# Part (II) Nitrogenous molecules metabolism

## Amino acids metabolism

1. Protein/amino acids catabolism:
   - Protein turnover
     - ◊ Normal cellular protein degradation
     - ◊ PEST sequence (rich in P, E, S, and T) target proteins for rapid degradation
     - ◊ In lysosome (ATP-independent processes): extracellular, membrane-associated and long-lived intracellular proteins.
     - ◊ ATP and Ubiquitin-tag → proteasome (abnormal and short-lived proteins in cytosol)
   - Dietary protein surplus
     - ◊ Provide up to 90% metabolic energy in carnivores after meal.
     - ◊ Amino acids can not be stored.
   - Starvation or diabetes mellitus
     - ◊ Protein is used as fuel
       - Kwashiorkor: results when a child is weaned onto a starchy diet poor in protein
       - Marasmus: both caloric intake and specific amino acids are deficient.
   - Nitrogen balance
     - ◊ Positive: an access of ingested over excreted, accompanies growth and pregnancy
     - ◊ Negative: output exceeds intake, may follow surgery, advanced cancer, and kwashiorkor or marasmus.


2. Amino acid catabolism:
   - Amino group: $NH_4^+$ → $(NH_3)_2CO$ (in mammal, urea cycle)
   - C-skeleton: all enter TCA cycle
     - ◊ Glucogenic a.a.
       - Degraded to pyruvate, a-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate → glucose and glycogen.
     - ◊ Ketogenic a.a.
       - Degraded to acetoacetyl-CoA and or acetyl-CoA (6 a.a.) → ketone bodies (acetone, acetoacetate, D-β-hydroxybutyrate).
       - Untreated diabetes: liver will produce large amounts of ketone bodies from fatty acids and ketongenic a.a.
       - Leu is an exclusively ketogenic a.a. that is common in proteins. Its degradation makes a substantial contribution to ketosis under starvation conditions.
   - Classification by biological function (glucogenic, ketogenic):

| Glucogenic | Ketogenic | Glucogenic and ketogenic |
|---|---|---|
| Ala, Arg, Asp<br>Cys<br>Glu, Gly<br>His<br>Met<br>Pro, (Hyp)<br>Ser<br>Thr<br>Val | Leu<br>Lys | Ile<br>Phe<br>Trp<br>Tyr |

3. Amino acid degradation in human:
   - Amino group:
     - Transamination (aminotransferase or transaminase; requires PLP-pyridoxal phosphate as a cofactor)
       - SALT test (alanine aminotransferase, or GPT)
       - SAST test (aspartate …, or GOT)
     - Transfer $NH_4^+$ to liver in the form of: Glu, Gln, Ala
       - In muscle tissue: pyruvate + $NH_4^+$ → alanine
       - Glucose-alanine cycle + Glucose-lactate cycle = Cori cycle
     - Deamination (trans-deamination) in liver by glutamate dehydrogenase
       - Requires $NAD^+$ or $NADP^+$
       - Allosterically regulated (reflects energy needs):
         - ✓ Activator: GDP, ADP
         - ✓ Inhibitor: GTP, ATP
       - Acidosis and Gln processing in kidney
     - N excretion: almost exclusively in liver:
       - $NH_4^+$ → urea (urea cycle)
       - 5 enzymatic steps (4 steps in urea cycle)
       - 2 cellular compartments involved
       - Urea → bloodstream → kidney → excreted into urine
     - Urea cycle enzyme defect → ammonia intoxication
       - Carbamoly phosphate synthetase I (hyperammonemia type I)
         - ✓ Supplement of carbamoyl glutamate (N-acetylglutamate analog)
       - Ornithine transcarbamoylase (hyperammonemia type II)
       - Argininosuccinate synthetase (citrullinemia)
         - ✓ Feeding arginine promotes N excretion
         - ✓ Feeding benzoate, phenylbutyrate (aromatic keto acids)
       - Argininosuccinate lyase (argininosuccinicaciduria)
         - ✓ Feeding arginine and benzoate
       - Arginase (hyperargininemia)
         - ✓ Low protein diet
   - C-skeleton: all enter mainstream metabolic pathway, TCA cycle.
     - Cofactor for one C-transfer:
       - Biotin (transfer $CO_2$)
       - Tetrahydrofolate ($H_4$ folate) (transfer –HC=O, -HCOH, or $–CH_3$)
         - ✓ $H_4$ folate deficiency and pernicious anemia
       - S-adenosylmethionine (adoMet, SAM) (transfer $–CH_3$)
     - BCAA (Val, Leu, and Ile)
       - Degraded in extrahepatic tissue (muscle, adipose tissue, kidney and brain)
       - Branched-chain aminotransferase
       - Branched-chain α-keto acid dehydrogenase complex
         - ✓ Maple syrup urine disease (MSUD)/branched-chain ketonuria
         - ✓ Diet restriction, branched-chain keto acids supplement.
     - Phenylalanine and tyrosine
       - Phe → Tyr: phenylalanine hydroxylase and phenylketouria (PKU)
         - ✓ The artificial sweetener: aspartame
       - Tyrosine degradation
         - ✓ Homogentisate dioxygenase defect → alkaptonuria

4. Principal serum enzymes used in clinical diagnosis: (from Harper's 26th ed. Table 7.2)

| Serum Enzyme | Major diagnostic use |
|---|---|
| Aminotransferases:<br>AST, or SGOT<br>ALT, or SGPT | Myocardial infarction<br>Viral hepatitis |
| Amylase | Acute pancreatitis |
| Ceruloplasmin | Hepatolenticular degeneration<br>(Wilson's disease) |
| Creatine kinase | Muscle disorders and myocardial infarction |
| γ-Glutamyl transpeptidase | Various liver diseases |
| Lactate dehydrogenase (isozymes) | Myocardial infarction |
| Lipase | Acute pancreatitis |
| Phosphatase, acid | Metastatic carcinoma of the prostate |
| Phosphatase, alkaline (isozymes) | Various bone disorders, obstructive liver diseases |

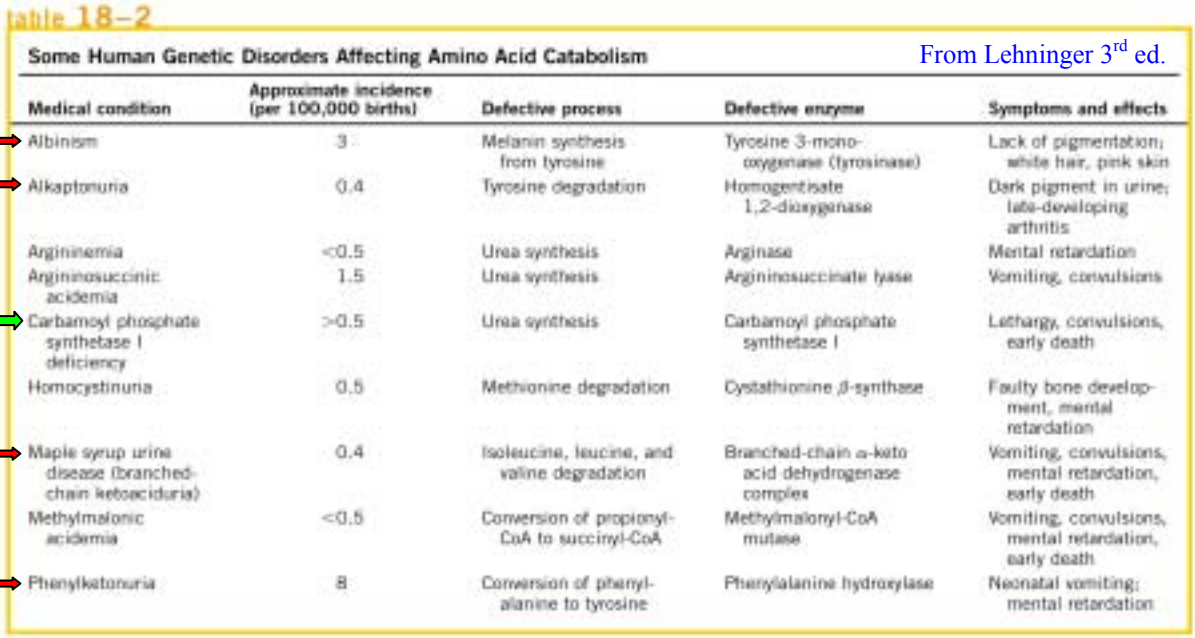


Table 18–2

**Some Human Genetic Disorders Affecting Amino Acid Catabolism** From Lehninger 3rd ed.

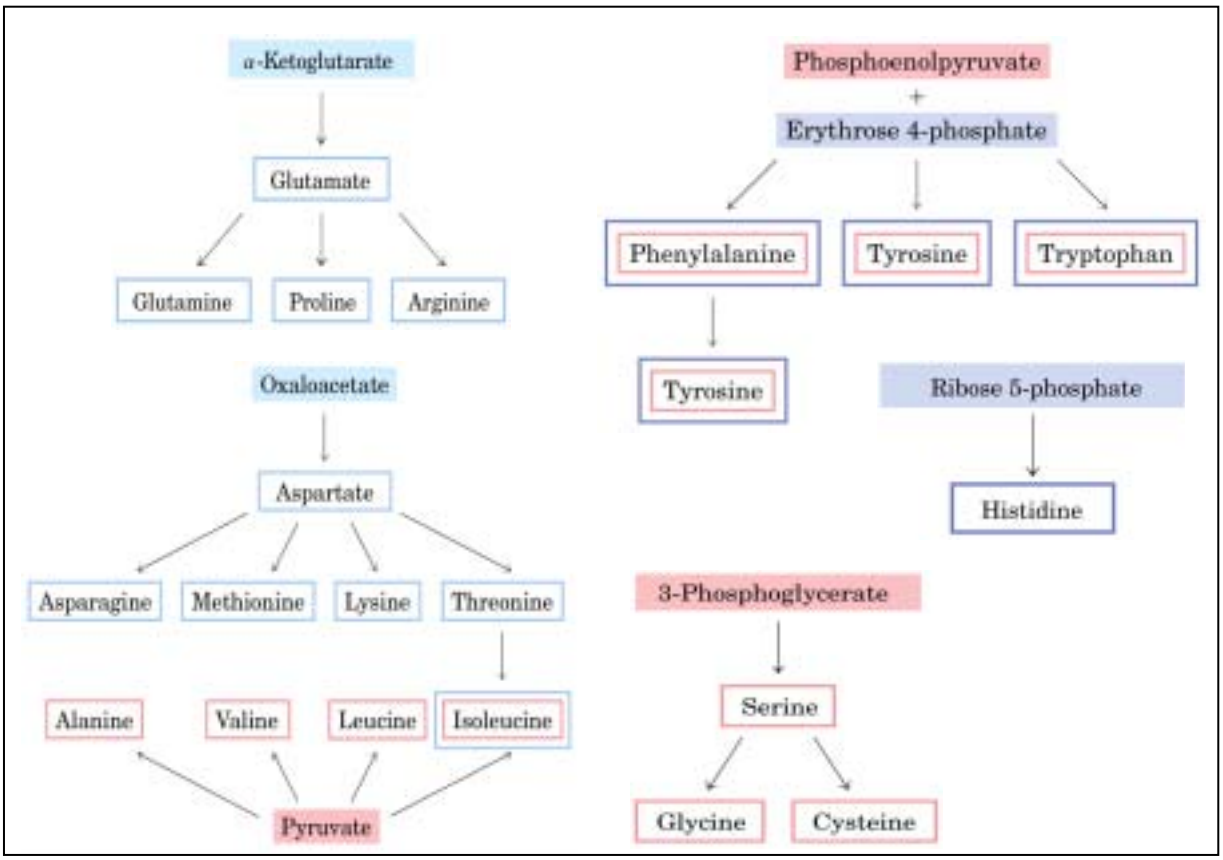
| Medical condition | Approximate incidence (per 100,000 births) | Defective process | Defective enzyme | Symptoms and effects |
|---|---|---|---|---|
| Albinism | 3 | Melanin synthesis from tyrosine | Tyrosine 3-monooxygenase (tyrosinase) | Lack of pigmentation; white hair, pink skin |
| Alkaptonuria | 0.4 | Tyrosine degradation | Homogentisate 1,2-dioxygenase | Dark pigment in urine; late-developing arthritis |
| Argininemia | <0.5 | Urea synthesis | Arginase | Mental retardation |
| Argininosuccinic acidemia | 1.5 | Urea synthesis | Argininosuccinate lyase | Vomiting, convulsions |
| Carbamoyl phosphate synthetase I deficiency | >0.5 | Urea synthesis | Carbamoyl phosphate synthetase I | Lethargy, convulsions, early death |
| Homocystinuria | 0.5 | Methionine degradation | Cystathionine β-synthase | Faulty bone development, mental retardation |
| Maple syrup urine disease (branched-chain ketoaciduria) | 0.4 | Isoleucine, leucine, and valine degradation | Branched-chain α-keto acid dehydrogenase complex | Vomiting, convulsions, mental retardation, early death |
| Methylmalonic acidemia | <0.5 | Conversion of propionyl-CoA to succinyl-CoA | Methylmalonyl-CoA mutase | Vomiting, convulsions, mental retardation, early death |
| Phenylketonuria | 8 | Conversion of phenylalanine to tyrosine | Phenylalanine hydroxylase | Neonatal vomiting; mental retardation |

5. Classification by nutrition: essential vs. nonessential amino acid: * semi-essential.

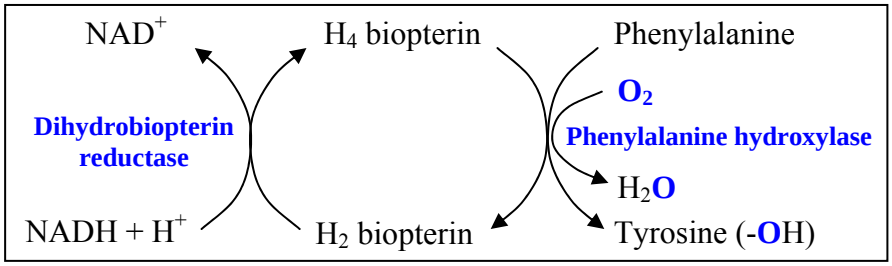
| Nutritionally essential | Nutritionally nonessential |
|---|---|
| Arginine* | Alanine |
| Histidine | Asparagine |
| Isoleucine | Aspartate |
| Leucine | Cysteine |
| Lysine | Glutamate |
| Methionine | Glutamine |
| Phenylalanine | Glycine |
| Threonine | Proline |
| Tryptophan | Serine |
| Valine | Tyrosine |

6. Amino acid biosynthesis:
   - N enters the pathway in the form of:
     - Glu (aminotransferase), Gln (amidotransferase)
   - C-skeleton is derived from:
     - Glycolysis (3-phosphoglycerate/3-PG, phosphoenolpyruvate/PEP, pyruvate)
     - Citric acid cycle (α-KG, OAA)
     - Pentose phosphate pathway (Ribose 5-phosphate, erythrose 4-phosphate)

7. Amino acid biosynthesis in human:
   - Essential a.a.: complex chemical structure, require multiple steps, human body has lost the ability to do the job…




   - Non-essential a.a.: short biosynthetic pathways (only few steps)
     - ◊ α-ketoglutarate → Glu, Gln, Arg, Pro
     - ◊ 3-phosphoglycerate → Ser, Gly, Cys
       - Cys from Met (S) and Ser (C-skeleton)
     - ◊ Oxaloacetate → Asp, Asn
     - ◊ Pyruvate → Ala
     - ◊ Tyr from Phe (phenylalanine hydroxylase)
       - Phenylalanine hydroxylase is a **mixed-function oxygenases**, which catalyze simultaneous hydroxylation of a substrate by an oxygen atom of $O_2$ and reduction of the other oxygen atom to $H_2O$.
       - Phenylalanine hydroxylase requires a cofactor tetrahydrobiopterin.
         - ✓ Dihydrobiopterin reductase defect: PKU, L-dopa…
         - ✓ Supplementing the diet with $H_4$ biopterin itself is ineffective because it is unstable and does not cross the BBB.



- ◊ Hydroxyproline and hydroxylysine (in collagen): no specialized tRNA, not from dietary intake (degraded completely)
  - Derived from Pro and Lys after incorporation into peptides (post-translational modification)
  - The hydroxylases are **mixed-function oxygenases** that require substrate, molecular $O_2$, ascorbate, $Fe^{2+}$, and α-ketoglutarate.
    - ✓ Pro + α-KG + $O_2$ (ascorbate, $Fe^{2+}$) → Hydroly-Pro + succinate
- ◊ BCAA (Val, Leu, Ile) can be formed by transamination with their corresponding α-keto acids (supplied in diet).
  - Ammonia intoxication….

■ Regulation
- ◊ Allosteric feedback inhibition
  - End product acts as a modulator for the allosteric enzyme.
  - Simple and concerted inhibition.
- ◊ Glutamine synthetase
  - Allosteric regulation
  - Covalent modification

8. S-adenosylmethionine (S-adoMet, SAM)

■ Cofactor for methyl group transfer: activated methyl cycle
- ◊ From ATP + Met (by methionine adenosyl transferase) (Fig 18-17)
  - Triphosphate of ATP is displaced by S from Met.
    - ✓ Similar reaction in coenzyme $B_{12}$ synthesis.
- ◊ Met is regenerated by addition of a methyl group to homocysteine (by methionine synthase)
  - The 1-carbon donor: $H_4$ folate or methylcobalamin derived from coenzyme $B_{12}$.
  - The methyl group of methylcobalamin is derived from $N^5$-methyl $H_4$ folate.
  - $B_{12}$ deficiency: may trap folate in $N^5$-methyl form → pernicious anemia.

## Molecules derived from amino acids:

9. Porphyrins (Gly + Succinyl-CoA)
   - Multiple steps
     - ALA synthestase (ALAS1, drug-induced ALAS1 de-repression)
     - ALA dehydratase (Zn containing enzyme), can be inhibited by Pb (lead).
     - Degraded to linear tetrapyrrole derivative: bilirubin (jaundice).

10. Creatine (Gly + Arg + Met/S-adoMet )
    - Cr + ATP ←→ CrP + ADP (by creatine kinase)
      - Creatine (Cr) and phosphocreatine (PCr, or CrP)
      - Energy buffer in skeletal muscle
    - Creatinine: from CrP by irreversible, nonenzymatic dehydration and loss of phosphate.
      - The 24-hour urinary excretion of creatinine is proportionate to muscle mass.

11. Glutathione (GSH), (Gly, Glu and Cys)
    - As a redox buffer.
      - Maintain Cys in the reduced form (-SH).
      - Iron of heme in the ferrous ($Fe^{2+}$) state.
      - Serve as a reducing agent for glutaredoxin in deoxyribonucleotide synthesis. (Fig 22-37)
      - Remove toxic peroxides under aerobic conditions.
    - Oxidized form: GSSG = two GSH linked by a disulfide bond.
      - 2 GSH + R-O-O-H → GSSH + $H_2O$ + R-OH
      - Catalyzed by glutathione peroxidase (containing selenium, Se, in the form of selenocysteine).

12. D-amino acids
    - Bacterial cell wall.
      - D-alanine and D-glutamate
      - Derived from L-isomers by racemase (PLP as coenzyme), which is the prime target for pharmaceutical agents (side-effect on other PLP-requiring enzymes)
        - L-fluoroalanine: tested as antibacterial drug
        - Cycloserine: to treat tuberculosis
    - Peptide antibiotics.

13. From aromatic a.a. to many plant substances
    - From Phe and Tyr
      - Tannins (單寧酸): inhibit oxidation in wines
      - Morphine: potent physiological effects
      - Flavor components: cinnamon oil, nutmeg (肉荳蔻), cloves (丁香), vanilla, and cayenne pepper (辣椒).

14. Amino acids are converted to biological amines by **decarboxylation** (PLP as a cofactor):
    - From Tyr
      - Dopa, dopamine (↓ Parkinson's disease, ↑ schizophrenia)
        - Dopa → melanin
      - Dopamine → norepinephrine (requires ascorbate, $Cu^{2+}$)
      - Norepinephrine → epinephrine (requires adoMet)
    - From Glu
      - GABA (γ-aminobutyrate): ↓ epileptic seizures

- GABA analogs to treat epilepsy and hypertension
- Or use inhibitors of GABA aminotransferase (GABA-degrading enzyme)

- From His
  - ◊ Hitamine (allergic reaction, stimulate gastric acid)
    - Cimetidine (Tagamet): histamine receptor antagonist: structural analog of histamine, it promotes healing of duodenal ulcers by inhibiting secretion of gastric acid
- From Trp
  - ◊ Nicotinate (niacin), a precursor of NAD and NADP.
  - ◊ Serotonin: a potent vasoconstrictor and smooth muscle stimulator.
  - ◊ Serotonin → → melatonin.

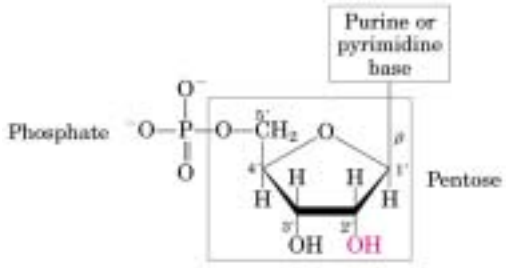
- From Met and ornithine (by ornithine decarboxylase, PLP-requiring enzyme)
  - ◊ Spermine and spermidine: used in DNA packaging.
    - Required in large amounts in rapidly dividing cells.
    - African sleeping sickness (trypanosome-caused disease, 錐蟲病): ornithine decarboxylase has a much slower turnover rate in trypanosome than in human (human, fast turnover, less side-effect of enzyme inhibitor)
    - DMFO (difluoromethylornithine): suicide inhibitor or mechanism-based inhibitor.

15. From Arg
- NO (nitric oxide), gas, unstable and can not be stored.
  - ◊ Nitric oxide synthase (NOS): 4 cofactors (FMN, FAD, $H_4$ biopterin, $Fe^{3+}$-heme)
  - ◊ Synthesis is stimulated by NOS with $Ca^{2+}$-CaM.
  - ◊ Neurotransmission, blood clotting, and the control of blood pressure.

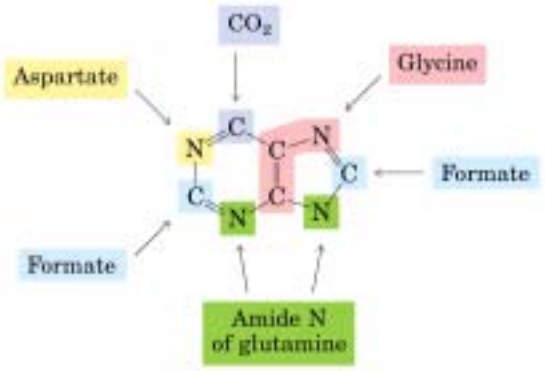
16. Summary of the biosynthesis of some important amines:

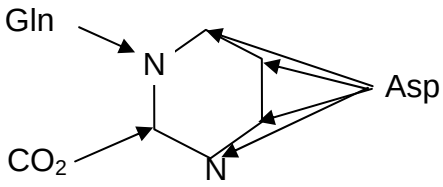
| **Amine** | **Amino acid precursor** | **Distinguishing features of pathways** |
|---|---|---|
| Acetylcholine | Ser, Met | S-adoMet is methylating agent |
| Norepinephrine | Tyr | L-dopa is intermediate and precursor of melanins |
| Epinephrine | Tyr, Met | S-adoMet-dependent tyrosine aminotransferase induced by glucocorticoids |
| Serotonin | Trp | 5-hydroxytryptophan intermediate |
| γ-aminobutyrate (GABA) | Glu | Decarboxylation reaction |
| Histamine | His | Decarboxylation reaction |
| Spermine | Ornithine, Met | Spermidine is intermediate |
| Creatine | Arg, Gly, Met | Guanidino group transferred to glycine |
| Purine nucleotide | ***Gly***, Asp, Gln | Gly → part of the carbon skeleton |
| Pyrimidine nucleotide | ***Asp***, Gln | Asp → part of the carbon skeleton |

## Nucleotide metabolism




17. Nucleotide
   - Chemical structure:
     - ◊ Phosphate group (monophosphate)
     - ◊ Pentose (ribose, deoxyribose)
     - ◊ Nitrogenous base (A, G, C, U, T)
   - Absorb UV light (max. ~ 260 nm)
   - Polynucleotide: $NT_1$ (5'-P) + $NT_2$ (3' OH- of ribose) ➔ 3'→5' phosphodiester bond.
     - ◊ RNA is less stable as the 2'-OH functions as a nucleophile during hydrolysis of the 3',5'-phosphodiester bond.
     - ◊ Directional molecules: 5' → 3'.
       - ▫ 5'-end: free or phosphorylated 5'-OH
       - ▫ 3'-end: free 3'-OH




18. Nucleotide synthesis: **de novo** pathways and **salvage** pathways:
   - Purine (two rings, shorter name) de novo synthesis:
     - ◊ **PRPP**, Gln x 2, Gly, Formate x 2, $CO_2$, Asp → inosine monophosphate (IMP)
     - ◊ IMP → AMP (GTP hydrolysis); IMP → GMP (ATP hydrolysis).
     - ◊ 1-C transfer (formate): requires $H_4$ folate (folic acid)
       - ▫ Deficiency of folic acid → purine deficiency state
       - ▫ Inhibition of $H_4$ folate formation → cancer chemotherapy.
       - ▫ e.g. azaserine, diazanorleucine, 6-mercaptopurine, and mycophenolic acid.
   - Purine salvage pathway (less energy required):
     - ◊ Purine base + PRPP → Purine nucleotide + PPi (pyrophosphate) or
     - ◊ Purine nucleoside + ATP → Purine nucleotide + ADP.
   - Liver is the major site of purine nucleotide biosynthesis.
   - Regulation (allosteric feedback + reciprocal energy use):
     - ◊ Ribose 5-phosphate → PRPP → … AMP, ADP, GMP, and GDP
     - ◊ IMP → AMP (GTP hydrolysis); IMP → GMP (ATP hydrolysis).
   - Ribonucleotide vs. deoxyribonucleotide. (reduction at the level of diphosphate).
     - ◊ Requires: thioredoxin, thioredoxin reductase, and NADPH.
   - Pyrimidine (one ring, longer name): orotate + PRPP → UMP → CMP
   - UDP → dUDP → dUMP → dTMP (thymidylate synthase + 1 C-transfer)
     - ◊ Dihydrofolate reductase is required and it is a target for the anticancer drug **methotrexate** (competitive inhibitor).
     - ◊ Disorders of folate and vitamin $B_{12}$ metabolism results in deficiencies of TMP.
     - ◊ Thymidylate synthase is inhibited by **fluorouracil** and **Aminopterin** (mechanism-based inhibitor).

- Pyrimidine catabolism: $NH_4^+$ → urea, all soluble compound
  - Thymine → → *β-aminoisobutyrate* (Harper 26th, p.300) → methylmalonylsemialdehyde (an intermediate of Val catabolism) → → succinyl-CoA (Lehninger 3rd, Fig 22-44).
    - Excretion of *β-aminoisobutyrate* increases in leukemia and severe x-ray radiationexposure due to increased destruction of DNA. However, many persons of Chinese or Japanese ancestry routinely excrete *β-aminoisobutyrate.*
  - Cytosine → uracil → → β-alanine.

- Disorders of purine catabolism. Purine is degraded to uric acid.
  - Gout
  - Lesch-Nyhan Syndrome:
    - Defect in **hypoxanthine-guanine phosphoribosyl transferase** (HPRT, HGPRTase, purine salvage enzyme)
  - Von Gierke's diseases
    - Glucose-6-phosphatase deficiency.
    - Enhanced PRPP precursor (R5P).
  - Hypouricemia
    - **Xanthine oxidase** deficiency (**allopurinol** is a competitive inhibitor)
  - **Immunodificiency**
    - Accumulation of dGTP and dATP, which inhibit **ribonucleotide reductase** and thereby deplete cells of DNA precursors.
    - Both T cells and B cells are sparse and dysfunctional: **adenosine deaminase** deficiency. → sterile "bubble" environment.
    - T cell deficiency but B cell normal: **purine nucleoside phosphorylase** deficiency.

- Many chemotherapeutic agents target enzymes in the nucleotide biosynthetic pathway.
  - **Cancer cells has a more active salvage pathway**
    - Compounds that inhibit glutamine amidotransferases (N donor)
      - Glutamine analogs: azaserine and acivicin.
    - **Thymidylate snythase** and **dihydrofolate reductase**: enzymes that provide the only cellular pthway for thymine synthesis.
      - **Fluorouracil → FdUMP**: acts on thymidylate synthase (mechanism-based).
      - **Methotrexate**: inhibits dihydrofolate reductase (competitive inhibitor)
      - Aminopterin: inhibits dihydrofolate reductase.
  - **Allopurinol (purine analog) used in against African trypanosomiasis.**
    - Allopurinol is also an alternative substrate for orotate phosphoribosyltransferase, competes with orotic acid.

19. Review of amino acids:

| **Amino acid** | **Features** |
|---|---|
| Gly | Break α-helix, to form β-turn;<br>Triple helix in collagen;<br>Creatine, heme/porphrin, purines. |
| α-alanine<br><br><br>β-alanine | L-Ala → pyruvate (by ALT or SGPT);<br>D-ala in bacterial wall and some antibiotics.<br><br>A metabolite of cysteine;<br>Present in coenzyme A as β-alanyl dipeptides (carnosine) (in pantotheinic acid → CoA);<br>Product of degradation of pyrimidine (cytosine and uracil). |
| Cys | The thioethanolamine portion of coenzyme A ($CO_2$ + β-mercaptoethylamine/Cys → CoA);<br>$CO_2$ + β-mercaptoethylamine/Cys → taurine → bile salt. (the taurine that conjugates with bile acids such as taurocholic acid). |
| Ser | Serine protease (trypsin, chymotrypsin, elastase); catalytic mechanism: covalent catalysis;<br>Irreversible inhibitor (**di**i**f**luoro**p**hosphate, DIFP);<br>Ser → ethanolamine → choline → phosphatidylcholine/Lecithin<br>choline → acetylcholine<br>Ser (palmitoyl-CoA) → Sphingosine<br>O-linked glycosylation site, phosphorylation site. |
| Thr | O-linked glycosylation site, phosphorylation site. |
| Asp | Asp protease (HIV-1 protease, inhibited by pepstatin); covalent catalysis;<br>General acid-base catalysis (lysozyme, trypsin, chymotrypsin);<br>Provide $NH_3^+$ in urea and purine (inosine) biosynthesis;<br>Provide C-skeleton in pyrimidine ring biosynthesis. |
| Glu | General acid-base catalysis (lysozyme)<br>Covalent catalysis (carboxypeptidase A)<br>Guutathione: GSH peroxidase/Se. |
| Pro | Break α-helix, induce β-turn;<br>Pro and HO-Pro in collagen (and HO-Lys): hydroxylation via oxidase and ascorbate. |
| Val, Leu, Ile | BCAA: contained β-oxidation;<br>Energy source of muscle, not degraded in liver |
| Met | Specific cleavaged by CNBr (cyanogens bromide) at C-terminus;<br>Precursor of S-adoMet, spermine, spermidine |
| Arg | Trypsin cleaves the carboxyl site of Arg and Lys residues in peptide;<br>Semi-essential a.a.;<br>Precursor of NO, creatine |
| Lys | Trypsin cleaves the carboxyl site of Arg and Lys residues in peptide;<br>Protein/Lys-$NH_3^+$ + $^-$OOC-ubiquitin → ubiquitin-dependent degradation. |
| Trp | Nicotinate (a precursor of NAD and NADP); Serotonin |
| His | Semi-essential a.a.;<br>General acid-base catalysis: chymotrypsin; trypsin. |