Ch 22: Nucelotide Metabolism

Saturday, August 23, 2025 4:00 PM

"Nucleotide Metabolism"

I. Overview

- Nucleotides = essential building blocks for life.
- Functions:
  - Nucleic acid synthesis → required for DNA & RNA → protein synthesis & cell proliferation.
  - Carriers of activated intermediates in biosynthesis:
    - UDP-glucose → glycogen synthesis.
    - CDP-choline → phospholipid synthesis.
  - Components of coenzymes:
    - Coenzyme A (CoA)
    - FAD(H_2)
    - NAD(H)
    - NADP(H)
  - Second messengers in signaling:

        - cAMP
        - cGMP

    - Energy sources:
        - ATP = universal energy currency.

    - Regulators of metabolism:
        - Allosteric inhibitors/activators of key enzymes.

- Sources of bases:

    - De novo synthesis (from scratch).
    - Salvage pathways (reuse of preformed bases).
    - Dietary nucleotides: rarely used → dietary nucleic acids degraded in GIT.

II. Structure of Nucleotides

- Components:

    1. Nitrogenous base (purine or pyrimidine)
    2. Pentose sugar (ribose in RNA, deoxyribose in DNA)
    3. Phosphate group(s) (mono-, di-, or triphosphate).

A. Nitrogenous Bases

Purines (Double-ring)

- Adenine (A)
- Guanine (G)
- Present in both DNA & RNA.

Pyrimidines (Single-ring)

- Cytosine (C) → DNA & RNA
- Thymine (T) → only in DNA
- Uracil (U) → only in RNA
- Difference between T & U: Thymine has a methyl group.

B. Unusual (Modified) Bases

- Found in:
    - Viral DNA
    - tRNA & rRNA (more common in RNA)
- Types of modifications:
    - Methylation

    - Glycosylation
    - Acetylation
    - Reduction

- Functions:

    - Recognition signals for specific enzymes.
    - Protection from nuclease degradation.

B. Nucleosides

- Definition: Base + Pentose sugar (ribose or deoxyribose) linked by N-glycosidic bond.

- Types:

    - Ribonucleosides (sugar = ribose):
        - Adenosine (A)
        - Guanosine (G)
        - Cytidine (C)
        - Uridine (U)

    - Deoxyribonucleosides (sugar = 2-deoxyribose):
        - Deoxyadenosine
        - Deoxyguanosine
        - Deoxycytidine

        - Thymidine (*note: "deoxy-" prefix usually omitted since thymine only exists in DNA*).

- Numbering:

    - Base atoms → numbered without prime (1,2,3...)
    - Sugar atoms → numbered with prime (1′ to 5′)
    - *Important in exam*: 5′-carbon refers to pentose, not the base.

C. Nucleotides

- Definition: Nucleoside + phosphate group(s).

- Phosphate attachment:

    - First phosphate esterified to 5′-OH of sugar → 5′-nucleotide.
    - Example: Adenosine monophosphate (AMP).

- Types:

    - Monophosphate → AMP
    - Diphosphate → ADP
    - Triphosphate → ATP

- High-energy bonds:
    - Between phosphate groups (2nd & 3rd).
    - Hydrolysis releases large $-\Delta G$ → drives cellular reactions.
- Key exam fact:
    - Phosphates confer negative charge → DNA & RNA = nucleic acids.

III. Purine Nucleotide Synthesis

Sources of Atoms for Purine Ring

- Amino acids → Glycine, Glutamine, Aspartate
- CO_2
- N^{10}-formyl tetrahydrofolate (THF)

🔑 *Exam tip*: "Purine = built on ribose step by step."

A. 5-Phosphoribosyl-1-pyrophosphate (PRPP) Synthesis

- Precursor: Ribose-5-phosphate (from PPP).

- Reaction:
    - ATP + Ribose-5-P → PRPP
    - Enzyme = PRPP synthetase (X-linked).
- Regulation:
    - Activated by inorganic phosphate (Pi).
    - Inhibited by purine nucleotides (feedback inhibition).
- Important: PRPP sugar = ribose → so de novo purine synthesis produces ribonucleotides.
    - Later converted to deoxyribonucleotides for DNA synthesis.

B. 5-Phosphoribosylamine Synthesis

- Committed step of purine biosynthesis.
- Reaction:
    - PRPP + Glutamine → 5-Phosphoribosylamine
    - Enzyme = Glutamine:PRPP amidotransferase (GPAT).

- Regulation:
    - Inhibited by AMP & GMP (end products).
    - Rate dependent on PRPP concentration.
    - PRPP normally below Km of GPAT → small increases strongly increase rate.

C. Inosine Monophosphate (IMP) Synthesis

- Pathway:
    - 9 enzymatic steps from 5-Phosphoribosylamine.
    - Requires:
        - 4 ATP molecules (energy).
        - 2 N^{10}-formyl-THF (one-carbon donors).
- Product: IMP (Inosine Monophosphate) → base = hypoxanthine.
- Importance:
    - IMP = parent nucleotide → precursor for AMP & GMP.
- Clinical note: Hypoxanthine also found in tRNA wobble base.

D. Synthetic Inhibitors of Purine Synthesis

1. Sulfonamides (Antibacterials)

- Mechanism:
    - Structural analogs of PABA (para-aminobenzoic acid).
    - Block bacterial synthesis of folic acid → ↓ nucleotide synthesis.
- Selectivity:
    - Humans do not synthesize folic acid (depend on diet) → selective for bacteria.

2. Methotrexate (Anticancer drug)

- Mechanism:
    - Structural analog of folic acid.
    - Inhibits dihydrofolate reductase (DHFR) → ↓ regeneration of tetrahydrofolate (THF).
    - Blocks purine + pyrimidine synthesis → ↓ DNA/RNA synthesis.

- Use:
    - Cancer chemotherapy.
- Toxicity (to rapidly dividing human cells):
    - Bone marrow suppression → anemia
    - GI mucosa → ulceration, diarrhea
    - Skin → scaly changes
    - Immune system → immunodeficiency
    - Hair follicles → alopecia

✅ Exam tip: "Methotrexate → inhibits DHFR → affects both purines & pyrimidines → anticancer drug but causes severe side effects."

E. AMP and GMP Synthesis from IMP

- IMP → AMP pathway:
    - Requires Aspartate (N donor).
    - Requires GTP (energy).
    - Inhibited by AMP (end-product feedback).
- IMP → GMP pathway:

    - Requires Glutamine (N donor).
    - Requires ATP (energy).
    - Inhibited by GMP.

- Regulation:

    - Balances purine pools → whichever nucleotide (AMP or GMP) is less abundant is preferentially synthesized.
    - If both AMP & GMP adequate → GPAT (committed step) inhibited.

Drug: Mycophenolic Acid

- Mechanism: Reversible inhibitor of IMP dehydrogenase (enzyme in GMP synthesis).

- Clinical use:

    - Immunosuppressant → prevents graft rejection (kidney, liver, heart).
    - Treats autoimmune disorders: lupus, Crohn's disease.

- Selectivity:

- T & B lymphocytes highly dependent on GMP → strongly affected.

F. Nucleoside Di- and Triphosphate Synthesis

1. Monophosphate → Diphosphate

- Enzyme: Base-specific nucleoside monophosphate kinases.
- Examples:
  - AMP → ADP (enzyme = adenylate kinase).
- Features:
  - Specific for base (A, G, C, U) but not for ribose vs deoxyribose.
  - Phosphate donor = usually ATP.

2. Diphosphate ↔ Triphosphate

- Enzyme: Nucleoside diphosphate kinase.
- Features:
  - Broad substrate specificity (can work with A, G, C,

U).
    - Maintains balance among NTPs.

Special role of Adenylate Kinase

- Highly active in muscle & liver.
- Maintains equilibrium among AMP, ADP, ATP.

G. Purine Salvage Pathway

- Importance:
    - Salvages purines from:
        - Normal nucleic acid turnover.
        - Small amount from diet.
    - Especially crucial in the brain (limited de novo synthesis).

I. Enzymes in Purine Salvage

- Adenine → AMP
    - Enzyme = Adenine phosphoribosyltransferase (APRT).
- Hypoxanthine + Guanine → IMP/GMP

    - Enzyme = Hypoxanthine-Guanine Phosphoribosyltransferase (HGPRT) (X-linked).

- Substrate: Both use PRPP (ribose donor).
- Irreversible: Due to pyrophosphate hydrolysis.

2. Special Note: Adenosine Salvage

- Adenosine → AMP
- Enzyme = Adenosine kinase.
- Adenosine = only purine nucleoside directly salvaged.

Purine Metabolism Disorders & Deoxyribonucleotide Synthesis

1. Lesch-Nyhan Syndrome

- Inheritance: X-linked recessive.
- Defect: HGPRT deficiency (Hypoxanthine-guanine phosphoribosyltransferase).

- Pathophysiology:
    - Failure of salvage pathway → hypoxanthine & guanine cannot be reused.
    - PRPP levels ↑ (excess substrate for de novo synthesis).
    - IMP & GMP levels ↓ (loss of negative feedback).
    - De novo purine synthesis ↑ → more purine degradation → hyperuricemia.

- Clinical features:
    - Hyperuricemia → uric acid stones (urolithiasis), gouty arthritis, urate deposits in soft tissue.
    - Neurological & behavioral symptoms:
        - Motor dysfunction
        - Cognitive impairment
        - Self-mutilation (biting lips, fingers — very high yield exam feature).

- Exam tip: Inherited cause of hyperuricemia + neurobehavioral symptoms = Lesch-Nyhan.

2. Deoxyribonucleotide Synthesis

- DNA synthesis requires deoxyribonucleotides (dNTPs).

- Enzyme: Ribonucleotide reductase (acts during S-phase of cell cycle).

- Reaction: Converts ribonucleoside diphosphates (ADP, GDP, CDP, UDP) → deoxy forms (dADP, dGDP, dCDP, dUDP).

A. Ribonucleotide Reductase Structure

- R1 (α) subunit: Catalytic + allosteric sites.

- R2 (β) subunit: Contains stable tyrosyl radical for catalysis.

- Hydrogen donor: Two –SH groups on R1 (form disulfide bond during reaction).

B. Regeneration Cycle

1. Enzyme regeneration:
    - Disulfide bond on R1 must be reduced.
    - Thioredoxin donates –SH groups.
2. Thioredoxin regeneration:

- Reduced by thioredoxin reductase (a selenoprotein).
- Uses NADPH + H^+ as electron donor.

3. Regulation of Ribonucleotide Reductase

- Ensures balanced supply of all dNTPs for DNA replication.

- Allosteric regulation (at R1 subunit):

➢ Activity sites:

- ATP binding → activates enzyme.
- dATP binding → inhibits enzyme (prevents all ribonucleotide reduction).
  - Explains toxicity of ↑ dATP in ADA deficiency → ↓ DNA synthesis → SCID.

➢ Substrate specificity sites:

- Binding of specific dNTPs regulates which ribonucleotide is reduced.
- Example: dTTP binding → stimulates GDP → dGDP conversion.

4. Clinical Application: Hydroxyurea

- Mechanism: Inhibits ribonucleotide reductase → ↓ dNTP synthesis → ↓ DNA synthesis.

- Uses:

    - Cancer therapy (melanoma, CML).
    - Sickle cell disease:
        - Increases fetal Hb (HbF) levels.
        - Mechanism: due to gene expression changes, not enzyme inhibition.

Purine Nucleotide Degradation & Gout

I. Overview

- Site:

    - Dietary nucleic acids degraded in small intestine.
    - De novo purines degraded in liver → free bases sent to peripheral tissues for salvage.

- Final product in humans: Uric acid (excreted in urine).

- Note:

- Other mammals: uric acid degraded further by uricase → allantoin (more soluble).
- Recombinant uricase now used clinically to lower urate.

2. Degradation in Small Intestine

i) Pancreatic nucleases (RNAse, DNAse) → oligonucleotides.

ii) Phosphodiesterases → mononucleotides.

iii) Nucleotidases → nucleosides.

iv) Nucleosidases (phosphorylases) → free bases + ribose-1-phosphate.

v) Dietary purine bases → degraded to uric acid (not reused for DNA/RNA).

vi) Most uric acid → absorbed → blood → urine.

3. Pathway of Uric Acid Formation (Enzymes & Steps)

i) AMP → IMP (by AMP deaminase) or adenosine → inosine (by ADA).

ii) IMP, GMP → inosine, guanosine (by 5′-nucleotidase).

iii) Inosine, guanosine → hypoxanthine, guanine (by purine nucleoside phosphorylase).

iv) Guanine → xanthine (by guanine deaminase).

v) Hypoxanthine → xanthine (by xanthine oxidase).

vi) Xanthine → uric acid (by xanthine oxidase).

- Xanthine oxidase (XO) = molybdenum-containing enzyme.

4. Diseases of Purine Degradation

A. Gout

- Definition: Disorder due to hyperuricemia → deposition of monosodium urate (MSU) crystals in joints & soft tissue.

- Pathogenesis:

  i. Hyperuricemia (> 6.8 mg/dL):

        - Overproduction of uric acid OR
        - Underexcretion by kidney (most common).

    ii. MSU crystals → deposit in joints.

    iii. Crystals trigger inflammatory response → acute gouty arthritis.

    iv. Progression → chronic tophaceous gout (nodular MSU deposits = tophi).

B. Clinical Features of Gout

- Acute gouty arthritis (red, swollen, painful joint; classic = 1st MTP = podagra).
- Tophi = nodular MSU crystal deposits in soft tissues.
- Urolithiasis = uric acid kidney stones.
- Diagnosis:
    - Synovial fluid aspiration + polarized light microscopy → needle-shaped crystals.

C. Causes of Hyperuricemia

i. Underexcretion of uric acid (>90% cases):

- Primary: idiopathic renal defects.
- Secondary:
  - Renal disease.
  - Lactic acidosis (lactate competes with urate for excretion).
  - Drugs: thiazide diuretics, lead toxicity (saturnine gout).

ii. Overproduction of uric acid (<10% cases):

- Primary:
  - PRPP synthetase mutation → ↑ Vmax, ↓ Km, or loss of feedback inhibition → ↑ PRPP → ↑ purine synthesis.
  - Lesch-Nyhan syndrome (HGPRT deficiency → salvage failure → ↑ PRPP availability).

- Secondary:
  - High cell turnover (e.g., chemotherapy, myeloproliferative disorders).
  - Metabolic diseases: Von Gierke disease, Hereditary fructose intolerance.

5. Clinical Pearls

- Hyperuricemia ≠ always gout, but gout always has hyperuricemia.
- Xanthine oxidase inhibitors (e.g., allopurinol, febuxostat) ↓ uric acid formation (important therapy).
- Urate oxidase therapy (rasburicase, pegloticase) used in tumor lysis syndrome.
- Lesch–Nyhan syndrome = inherited cause of hyperuricemia + neurobehavioral features.

Dietary Risk Factors for Gout

- Increases risk:
    - Meat (esp. organ meat)
    - Seafood (shellfish)
    - Alcohol (esp. beer & spirits → ↑ lactic acid → ↓ urate excretion)
- Decreases risk:

- Low-fat dairy products

D. Treatment of Gout

➢ Acute Gout Attack

- Aim: ↓ inflammation (no effect on uric acid levels).
- Drugs:
  - Colchicine → inhibits microtubule polymerization → ↓ neutrophil migration.
  - NSAIDs (e.g., indomethacin).
  - Corticosteroids (e.g., prednisone).

➢ Chronic / Long-Term Management

- Aim: Lower serum uric acid < 6.5 mg/dL (below saturation).
- In underexcretors:
  - Uricosuric drugs: probenecid, sulfinpyrazone → ↑ renal excretion.
- In overproducers:

    - Allopurinol (hypoxanthine analog):
        - Converted → oxypurinol = long-lived xanthine oxidase inhibitor.
        - ↓ uric acid synthesis.
        - Hypoxanthine & xanthine accumulate (more soluble than uric acid).
        - Salvage of hypoxanthine by HGPRT → ↓ PRPP → ↓ de novo purine synthesis.
    - Febuxostat: non-purine xanthine oxidase inhibitor (alternative).

Additional Notes

- Uric acid normally near saturation point in plasma → believed to have antioxidant role.

- Tumor lysis syndrome (chemotherapy): risk of uric acid nephropathy → treat with rasburicase/pegloticase (urate oxidase).

Adenosine Deaminase (ADA) Deficiency

- Normal role: ADA deaminates adenosine → inosine.

- Deficiency (autosomal recessive):

    - Adenosine & dATP accumulate.

    - High dATP → inhibits ribonucleotide reductase → ↓ dNTP synthesis → ↓ DNA replication.
    - Lymphocytes (T, B, NK) most affected → severe combined immunodeficiency (SCID).

Clinical Features

- Onset in infancy.
- Severe, recurrent infections (bacterial, viral, fungal).
- Failure to thrive.
- Without treatment → death by 2 years.

Treatment

- Bone marrow transplantation (curative).
- Enzyme replacement therapy (PEG-ADA).
- Gene therapy (ADA gene transfer into stem cells).

Purine Nucleoside Phosphorylase (PNP) Deficiency

- Rarer, less severe than ADA deficiency.

- Affects primarily T cells → partial immunodeficiency.

Pyrimidine Synthesis and Degradation

General Features

- Purine vs. Pyrimidine synthesis:
    - Purine ring → built on ribose-5-phosphate.
    - Pyrimidine ring → synthesized first, then attached to ribose-5-phosphate (from PRPP).
- Sources of atoms for pyrimidine ring:
    - Glutamine
    - CO_2
    - Aspartate

A. Carbamoyl Phosphate Synthesis (Regulated Step)

- Enzyme: Carbamoyl phosphate synthetase II (CPS II).
- Reaction: Glutamine + CO_2 → Carbamoyl phosphate.
- Location: Cytosol.

- Regulation:
    - Inhibited by UTP (end product).
    - Activated by PRPP.
- Clinical correlation:
    - Defects in ornithine transcarbamylase (OTC) in urea cycle → ↑ carbamoyl phosphate → shunted into pyrimidine synthesis → ↑ orotic acid.
- Comparison: CPS I vs. CPS II

Feature	CPS I	CPS II
Location	Mitochondria	Cytosol
Pathway	Urea cycle	Pyrimidine synthesis
N source	Ammonia	Amide group of glutamine
Activator	N-acetylglutamate	PRPP

Inhibitor	—	UTP

B. Orotic Acid Synthesis

- Enzyme 1: Aspartate transcarbamoylase → forms carbamoylaspartate.
- Enzyme 2: Dihydroorotase → closes the ring → dihydroorotate.
- Step: Dihydroorotate oxidized → orotic acid (orotate).
- Cofactor: FMN reduced in this reaction.

C. Pyrimidine Nucleotide Synthesis

- Attachment of ribose-5-phosphate:
  - Enzyme: Orotate phosphoribosyltransferase.
  - Reaction: Orotate + PRPP → OMP (orotidine monophosphate).
  - Releases PPi → irreversible.
- Conversion to UMP:
  - Enzyme: Orotidylate decarboxylase.

    - OMP → UMP.
    - Both enzymes (transferase + decarboxylase) = domains of UMP synthase.

- Clinical correlation:

    - Hereditary orotic aciduria: deficiency of UMP synthase → ↑ orotic acid in urine + megaloblastic anemia.
    - Treatment: Uridine (bypasses block, feedback inhibits CPS II).

- OTC deficiency (urea cycle) → ↑ carbamoyl phosphate → ↑ orotate in urine (but no anemia).

- Further conversions:

    - UMP → UDP → UTP.
    - UDP → substrate for ribonucleotide reductase → dUDP → dUTP → rapidly hydrolyzed to dUMP (by dUTPase, prevents misincorporation of U into DNA).

D. Cytidine Triphosphate (CTP) Synthesis

- Enzyme: CTP synthetase.

- Reaction: UTP + glutamine → CTP.

- Fates:

    - CTP → CDP → substrate for ribonucleotide reductase → dCDP → dCTP (DNA synthesis).
    - dCDP → dCMP → deaminated → dUMP.

E. Deoxythymidine Monophosphate (dTMP) Synthesis

- Reaction: dUMP → dTMP.

- Enzyme: Thymidylate synthase.

- Cofactor: N^5,N^{10}-methylene tetrahydrofolate (THF).

    - Provides both methyl group + 2 hydrogens.
    - THF → oxidized to DHF.

- DHF → THF (by DHF reductase).

- Inhibitors:

    - 5-Fluorouracil (5-FU) → converted to 5-FdUMP → suicide inhibitor of thymidylate synthase → ↓ dTMP → ↓ DNA synthesis → used in cancer

therapy.

- Methotrexate (MTX) and other folate analogs → inhibit DHF reductase → ↓ THF → ↓ purine + ↓ dTMP synthesis → ↓ DNA synthesis → anticancer effect.

- Other analogs:

  - Acyclovir (purine analog) → HSV infections.
  - AZT (zidovudine, pyrimidine analog) → HIV infections.

F. Pyrimidine Salvage and Degradation

1. Degradation of Pyrimidines

- Unlike purines:

  - Purines → not cleaved in humans → excreted as uric acid (poorly soluble).
  - Pyrimidines → ring is opened → degraded into soluble products.

- End products:

    - CMP & UMP → degraded to β-alanine.
    - TMP → degraded to β-aminoisobutyrate.

- Other products formed: Ammonia (NH_3) and CO_2.

- Clinical note: Since products are highly soluble, pyrimidine degradation does not cause gout-like disorders.

2. Pyrimidine Salvage Pathway

- Process: Pyrimidine bases → converted to nucleosides → phosphorylated to nucleotides.

- Clinical significance:

    - Less critical than purine salvage (because pyrimidine degradation products are soluble and easily excreted).

    - Exception:
        - Uridine salvage is clinically important.
        - Basis of treatment for hereditary orotic aciduria (supplemented uridine bypasses UMP synthase deficiency and inhibits CPS II by feedback).