بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Final 21

Nucleotide Metabolism pt. 2

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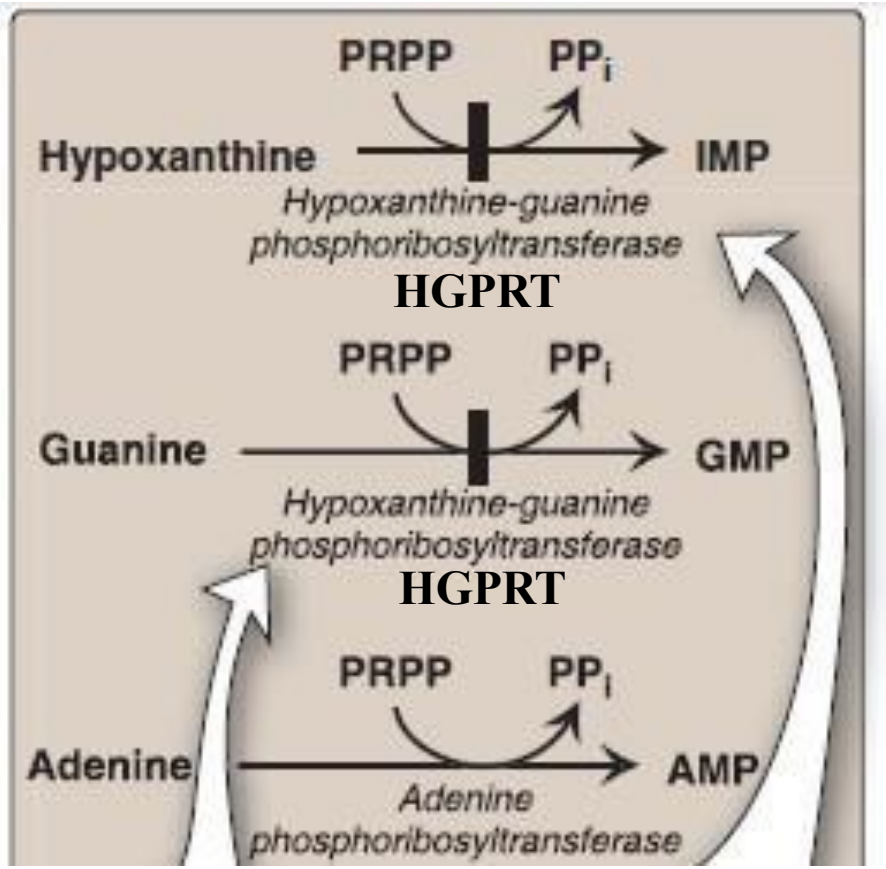
Reviewed by : NST

Salvage pathway for purines



Salvage pathway for purines is purine synthesis from:

1. The normal turnover of cellular nucleic acids
2. Diet purines that are not degraded (small amounts)

Conversion of purine bases to nucleotides:

- Both APRT and HGPRT use PRPP as the source of the ribose 5-phosphate group.
- PP is released and hydrolyzed by pyrophosphatase making these reactions irreversible.
- Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.

Salvage Pathway for Purines: Recycling Preexisting Nitrogenous Bases

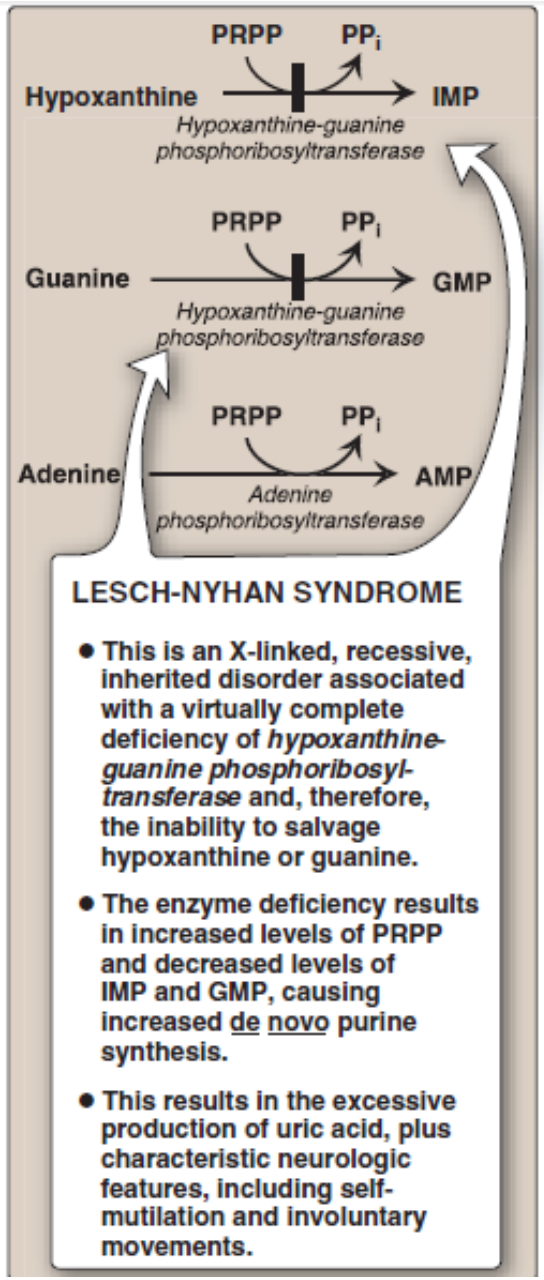
- The **salvage pathway** is a simple and efficient process that recycles preexisting nitrogenous bases and nucleosides to synthesize nucleotides, reducing the need for de novo synthesis.

1. **Salvaging Adenine:**
   - Adenine, a nitrogenous base, can be salvaged by adding a ribose sugar and phosphate group, both provided by **PRPP (5-phosphoribosyl-1-pyrophosphate).** This reaction, catalyzed by **adenine phosphoribosyltransferase (APRT),** produces **AMP (adenosine monophosphate)** while releasing **PPi (pyrophosphate).**
2. **Salvaging Guanine and Hypoxanthine:**
   - Guanine and hypoxanthine are salvaged by the enzyme **hypoxanthine-guanine phosphoribosyltransferase (HGPRT).** Similar to adenine salvage, PRPP donates ribose and phosphate to form **GMP (guanine monophosphate)** from guanine and **IMP (inosine monophosphate)** from hypoxanthine. This enzyme can act on multiple nitrogenous bases, unlike APRT, which is specific to adenine.

Salvage Pathway for Purines: Recycling Preexisting Nitrogenous Bases

3. Recycling IMP:
   - IMP, synthesized during de novo purine synthesis, can re-enter the pathway through salvage after hypoxanthine is recycled, ensuring efficient use of cellular resources.
4. Alternative Salvage of Adenine via Adenosine:
   - Adenine can also be salvaged indirectly by phosphorylating adenosine (a nucleoside) using adenosine kinase. This pathway is exclusive to adenine and converts adenosine into AMP by adding a phosphate group.

Application: Salvage pathway for purines- Lesch-Nyhan syndrome



- A rare, X-linked, recessive
- HGPRT deficiency.
- **Inability to salvage hypoxanthine or guanine** resulting in high amounts of uric acid (the end product of purine degradation)

- Increased PRPP levels and decreased IMP and GMP levels.
- The committed step in purine synthesis has excess substrate and decreased inhibitors available, and **de novo purine synthesis is increased.**

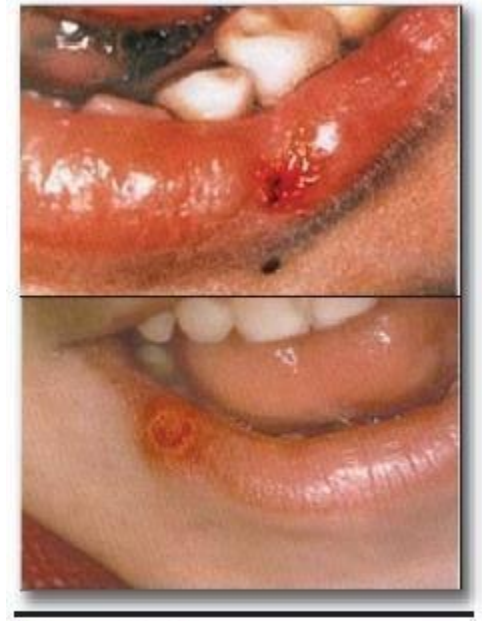


Figure 22.11
Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.

- The decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid (hyperuricemia)

- Hyperuricemia results in uric acid stones in the kidneys (urolithiasis) and the deposition of monosodium urate crystals in the joints (gouty arthritis) and soft tissues.

- The syndrome is characterized by motor dysfunction, cognitive deficits and behavioral disturbances that include self-mutilation (biting of lips and fingers)

3G

Lesch-Nyhan Syndrome: HGPRT Deficiency and Clinical Manifestations

- **Lesch-Nyhan syndrome** is a rare, hereditary, **X-linked recessive disorder**, making males more susceptible because they require only one copy of the defective gene to express the disease. This syndrome is caused by a deficiency of **hypoxanthine-guanine phosphoribosyltransferase (HGPRT),** the enzyme responsible for salvaging guanine and hypoxanthine.
- Due to HGPRT deficiency, **guanine salvaging is disrupted,** leading to increased reliance on **de novo purine synthesis.** AMP, however, can still be synthesized through both de novo and salvage pathways, resulting in a higher concentration of AMP compared to GMP. The excess AMP is targeted for degradation, increasing purine breakdown.
- Purine degradation produces **uric acid** as the final product, which is excreted in urine. High levels of uric acid cause **hyperuricemia,** leading to **gout-like symptoms.** Uric acid can form **monosodium urate crystals,** which accumulate in the **synovial fluid of joints,** causing **gouty arthritis.** Additionally, uric acid in high concentrations may exceed its solubility in urine, leading to **precipitation and kidney stone formation.**
- Apart from metabolic symptoms, Lesch-Nyhan syndrome is characterized by **neurological and behavioral abnormalities,** including impaired cognitive skills, self-mutilating behaviors, and other **severe behavioral disturbances.** These symptoms arise from the toxic effects of elevated uric acid and associated metabolic disruptions in the central nervous system.

Degradation of Purine Nucleotides

A. Degradation of dietary nucleic acids in the small intestine

-Ribonucleases and deoxyribonucleases, secreted by the **pancreas**, hydrolyze dietary RNA and DNA to oligonucleotides.

-Oligonucleotides are further hydrolyzed by **pancreatic** phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides.

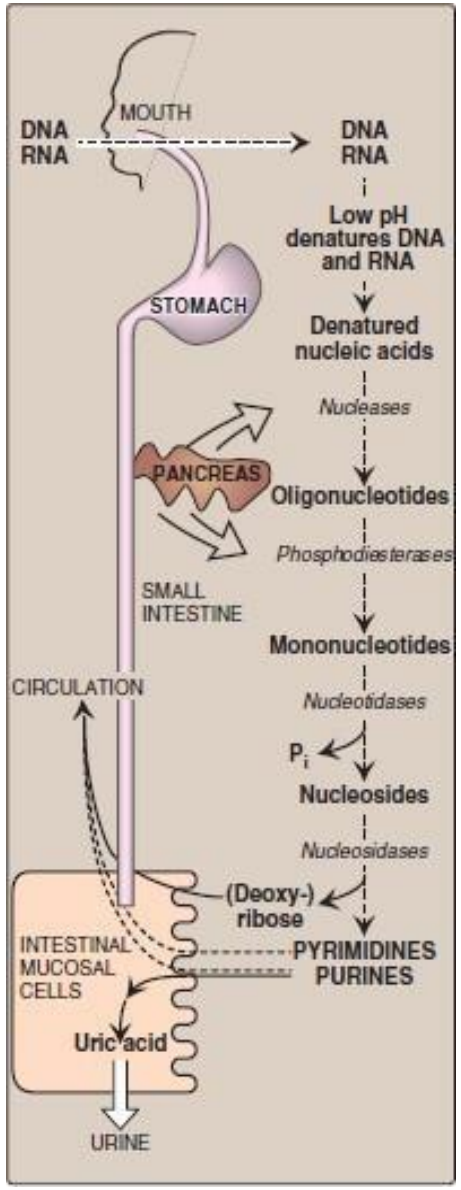
-In the intestinal mucosal cells, nucleotidases remove the phosphate groups hydrolytically, releasing nucleosides that are further degraded to free bases.

-Dietary purine bases are not an appreciable source for the synthesis of tissue nucleic acids.

-Dietary purines are generally converted to uric acid (excreted in urine) in intestinal mucosal cells.

-Purine nucleotides <u>from de novo synthesis</u> are degraded in the <u>liver</u> primarily.

-The free bases are sent out from liver and salvaged by peripheral tissues



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Degradation of Purine Nucleotides

Dietary nucleotides serve as a minor source of nucleotides in our bodies. These nucleotides are present in the diet in the form of DNA and RNA , which are nucleic acids. Their digestion begins in the small intestine , where pancreatic enzymes **ribonuclease** and **deoxyribonuclease** break down **RNA** and **DNA** , respectively , into oligonucleotides

Then , the pancreas secretes additional enzymes known as **phosphodiesterases** , which further degrade oligonucleotides into mononucleotides. These mononucleotides are then ready for absorption. They cross the brush border of the intestinal cells and enter the cells for further processing

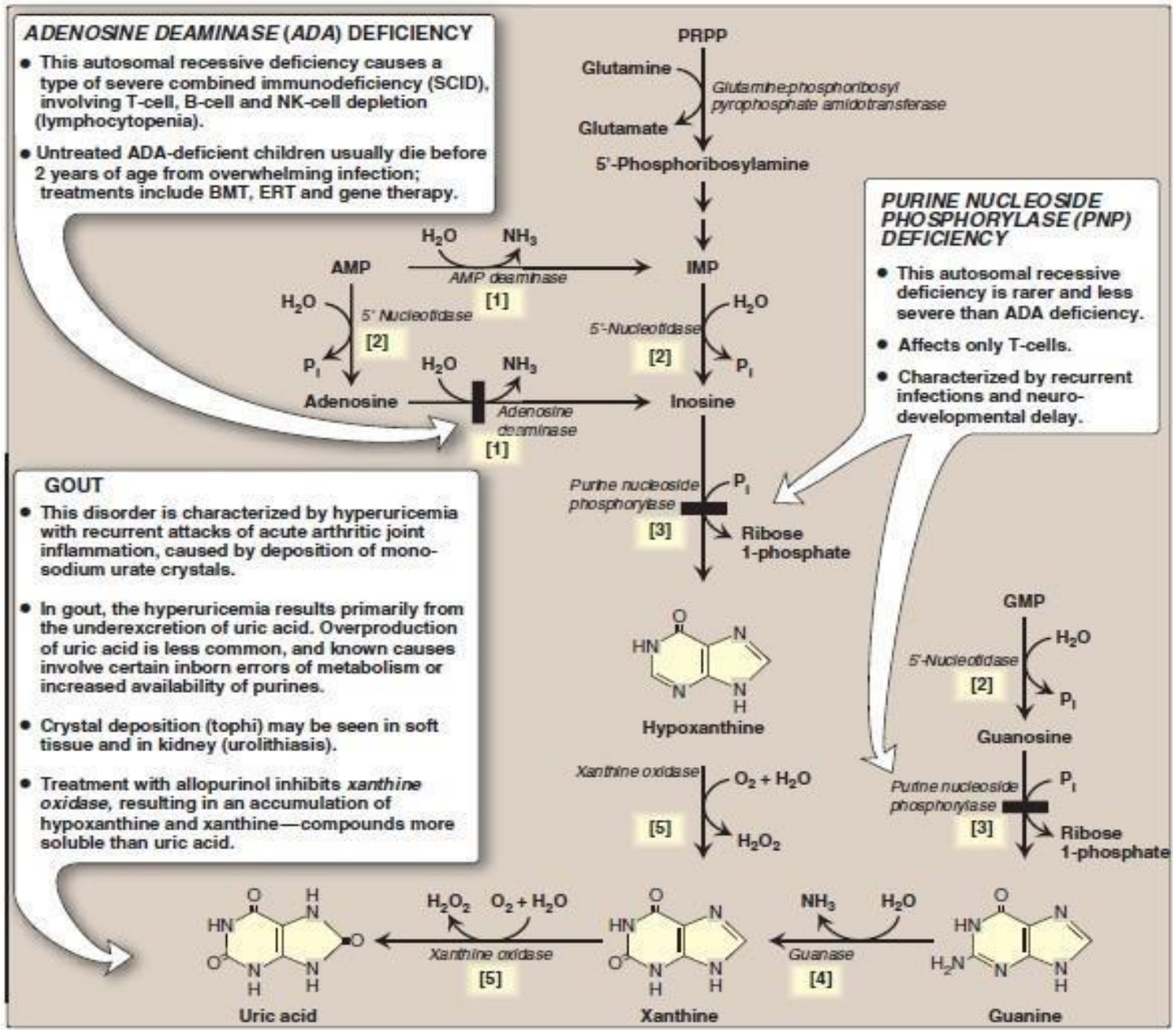
Most of the degradation process occurs within the intestinal cells and the liver , depending on the source of the nucleotides. **Dietary** nucleotides are primarily degraded in the **intestinal cells** , while purines synthesized **within the body** are metabolized in **hepatocytes**

The degradation process involves the removal of the phosphate group from nucleotides by the enzyme **nucleotidase** , converting them into nucleosides. Next , **nucleosidase** enzymes remove the sugar , leaving only the nitrogenous base. The fate of the nitrogenous base depends on its **type**. For instance , purines (adenine and guanine) are generally degraded into **uric acid** (not urea) , which is excreted through urine

There is no information in this explanation that has not been mentioned in the slide above , it is merely a clarification and organization of the information mentioned

Degradation of Purine Nucleotides

1 An amino group is removed from AMP to produce IMP by AMP deaminase, or from adenosine to produce inosine (hypoxanthineribose) by adenosine deaminase.
2 IMP and GMP are converted into their nucleoside forms—inosine and guanosine—by the action of 5'-nucleotidase.
3 Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine.
Note: A mutase interconverts ribose 1- and ribose 5-phosphate.
4 Guanine is deaminated to form xanthine.
5 Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is further oxidized by xanthine oxidase to uric acid, the final product of human purine degradation.



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Degradation of Purine Nucleotides /1

- **GMP Degradation Pathway :**
- GMP undergoes dephosphorylation by **nucleotidase** , which removes the phosphate group through hydrolysis , converting GMP into the nucleoside guanosine
- **Purine nucleoside phosphorylase** removes the sugar (ribose) as **ribose-1-phosphate** , leaving the nitrogenous base guanine
- Guanine is then deaminated by **guanase** , removing an amino group (which was originally added during GMP synthesis when IMP was converted to GMP) and producing **xanthine**. H_2O molecule is consumed as a source of
  oxygen.
- Xanthine is subsequently oxidized by **xanthine oxidase** , utilizing **oxygen** and generating hydrogen peroxide (H_2O_2) as a byproduct. This oxidation yields **uric acid (final product)**

- **AMP Degradation Pathway:**

AMP can follow two pathways for degradation into uric acid , **First Pathway :**

- AMP is first dephosphorylated by **nucleotidase** , forming the nucleoside adenosine
- Adenosine is then deaminated by adenosine deaminase , removing the amino group and converting it to inosine
- Inosine is processed by **purine nucleoside phosphorylase** , which removes the sugar (ribose) as **ribose-1-phosphate** , producing **hypoxanthine**
- Hypoxanthine is oxidized by **xanthine oxidase** to form xanthine utilizing oxygen and generating hydrogen peroxide (H_2O_2) as a byproduct , and further oxidation by the same enzyme generates **uric acid**

Degradation of Purine Nucleotides /2

Second Pathway :

- AMP is directly deaminated by **AMP deaminase** , removing the amino group and converting it into **IMP**
- IMP is then dephosphorylated to **inosine** by **nucleotidase**
- Inosine undergoes sugar removal by **purine nucleoside phosphorylase** , releasing **ribose-1-phosphate** and forming **hypoxanthine**
- Hypoxanthine is subsequently oxidized to xanthine and then to uric acid by xanthine oxidase (same as the first pathway)

✓ Both AMP degradation pathways meet at **inosine** .
✓ GMP and AMP pathways meet at **xanthine**.
✓ Both pathways result in the formation of **uric acid** as the final product

There is no information in this explanation that has not been mentioned in slide 4 , it is merely a clarification and organization of the information mentioned

Application: Diseases associated with purine degradation

- ✓ **Gout:** high levels of uric acid in blood (hyperuricemia)
- ✓ Hyperuricemia due to either the overproduction or underexcretion of uric acid.
- ✓ Hyperuricemia lead to the deposition of monosodium urate crystals in the joints, leading to inflammation, causing first acute and then chronic gouty arthritis.
- ✓ Nodular masses of monosodium urate crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout

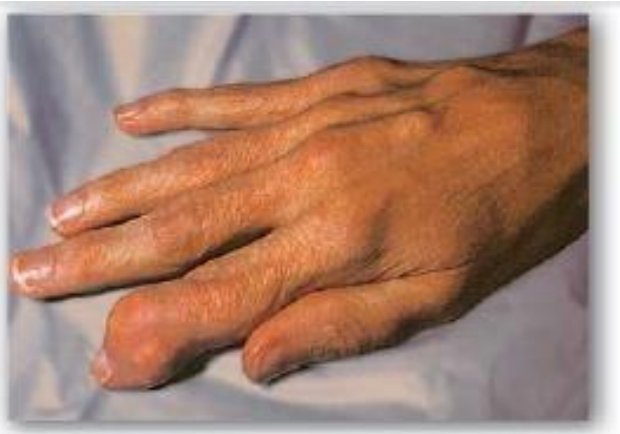


Figure 22.16
Tophaceous gout.

- ✓ Formation of uric acid stones in the kidney (urolithiasis)
- ✓ Hyperuricemia is typically asymptomatic and does not lead to gout, but gout is preceded by hyperuricemia.

Diagnosis requires aspiration and examination of synovial fluid from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped monosodium urate crystals

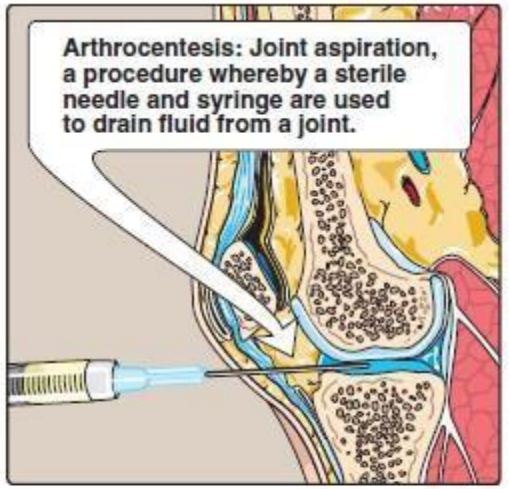


Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling or arthritis, such as infection, gout, and rheumatoid disease.

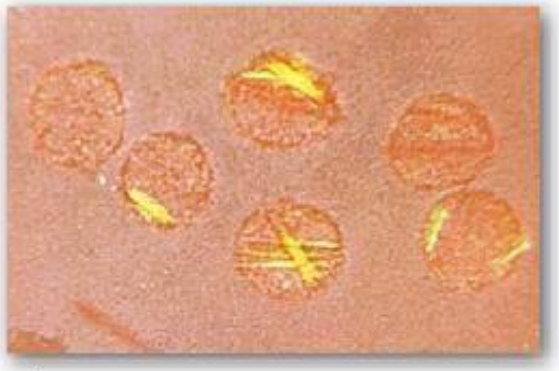


Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within poly-morphonuclear leukocytes.

- ✓ **Causes of hyperuricemia**

1. Underexcretion of uric acid (in most gout patients)

-Primary (due to unidentified inherent excretory defects)

-Or secondary to: a. A known disease that affects the kidney function in handling urate, such as lactic acidosis (lactate and urate compete for the same renal transporter), b. Environmental factors such as drugs (thiazide diuretics), c. Exposure to lead (saturnine gout)

2. Overproduction of uric acid: less common.

Several identified mutations in the X-linked PRPP synthetase gene that increase PRPP production

Application: Diseases associated with purine degradation

When the amount of uric acid in the body increases -whether due to overproduction or decreased excretion in urine- it can lead to various diseases , such as **gout**. Uric acid is normally soluble and easily excreted through urine , but when its levels rise , it can precipitate in the fluids forming urine , leading to kidney stones. Most patients with gout develop **hyperuricemia** primarily due to **reduced excretion** rather than overproduction. This condition results in the formation of uric acid kidney stones , a type of **urolithiasis**. Kidney stones vary in type depending on their composition , such as uric acid stones or calcium oxalate stones

Additionally , uric acid may precipitate as **monosodium urate crystals** in the **synovial fluid** of joints -both small and large- or in the soft tissues . These crystals trigger an inflammatory response in the affected organs , causing arthritis and swelling known as **tophus** (or tophi in plural). To diagnose gout , a needle is used to withdraw synovial fluid , which is then examined under a microscope for the presence of these crystals

To manage (not treat) this condition , patients are often given **xanthine oxidase** inhibitors to reduce uric acid production. Gout may also have genetic causes , such as **Lesch-Nyhan syndrome** , a genetic disorder linked to the purine salvage pathway. This syndrome can present symptoms similar to gout due to excessive uric acid accumulation

Pyrimidine Synthesis

The pyrimidine ring is synthesized before being attached to ribose 5-phosphate

Ribose 5-phosphate is donated by PRPP.

Pyrimidine synthesis is simpler than purine synthesis due to its simpler structure. Pyrimidines consist of a six-membered ring , with four of the six atoms directly derived from **aspartic acid** (specifically , the amino group , alpha carbon , carboxyl group carbon , and the carbon from the side chain). The remaining atoms are contributed by the **amide nitrogen** from the side chain of **glutamine** and a carbon atom from a CO_2 molecule.

Thus, pyrimidine synthesis requires two amino acids (aspartic acid and glutamine) and a CO_2 molecule.

Additionally,**PRPP**(phosphoribosyl pyrophosphate) is needed to provide the sugar and phosphate required to construct the nucleotide structure

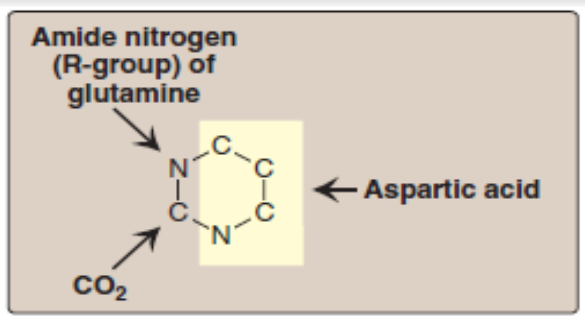


Figure 22.19
Sources of the individual atoms in the pyrimidine ring.

Pyrimidine Synthesis

A. Synthesis of carbamoyl phosphate

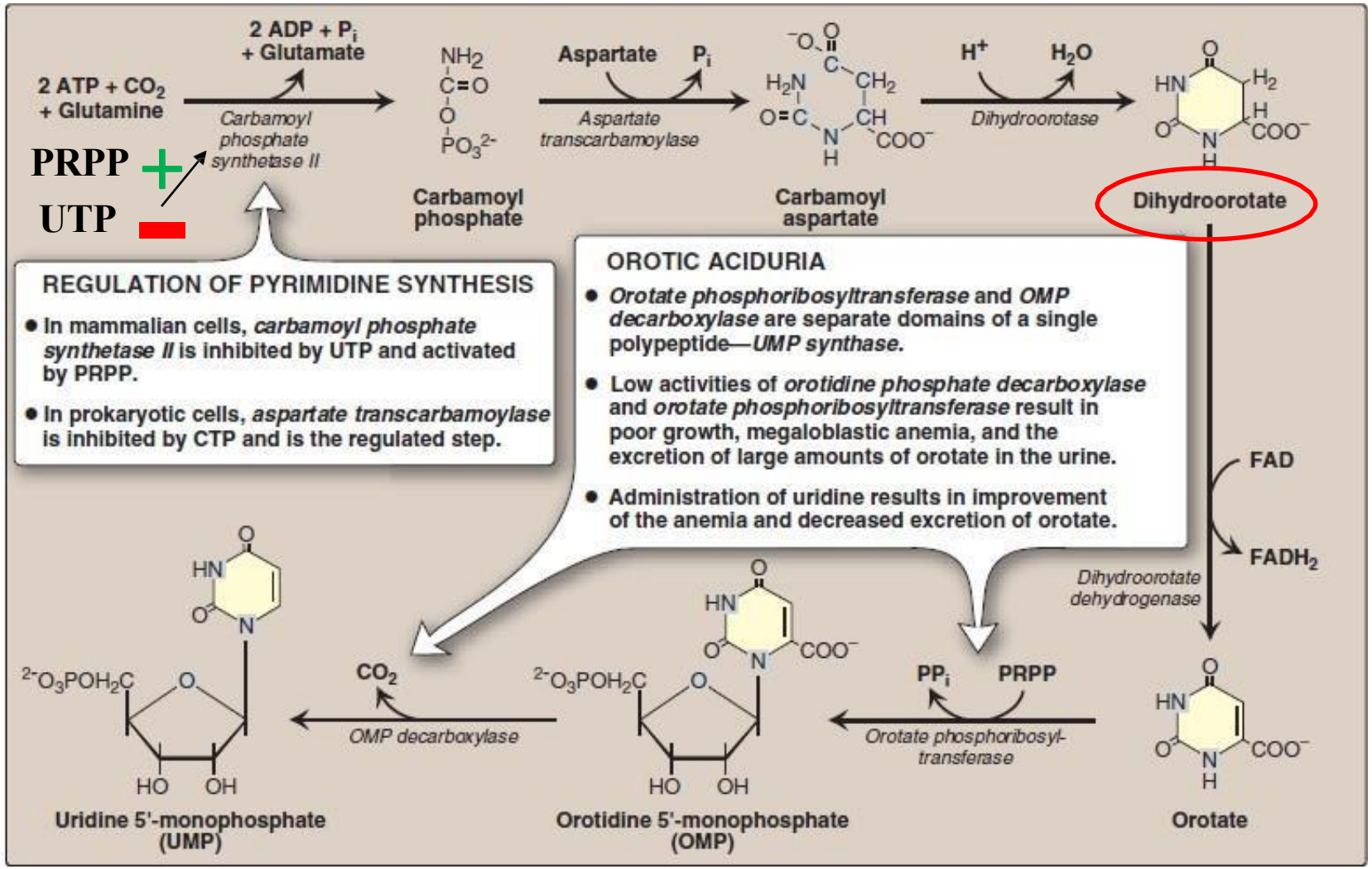
-The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO2

B. Synthesis of orotic acid

-The enzyme that produces orotate, dihydroorotate dehydrogenase, is associated with the **inner mitochondrial membrane**.

-All other enzymes in pyrimidine biosynthesis are **cytosolic**.

-The first three enzymic activities in this pathway (**CPS II, aspartate transcarbamoylase, and dihydroorotase**) are three different catalytic domains of a single polypeptide chain



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Pyrimidine Synthesis

I. The pathway begins with carbon dioxide (CO_2) as a source of carbon and the amino group from **glutamine** (from its side chain) . This process requires **two ATP molecules** , one provides a phosphate group while also supplying **energy** for the reaction. The product of this reaction is **carbamoyl phosphate** , similar to the urea cycle, but in this case , the reaction is catalyzed by the enzyme **carbamoyl phosphate synthetase 2** (CPS2 , unlike CPS1 in the urea cycle)

II. Next , **carbamoyl phosphate** combines with the amino acid **aspartate** in a reaction catalyzed by **aspartate transcarbamoylase**. This reaction releases phosphate as a byproduct and forms **carbamoyl aspartate**

III. The product , **carbamoyl aspartate** , then converted into **dihydroorotate** by the enzyme **dihydroorotase** , which forms a ring structure

IV. **Dihydroorotate** contains two extra hydrogens that are removed via an **oxidation-reduction** reaction catalyzed by **dihydroorotate dehydrogenase**. This reaction oxidizes dihydroorotate into **orotate** while reducing **FAD** to **$FADH_2$**

V. **Orotate** is then combined with a **sugar-phosphate** group derived from **PRPP** (phosphoribosyl pyrophosphate) in a reaction catalyzed by a **transferase** enzyme. This reaction releases **pyrophosphate** and produces the first pyrimidine nucleotide , **orotidine monophosphate** (OMP)

VI. OMP is subsequently modified to produce the pyrimidines **cytosine** (C), **uracil** (U), and **thymine** (T). First , OMP is converted into **uridine monophosphate** (UMP) by **OMP decarboxylase** , which removes a carboxyl group. UMP is the first pyrimidine nucleotide to appear as it has the simplest structure. It is then further modified to form **cytosine** and **thymine**

CPS I Versus CPS II

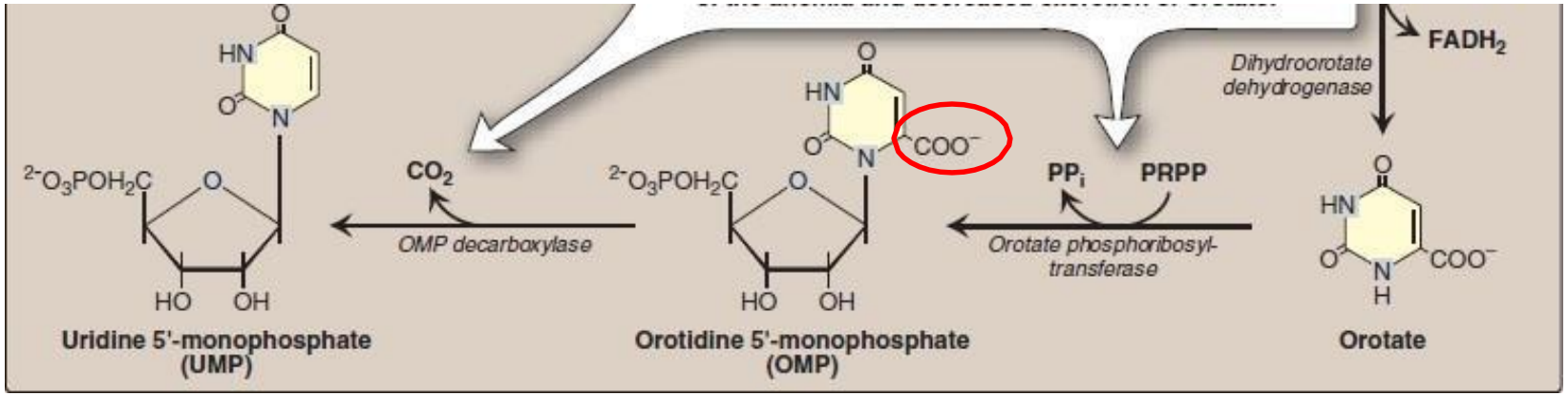
Carbamoyl phosphate, which is synthesized by CPS I, is a precursor of urea.
Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate.

	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia Free ammonia	γ-Amide group of glutamine
Regulators	Activator: N–acetyl-glutamate Its synthesis is activated by arginine (meta 14)	Activator: PRPP Inhibitor: UTP

Although both occur in **hepatocytes** , they are spatially separated

Pyrimidine Synthesis

C. Formation of a pyrimidine nucleotide



-The completed pyrimidine ring is converted to the nucleotide orotidine 5'-monophosphate (OMP), or the parent pyrimidine mononucleotide.

-The reaction releases pyrophosphate, thus, it is irreversible.

-Both purine and pyrimidine synthesis require Gln, Asp, and PRPP as essential precursors.

-Orotate phosphoribosyl transferase and orotidylate decarboxylase are catalytic domains of a single polypeptide chain called UMP synthase.

-UMP is phosphorylated to UDP and then UTP.

-The UDP is a substrate for ribonucleotide reductase, which generates dUDP.

-The dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase (dUTPase).

-dUTPase reduces the available dUTP for DNA synthesis, thus preventing incorporation of uracil into DNA.

PLEASE SEE NEXT SLIDE

Pyrimidine Synthesis

- ✓ After the synthesis of UMP , it can be **phosphorylated** to form UDP or UTP. The phosphorylation process is similar to that of purines. The addition of the second phosphate is catalyzed by **nucleoside monophosphate-specific kinases** (one of which is specific for uracil). The third phosphate is added by a non-specific enzyme that works on both purines and pyrimidines

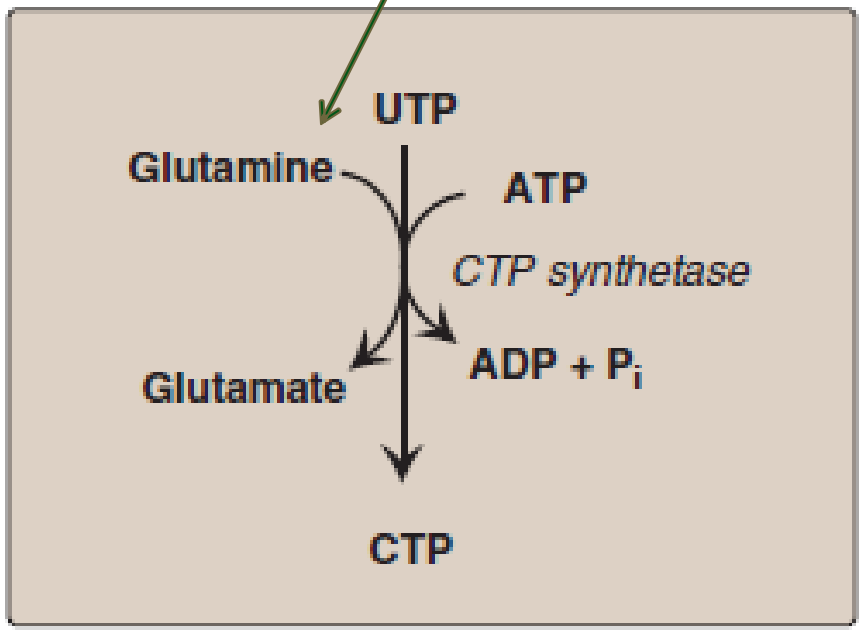
- ✓ UMP can also be converted into a **deoxyribonucleotide** by the enzyme **ribonucleotide reductase** , which functions on both purines and pyrimidines. However , UMP must first be **phosphorylated** to UDP before this conversion

- ✓ To prevent the incorporation of **uracil** into DNA -especially since uracil is produced before cytosine and thymine- the enzyme **dUTPase** hydrolyzes **deoxyUTP**. This prevents them from being incorporated into DNA and ensures they are only used for the synthesis of other pyrimidine nucleotides

Pyrimidine Synthesis

synthetase = energy

Add amino group from glutamine to UTP



Cytosine is not synthesized with deoxy subunit Transformed to deoxy after synthesis

D. Synthesis of UTP and cytidine triphosphate (CTP)

Some CTP is dephosphorylated to CDP (a substrate for ribonucleotide reductase)

dCDP can be phosphorylated to dCTP for DNA synthesis.

Figure 22.22
Synthesis of CTP from UTP. [Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.]

Pyrimidine Synthesis

E. Synthesis of thymidine monophosphate (TMP) from dUMP

Thymidylate synthase inhibitors include thymine analogs such as 5-fluorouracil (antitumor agents).

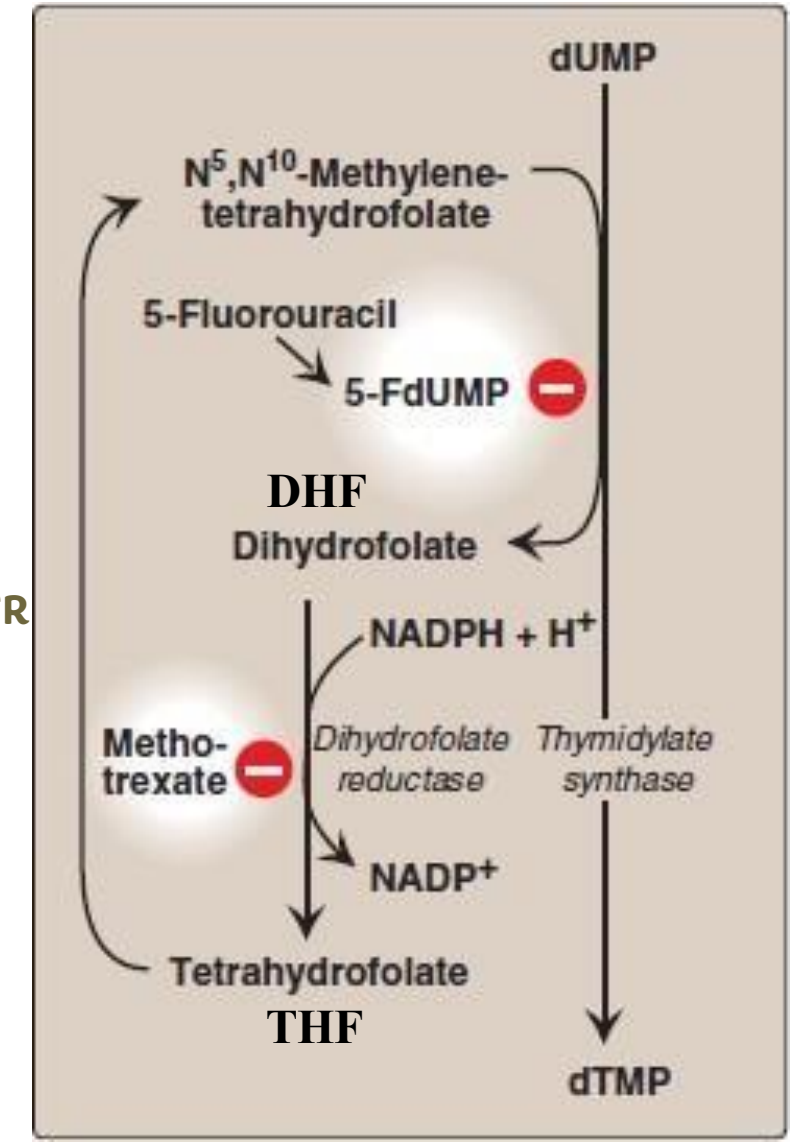
5-Fluorouracil (**suicide inhibitor**) is converted to 5-FdUMP that **permanently** binds to the inactivated thymidylate synthase

Methotrexate inhibits dihydrofolate reductase **(Methotrexate inhibits DHFR → ↓ THF → ↓ purines + ↓ dTMP)**

Methotrexate inhibits steps in purine de novo synthesis that transfers formyl from formyltetrahydrofolate by formyltransferase.

Methotrexate reduces THF, inhibits purine synthesis and prevents methylation of dUMP to dTMP, resulting in **DNA synthesis inhibition and cell growth slow down .**

5-Fluorouracil and Methotrexate are **anti cancerous agents**

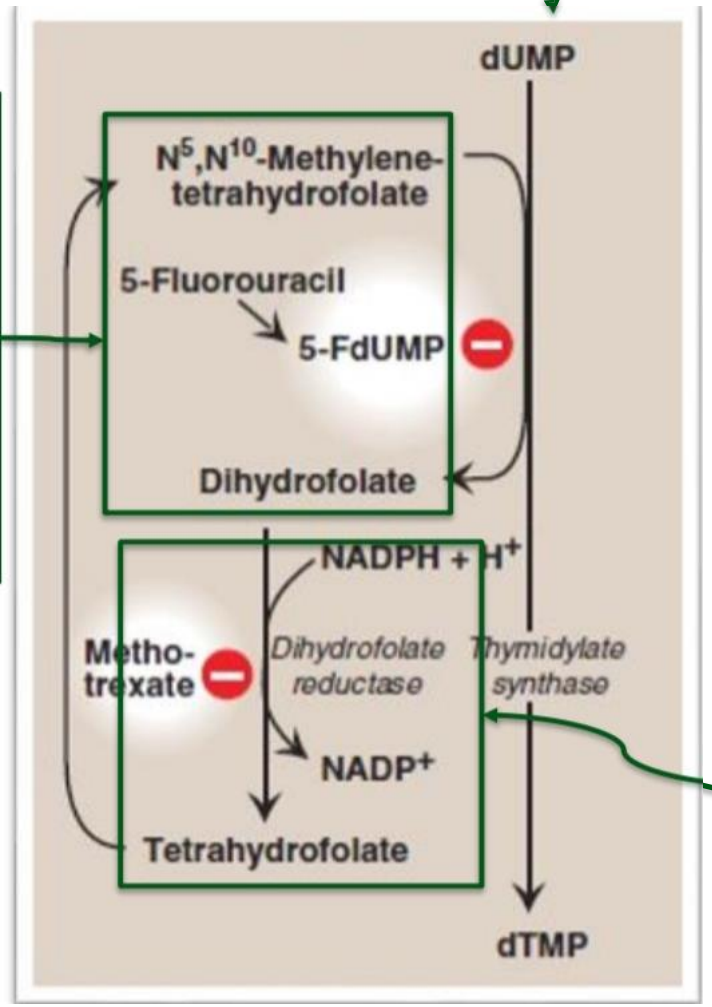


Formed from deoxy unit directly - not produced as ribonucleotide then converted like cytosine, rather produced as deoxyribonucleotide directly.

UMP phosphorylated to UDP changes to dUDP by ribonucleotide reductase remove phosphate to produce dUMP add single carbon unit -methyl group- to uracil to produce thyamine dTMP

- This reaction is targeted with various anti- cancerous drugs.
- **Methotrexate** inhibits both purine & pyrimidine De novo synthesis and both anti-cancerous drugs (methotrexate & **5-fluorouracil**) impair formation of dTMP which impairs DNA replication in cancer cells but it also affects normal cells - why different affects of chemotherapy happen.

Single carbon unit is taken from N5, N10 - Methylene tetrahydrofolate and because it is a mythlene - not a methyl- it will require extra hydrogens taken from tetrahydrofolate, this is why N5, N10 - Methylene tetrahydrofolate changes to dihydrofolate and not just tetrahydrofolate.



Dihydrofolate is unable to carry single carbon units has to be reduced to tetrahydrofolate by dihydrofolate reductase that oxidizes NADPH
tetrahydrofolate is now ready to be loaded with single carbon units of different types like methylene, methyl, etc.

Pyrimidine Salvage

Pyrimidine phosphorylase is also called uracil phosphorylase

Few pyrimidine bases are salvaged in human cells because of the very low concentration of the bases in plasma and tissues.

Mechanism: Phosphorylase then kinase

Thymidine phosphorylase adds deoxyribose sugar to thymine producing a deoxy sugar right way – ready for being used in DNA.

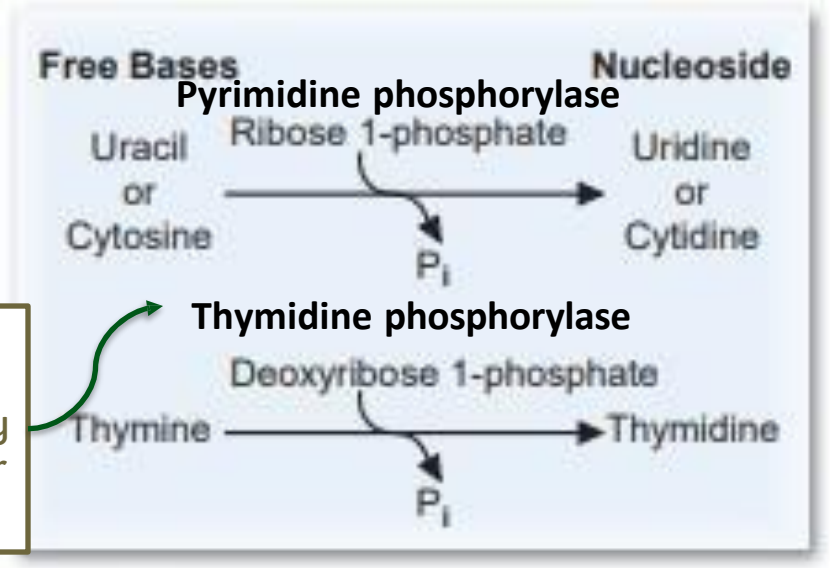


FIG. 41.17. Salvage reactions for pyrimidine nucleoside production. Thymine phosphorylase uses deoxyribose 1-phosphate as a substrate, so ribothymidine is rarely formed.

Specific Nucleoside kinases

Pyrimidine nucleosides → Nucleotides

ATP

Each base has its own kinase that takes the phosphate for the reaction from an ATP molecule

Thymidine kinase (TK) activity is closely related to the proliferative state of the cell as TK levels and activity increase dramatically as cells enter S-phase and in rapidly dividing cells.

Labelling of thymidine follow its consumption indicating DNA replication activity in S phase which in turn indicates the proliferative activity of the cell.

Radiolabeled thymidine is widely used for isotopic labeling of DNA, in radioautographic investigations or to estimate rates of intracellular DNA synthesis.

Pyrimidine Degradation

- ✓ The pyrimidine nucleotides are dephosphorylated
- ✓ The nucleosides are cleaved to produce ribose 1-phosphate and the free pyrimidine bases cytosine, uracil, and thymine.
- ✓ Cytosine is deaminated, forming uracil, which is converted to CO2, NH3, and beta-alanine.
- ✓ Thymine is converted to CO2, NH3, and beta-aminoisobutyrate

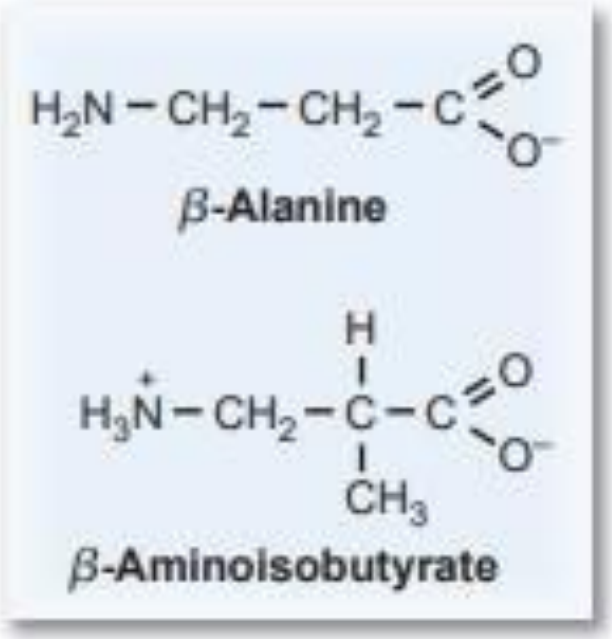


FIG. 41.20. Water-soluble end products of pyrimidine degradation.

- ✓ The highly soluble products β-alanine and β-aminoisobutyrate are excreted in the urine or converted to CO2, H2O, and NH3 (which forms urea).
- ✓ The products do not cause any problems for the body, in contrast to urate
- ✓ As with the purine degradation pathway, little energy can be generated by pyrimidine degradation

Similar start to purine degradation. Nucleotidases that remove phosphate forming nucleosides use nucleosidases to remove sugar as ribose 1-P nitrogenous bases are degraded

Cytosine &uracil have same final product

Beta-alanine is different than alpha-alanine of amino acids that is incorporated into protein structure, alpha-alanine has all groups around alpha-carbon while beta-alanine has carboxyl on one carbon while the amine group is on the other (isomers).

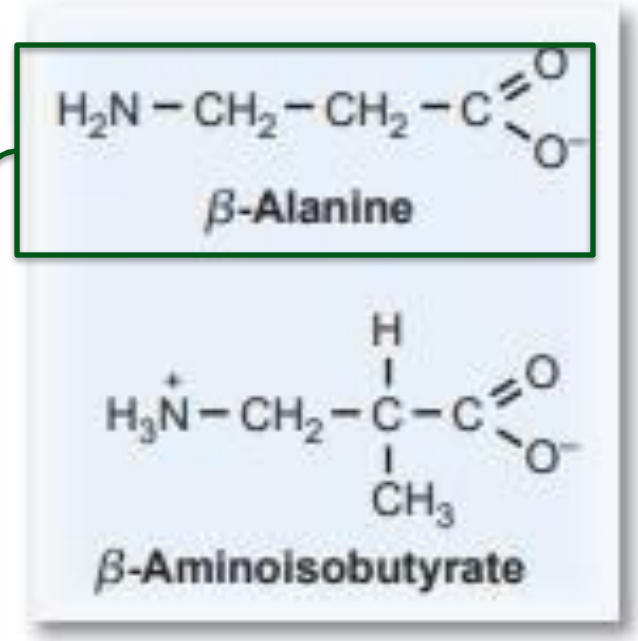


FIG. 41.20. Water-soluble end products of pyrimidine degradation.

Beta-alanine & beta-aminoisobutyrate are hydrophilic and polar excreted through urine.

Additional Resources:

رسالة من الفريق العلمي:

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			