CHAPTER 22 SUMMARY

BY

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Nucleotides Metabolism

Overview

1) Nucleotides are essential for DNA and RNA synthesis, and therefore ⟶ Protein synthesis
2) They can serve as carriers of activated intermediates in the synthesis of carbohydrates, lipids, or conjugated proteins.
e.g. UDP-Glucose & CDP-Choline
3) Are structural components of important enzymes
e.g. co-enzyme A, FAD, NAD^+, NADP+
4) Can act as second messengers in signal transduction pathways
5) Play an important role as "energy currency" e.g. ATP
6) Act as regulatory compounds for pathway intermediates (by activation or inhibition)

Purine + Pyrimidine bases are found in nucleotides, they can be:

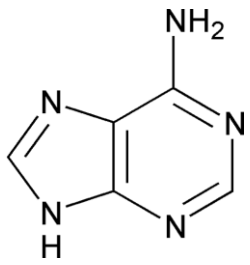
- synthesized de novo
- obtained through salvage pathways
- obtained from degradation of nucleotides in the GIT ⟶ base of nucleoside
  ↘ Uric Acid

Structure of a Nucleotide

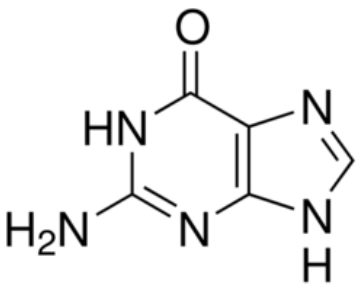
It is composed of:

1) Nitrogenous base (either purine or pyrimidine)
2) pentose monosaccharide
3) one/two/three phosphate groups
Nucleotide - Phosphate group = Nucleoside

DNA and RNA both have the same purine bases:

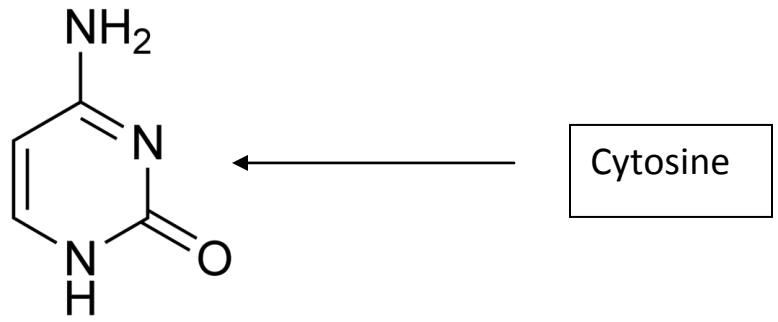


Adenosine



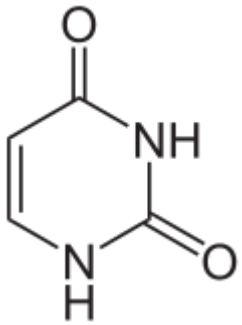
Guanine

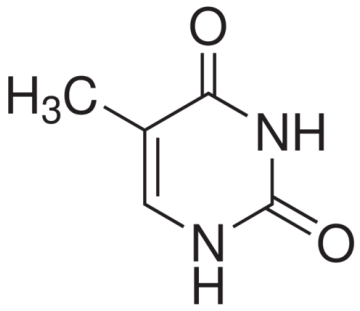
Both DNA and RNA contain a pyrimidine called **cytosine** :



However, they differ in the following:

RNA contain a **Uracil** nitrogenous base while DNA contain **Thymine**



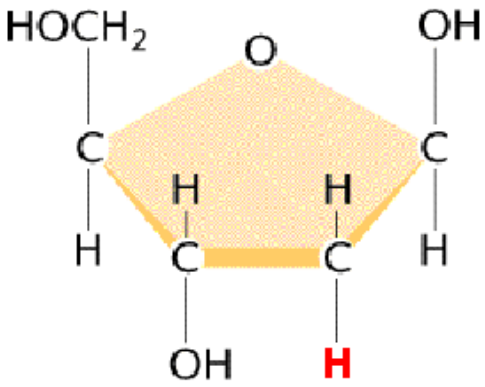


Thymine is basically Uracil with <u>an extra methyl group</u>

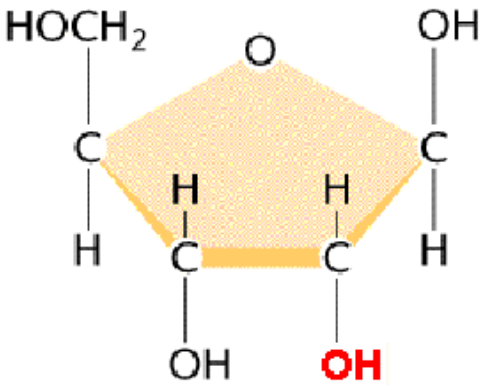
Nitrogenous bases can undergo various modifications in order to be <u>*recognized*</u> by specific enzymes or <u>*protected*</u> from degradation by nucleases. Those modifications may include:

- methylation
- glycosylation
- acetylation
- reduction

A nucleoside is called "deoxyribonucleoside if there is an "H" instead of an "OH" on the second carbon of the ribose ring.



2-Deoxyribose



Ribose

Nucleosides can be attached to one two or three phosphate groups, e.g.

- Adenine + Ribose + one phosphate = AMP
- Adenine + ribose + two phosphates = ADP
- Adenine + Ribose + three phosphates = ATP

The second and third phosphates are connected to the nucleotide by a "*high-energy*" bond. They are responsible for the negative charges associated with the nucleotides.

Synthesis of purine nucleotides

The purine ring is primarily constructed in the liver by adding donated carbons and nitrogens to ribose 5-phosphate. (Which was synthesized in the pentose phosphate pathway)

Atoms are taken from:
1- Amino Acids
2- CO2
3) N^{10} formyltetrahydrofolate

STEPS:

First step; **PRPP** is an activated pentose, its synthesis is catalyzed in the first step by the enzyme **PRPP synthetase**, it is activated by inorganic phosphate and inhibited by purine nucleotides. (end-product inhibition)

1- Ribose 5-Phosphate + ATP ⟶ 5-phosphoribosyl-1-pyrophosphate

PRPP Synthetase

Mg^{2+} ; coenzyme

The second step is the committed step, the amide group of glutamine displaces the pyrophosphate group on carbon 1 of the ring.

2- PRPP + Glutamine + H_2O ⟶ 5-phosphoribosylamine + Glutamate + Pyrophosphate

Glutamine: phosphoribosyl pyorphosphate amidotransferase

The intracellular concentration of PRPP is normally below the K_m for the enzyme, therefore any small change in the PRPP concentration causes a proportional change in the rate of reaction.

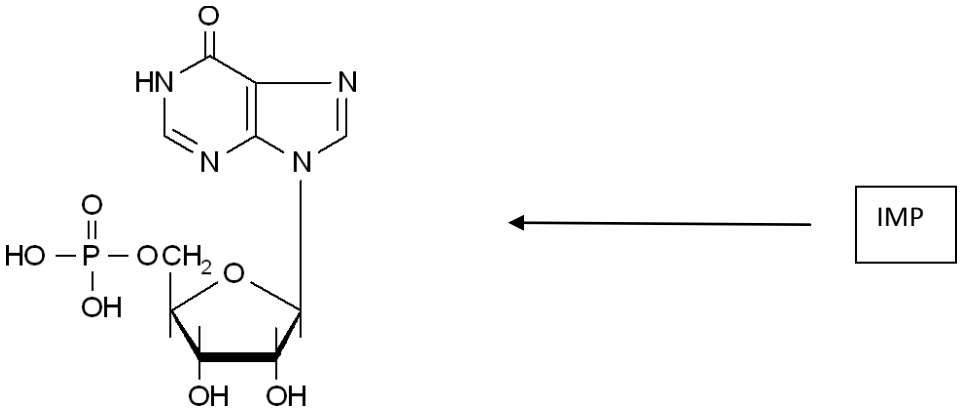
The next 9 steps aren't fairly important, but it should be kept in mind that:

ATP is used up 3 times

There is an enzyme called formyltransferase that converts N^{10} formyltetrahydrofolate to **tetrahydrofolate** twice in the reaction.

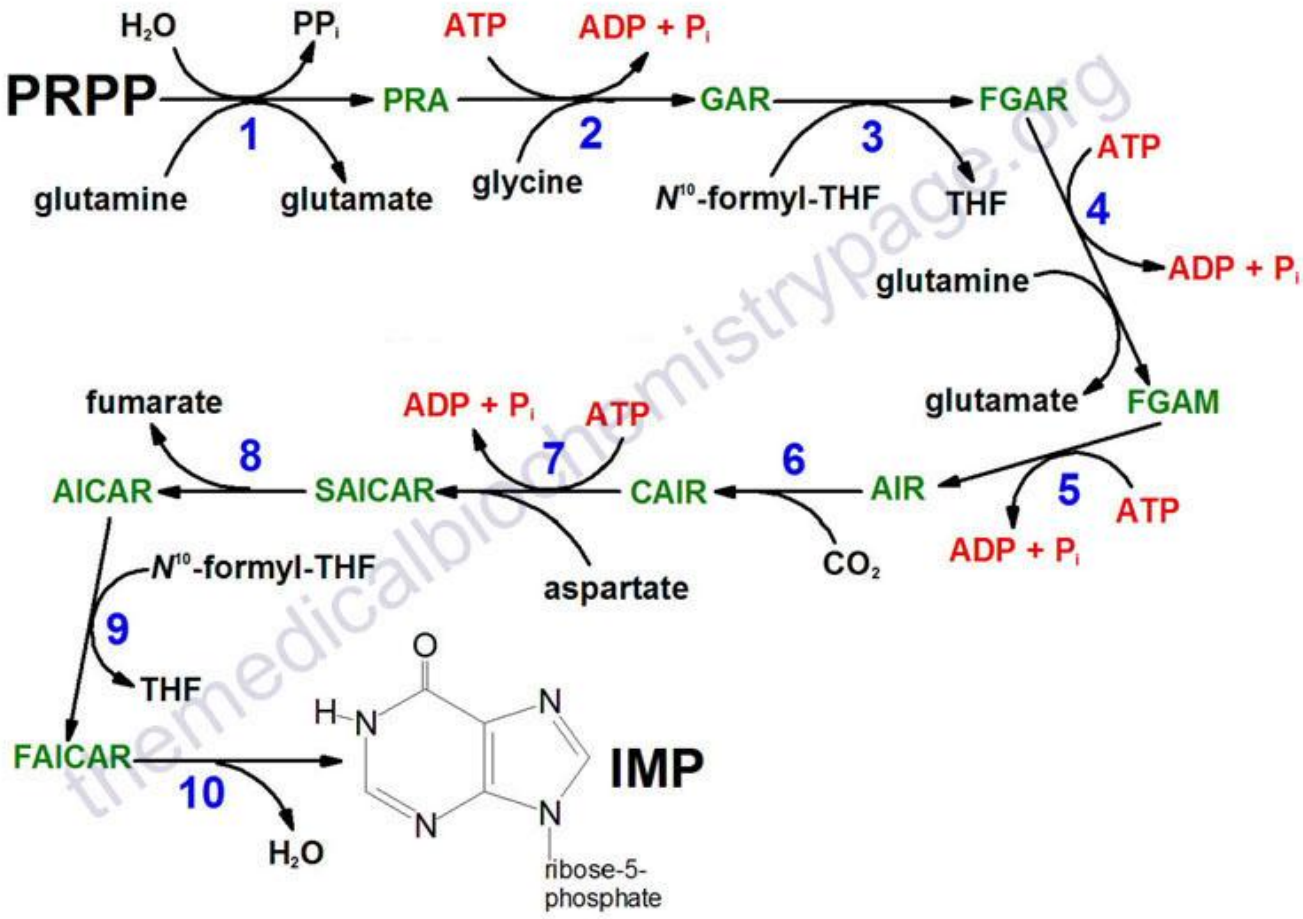
Amino acids such as glycine, glutamine and aspartate are used.

In the last step we end up with **Inosine 5-monopohsphate (IMP)**



IMP

Summary of the steps:



<u>Synthetic inhibitors of purine synthesis</u>

Sulfanomides inhibit growth of rapidly dividing cells without interfering with human cell functions, how?

Sulfanoamides <u>competitively inhibit bacterial synthesis of folic acid</u>, humans cannot synthesize folic acid, thus they are not affected in the human purine synthesis. Since *<u>purine synthesis needs tetrahydrofolate as a co-enzyme</u>*, sulfa drugs can slow down this process in bacteria.

Methoxtrexate and related compounds <u>inhibit the reduction of dihydrofolate</u> to tetrahydrofolate, which is catalyzed **by dihydrofolate reductase**, this limits the amount of tetrahydrofolate available for use in purine synthesis.

Conversion of IMP to AMP and GMP

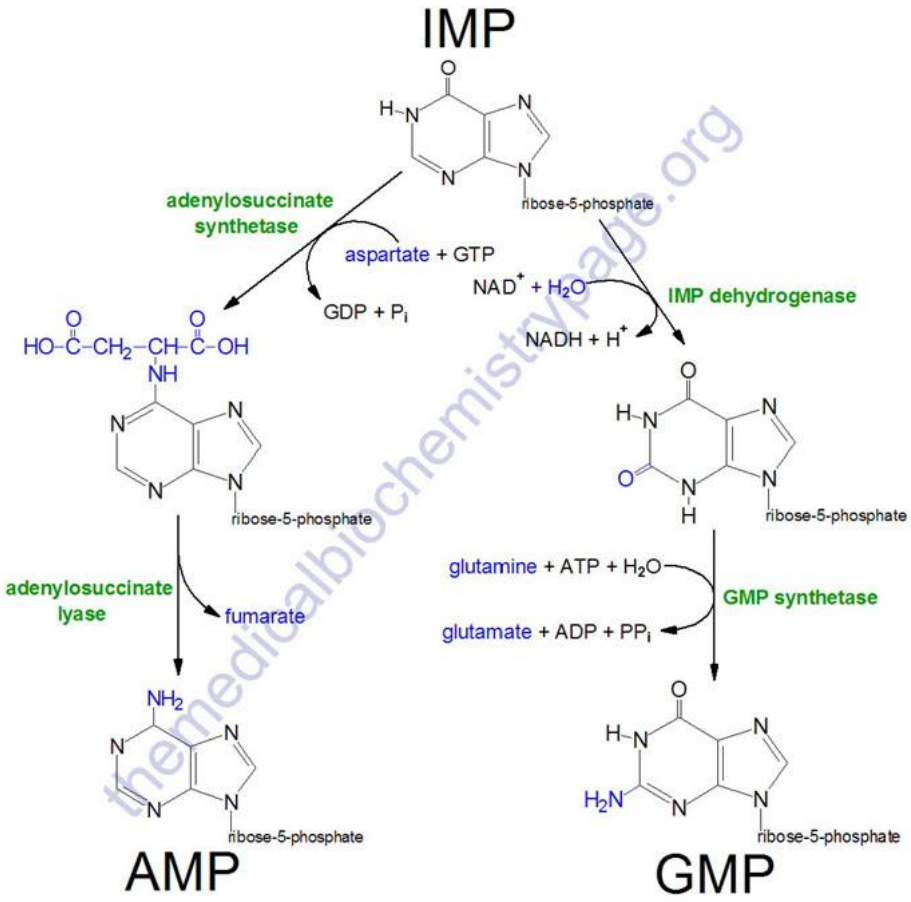
IMP → GMP (requires ATP as an energy source)

IMP → AMP (requires GTP as an energy source)

The conversion is a two step-process

The first reaction of each is inhibited by the end product of the pathway, this mechanism is important for *diverting* to the synthesis of a purine base which is found in lesser amounts.

If both AMP and GMP are present in significant amounts, de novo pathway of purine synthesis is TURNED OFF at the *aminotransferase step. (step 2)*



Note: Hypoxanthine is IMP but without the ribose 5-phosphate attached.

Mycophenolic acid is a reversible inhibitor of *IMP dehydrogenase* and an immunosuppressant.

Base-specific nucleoside monophosphate kinases produce *nucleoside diphosphates* from their corresponding *nucleoside monophosphate*, ATP is the general phosphate donor.

AMP + ATP ⟷ 2ADP Adenylate kinase

GMP + ATP ⟷ GDP + ADP

Guanylate kinase

Nucleoside diphosphates and triphosphates are **interconverted** by nucleoside diphosphate kinase (this enzyme has broad specificity)

GDP + ATP ⟷ GTP + ADP

CDP + ATP ⟷ CTP + ADP

Adenylate kinase's activity is high in the liver and muscles due to high turn-over of energy from ATP.

Salvage pathway of Purines

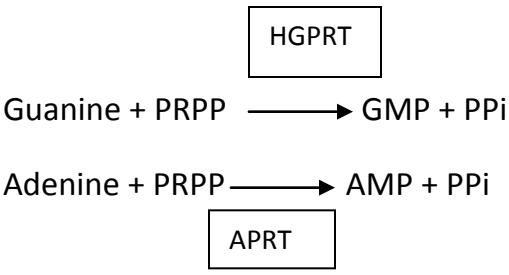
Salvage means to "reuse"

Purines can be obtained from normal turnover of cellular nucleic acids, a small amount can be obtained from the diet, when this small amount isn't degraded it can be converted to nucleoside triphosphates and used by the body in this pathway.

Adenine phosphoribosyltransferase (APRT) and hypoxanthine-guanine phosphoribosyl transferase (HGPRT) are two important enzymes involved in the conversion of pure bases to nucleotides, they use *PRPP as a source of ribose 5-phosphate* and release a pyrophosphate, when this pyrophosphate is hydrolyzed by pyrophosphatase, the reaction becomes irreversible.

Hypoxanthine + PRPP ⟶ IMP + PPi

HGPRT



Lesche-nyan syndrome

- Rare, x-linked autosomal recessive disease
- Heritable cause of hyperuricemia
- Complete deficiency of HGPRT
- Inability to salvage hypoxanthine or guanine ⟶ Increased level of PRPP + Decreased GMP + IMP
- Glutamine:phosphoribosylpyrophosphate aminotransferase has excess substrate ⟶ De novo synthesis increased + Increased purine synthesis
- Increased degradation of purines + Decreased purine reutilization ⟶ production of large amounts of uric acid
- Uric acid stones in kidney (urolithiasis)
- Deposition of uric acid crystals in joints and soft tissues
- Motor dysfunction, behavioral disturbances (biting of lips and fingers; self-mutilation)

Synthesis of deoxyribonucleotides

- DNA synthesis requires deoxyribonucleotides NOT ribonucleotides
- 2-deoxy-ribonucleotides are produced during the S-phase by an enzyme called **ribonucleotide reductase**, this enzyme has 2 non-identical dimeric subunits, R1 and R2, which are specific for purine nucleoside diphosphates (ADP + GDP), and for pyrimidine nucleoside diphosphates (CDP + UDP).
- *ADP + GDP + CDP + UDP are converted to dADP + dGDP + dCDP + dUDP*
- The donors of the hydrogen atoms are two sulfhydryl (SH) groups on the enzyme itself.

So what exactly happens?

Ribonucleotide reductase (an enzyme with 2 SH groups) donates its 2 H atoms to the purine/pyrimidine diphosphate, forming a disulfide bond (with itself) and a

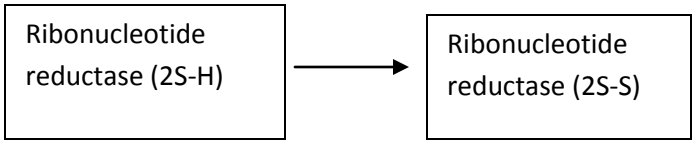
deoxyribonucleotide diphosphate molecule, now the enzyme isn't in its original form and has to be regenerated ... how?

Thioredoxin is a co-enzyme which has two cysteine residues separated by 2 amino acids in a peptide chain, it donates 2Hs atoms to the enzyme forming a disulfide bond and restoring the enzyme back to its original form.

The co-enzyme thioredoxin is *converted back* to its original form **by NADPH** + H^+ , this is catalyzed by **thioredoxin reductase**.

e.g.

1) ADP ⟶ dADP



2) Thioredoxin (2S-H) ⟶ Thioredoxin 2S-S (oxidized) + H2O

3) NADPH + H^+ ⟶ $NADP^+$ + Thioredoxin (reduced) 2S-H

Regulation of deoxyribonucleotide synthesis

There are **allosteric** sites on the enzyme ribonulceotide reductase in order to regulate its activity. Binding of dATP in this site *inhibits* the reaction and subsequently prevents the synthesis of any of the 4 deoxyribonucleotides.

ATP in contrast activates the enzyme.

In adenosine deaminase deficiency, increased levels of dATP is seen which prevents DNA synthesis.

Binding of a nucleoside triphosphate to a "substrate specificity site" on the enzyme, **regulates the substrate specificity**, in other words if there is an increase of a specific deoxyribonucleotide, a conformational change takes place which allows the reduction of a different type of ribonucleotide. (e.g. too much dATP results in the diversion to the reduction of GDP to dGDP)

Hydroxyurea is a drug which destroys free radicals required for the enzymic activity of ribonucleotide, thus inhibiting generation of substrates for DNA synthesis. (this method is used to treat cancer cells or sickle cell anemia)

Degradation of purine nucleotides (occurs in liver)

Degradation of dietary nucleic acids occurs in the small intestine, where nucleic acids are converted to **nucleotides** by pancreatic enzymes, and then to **nucleosides + free bases + uric acid.**

Pancreas secrets **ribonucleases** and **deoxyribonucleases** → DNA + RNA hydrolyzed → oligonucelotides formed → phosphodiesterases digest oligonucleotides to 3 & 5-mononucleotides → nucleotidases remove phosphate group → nucelosides → free bases.

Dietary purine bases are generally converted to uric acid crystals which enter the blood and are excreted in urine.

Urate oxidases cleave the purine ring forming **allantoin**. (this can be used in therapy to lower Urate levels)

Formation of uric acid:

1) amino group is removed from either - AMP → IMP

AMP deaminase

Or from - Adenosine → Inosine (by adenosine deaminase)

2) IMP and GMP → Inosine + Guanosine → Hypoxanthine + guanine

5-nucleotidase Nucleoside phosphorylase

3)Guanine is then deaminated to xanthine

4) Hypoxanthine → xanthine → Uric acid (after further oxidation)

Xanthine oxidase

Diseases associated with purine degradation:

Gout, can be due to overproduction of uric acid or underexcretion

Hyperuricemia results *in deposition* of monosodium urate crystals in joints (cause an inflammation) which in turn causes chronic gouty arthritis.

It can be also deposited in soft tissues resulting in **chronic tophaceous gout.**

Deposition in kidney = kidney stones = urlithiasis

** gout crystals look needle-shaped

Over production:

Primary hyperuricemia

- Mutations in x-linked **PRPP synthetase** leading to increased Vmax and a lower Km for ribose 5-phosphate, and decreased sensitivity to inhibition by purine nucleotides.
- Decreased salvage pathway; decreased HGPRT activity (also known as Lesch-Nyam syndrome)

Secondary Hyperuricemia

- Caused by a variety of disorders & lifestyles
- Chronic renal insufficiency
- Treatment with chemotherapy (high cell turnover rate)
- Excessive consumption of alcohols and purine-rich foods
- Von-Gierk's disease

Underexcretion of Uric acid

- 90% of cases of hyperuricemia are due to underexcretion of uric acid
- Primary cause is idiopathic (unknown), inherited
- Secondary cause is from kidney-related malfunctions, e.g. in lactic acidosis, lactate and Urate compete for the renal reabsorbtion tubules
- Use of drugs, e.g. thiazide diuretics
- Exposure to lead

Treatment of gout

For acute attacks: anti-inflammatory agents

For long term therapy: lowering uric acid level below saturation point

Undersecretors are give uricsuric agents increase renal excretion. E.g. probenacid / sulfipyrazone.

Allopurinol inhibits uric acid synthesis for overproducers. Its converted in the body to oxypurinol which inhibits xanthine oxidase ⟶ accumulation of hypoxanthine + xanthine which can be salvaged.

Adenosine deaminase deficiency

Lymphocytes have the highest cytoplasmic activity of this enzyme

Accumulation of adenosine results in conversion to ribose/deoxyribose nucleotides by cellular kinases.

When dATP levels increase, ribonucleotide reductase is inhibited, preventing production of all other dNucleotides ⟶ No DNA ⟶ No division

In the most severe form, it is an autosomal recessive disorder which causes severe combined immunodeficiency disease (SCID), T-cells, B-cells and natural killer cells decrease.

Treatment needs bone marrow transplantation or enzyme replacement therapy

Children die before the age of 2

Purine nucleoside phosphorylase (PNP) deficiency is an autosomal recessive disease affecting t-cells only, it is characterized by recurrent infections and neuro-developmental delays.

A diet rich in meat and sea food increases the risk of gout.

Pyrimidine synthesis and degradation

Unlike the synthesis of the purine ring, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate.

Source of atoms:

- CO_2
- Glutamine

- Aspartic acid

Steps

The first step is the regulated step

1)Glutamine + CO_2 + 2ATP ⟶ carbamoyl phosphate + 2ADP + Glutamate + Pi

carbamoyl phosphate synthetase II (CPS)

CPSII is inhibited by UTP (the end product of rxn) and is activated by PRPP

Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate (CPS I is involved in urea cycle)

2) carbamoyl phosphate + aspartate ⟶ carbamoyl aspartate + Pi

Aspartate transcarbamyloase

3) the pyrimidine ring is then closed hydrolytically by dihydroorotase

4) the closed ring (hydroorate) is oxidized by FAD and dihydroorotate dehydrogenase to ortate

5) ortotate + PRPP ⟶ OMP + PPi ⟶ UMP + CO_2

Orotate phosphoribosyl transferase

OMP decarboxylase

- **Dihydroorotate dehydrogenase** is the <u>only enzyme</u> in this pathway present in the **<u>inner mitochondria</u>**, the rest are **cytosolic enzymes**
- The first 3 enzymes : CPS II , aspartate transcarbamyolase and dihydroorotase form a complex called CAD
- Orotate phosphoribosyl transferase + OMP decarboxylase form the catalytic domains of UMP synthase.
- Orotic aciduria is a rare genetic disease where orotic acid is found in urine.
- The release of pyrophosphate in any reaction makes it biologically irreversible.

- ❖ UMP is phosphorylated to UDP and UTP.
- ❖ UDP is a substrate for ribonucleotide reductase which generates dUDP, the dUDP can be phosphorylated to dUTP which is raptly hydrolyzed to dUMP by UTP diphosphatase.

Synthesis of UTP and CTP

CTP is produced by amination of UTP by **CTP synthetase** with glutamine providing the nitrogen.

UTP + Glutamine + ATP $\xrightarrow{\text{CTP synthetase}}$ CTP + Glutamate + ADP + Pi

Synthesis of thymidine monophosphate from dUMP

dUMP + n5,n10 methylene-tetrahydrofolate $\xrightarrow{\text{Thymidylate synthase}}$ dTMP + dihydrofolate

dihydrofolate $\xrightarrow{\text{Dihydrofolate reductase + NADPH + } H^+}$ Tetrahydrofolate + $NADP^+$

- ✓ This is an "unusual" reaction as THF (tetrahydrofolate) donates only one carbon, and two hydrogens from its pteridine ring, resulting in the oxidation of THF to DHF
- ✓ Inhibitors of thymidylate synthase include thymine analogs e.g. 5-flurouracil (antitumor agent)
- ✓ 5-FdUMP permanently binds to the inactivated thymidylate synthase (so this drug is a suicide inhibitor)
- ✓ Dihydrofolate reductase can be inhibited by methotrexate, this decreases THF concentration, thus inhibiting purine synthesis and DNA synthesis. (those drugs are used to decrease rate of growth of cancer cells)
- ✓ Trimethoprim is a folate analog and has a potent antibacterial activity due to its selective inhibition of bacterial dihydrofolate reductase enzyme.

Salvage of pyrimidines

- Few can be salvaged
- Done by nucleoside kinases that utilize ATP in the phosphorylation of nucleosides to nucleotides

Degradation of pyrimidine nucleotides

Pyrimidine can be cleaved by human cells (unlike the purine ring) and degraded to highly soluble products, b-alanine and b-aminoisobutyrate, producing NH_3 and CO_2.