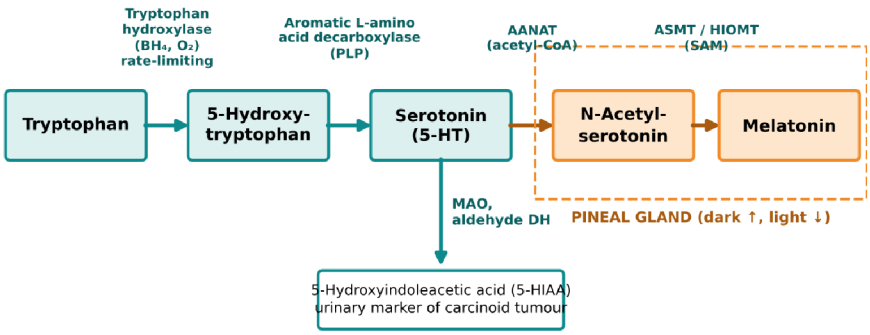

Figure 7: Figure 8: Synthesis of serotonin and melatonin from tryptophan; catabolism of serotonin to 5-HIAA

Biosynthesis

- **Step 1: hydroxylation.** Tryptophan → **5-hydroxytryptophan (5-HTP)** by **tryptophan hydroxylase** (needs O_2, **tetrahydrobiopterin**). This is the **rate-limiting step**. Isoforms: TPH1 (gut, pineal) and TPH2 (brain neurons).
- **Step 2: decarboxylation.** 5-HTP → **serotonin** by **aromatic L-amino acid decarboxylase** (the same enzyme as DOPA decarboxylase; **PLP**-dependent).
- **Availability of tryptophan** (competition with other large neutral amino acids for transport across the blood-brain barrier, boosted after carbohydrate-rich meals through insulin) influences brain serotonin.

Catabolism

Serotonin is oxidatively deaminated by **monoamine oxidase A (MAO-A)** to 5-hydroxyindoleacetaldehyde, which **aldehyde dehydrogenase** converts to **5-hydroxyindoleacetic acid (5-HIAA)**, excreted in urine (normal **2–8 mg/24 h**). Serotonin is also removed from the synaptic cleft by the **serotonin transporter (SERT)**, which is the target of antidepressants.

14. Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone made mainly by the **pineal gland** from serotonin. It is the chemical signal of **darkness** and regulates the **circadian rhythm** (24-hour sleep–wake cycle).

Biosynthesis

- **Tryptophan → 5-HTP → serotonin** (as in Section 13).
- **Serotonin → N-acetylserotonin** by **arylalkylamine N-acetyltransferase (AANAT)**, which uses **acetyl-CoA**. AANAT is the **rate-limiting enzyme** and shows a large night-time rise.
- **N-Acetylserotonin → Melatonin** by **acetylserotonin O-methyltransferase (ASMT, formerly HIOMT)**, which uses **S-adenosylmethionine (SAM)** as the methyl donor.

Actions

- **Circadian regulation:** synchronises the body clock with the light–dark cycle; **induces sleepiness** and lowers body temperature; acts on **MT_1 and MT_2 receptors** (G-protein-coupled) in the SCN.
- **Reproduction:** inhibits gonadotropin release in animals (seasonal breeding); melatonin falls at puberty.
- **Antioxidant:** a powerful free radical scavenger that protects neurons and mitochondria.
- Other actions: modulates immunity, retinal physiology and blood pressure (nocturnal fall).

15. Catecholamines: Dopamine, Noradrenaline and Adrenaline

Catecholamines are compounds with a catechol nucleus (benzene ring with two adjacent hydroxyl groups) and an amine side chain. **Dopamine, noradrenaline (norepinephrine) and adrenaline (epinephrine)** are made from **tyrosine** and act as neurotransmitters (CNS and sympathetic nerves) and hormones (adrenal medulla). They mediate the **fight-or-flight** response.

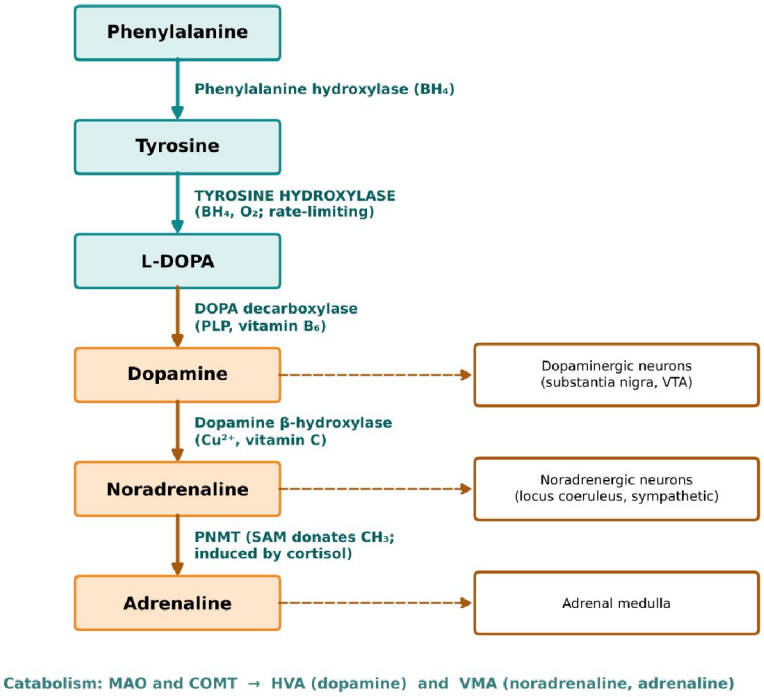

Figure 8: Figure 7: Biosynthesis of catecholamines from phenylalanine and tyrosine

Biosynthesis

Step	Reaction	Enzyme and cofactors	Site / regulation
1	Phenylalanine → **Tyrosine**	Phenylalanine hydroxylase (BH_4)	Liver
2	Tyrosine → **L-DOPA** (3,4-dihydroxyphenylalanine)	**Tyrosine hydroxylase** (O_2, Fe^{2+}, **BH_4**)	**Rate-limiting step.** Inhibited by end-product catecholamines (feedback), activated by phosphorylation; blocked by **α-methyl-p-tyrosine (metyrosine)**
3	L-DOPA → **Dopamine**	**Aromatic L-amino acid (DOPA) decarboxylase** (**PLP**)	Cytosol; inhibited by carbidopa
4	Dopamine → **Noradrenaline**	**Dopamine β-hydroxylase** (**Cu^{2+}**, **vitamin C**, O_2)	Inside **storage vesicles** (chromaffin granules)
5	Noradrenaline → **Adrenaline**	**PNMT (phenylethanolamine N-methyltransferase)**; methyl donor **SAM**	Cytosol of adrenal medulla; **induced by cortisol** from the adrenal cortex (through the portal circulation)

- **Sites:** dopamine in dopaminergic neurons (substantia nigra, ventral tegmental area, hypothalamus); noradrenaline in noradrenergic neurons (**locus coeruleus**) and **postganglionic sympathetic nerve endings**; adrenaline in the **adrenal medulla** (about 80% adrenaline and 20% noradrenaline).
- **Storage and release:** amines are taken into vesicles by **VMAT2** and released by **calcium-dependent exocytosis** (acetylcholine acts on chromaffin cells through nicotinic receptors). Action is ended mainly by **reuptake** (uptake-1: dopamine transporter DAT and noradrenaline transporter NET) and then metabolism.

Receptors and actions

Receptor	Signalling	Main effects
α_1	Gq → IP_3/DAG, ↑ Ca^{2+}	Vasoconstriction (↑ blood pressure), pupil dilation, sphincter contraction, glycogenolysis in liver
α_2	Gi → ↓ cAMP	Inhibition of noradrenaline release (presynaptic), ↓ insulin secretion, platelet aggregation

Receptor	Signalling	Main effects
β_1	Gs → ↑ cAMP	**Heart:** ↑ rate, ↑ contractility; renin release
β_2	Gs → ↑ cAMP	Bronchodilation, vasodilation in muscle, glycogenolysis in muscle and liver, lipolysis, uterine relaxation
β_3	Gs → ↑ cAMP	Lipolysis, bladder relaxation
D_1, D_5	Gs → ↑ cAMP	Renal and mesenteric vasodilation, CNS motor control
D_2, D_3, D_4	Gi → ↓ cAMP	Inhibit prolactin release, CNS reward and motor control; blocked by antipsychotics

Effect	Adrenaline	Noradrenaline	Dopamine
Heart rate and contractility	↑↑ (β_1)	↑ (reflex bradycardia may mask)	↑ at medium dose
Blood pressure	Systolic ↑, diastolic ↓ or no change	Systolic and diastolic ↑↑	↑ at high dose
Peripheral vascular resistance	Variable (β_2 dilation in muscle, α_1 constriction in skin)	↑↑ (α_1)	Renal vasodilation at low dose
Bronchi	Dilation (β_2)	Slight	—
Metabolic	↑ **Glycogenolysis, gluconeogenesis, lipolysis**; ↑ blood glucose, fatty acids, lactate; ↑ metabolic rate	Similar, smaller	—
CNS	Alertness and anxiety	Arousal, attention	Reward, movement, motivation, prolactin inhibition

Catabolism of catecholamines

- Two enzymes inactivate catecholamines: **monoamine oxidase (MAO)**, on the outer mitochondrial membrane (MAO-A: noradrenaline, serotonin; MAO-B: dopamine), and **catechol-O-methyltransferase (COMT)**, which uses SAM and acts in the cytosol (mainly in liver, kidney, and post-synaptic tissues).
- **Dopamine** → DOPAC / 3-methoxytyramine → **homovanillic acid (HVA)**.
- **Noradrenaline and adrenaline** → normetanephrine and metanephrine (COMT) and 3,4-dihydroxymandelic acid (MAO) → **vanillylmandelic acid (VMA, 3-methoxy-4-hydroxymandelic acid)**, the main urinary end-product (normal about **2–7 mg/24 h**); only about 1% of adrenaline is excreted unchanged.

PART E: HEME CATABOLISM AND JAUNDICE

16. Heme: Structure and Synthesis (Background)

Heme is an iron-containing **porphyrin**. Protoporphyrin IX is a ring of four pyrrole rings joined by methene bridges, with four methyl, two vinyl and two propionate side chains; **ferrous iron (Fe^{2+})** is held at the centre by four nitrogens. Heme is the prosthetic group of **haemoglobin, myoglobin, cytochromes (a, b, c, P450), catalase, peroxidase, tryptophan dioxygenase and nitric oxide synthase**. About **85% of heme is made in the bone marrow** (developing red cells) and most of the rest in the **liver**.

Steps of heme biosynthesis

Step	Reaction	Enzyme	Location
1	**Succinyl-CoA + Glycine → δ-Aminolevulinic acid (ALA)**	**ALA synthase (ALAS; PLP)**	Mitochondria. **Rate-limiting.** ALAS1 (liver) is repressed by heme and glucose and induced by drugs; ALAS2 (erythroid) is controlled by iron
2	2 ALA → **Porphobilinogen (PBG)**	**ALA dehydratase** (zinc enzyme)	Cytosol. **Inhibited by lead**
3	4 PBG → **Hydroxymethylbilane**	PBG deaminase (uroporphyrinogen I synthase)	Cytosol
4	→ **Uroporphyrinogen III**	Uroporphyrinogen III cosynthase	Cytosol
5	→ **Coproporphyrinogen III**	Uroporphyrinogen decarboxylase	Cytosol
6	→ **Protoporphyrinogen IX**	Coproporphyrinogen oxidase	Mitochondria
7	→ **Protoporphyrin IX**	Protoporphyrinogen oxidase	Mitochondria
8	Protoporphyrin IX + Fe^{2+} → **Heme**	**Ferrochelatase (heme synthase)**	Mitochondria. **Inhibited by lead**

- **Regulation: heme inhibits ALAS1** by feedback (repressing synthesis and blocking mitochondrial import of the enzyme). **Drugs** (barbiturates, griseofulvin, sulphonamides, rifampicin, phenytoin), alcohol and steroids induce cytochrome P450 and so increase the use of heme, which **induces ALAS1** and can precipitate attacks of acute porphyria. **Glucose** and **hemin** reduce ALAS1 (treatment of acute attacks).
- **Sideroblastic anaemia:** defective ALAS2 (X-linked) or lack of vitamin B_6, lead poisoning or alcohol: iron enters mitochondria but cannot be incorporated into heme and forms **ring sideroblasts**; some respond to pyridoxine.

17. Catabolism of Heme and Bilirubin Metabolism

A normal adult breaks down about **6–7 g of haemoglobin per day** (about 1% of red cells; red-cell life span is **120 days**), which gives about **250–350 mg of bilirubin per day** (about 4 mg/kg). About 80% comes from haemoglobin of senescent red cells and the rest from ineffective erythropoiesis and other heme proteins (myoglobin, cytochromes, catalase). Haemoglobin is degraded in the **reticuloendothelial (macrophage) system** of the spleen, liver (Kupffer cells) and bone marrow.

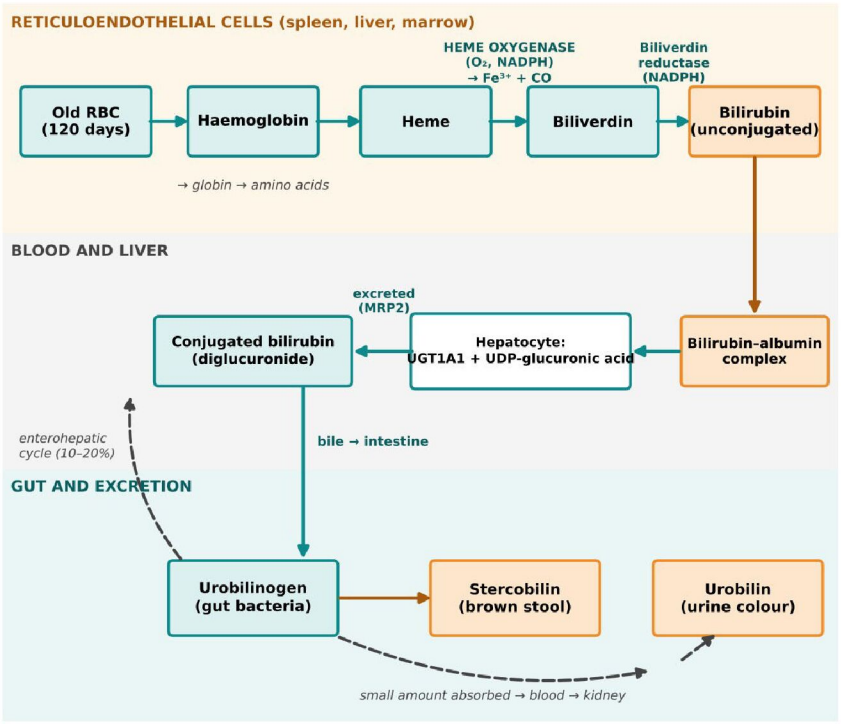

Figure 9: Figure 9: Catabolism of heme: formation, hepatic uptake, conjugation and excretion of bilirubin

Step 1: Formation of bilirubin (in the reticuloendothelial cells)

- Haemoglobin is split into **globin** (hydrolysed to amino acids, reused) and **heme**. Iron is released and recycled (stored as ferritin or carried by transferrin to the marrow).
- **Heme oxygenase** (a microsomal enzyme; uses **O_2 and NADPH**; induced by heme itself) opens the porphyrin ring at the **α-methene bridge**, producing the green pigment **biliverdin**, free **Fe^{3+}** and **carbon monoxide (CO)**. This is the **only endogenous source of CO** in the body (carboxyhaemoglobin about 1%, a marker of haemolysis). The enzyme is the rate-limiting step.
- **Biliverdin reductase** (cytosol; uses **NADPH**) reduces the central methene bridge of biliverdin to give the yellow-orange pigment **bilirubin (unconjugated, indirect bilirubin)**.

Step 3: Uptake, conjugation and excretion by the liver

- **Uptake:** at the sinusoidal surface bilirubin separates from albumin and enters the hepatocyte by facilitated transport (**OATP1B1 and OATP1B3**). In the cell it binds to **ligandin (glutathione S-transferase B, Y protein)** and Z protein, which prevents its backflow into blood.
- **Conjugation:** in the smooth endoplasmic reticulum, **UDP-glucuronosyltransferase 1A1 (UGT1A1)** transfers glucuronic acid from **UDP-glucuronic acid** to the two propionate groups of bilirubin, forming first **bilirubin monoglucuronide** and then **bilirubin diglucuronide** (**conjugated, direct bilirubin**). This makes bilirubin **water-soluble**. UGT1A1 is induced by **phenobarbital** (used in Crigler–Najjar type II) and low in newborns.
- **Excretion:** conjugated bilirubin is actively secreted into the **bile canaliculus** against a concentration gradient by **MRP2 (multidrug resistance-associated protein 2, ABCC2)**, an ATP-dependent transporter. This is the **rate-limiting step** of the whole process, and it is impaired first in liver damage. Some conjugated bilirubin leaks back into blood (OATP1B3 or MRP3) in cholestasis.

Step 4: Fate in the intestine

- Bile carries conjugated bilirubin into the intestine. **Bacterial β-glucuronidases** in the terminal ileum and colon hydrolyse the glucuronides, and the free bilirubin is reduced to the colourless **urobilinogens (mesobilirubinogen, stercobilinogen)**.
- Most urobilinogen is oxidised in the colon to the brown pigment **stercobilin**, which gives faeces its normal colour (**faecal stercobilinogen 40–280 mg/day**).
- About **10–20% of urobilinogen is reabsorbed** into the portal blood (**enterohepatic urobilinogen cycle**); most is taken up again by the liver and re-excreted in bile, and a small amount escapes into the systemic blood and is filtered by the kidney: **urinary urobilinogen 0.5–4 mg/day**, which oxidises to yellow **urobilin** (colour of urine).
- **Antibiotics** that kill gut bacteria and **obstruction of the bile duct** lower or abolish urobilinogen formation; faeces become pale (clay-coloured) in obstruction.

18. Jaundice (Hyperbilirubinaemia)

Jaundice (icterus) is the **yellow discolouration of the skin, sclera and mucous membranes** caused by a raised level of bilirubin in the blood (**hyperbilirubinaemia**, above 2–2.5 mg/dL). It is a sign of disease and not a disease in itself. According to the site of the defect in bilirubin metabolism it is classified as **pre-hepatic (haemolytic), hepatic (hepatocellular) or post-hepatic (obstructive, cholestatic)**. Also by the type of bilirubin raised: unconjugated or conjugated hyperbilirubinaemia.

Types of jaundice

- **Pre-hepatic (haemolytic) jaundice: excess production** of bilirubin by haemolysis (haemolytic anaemias such as sickle cell, thalassaemia, G6PD deficiency, malaria, autoimmune haemolysis, incompatible transfusion, Rh incompatibility) exceeds the capacity of the liver to conjugate and excrete it. **Unconjugated** bilirubin rises.
- **Hepatic (hepatocellular) jaundice:** damage to liver cells (viral hepatitis A–E, alcoholic hepatitis, cirrhosis, drugs and toxins such as paracetamol, isoniazid, Wilson's disease, ischaemia) or an enzyme defect impairs **uptake, conjugation and excretion**. Both types of bilirubin are raised; conjugated bilirubin enters blood as it cannot be secreted.
- **Post-hepatic (obstructive or cholestatic) jaundice: blockage of the bile flow** (intrahepatic: drugs, primary biliary cholangitis; extrahepatic: **gallstones in the common bile duct, carcinoma of head of pancreas, cholangiocarcinoma, biliary stricture, biliary atresia**). Conjugated bilirubin regurgitates into blood and is excreted in urine.

Laboratory differentiation

Finding	Pre-hepatic (haemolytic)	Hepatic (hepatocellular)	Post-hepatic (obstructive)
Serum bilirubin	**Unconjugated** ↑↑ (usually under 5 mg/dL)	**Both** ↑ (conjugated and unconjugated)	**Conjugated** ↑↑ (often over 10 mg/dL)
Urine bilirubin	**Absent** (acholuric)	Present	**Present** (dark, frothy urine)
Urine urobilinogen	**Increased**	Variable (normal or ↑, ↓ if cholestasis)	**Decreased or absent**
Stool colour (stercobilin)	**Dark** (excess stercobilin)	Normal or pale	**Pale, clay-coloured**
ALT, AST	Normal	**Very high** (hundreds to thousands)	Mildly raised
Alkaline phosphatase, GGT	Normal	Mildly raised	**Markedly raised** (3–10 times)
Serum albumin; prothrombin time	Normal	Albumin ↓; PT prolonged (not corrected by vitamin K in severe damage)	PT prolonged, **corrected by vitamin K** injection (fat-soluble vitamin malabsorption)
Other features	Anaemia, reticulocytosis, raised LDH, low haptoglobin, splenomegaly, pigment gallstones	Fever, malaise, hepatomegaly, spider naevi, ascites in cirrhosis	**Pruritus** (bile salts), steatorrhoea, palpable gallbladder (Courvoisier's law)

QUICK WAY TO REMEMBER

Dark urine + pale stool = obstruction. Normal urine colour + dark stool + anaemia = haemolysis. Bilirubin in urine proves conjugated hyperbilirubinaemia, because unconjugated bilirubin is bound to albumin and cannot be filtered.

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NOTE FOR STUDENTS

These notes are prepared for study and revision. Normal values and drug doses vary between laboratories and textbooks; always follow your prescribed textbook and the reference range of your laboratory.

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