Ministry of Public Health of Ukraine
Poltava State Medical University

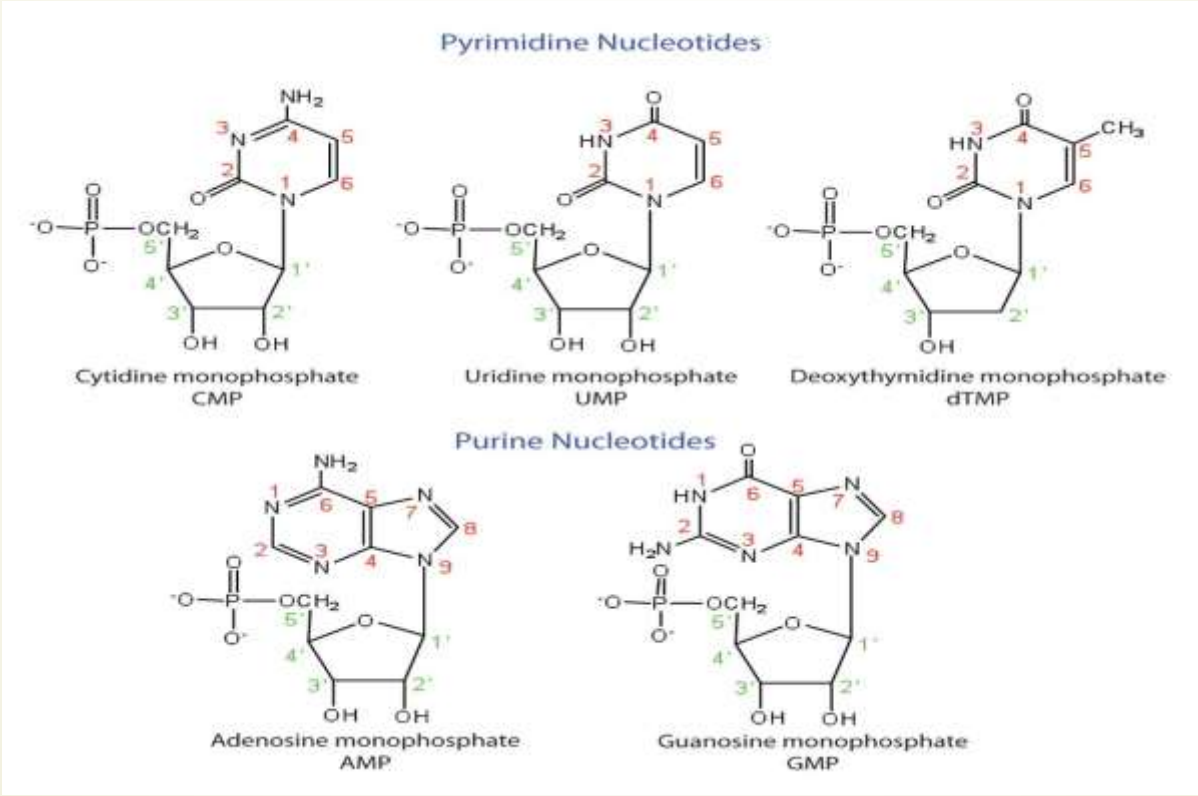
Biosynthesis and catabolism of purine and pirimidine nucleotides. Analysis for the end products of their metabolism.

Assoc. Prof. Bilets M.V.

Lecture plan

- Biosynthesis of purine nucleotides.
- Biosynthesis of pyrimidine nucleotides.
- Biosynthesis of deoxyribo-nucleotides.
- Catabolism of purine nucleotides.
- Catabolism of pyrimidine nucleotides.

A **nucleotide** is made up of three parts: a nitrogenous base (purine or pyrimidine), a 5-carbon sugar (ribose or dioxyribose) and phosphate group (1-3 groups), a 5-carbon sugar.

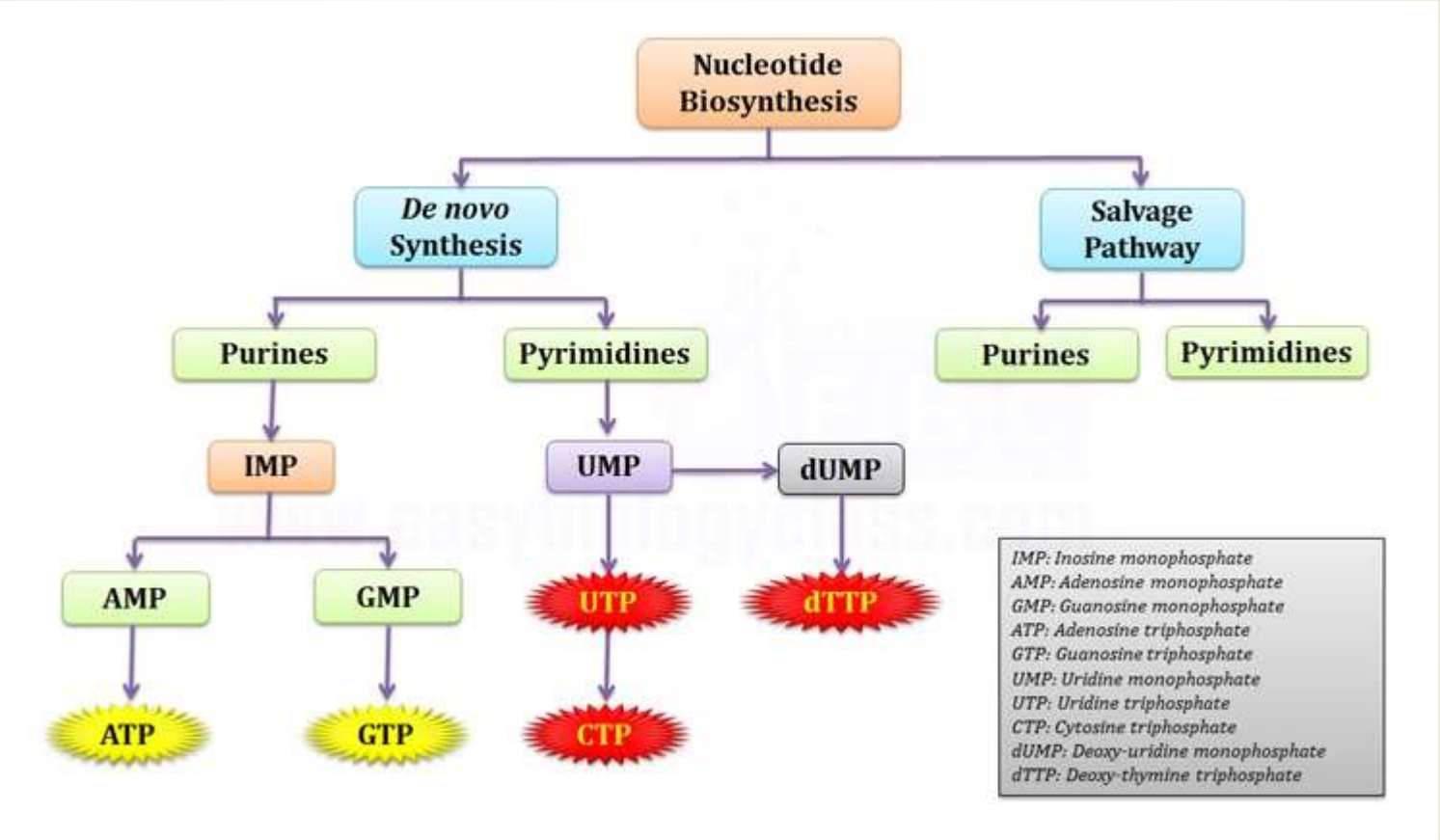


https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(McMurry)/28%3A_Biomolecules_-_Nucleic_Acids/28.02%3A_Nucleotides_and_Nucleic_Acids

Biosynthesis of nucleotides.

- There are two principal pathways for the synthesis of nucleotides: the **de novo** and the **salvage pathways.**
- Using **5-phosphoribosyl-1-pyrophosphate (PRPP),** the **de novo** pathway enzymes build purine and pyrimidine nucleotides from "scratch" using **simple molecules** such as CO2, amino acids and tetrahydrofolate. This pathway of nucleotide synthesis has a high requirement for energy as compared that of the salvage pathway.
- The **salvage pathway** interconverts nitrogenouse bases, nucleosides and nucleotides released as by-products of cellular metabolism or from the catabolism of nucleic acids or nucleotide cofactors.
- For example, five of the 12 steps of de novo purine synthesis require hydrolysis of ATP or GTP but only one salvage cycle reaction uses ATP.
- The enzymes of both of these biosynthetic pathways are classified as "housekeeping" enzymes because they perform basic, cellular activities and are assumed to be present in low, constitutive levels in all cells. Whereas the de novo pathway is thought to reside in plastids, salvage cycle enzymes may be localized in more than one compartment.

Biosynthesis of nucleotides.



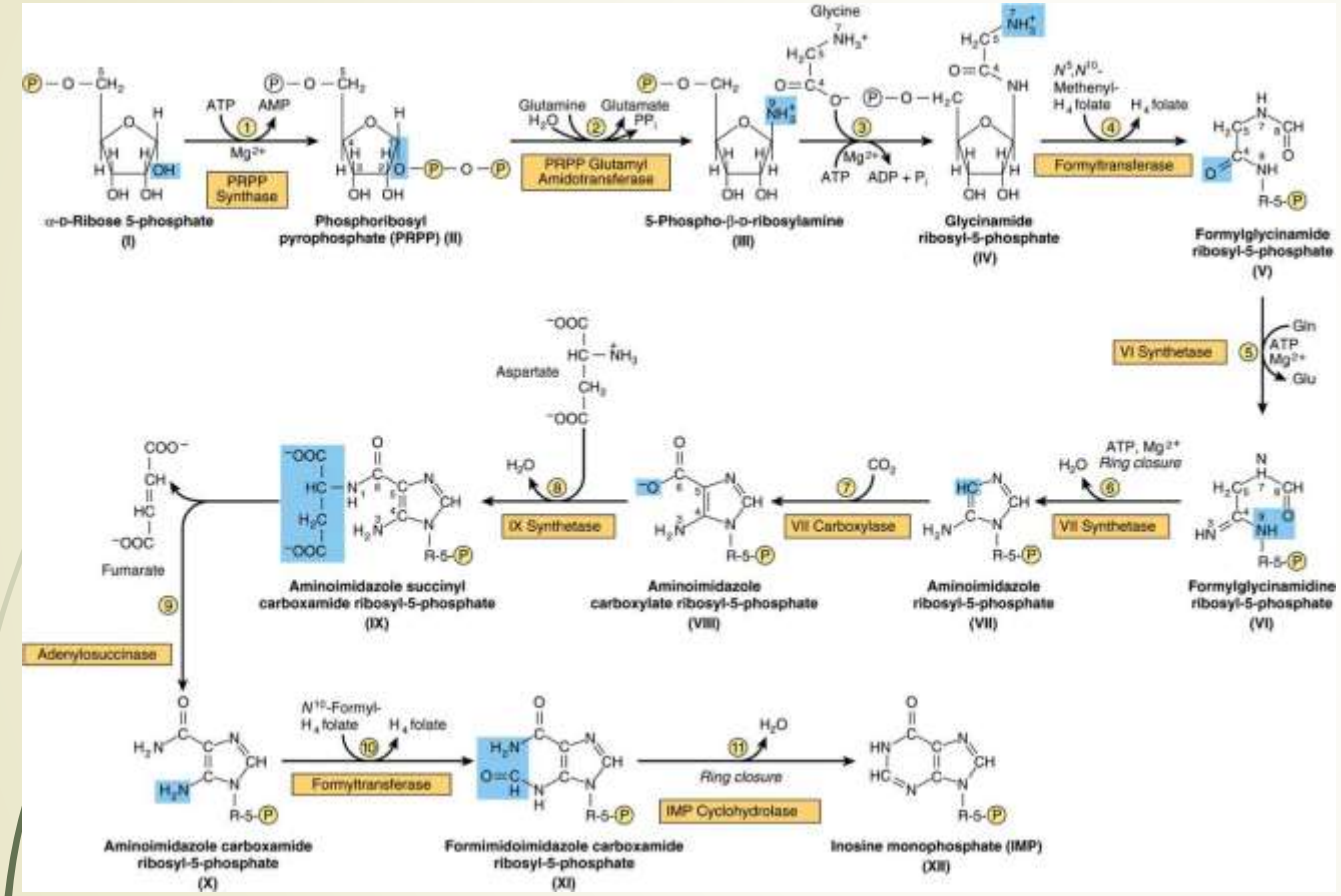
https://www.easybiologyclass.com/nucleotide-biosynthesis-de-novo-salvage-purine-pyrimidine-nucleotides-cells/

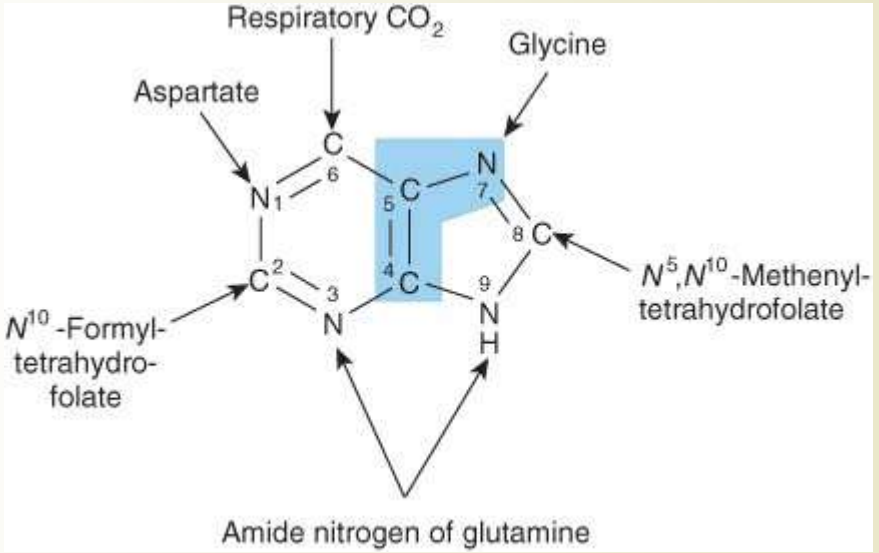
Biosynthesis of purine nucleotides (de novo pathway).

- Purines are biologically synthesized as nucleotides and in particular as ribotides, i.e. bases attached to ribose 5-phosphate. Both adenine and guanine are derived from the nucleotide inosin monophosphate (IMP), which is the first compound in the pathway to have a completely formed purine ring system.
- We use for purine nucleotides the entire glycine molecule (atoms 4, 5,7), the amino nitrogen of aspartate (atom 1), amide nitrogen of glutamine (atoms 3, 9), components of the folate-one-carbon pool(atoms 2, 8), carbon dioxide, ribose 5-P from glucose and a great deal of energy in the form of ATP. In de novo synthesis, IMP is the first nucleotide formed. It is then converted to either AMP or GMP.
- Since the purines are synthesized as the ribonucleotides, (not as the free bases) a necessary prerequisite is the synthesis of the activated form of ribose 5-phosphate. Ribose 5-phosphate reacts with ATP to form **5-Phosphoribosyl-1-pyrophosphate (PRPP)**.
- his reaction occurs in many tissues because PRPP has a number of roles - purine and pyrimidine nucleotide synthesis, salvage pathways, NAD and NADP formation. The enzyme is heavily controlled by a variety of compounds (di- and tri-phosphates, 2,3-DPG), presumably to try to match the synthesis of PRPP to a need for the products in which it ultimately appears.

- Purine synthesis occurs in all tissues. The major site of purine synthesis is in the liver and, to a limited extent, in the brain.
- **Substrates:** Ribose-5-phosphate; glycine; glutamine; H_2O; ATP; CO_2; aspartate.
- **Products:** GMP; AMP; glutamate; fumarate; H_2O.

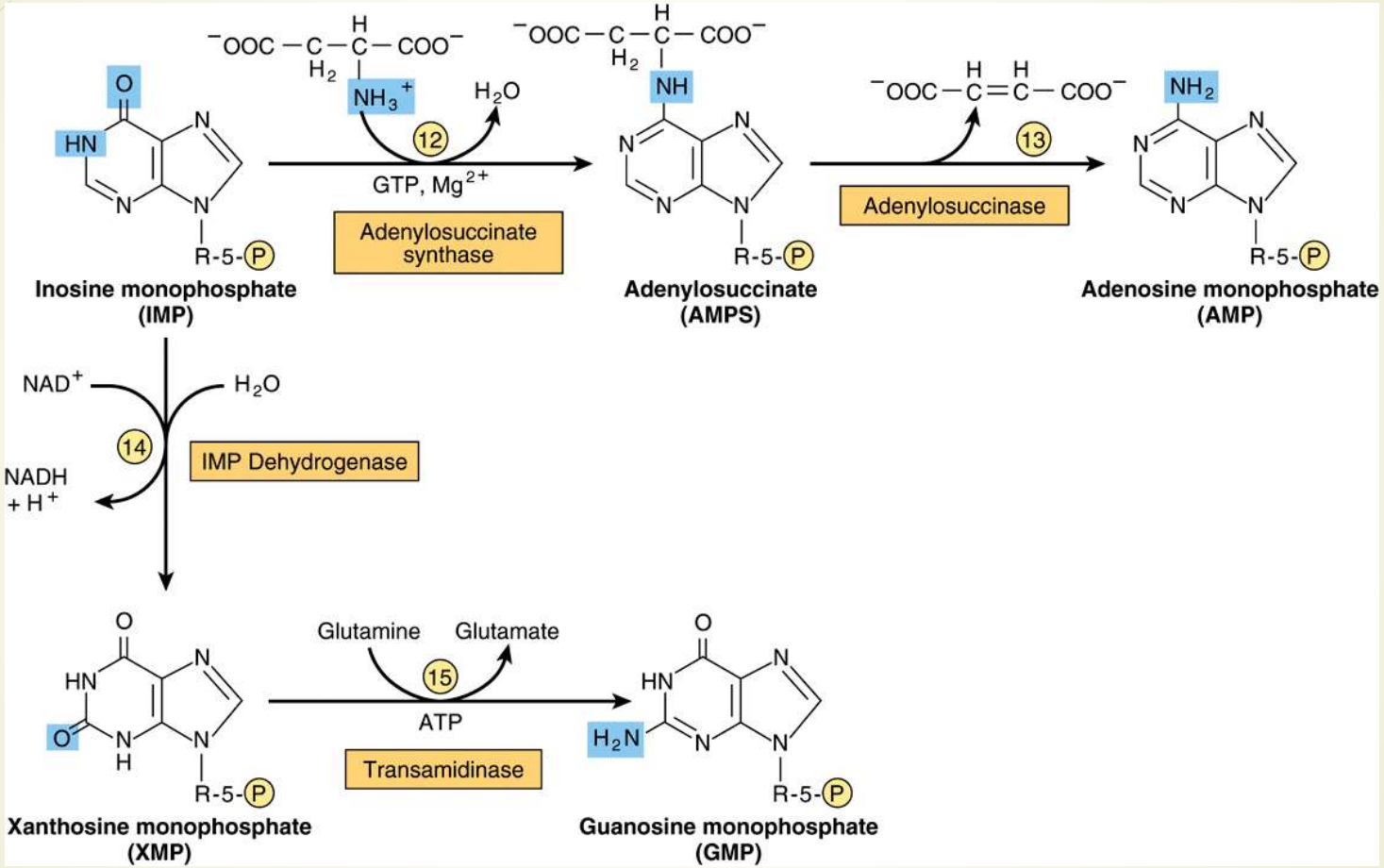
Biosynthesis of purine nucleotides (de novo pathway).





https://basicmedicalkey.com/metabolism-of-purine-pyrimidine-nucleotides/

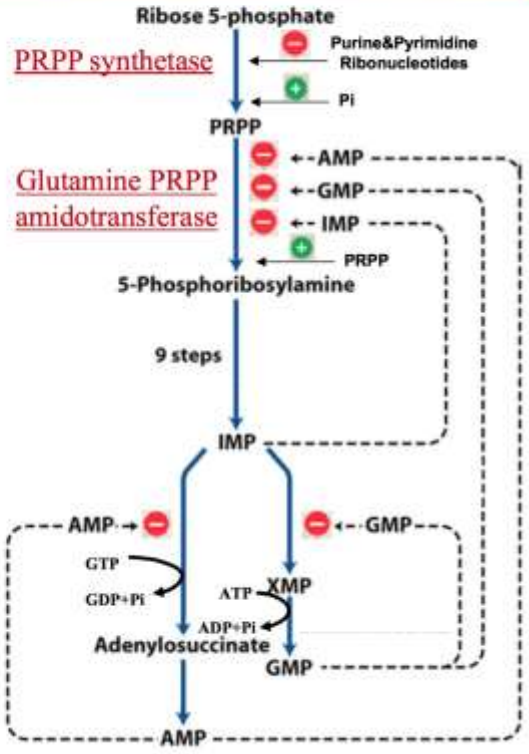
Biosynthesis of purine nucleotides (de novo pathway).



https://basicmedicalkey.com/metabolism-of-purine-pyrimidine-nucleotides/

De novo synthesis of purine nucleotides

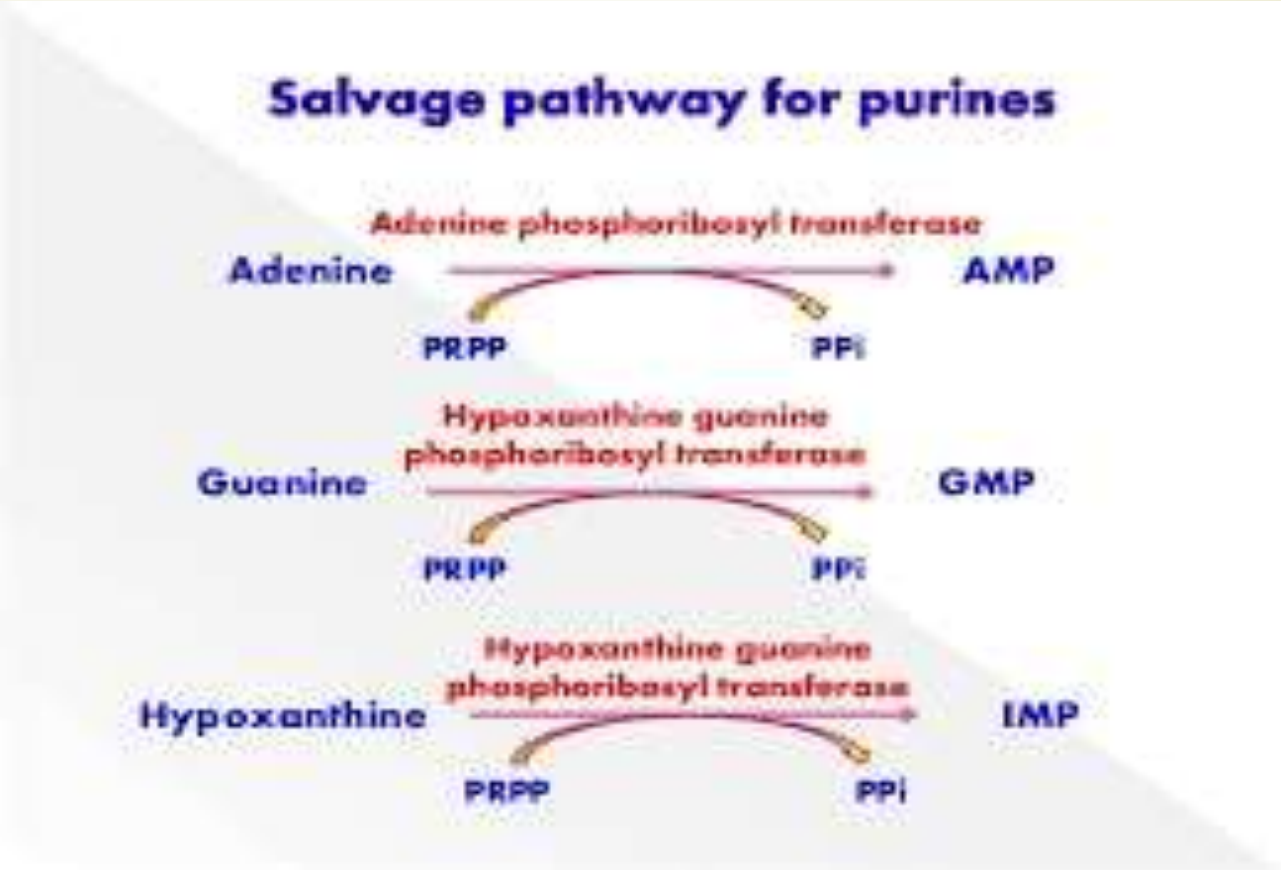
- **PRPP synthetase** is the rate-limiting step in the synthesis of both purines and pyrimidines.
- **Glutamine:PRPP amidotransferase** catalyzes the first-committed step in purine synthesis.
- **IMP branch to AMP**
  - Inhibitor: AMP
  - Need for GTP
- **IMP branch to GMP**
  - Inhibitor: GMP
  - Need for ATP



https://microbenotes.com/purine-synthesis/

Biosynthesis of purine nucleotides (salvage pathway).

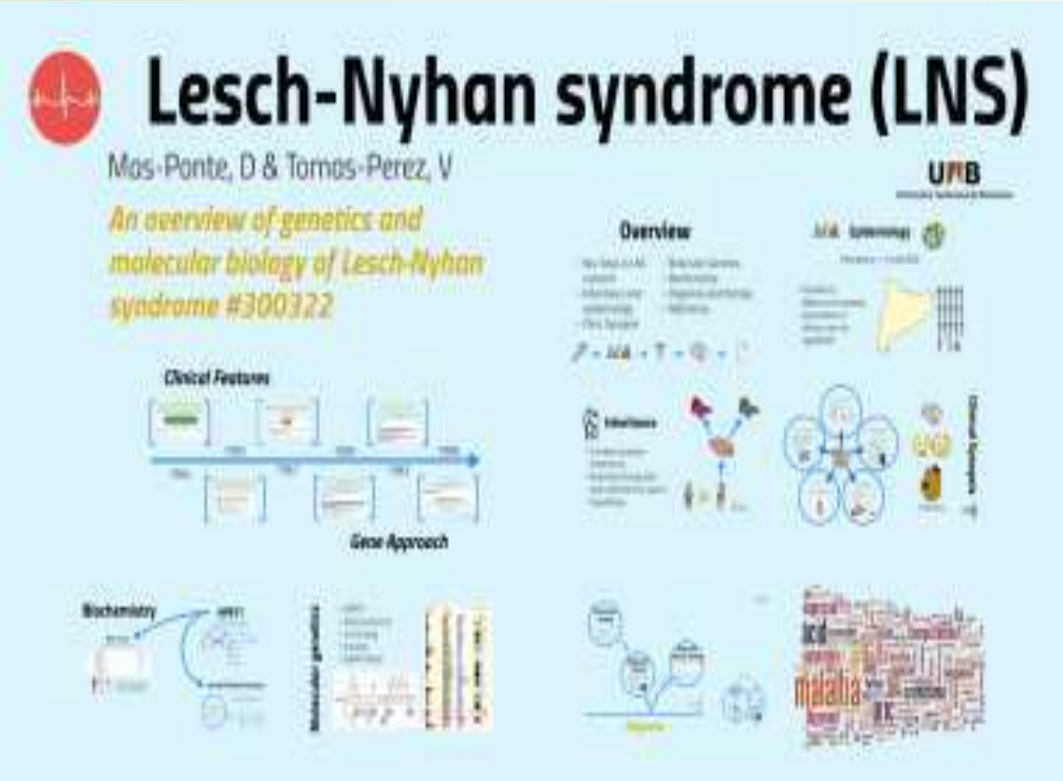
- This pathway ensures the recycling of purines formed by degradation of nucleotides.
- Nucleosides & deoxy-nucleosides can also be salvaged.
- **Salvage pathway of purine nucleotide synthesis are used by Brain, RBC, Leukocytes**
- The purines can be directly converted to the corresponding nucleotides & this process is known as ‘salvage pathway’.
- **PRPP is the starting material in this pathway.**
- It is also a substrate for de novo synthesis.
- **The purines salvage pathway is for Hypoxanthine and Adenine**
- The free purines are salvaged by two different enzymes.
  - Adenine phospho ribosyl transferase (APRTase).
  - Hypoxanthine guanine phosphoribosyl transferase (HGPRTase).
- Adenine phosphoribosyl transferase catalyses the formation of AMP from adenine.
- **First purine nucleotide, which is synthesized in purine biosynthesis is IMP**
- Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) converts guanine & hypoxanthine to GMP & IMP.
- Phosphoribosyl pyrophosphate (PRPP) is the donor of ribose 5-phosphate in the salvage pathway.



https://medicoapps.org/m-purine-salvage-pathway/

Lesch–Nyhan syndrome

- **Lesch–Nyhan syndrome** (**LNS**) is a rare inherited disorder caused by a deficiency of the enzyme **hypoxanthine-guaninephosphoribosyltransferase** (HGPRT).
- LNS is characterized by three major hallmarks: neurologic dysfunction, cognitive and behavioral disturbances including self-mutilation, and uric acid overproduction (hyperuricemia). Damage to the basal ganglia causes sufferers to adopt a characteristic fencing stance due to the nature of the lesion. Some may also be afflicted with macrocytic anemia. Virtually all patients are male; males suffer delayed growth and puberty, and most develop shrunken testicles or testicular atrophy. Female carriers are at an increased risk for gouty arthritis but are usually otherwise unaffected.
- Persons affected are cognitively impaired and have behavioral disturbances that emerge between two and three years of age. The uncontrollable self-injury associated with LNS also usually begins at three years of age. The self-injury begins with biting of the lips and tongue; as the disease progresses, affected individuals frequently develop finger biting and head banging. The self-injury can increase during times of stress. Self-harm is a distinguishing characteristic of the disease and is apparent in 85% of affected males.



https://prezi.com/yh1v9feiwbb8/lesch-nyhan-syndrome-lns/

https://en.wikipedia.org/wiki/Lesch–Nyhan_syndrome

- It affects only the males.
- Characterized by excessive uric acid production (Gouty arthritis) & neurological abnormalities.
- Mental retardation, aggressive behavior, learning disability etc.
- The patients of this disorder have an irresistible urge to bite their fingers & lips, often causing self-mutilation.

https://www.slideshare.net/YESANNA/purine-degradation-gout-44397572

Biosynthesis of pyrimidine nucleotides.

- Since pyrimidine molecules are simpler than purines, so is their synthesis simpler but is still from readily available components. Glutamine's amide nitrogen and carbon dioxide provide atoms 2 and 3 or the pyrimidine ring. They do so, however, after first being converted to carbamoyl phosphate. The other four atoms of the ring are supplied by aspartate. As is true with purine nucleotides, the sugar phosphate portion of the molecule is supplied by PRPP.

- **Carbamoyl Phosphate**

Pyrimidine synthesis begins with **carbamoyl phosphate** synthesized in the cytosol of those tissues capable of making pyrimidines (highest in spleen, thymus, GItract and testes). This uses a different enzyme than the one involved in urea synthesis. **Carbamoyl phosphate synthetase II (CPS II)** prefers glutamine to free ammonia and has no requirement for N-Acetylglutamate.

- **Formation of Orotic Acid**

Carbamoyl phosphate condenses with aspartate in the presence of **aspartate transcarbamylase** to yield N-carbamylaspartate which is then converted to dihydroorotate.

- **Formation of the Nucleotides**

Orotic acid is converted to its nucleotide with PRPP. **OMP** is then **converted sequentially** - not in a branched pathway - to the other pyrimidine nucleotides. Decarboxylation of OMP gives **UMP**. **O-PRT and OMP** decarboxylase are also a **multifunctional protein**. After conversion of UMP to the triphosphate, the amide of glutamine is added, at the expense of ATP, to yield **CTP**.

- In human, **CPSII, asp-transcarbamylase, and dihydroorotase activities** are part of a **multifunctional protein**.

Oxidation of the ring by a complex, poorly understood enzyme produces the free pyrimidine, orotic acid. This enzyme is located on the outer face of the inner mitochondrial membrane, in contrast to the other enzymes which are cytosolic. Note the contrast with purine synthesis in which a nucleotide is formed first while pyrimidines are first synthesized as the **free base**.

- **Formation of the Nucleotides**

Orotic acid is converted to its nucleotide with PRPP. **OMP** is then **converted sequentially** - not in a branched pathway - to the other pyrimidine nucleotides. Decarboxylation of OMP gives **UMP**. **O-PRT and OMP** decarboxylase are also a **multifunctional protein**. After conversion of UMP to the triphosphate, the amide of glutamine is added, at the expense of ATP, to yield **CTP**.

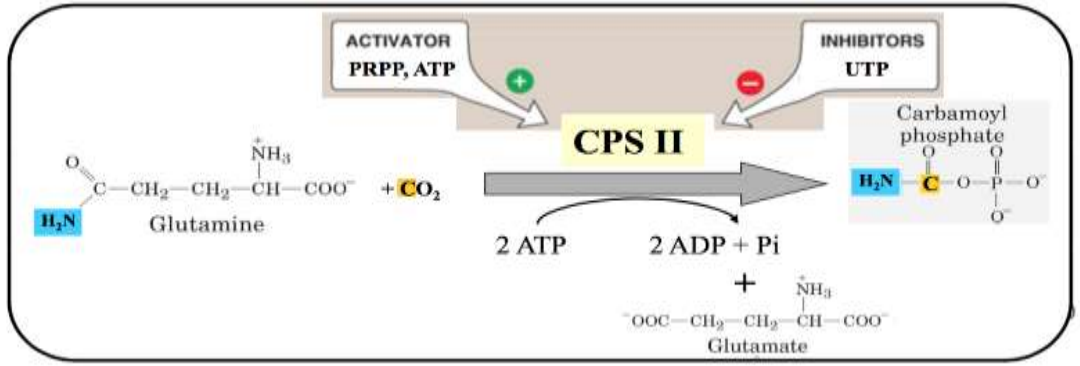
- **Control**

The control of pyrimidine nucleotide synthesis in man is exerted primarily at the level of **cytoplasmic CPS II**. **UTP inhibits** the enzyme, competitively with ATP. **PRPP activates** it. Other secondary sites of control also exist (e.g. OMP decarboxylase is inhibited by UMP and CMP). These are probably not very important under normal circumstances.

Biosynthesis of pyrimidine nucleotides

Carbamoyl Phosphate Synthetase II (CPS II)

- CPS II catalyzes the first-committed step in pyrimidine biosynthesis.
- PRPP and ATP are positive allosteric effectors of CPS II.
  - PRPP stimulates the synthesis of both purines and pyrimidines.
  - ATP provides coordination between both purine and pyrimidine biosynthetic pathways.
- UTP is a negative allosteric effector of CPS II.



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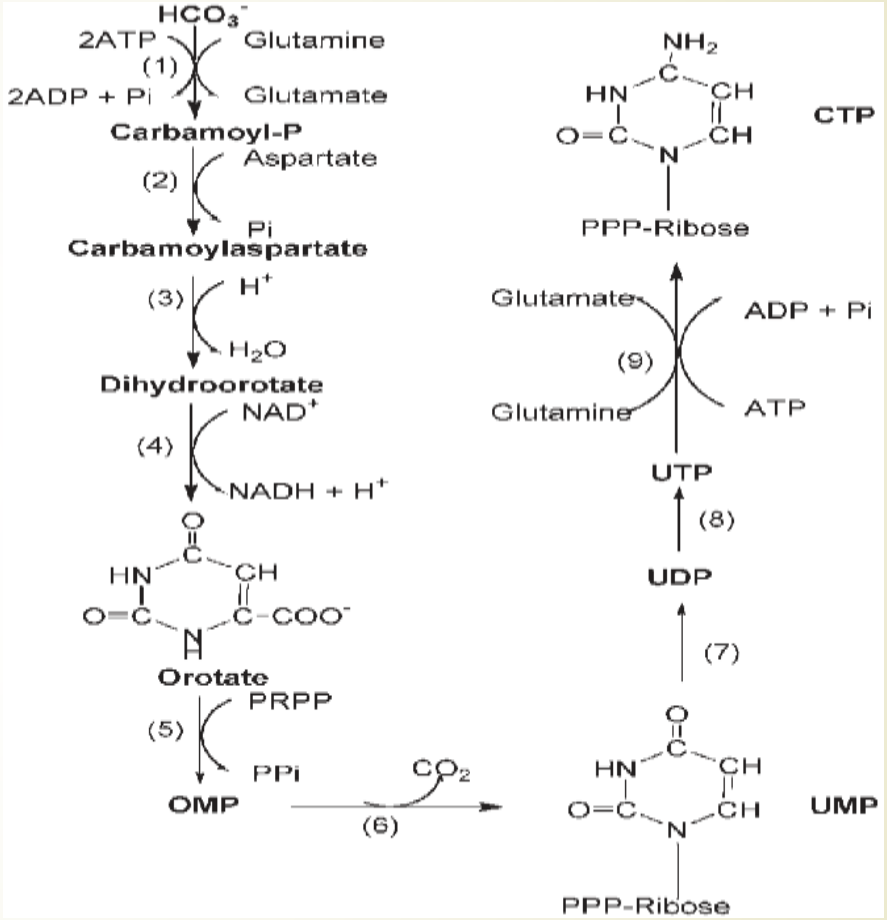


https://www.researchgate.net/figure/De-novo-biosynthetic-pathway-of-pyrimidine-nucle
in-plants-Enzymes-shown-are-1_fig1_221803893

Orotic aciduria

- The **orotic aciduria** is a disease caused by an enzymes (orotate hosphoribosyltransferase and orotidine-5′-monophosphate decarboxylase) activities deficiency resulting in a decreased ability to *de novo* pyrimidine synthesis pathway.
- Orotic aciduria is characterized by failure of normal growth and by the presence of hypochromic erythrocytes and megaloblastic bone marrow, none of which is improved by the usual hematinic agents (e.g., iron, pyridoxine, vitamin B_{12}, and folate). Leukopenia is also present. Treatment with uridine (2–4 g/d) results in marked improvement in the hematological abnormalities, in growth and development, and in decreased excretion of orotic acid.

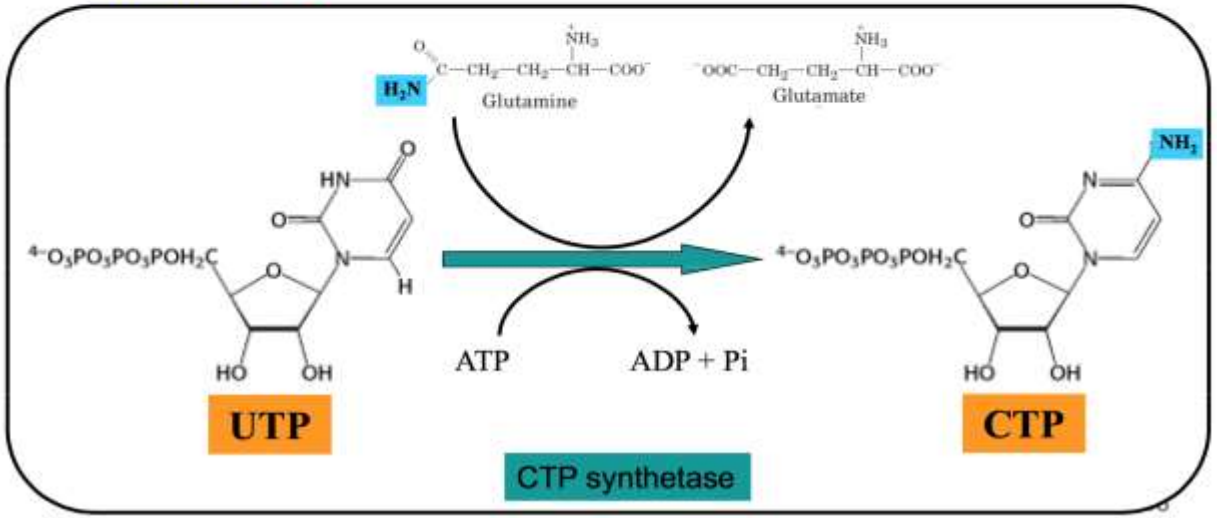
In addition to genetically determined reasons, orotic aciduria can be observed:

- with hyperammonemia caused by a defect in any of the enzymes of the ornithine cycle, except for carbamoyl phosphate synthetase I. In this case, carbamoyl phosphate synthesized in mitochondria enters the cytosol of cells and begins to be used for the formation of pyrimidine nucleotides. The concentration of all metabolites, including orotic acid, increases. The most significant excretion of ornithine is noted with a deficiency of ornithinecarbamoyltransferase (the second enzyme of the ornithine cycle);

- during the treatment of gout with allopurinol, which is converted to oxypurinol mononucleotide and becomes a potent inhibitor of UMP synthase. This leads to the accumulation of orotic acid in tissues and blood.

Ⓓ Formation of CTP

- CTP, the second of the pyrimidine nucleotides, is made by transferring the amide group from glutamine to UTP in a reaction catalyzed by CTP synthetase.
- CTP synthetase is regulated by one activator and one inhibitor:
  - * Activator: UTP
  - * Inhibitor: CTP



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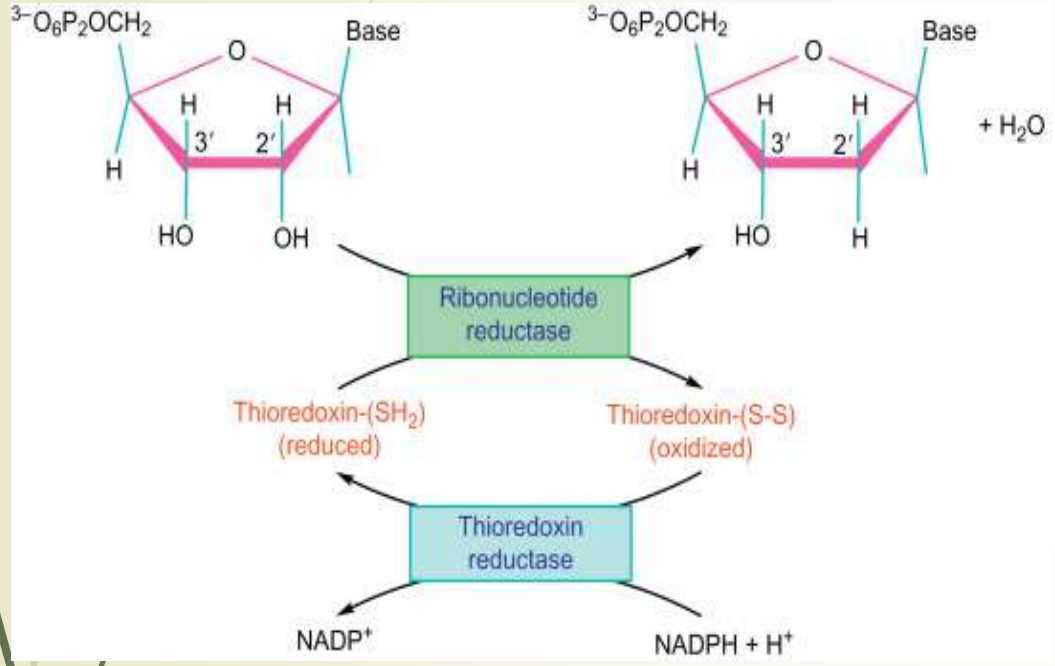
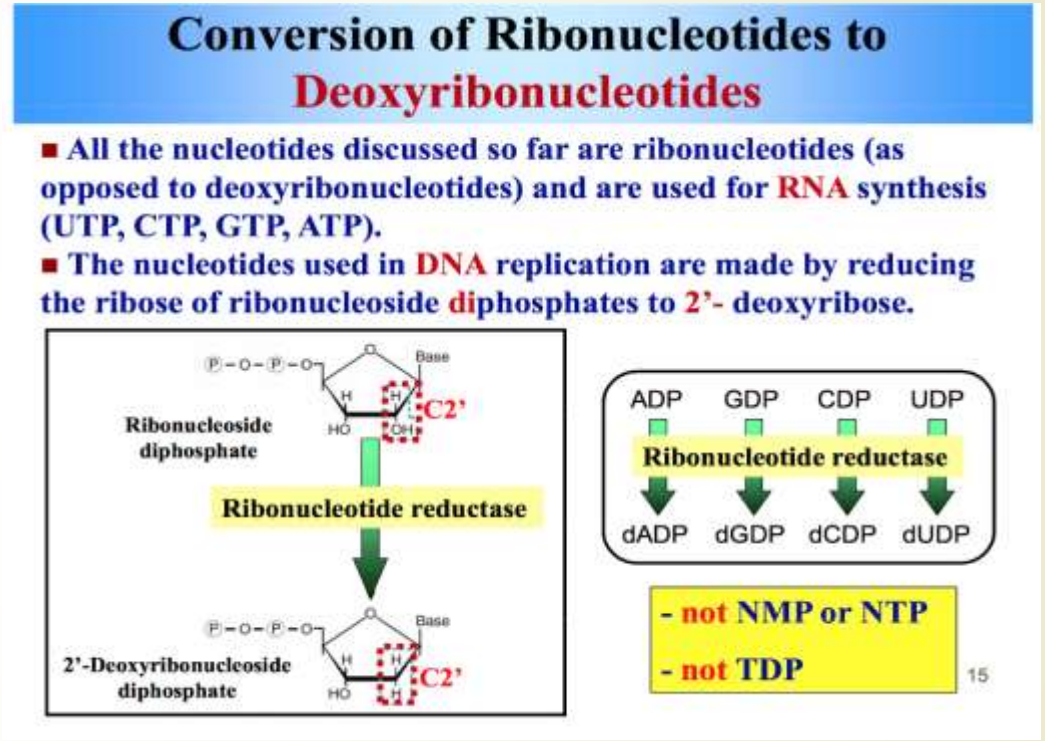
Biosynthesis of deoxyribo-nucleotides.





https://www.sciencedirect.com/topics/medicine-and-dentistry/ribonucleoside-diphosphate reductase

https://www.memorangapp.com/flashcards/68888/GS+L47+Pyrimidine+Biosynthesis/

Biosynthesis of dTMP.

Conversion of Ribonucleotides to Deoxyribonucleotides

- Ribonucleotide reductase (RNR) catalyzes the rate-limiting step and only reaction needed for the synthesis of dADP, dGDP, dCDP and dUPD.
- The substrates for the enzyme are any of the four ribonucleoside diphosphates (ADP, GDP, CDP and UDP).
  - Note: Thymidine diphosphate (TDP) is NOT a substrate for ribonucleotide reductase.
- Ribonucleotide reductase (RNR) requires a peptide coenzyme, thioredoxin, which ultimately donates the electrons used to reduce the hydroxyl group in ribose.
- The oxidized thioredoxin contains a disulfide which is reduced by a second enzyme, thioredoxin reductase. NADPH is the electron donor.

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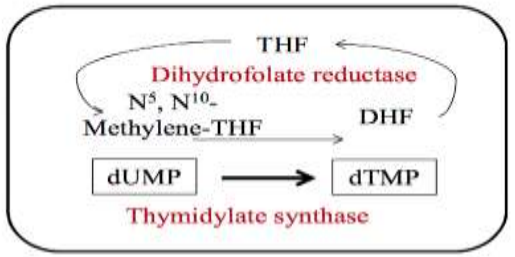
Thymidylate Synthase

- Thymidylate synthase catalyzes the formation of thymidine monophosphate (dTMP) from dUMP.
  - ➢ Thymidine never exists with a ribose. It is always found attached to deoxyribose.
  - ➢ Thymidylate synthase is the rate-limiting step for DNA synthesis.
- What is the source of dUMP?



- ➢ dUTPase: $dUTP \rightarrow dUMP + PP_i$
- ➢ dCMP deaminase: $dCMP \rightarrow dUMP + NH_3$

- Tetrahydrofolate is required for the synthesis of all purines (AMP, GMP) and thymidine monophosphate (dTMP), but not for the synthesis of UTP or CTP.

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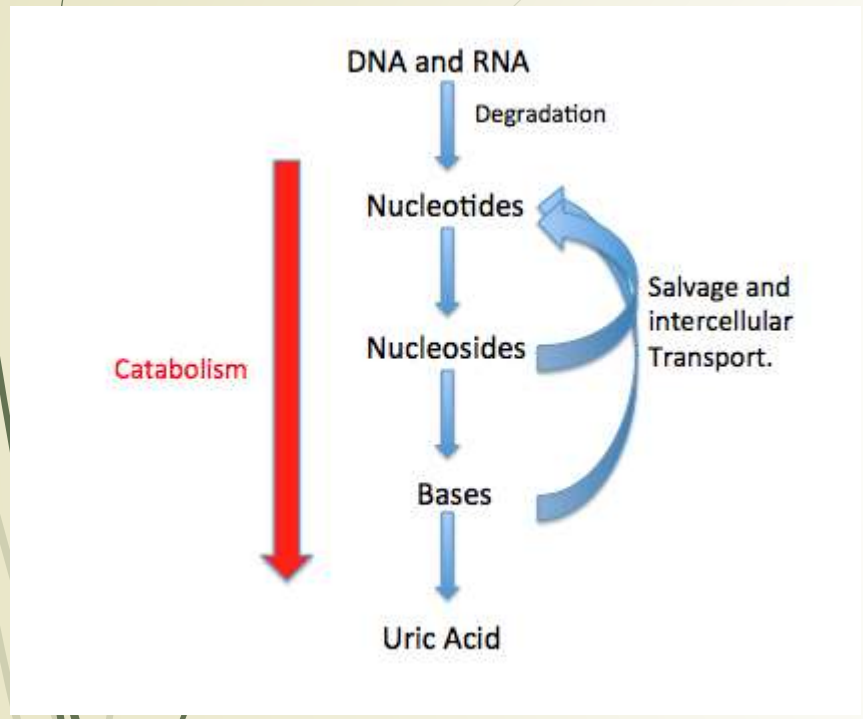
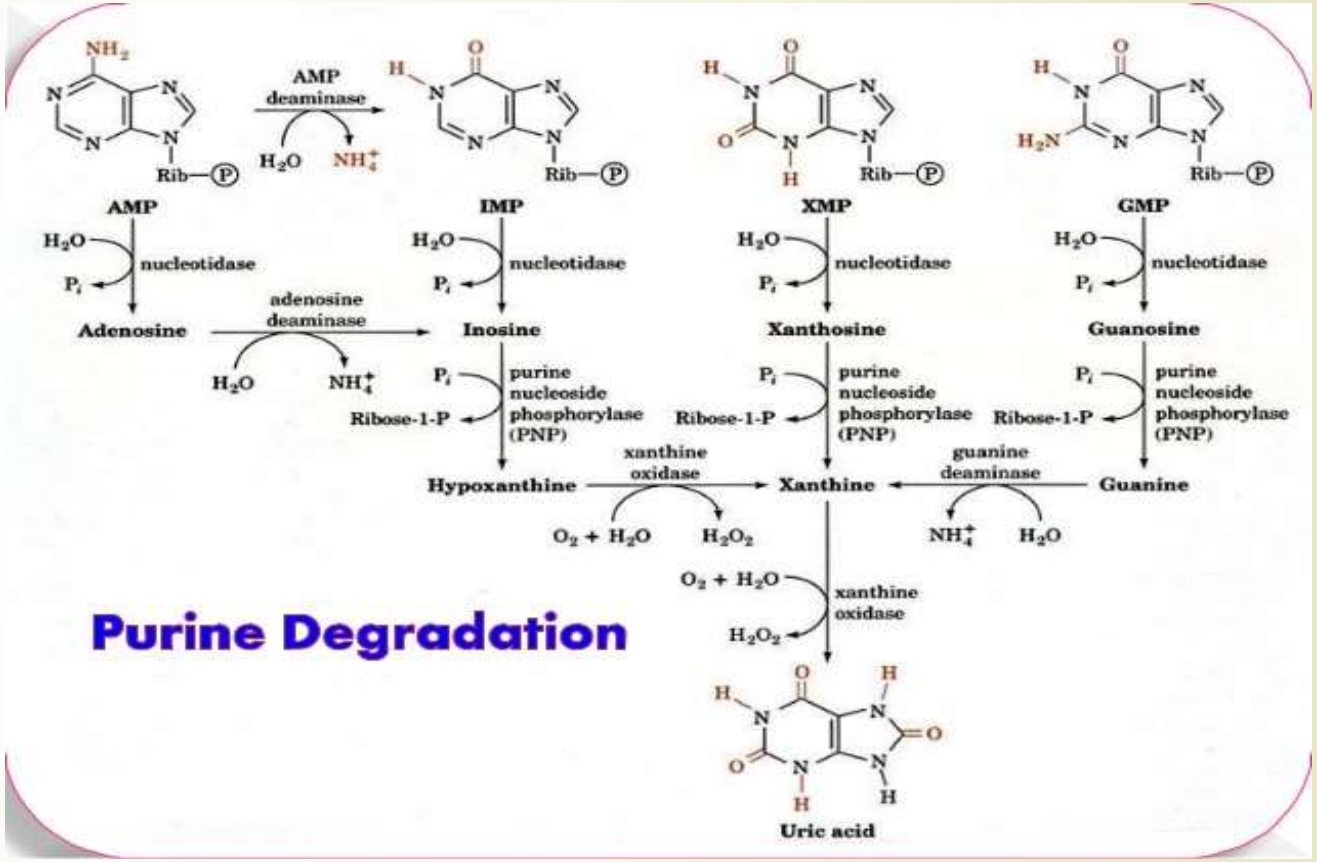
Catabolism of purine nucleotides.



https://www.wikiwand.com/en/Nucleic_acid_metabolism



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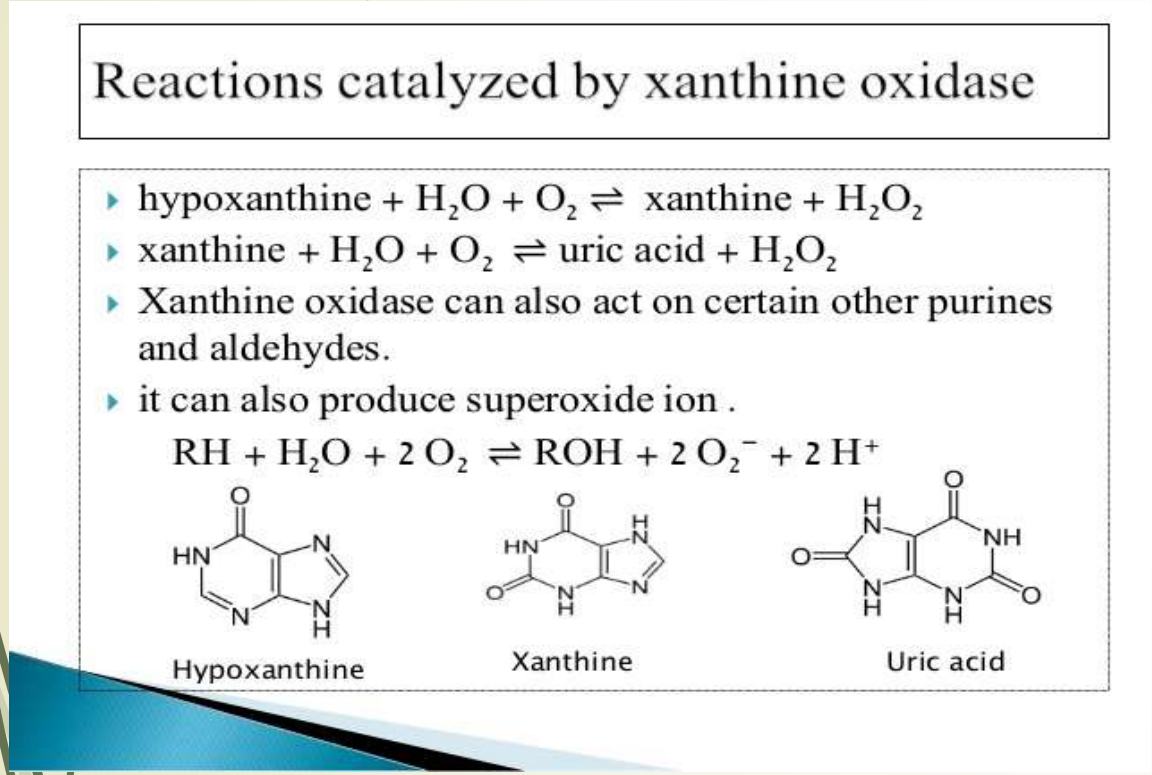
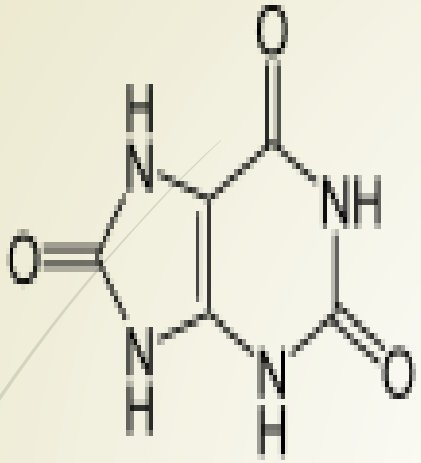
Xanthine oxidase



- The protein is large, having a molecular weight of 270 kDa, and has 2 flavin molecules (bound as FAD), 2 molybdenum atoms, and 8 iron atoms bound per enzymatic unit. The molybdenum atoms are contained as molybdopterin cofactors and are the active sites of the enzyme. The iron atoms are part of [2Fe-2S] ferredoxin iron-sulfur clusters and participate in electron transfer reactions.

https://www.slideshare.net/DeepakKumar1974/xanthine-oxidase-enzyme-74073690

Uric acid



https://en.wikipedia.org/wiki/Uric_acid

Uric acid (its keto form) is the final product of purine nucleotides catabolism and is characterized by low solubility in water.

It forms ions and salts known as **urates** and **acid urates**, its sodium salt is distinguished by its higher solubility.

The form in which uric acid is found in biological fluids (blood, urine, cerebrospinal fluid) depends on the pH of this liquid.

At normal pH - uric acid and its monosodium salt (sodium urate).At a higher pH, its dominant form is sodium uric acid.

At a lower value (especially if the pH is <5.75) – (in tissues, but more often in the renal tubules and urine),

the main molecular form is sparingly soluble urine acid in keto form.

- **Uric acid blood concentration: 0.12-0.46 mmol/l. Urine daily excretion – 0.3-0.8 g/day.**
- An increase in the concentration of uric acid in the blood – **hyperuricemia**.

Causes of hyperuricemia: hereditary purine metabolism defects, for example, (Lesh-Nyhan syndrome), a diet rich in purines (meat products, fish, liver), increased catabolism of nucleoproteins, blood, kidney diseases, lead poisoning and other conditions.

GOUT

Hyperuricemia often leads to the development of **gout.**
This disease is characterized by the deposition of crystals of uric acid salts (urates) in the joints (mainly of the metatarsophalangeal thumb) and around them, in soft tissues, places of attachment of ligaments, tendons.
Polyarthritis gradually develops and gouty nodes appear.
Chronic gouty arthritis leads to joint deformation.
When crystals are deposited in the kidneys, urolithiasis develops.

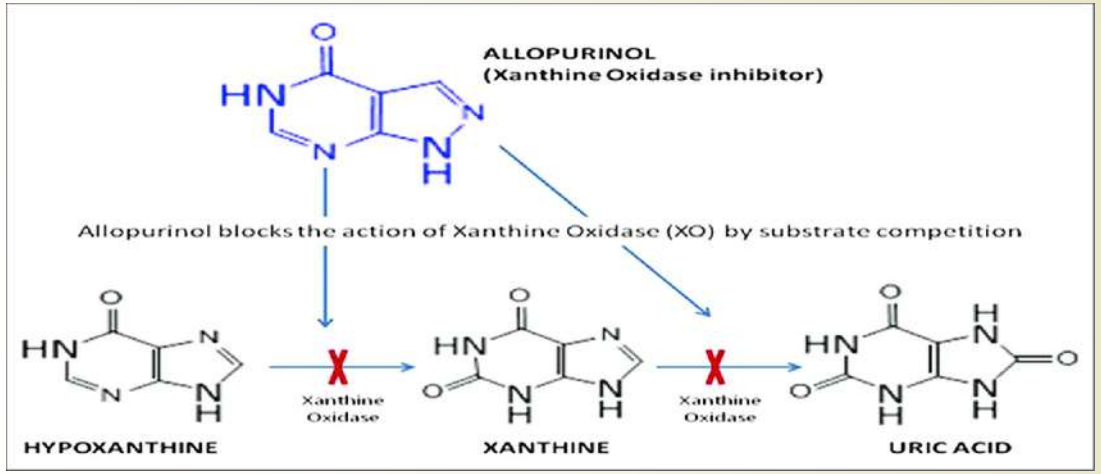
In the blood, uric acid is in the form of its salts - sodium urate. Due to their low solubility, urates can settle in areas with low temperatures, for example, in the small joints of the feet and toes. Urates accumulating in the intercellular substance are phagocytized for some time, but phagocytes are not able to destroy the purine ring. As a result, this leads to the death of the phagocytes themselves, to the release of lysosomal enzymes, activation of free radical oxidation and the development of an acute inflammatory reaction - gouty arthritis develops.
In 50-75% of cases, the first sign of the disease is excruciating night pain in the big toes.

Urolithiasis consists in the formation of salt crystals (stones) of a different nature in the urinary tract. The formation of uric acid stones directly makes up about 15% of all cases of this disease. Uric acid stones in the urinary tract are deposited in about half of patients with gout.

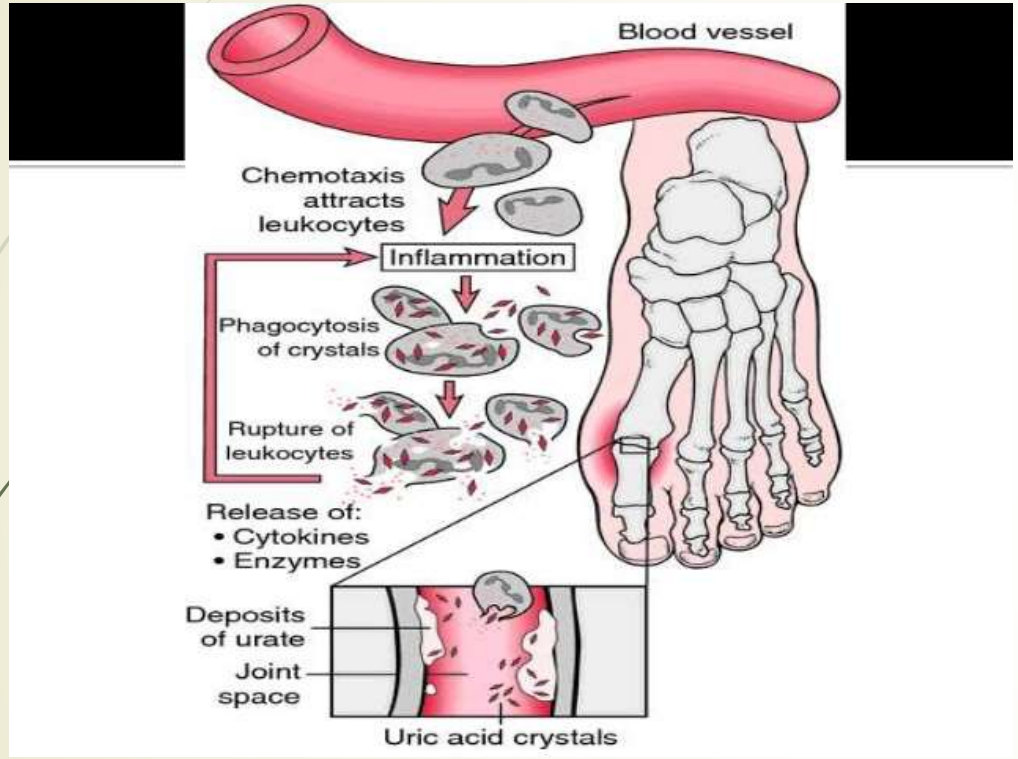
Most often, these stones are present in the distal tubules and collecting ducts. The reason for the deposition of crystals of uric acid is hyperuricemia and increased excretion of sodium urate in the urine. The main provoking factor of crystallization is an increase in urine acidity. With a decrease in urine pH below 5.75, urates (enol form) pass into a less soluble keto form and crystallize in the renal tubules.

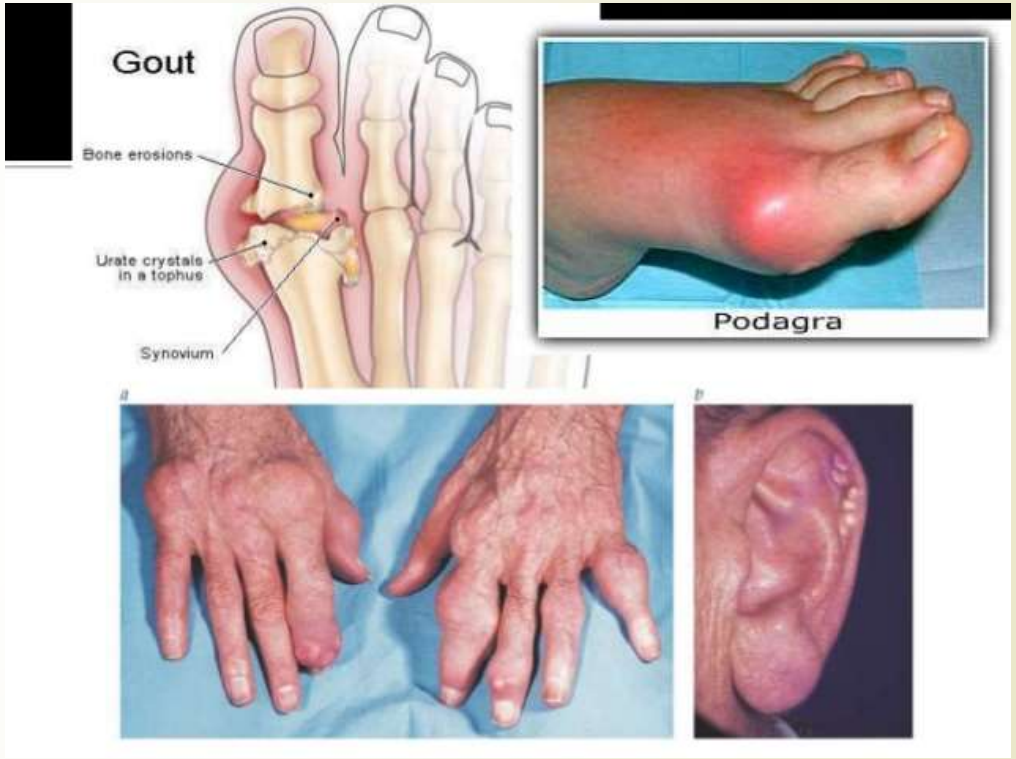
GOUT

The mainstay of gout treatment is a purine-free diet and the use of allopurinol. In addition, a plant-based diet is recommended, leading to urine alkalization, which increases the proportion of the more water-soluble enolic form of uric acid in the urine. Along with this, already existing crystals of uric acid (as well as crystals of oxalates) are able to dissolve when urine alkalizes. Medicinal treatment must certainly be accompanied by a purine-free diet with plenty of pure water, otherwise the appearance of xanthine crystals in tissues and xanthine kidney stones is inevitable.



https://www.researchgate.net/figure/The-reaction-cascade-of-allopurinol-in-xanthine-oxidase-inhibition-mechanism-and-uric_fig4_306332564



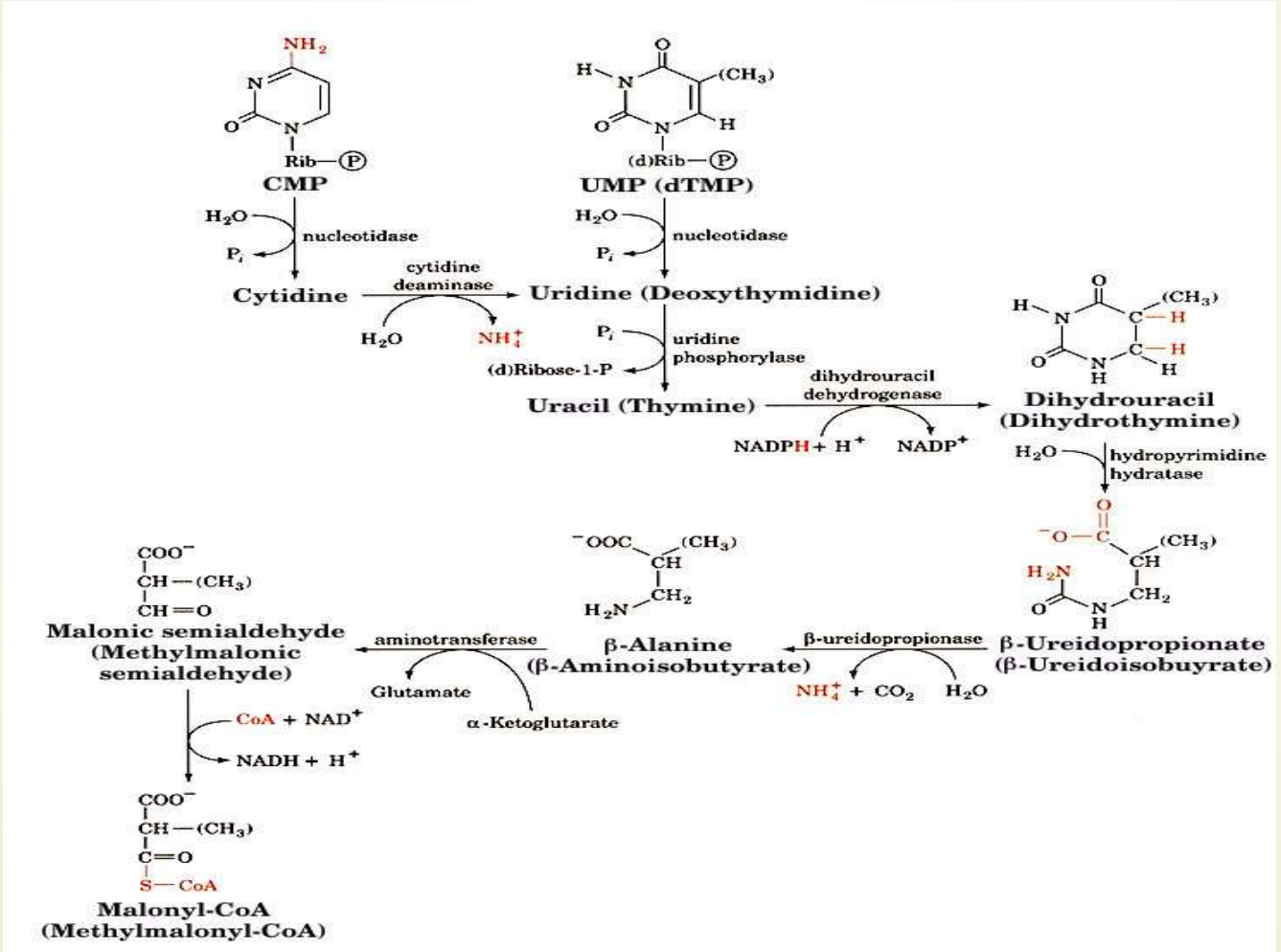


https://www.slideshare.net/drangelosmith/gout-and-pseudogout

Uric acid crystals in urine

https://ua.depositphotos.com/136760968/stock-photo-uric-acid-crystal-in-urine.html

Catabolism of pyrimidine nucleotides.



https://biochemden.com/pyrimidine-catabolism

Sources of information

- https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(McMurry)/28%3A_Biomolecules_-_Nucleic_Acids/28.02%3A_Nucleotides_and_Nucleic_Acids
- https://www.mdpi.com/2072-6694/11/5/688/htm
- https://basicmedicalkey.com/metabolism-of-purine-pyrimidine-nucleotides/
- https://microbenotes.com/purine-synthesis/
- https://medicoapps.org/m-purine-salvage-pathway/
- https://www.slideshare.net/YESANNA/purine-degradation-gout-44397572
- https://en.wikipedia.org/wiki/Lesch–Nyhan_syndrome
- https://prezi.com/yh1v9feiwbb8/lesch-nyhan-syndrome-lns/
- https://www.researchgate.net/figure/De-novo-biosynthetic-pathway-of-pyrimidine-nucleotides-in-plants-Enzymes-shown-are-1_fig1_221803893
- https://www.sciencedirect.com/topics/medicine-and-dentistry/ribonucleoside-diphosphate-reductase
- https://www.memorangapp.com/flashcards/68888/GS+L47+Pyrimidine+Biosynthesis/
- https://www.slideshare.net/YESANNA/purine-degradation-gout-44397572
- https://www.wikiwand.com/en/Nucleic_acid_metabolism
- https://www.slideshare.net/DeepakKumar1974/xanthine-oxidase-enzyme-74073690
- https://en.wikipedia.org/wiki/Uric_acid
- https://www.researchgate.net/figure/Human-serum-uric-acid-SUA-homeostasis-and-abnormal-changes-Normal-SUA-levels-in-humans_fig2_305392784
- https://www.slideshare.net/YESANNA/purine-degradation-gout-443
- https://www.slideshare.net/YESANNA/purine-degradation-gout-44397572
- https://www.slideshare.net/drangelosmith/gout-and-pseudogout

- Halkerston I.D.K. Biochemistry: 2nd edition. The National medical series for independent study / Halkerston I.D.K. - 1988. - 522 p.
- Harper`s Biochemistry. R.K.Murray, D.K.Granner, P.A.Mayes, V.W.Rodwell. Prentice-Hall International Inc., 2010. – 1134 p.
- Koolman J. Color Atlas of Biochemistry / J.Koolman, K.-H. Rom. – Stuttgart. New York. – Thieme Verlag. — 1996. – 435 p.
- Lehninger A. Principles of Biochemistry / Lehninger A. – New York. – W.H.Freeman and Company. – 2005. – 1010 p.
- Pamela C.Champe Lippincott's Illustrated Reviews: Biochemistry, 3rd Edition / Pamela C.Champe and Richard A.Harvey. – Baltimore, Lippincott Williams & Wilkins, MD ©, 2005. – 534p.