VNIVERSITAT DE VALÈNCIA

LESSON 30. NUCLEOTIDE METABOLISM

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Degree in Medicine

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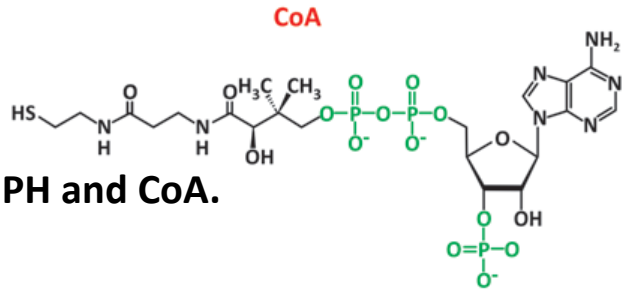
1. NUCLEOTIDE FUNCTIONS IN CELLS

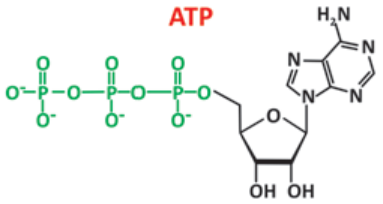
1. Structural components of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are essential to the maintenance, protein synthesis and inheritance of the cell. Nucleic acid precursors.

2. Activated intermediate transporters in the synthesis of UDP-glucose molecules or CDP-choline conjugated proteins.



3. Structural elements of essential coenzymes: FADH2, NADH, NADPH and CoA.

4. Second messengers in signal transduction: cAMP and cGMP.

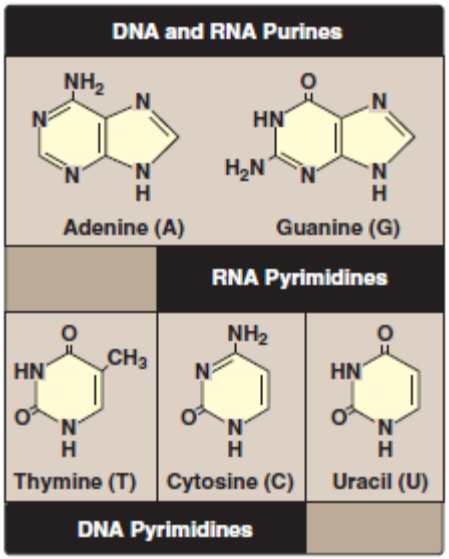


5. Allosteric regulators of metabolic pathways.

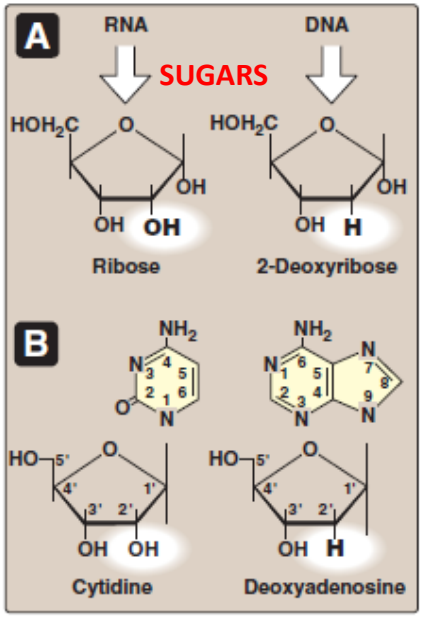
6. ATP donor phosphoryl groups for protein kinases. ATP universal energy currency, GTP.

NOMENCLATURE

NITROGEN BASES

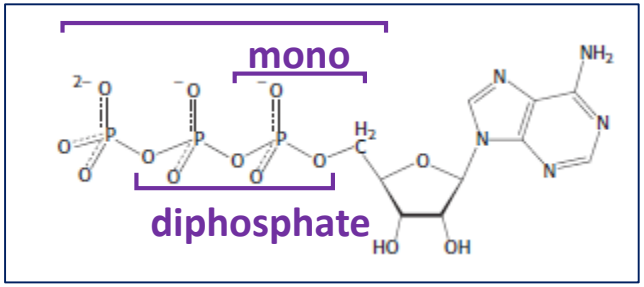


NUCLEOSIDES



NUCLEOTIDES:
Mono, di, triphosphate

Adenosin-triphosphate



ribonucleoside

BASES

Bases can be methylated, glycosylated, acetylated and reduced

NUCLEOSIDES

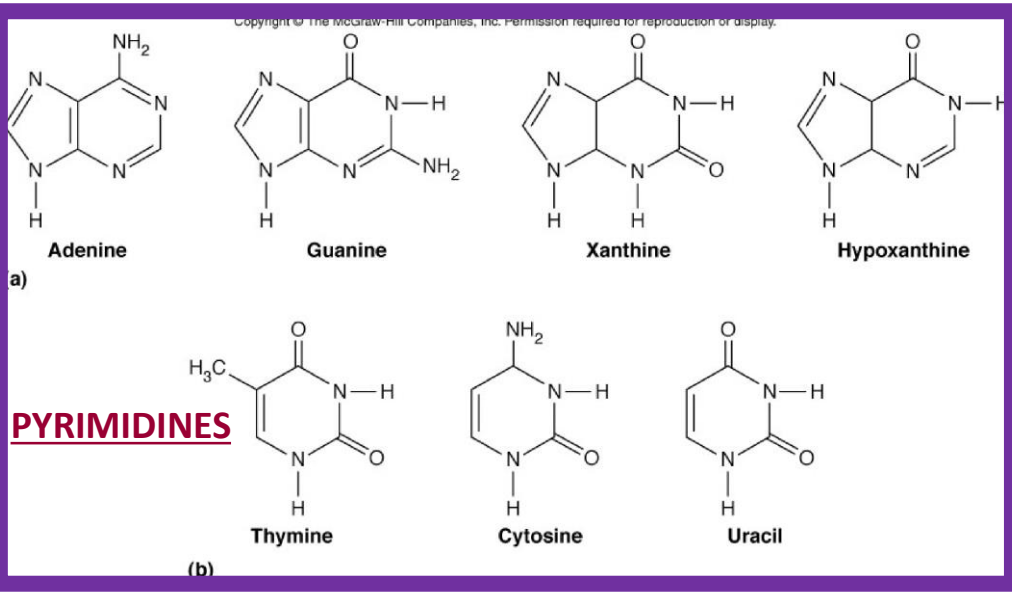
The carbon numbers of the ribose and deoxyribose are identified with a prime (‘): phosphate ester linkage occurs at C5

NUCLEOTIDES

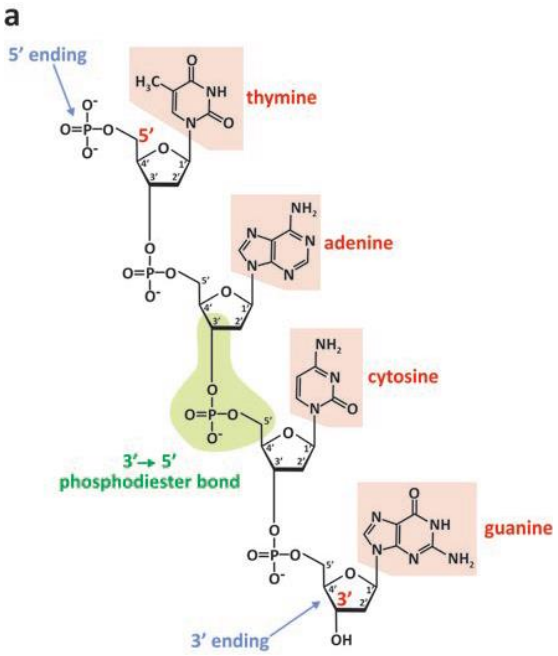
1. NUCLEOTIDE FUNCTIONS IN CELLS

BASE	NUCLEOSIDE (BASE + SUGAR)	NUCLEOTIDE (BASE + SUGAR + Pi)
PURINES		
Adenine (DNA, RNA)	Adenosine	AMP
Guanine (DNA, RNA)	Guanosine	GMP
Hypoxanthine	Inosine	IMP
PYRIMIDINES		
Cytosine (DNA, RNA)	Cytidine	CMP
Uracil (RNA)	Uridine	UMP
Thymine (DNA)	Thymidine	dTMp

PURINES



1. NUCLEOTIDE FUNCTIONS IN CELLS



Natural polymers of nucleotides—nucleic acids.

(a) Deoxyribonucleic acid (DNA) has 2-deoxyribose residues and uses thymine but not uracil. Ribonucleic acid (RNA) has ribose residues and uses uracil but not thymine.
Both polymers are formed by C3′–C5′ phosphodiester bonds.

(b–d) For the sake of simplicity, the chemical structure of the monomers is usually omitted and other ways to present the nucleotide residues sequence are preferred.
The simplest and most common form represents the nucleotides by a one-letter code (the first letter of the base name: T, A, C, or G).
Although it is not explicitly mentioned which ending is free, C5′ or C3′, it is established by convention that the sequences are presented in the direction 5′ to 3′.

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2. ORIGIN OF NUCLEOTIDES

BASE	NUCLEOSIDE (BASE + SUGAR)	NUCLEOTIDE (BASE + SUGAR + Pi)
PURINES		
Adenine (DNA, RNA)	Adenosine	AMP
Guanine (DNA, RNA)	Guanosine	GMP
Hypoxanthine	Inosine	IMP
PYRIMIDINES		
Cytosine (DNA, RNA)	Cytidine	CMP
Uracil (RNA)	Uridine	UMP
Thymine (DNA)	Thymidine	dTMp

ORIGIN OF NUCLEOTIDES

Purine and pyrimidine bases:

-***De novo*** DE NOVO PATHWAY **5%**

Activated ribose (PRPP) + amino acids + ATP + CO_2 + . . .

↓

Nucleotide

-Nucleotides salvage pathway SALVAGE PATHWAY **95%**

Activated ribose (PRPP) + base

↓

Nucleotide

2.1 *DE NOVO* SYNTHESIS OF NUCLEOTIDES

- Several important precursors are shared by the *de novo* pathways for the synthesis of pyrimidines and purines.
- Phosphoribosyl pyrophosphate (PRPP) is required.
- The structure of ribose is retained in the product nucleotide.

There is a specific amino acid precursor for each type of nucleotide synthesis pathway:

Glycine → Purines

Aspartate → Pyrimidines

Glutamine is the most important source of amino groups. It is involved in five different steps in the *de novo* synthesis pathway.

Aspartate is also used as the source of an amino group in the purine pathway in two steps.

There is evidence, especially in the *de novo* purine pathway, that the enzymes are present as large, multienzyme complexes in the cell.

The cellular pools of nucleotides (other than ATP) are quite small, roughly 1% or less of the amounts required to synthesize the cell's DNA. Cells must therefore continue to synthesize nucleotides during nucleic acid synthesis and, in some cases, nucleotide synthesis may limit the rates of DNA replication and transcription. Because of the importance of these processes in dividing cells, agents that inhibit nucleotide synthesis have become particularly important in medicine.

De novo synthesis of purine nucleotides: IMP, AMP and GMP

This process takes place mainly in the liver.

-**Purine ring**:

- 3 amino acids required (aspartate, glycine and glutamine)
- Carbon dioxide (CO_2)
- N10-formyl-THF (tetrahydropholate)

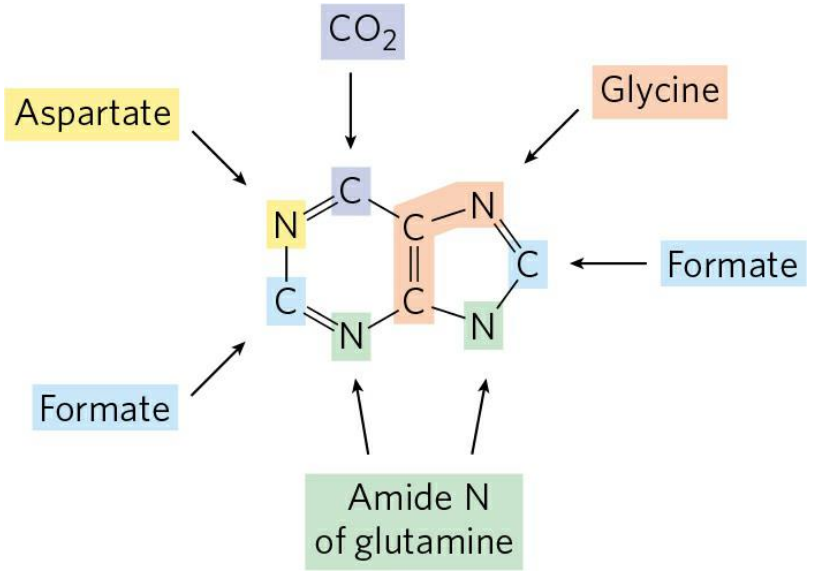
-**Process:**

- Addition of N and C to 5-phosphate ribose preformed
- Highly complex process

-**Requires:**

- 5-phosphorybosil-1- pyrophosphate (PRPP)
- IMP

-**High allosteric regulation**



Origin of purine rings

With the exception of 3 atoms from glycine, each C atom of the purine comes from a different precursor in a different reaction.

De novo synthesis of purine nucleotides: IMP, AMP, GMP

IMP (inosin-5'-monophosphate) synthesis from 5-phosphorybosilamine

IMP is the purine nucleotide precursor for AMP and GMP and contains hypoxanthine as base

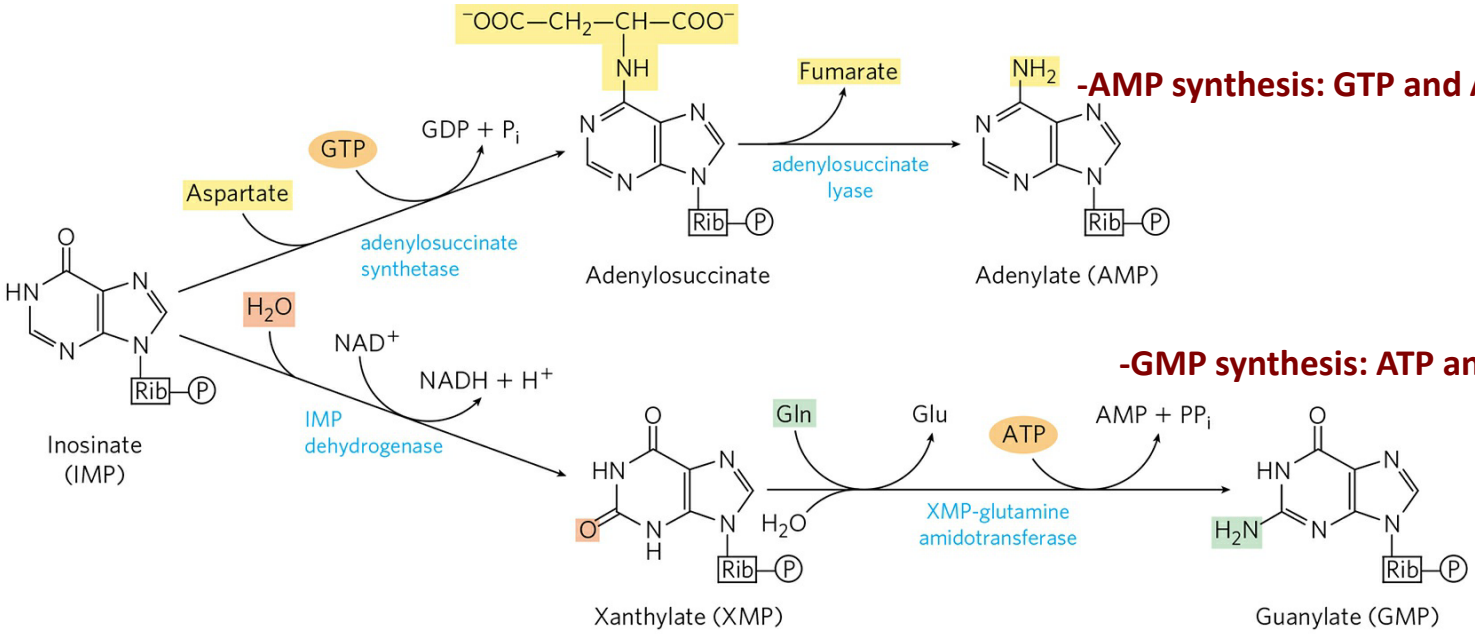
A huge number of enzymatic reactions are involved

Glycine precursor

PRPP is required

Glutamine and Aspartate are required

ATP is consumed



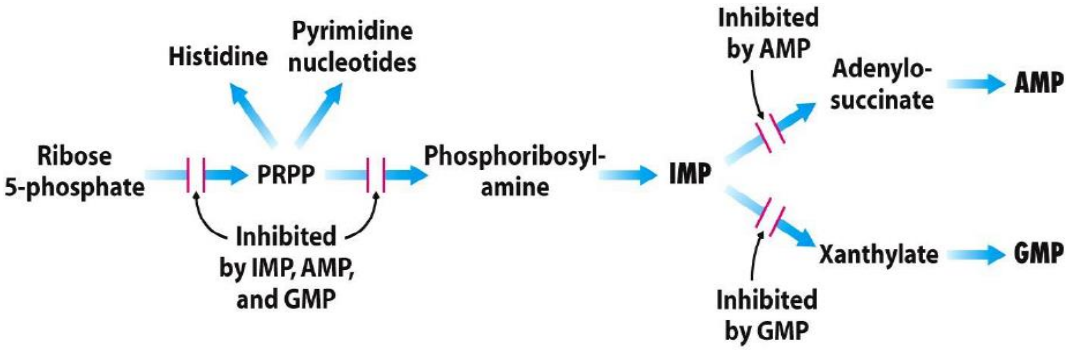
-AMP synthesis: GTP and Asp are required

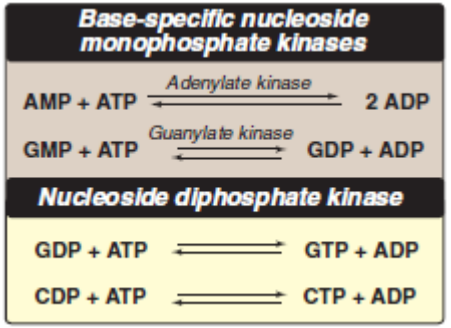
-GMP synthesis: ATP and Gln are required

Control mechanisms in purines supply

De novo synthesis of purine nucleotides: IMP, AMP, GMP

Purine nucleotide synthesis





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Purine nucleotide synthesis regulation:

-Negative Feedback Regulation by:

1. PRPP synthetase AMP, ADP and GMP

2. PRPP amide transferase AMP, ADP, GMP, GDP

3. AMP synthesis requires GTP, and GMP requires ATP: Cell mechanism to keep both nucleotides balanced

ATP accumulation→→→ Increase in GMP synthesis →→→ Decrease in AMP

GTP Accumulation →→→ Increase in AMP synthesis→→→ Decrease in GMP

***Purine synthesis inhibitors: antitumoural treatments**

De novo synthesis of pyrimidine nucleotides: UMP, TMP, CTP

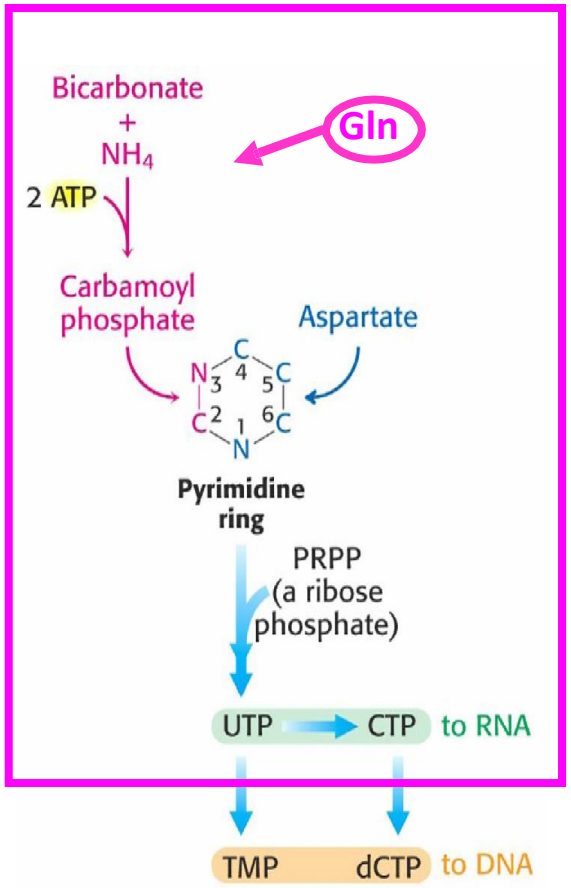
-Synthesis of the pyrimidine ring before binding to the PRPP that donates 5'ribose phosphate
- Pyrimidine ring: Ammonia from glutamine, carbon from CO_2 and aspartate
-Step is regulated by carbamoyl phosphate synthetase (CPS) II:
Carbamoyl phosphate synthesis from CO_2 and glutamine

Pyrimidine nucleotides are made from aspartate, PRPP, and carbamoyl phosphate

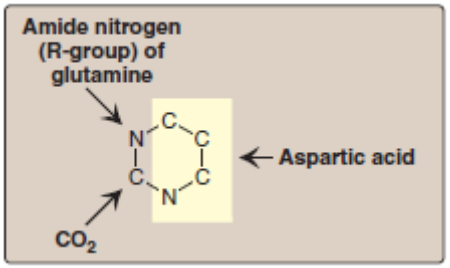
1: Synthesis of carbamoyl phosphate: Carbamoyl Pi + aspartate

2: Synthesis of orotic acid: Orotic acid + PRPP

3: Formation of a pyrimidine nucleotide: UTP synthesis



Pyrimidine ring



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De novo synthesis of pyrimidine nucleotides: UMP, TMP, CTP

Summary of the differences between carbamoyl phosphate synthetase (CPS) I and II

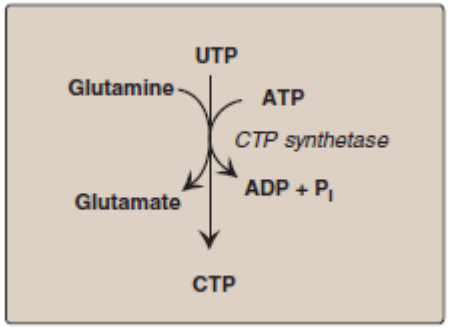
	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ-Amide group of glutamine
Regulators	Activator: N–acetyl-glutamate	Activator: PRPP Inhibitor: UTP

CPSII is inhibited by UTP and activated by PRPP

UTP (-) (+) **PRPP**

UMP → **UTP** → **CTP** → **TTP**

Synthesis of CTP from UTP



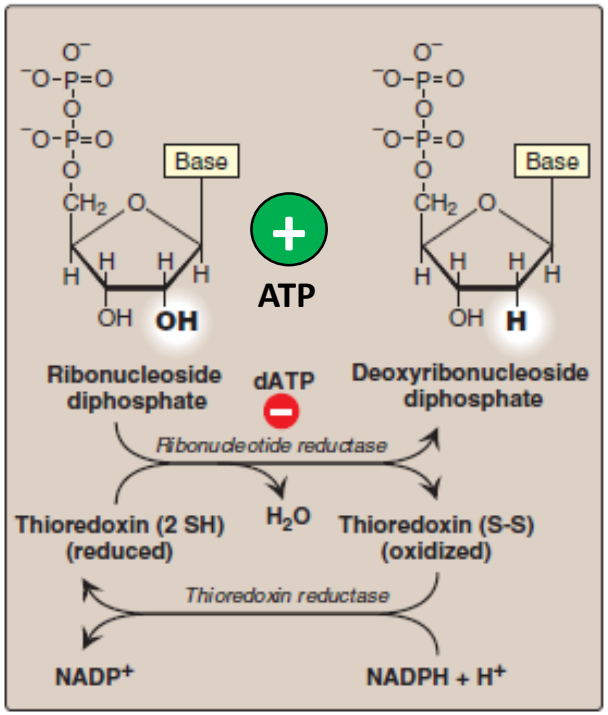
Pyrimidine nucleotide biosynthesis is regulated by feedback inhibition

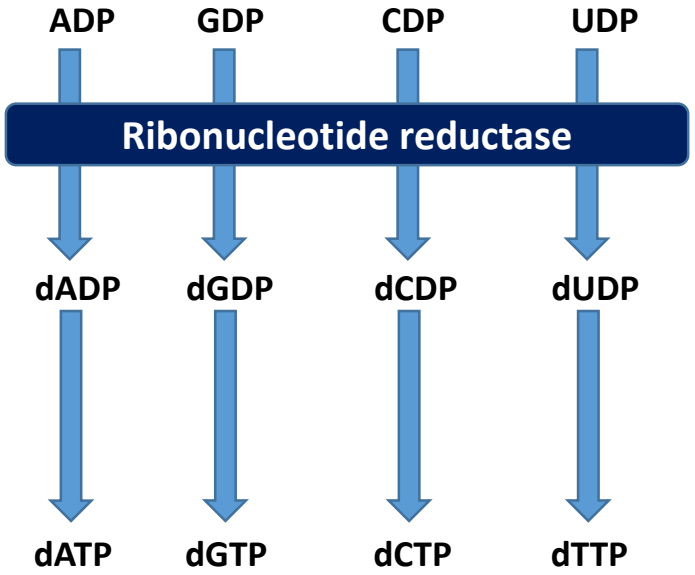
Deoxyribonucleotide synthesis

Reduction of NTPs to dNTPs:

Ribonucleotide reductase uses ATP as a donor of Pi groups as it is the most abundant energy donor.

Conversion of ribonucleotides to deoxyribonucleotides





2.2 SALVAGE PATHWAYS

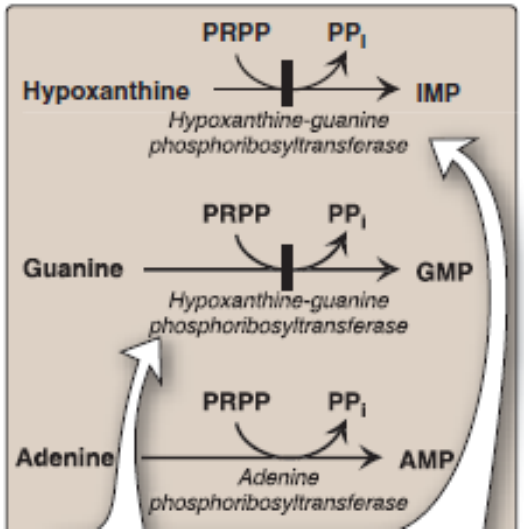
Purine and pyrimidine bases are recycled by salvage pathways

Free purine and pyrimidine bases are constantly released in cells during the metabolic degradation of nucleotides.

PURINES

Free purines are in large part salvaged and reused to make nucleotides, in a pathway much simpler than the *de novo* synthesis of purine nucleotides described earlier.

One of the primary salvage pathways consists of a single reaction catalyzed by hypoxanthine-guanine phosphoribosyl transferase (HPGRT) to release free guanine and hypoxanthine (the deamination product of adenine). The other enzyme participating in catalysis is adenine phosphoribosyl transferase (APRT), in which free adenine reacts with PRPP to yield the corresponding adenine nucleotide.



2 Enzymes: adenine phosphoribosyl transferase (APRT) and hypoxanthine-guanine phosphoribosyl transferase (HGPRT).

These reactions use PRPP as the source of ribose 5-phosfate and are irreversible.

If there is hypoxanthine, purine nucleotides are always synthesized by the salvage pathway.

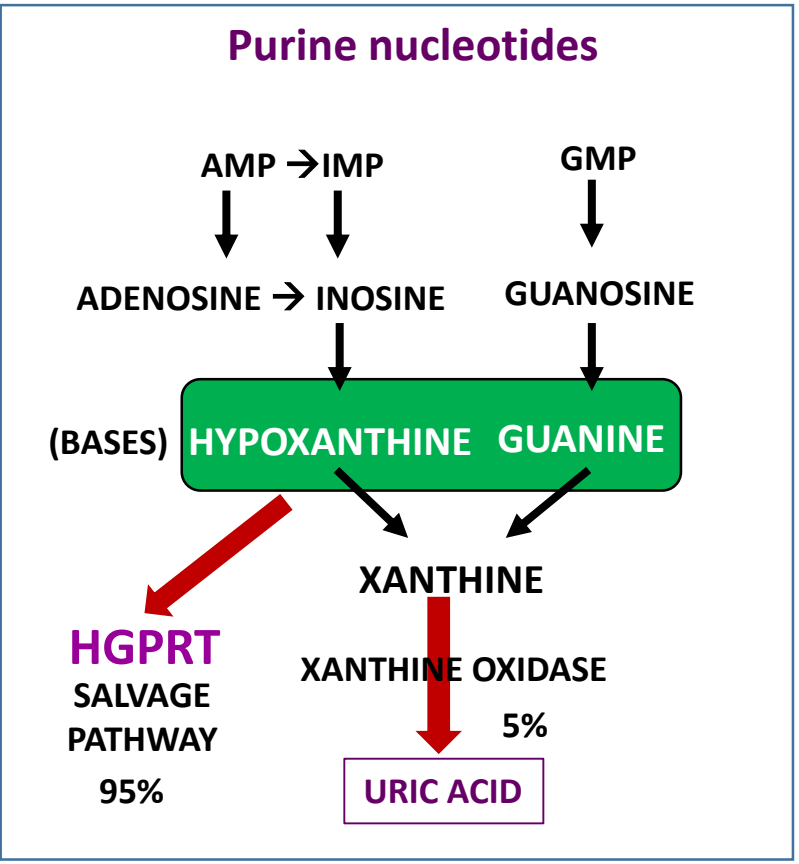
Hypoxanthine inhibits de novo synthesis.

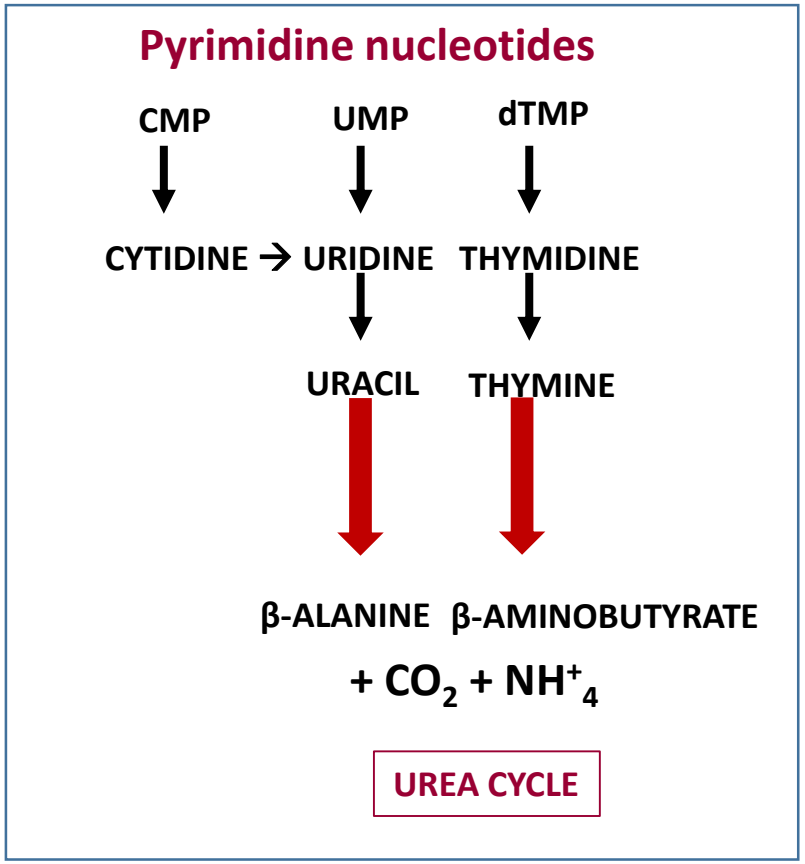
*HGPRT Deficiency: Lesch-Nyhan Syndrome

3. CATABOLISM OF NUCLEOTIDES

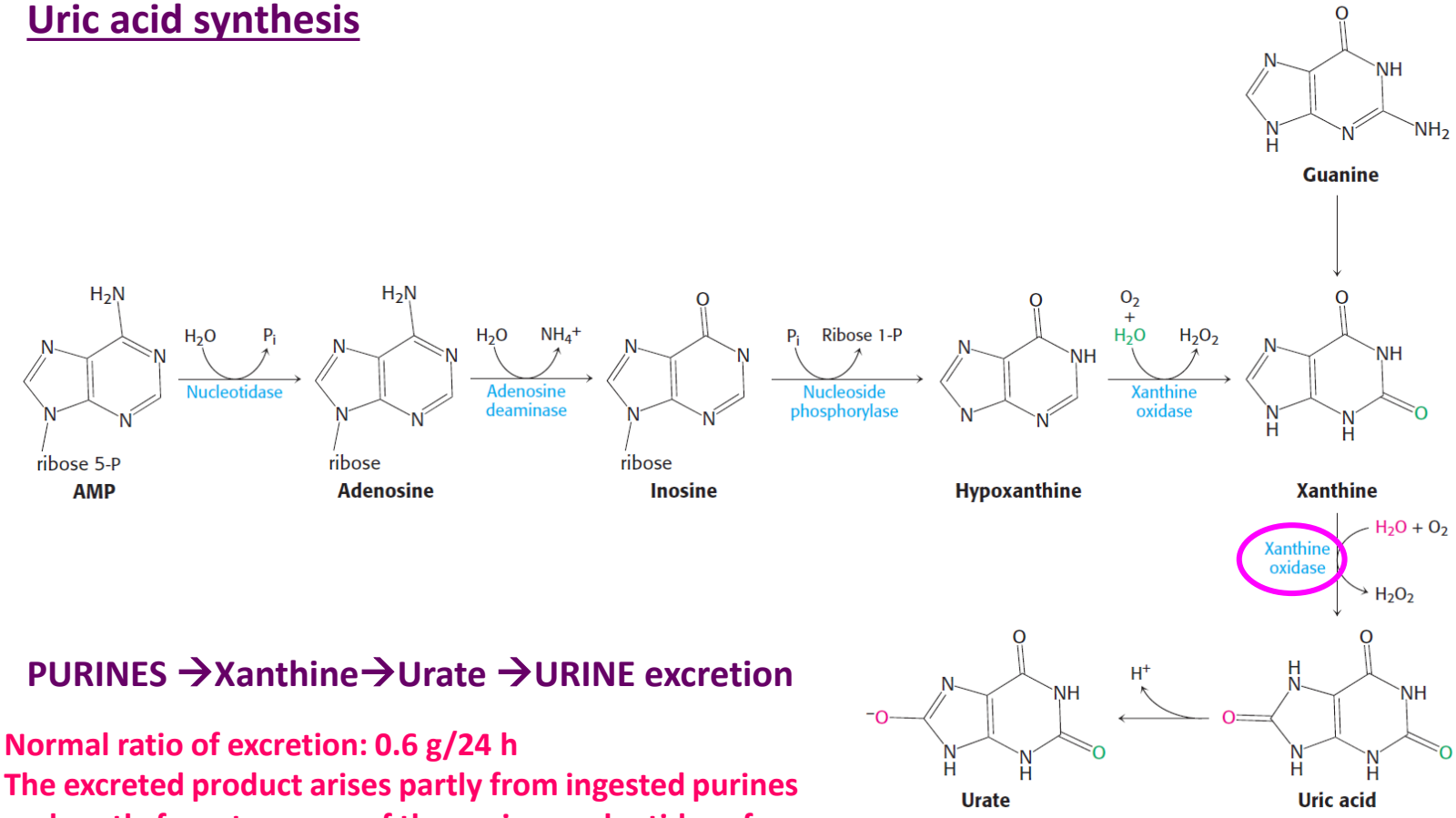
Purines: These are degraded to uric acid. Uric acid is the final product of purine degradation. It enters the bloodstream and is excreted in urine.

Pyrimidines: These are completely degraded to β-alanine and β-aminobutyric acid to enter the urea cycle.





Uric acid synthesis



PURINES →Xanthine→Urate →URINE excretion

Normal ratio of excretion: 0.6 g/24 h
The excreted product arises partly from ingested purines and partly from turnover of the purine nucleotides of nucleic acids.

PURINES

Alterations in the salvage pathways of purine nucleotide synthesis

Lesch-Nyhan syndrome

HGPRT deficiency: Lesch-Nyhan syndrome: a rare recessive X-linked disorder.

Consequences:

-Inability to rescue hypoxanthine or guanine.

-Increased degradation of purines→ Too much uric acid (hyperuricaemia)

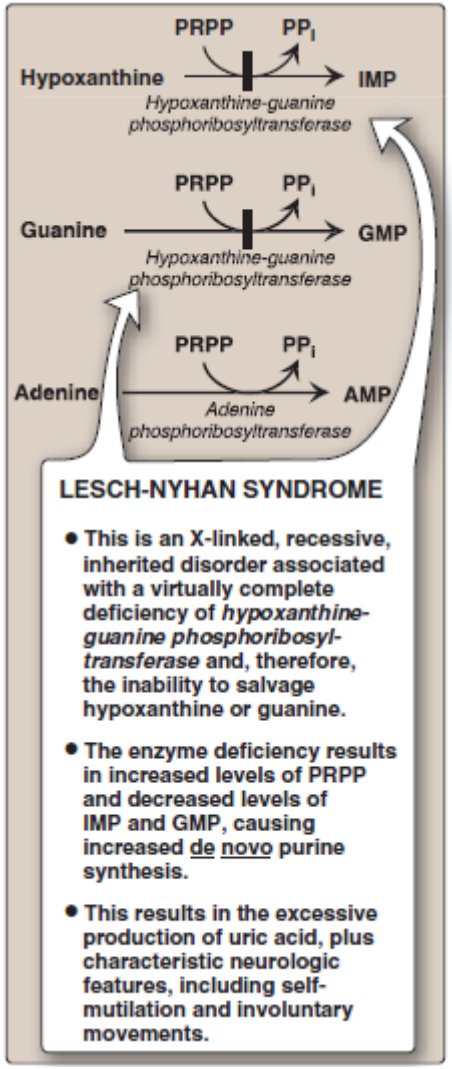
-Increase in PRPP levels and decrease in IMP and GMP levels.

-Increased de novo purine synthesis (no synthesis inhibitor).

-Formation of uric acid deposits in the kidneys, deposition of urate crystals in the joints (gouty arthritis).

-Motor dysfunction, cognitive deficits and behavioural disorders (self-mutilation).

Children with this genetic disorder, which becomes manifest by the age of two, are sometimes poorly coordinated and have intellectual deficits.
They are also extremely hostile and show compulsive self-destructive tendencies: they mutilate themselves by biting off their fingers, toes, and lips.
This syndrome is a potential target for gene therapy.



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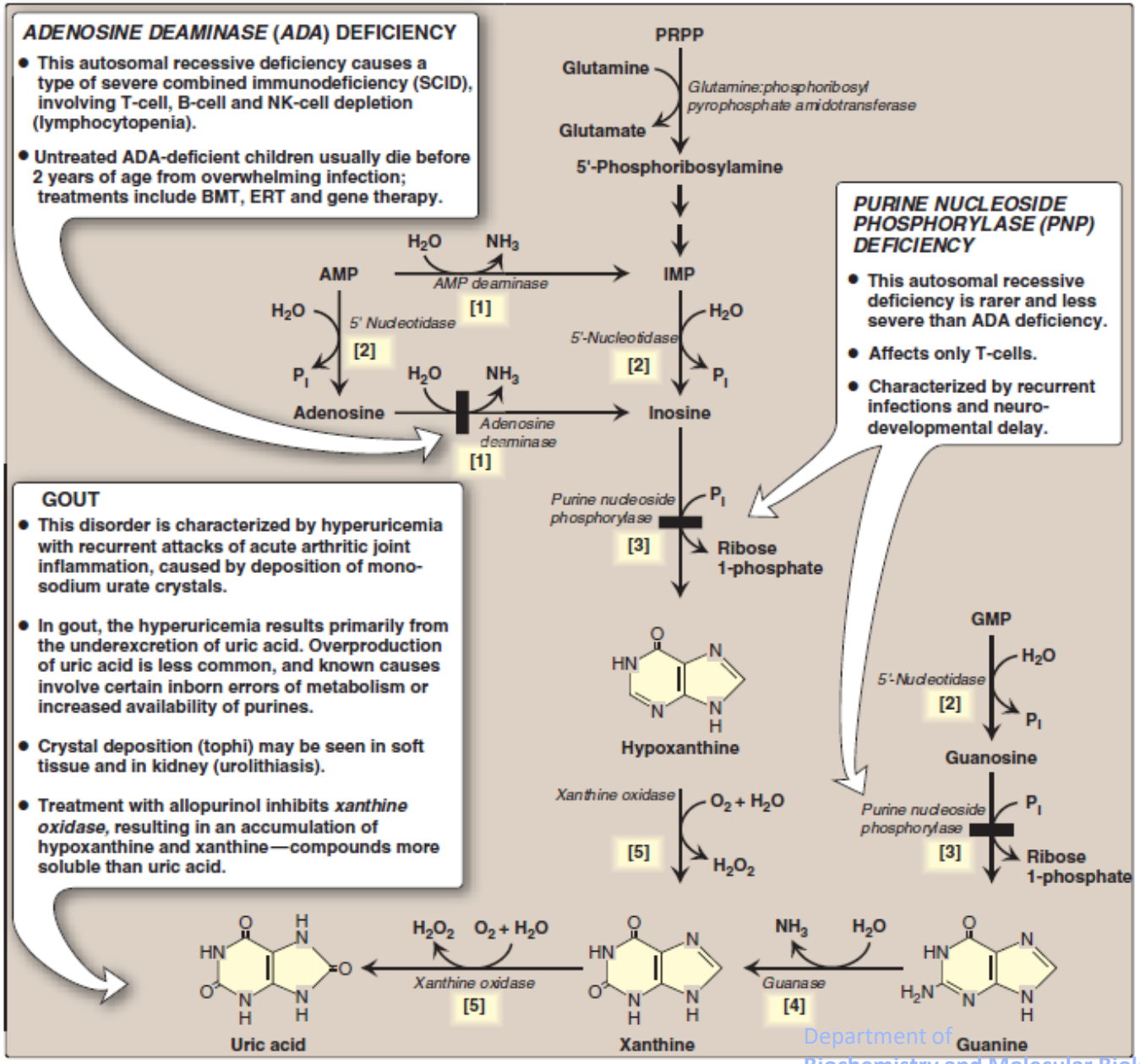
PURINES

Biosynthesis and degradation of purine nucleotides to uric acid illustrating some of the genetic diseases associated with these pathways.



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4. NUCLEOTIDE METABOLISM ALTERATIONS

PURINES

Alterations in the biosynthesis and degradation of purine nucleotides.

Immunodeficiciency syndrome (ADA)

Adenosine deaminase (ADA) deficiency leads to severe immunodeficiency disease in which T and B lymphocytes do not develop properly.

A lack of ADA leads to a 100-fold increase in the cellular concentration of dATP, a strong inhibitor of ribonucleotide reductase.
High levels of dATP produce a general deficiency of other dNTPs in lymphocytes.
Individuals with ADA deficiency lack an effective immune system and do not survive unless treated.

Current therapies include bone marrow transplant from a matched donor to replace the hematopoietic stem cells that mature into B and T lymphocytes. However, transplant recipients often suffer a variety of cognitive and physiological problems.

Enzyme replacement therapy, requiring once- or twice-weekly intramuscular injection of active ADA, is effective but the therapeutic benefit often declines after 8 to 10 years and complications arise, including malignancies.
For many people, a permanent cure requires replacing the defective gene with a functional one in bone marrow cells.
ADA deficiency was one of the first targets of human gene therapy trials first assayed in 1990.

4. NUCLEOTIDE METABOLISM ALTERATIONS

PURINES

Alterations in the degradation of purine nucleotides to uric acid.

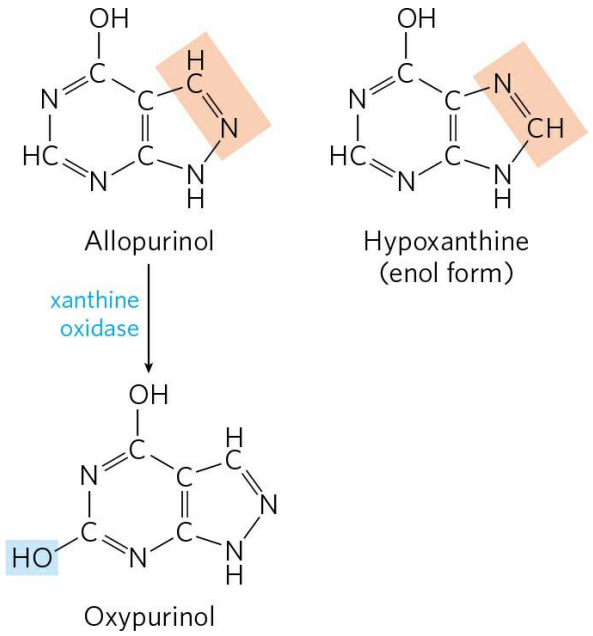
Excess uric acid causes gout

Gout is a disease of the joints caused by an elevated concentration of uric acid in the blood and tissues.
The joints become inflamed, painful and arthritic, owing to the abnormal deposition of sodium urate crystals.

The kidneys are also affected, as excess uric acid is deposited in the kidney tubules. Gout occurs predominantly in males. Its precise cause is not known but it often involves an underexcretion of urate.
A genetic deficiency of one or another enzyme of purine metabolism may also be a factor in some cases.

Gout is effectively treated by a combination of nutritional and drug therapies. Patients exclude foods especially rich in nucleotides and nucleic acids from the diet.

Major alleviation of the symptoms is provided by the drug allopurinol, which inhibits xanthine oxidase, the enzyme that catalyzes the conversion of purines to uric acid. When xanthine oxidase is inhibited, the excreted products of purine metabolism are xanthine and hypoxanthine, which are more water-soluble than uric acid and less likely to form crystalline deposits.



Allopurinol, an inhibitor of xanthine oxidase.
Hypoxanthine is the normal substrate of xanthine oxidase.
Allopurinol is slightly different to hypoxanthine and acts as an enzyme inhibitor.
At the active site, allopurinol is converted to oxypurinol, a strong competitive inhibitor that remains tightly bound to the reduced form of the enzyme.

PURINES

Other related pathologies

Roughly 10% of the human population (and up to 50% of people in impoverished communities) suffer from folic acid deficiency.
When the deficiency is severe, the symptoms can include heart disease, cancer, and some types of brain dysfunction.

At least some of these symptoms arise from a reduction of thymidylate synthesis, leading to an abnormal incorporation of uracil into DNA. Uracil is recognized by DNA repair pathways and is cleaved from the DNA. The presence of high levels of uracil in DNA leads to strand breaks that can greatly affect the function and regulation of nuclear DNA, ultimately causing the observed effects on the heart and brain, as well as increased mutagenesis that leads to cancer.

Many chemotherapeutic agents target enzymes in nucleotide biosynthetic pathways

The growth of cancer cells is not controlled in the same way as cell growth in most normal tissues. Cancer cells have greater requirements for nucleotides as precursors of DNA and RNA, and consequently are generally more sensitive than normal cells to inhibitors of nucleotide biosynthesis.

A growing array of important chemotherapeutic agents – for cancer and other diseases – act by inhibiting one or more enzymes in these pathways.

PURINE SYNTHESIS INHIBITORS
- are used to treat several types of cancer
- are extremely toxic for tissues with a high rate of replication, including bone marrow, the skin, the gastrointestinal tract, the immune system and follicular hair.
Folic acid analogues (i.e. methotrexate):
individuals taking such anticancer drugs may suffer from:
anaemia, scaly skin, gastrointestinal tract disorders, immunodeficiency, and hair loss.

BIOSYNTHESIS AND DEGRADATION OF NUCLEOTIDES. SUMMARY.

■ The purine ring system is built up step by step, beginning with 5-phosphoribosylamine. The amino acids glutamine, glycine and aspartate furnish all the nitrogen atoms of purines. Two ring-closure steps form the purine nucleus.

■ Pyrimidines are synthesized from carbamoyl phosphate and aspartate, and ribose 5-phosphate is then attached to yield the pyrimidine ribonucleotides.

■ Nucleoside monophosphates are converted into their triphosphates by enzymatic phosphorylation reactions. Ribonucleotides are converted into deoxyribonucleotides by ribonucleotide reductase, an enzyme with novel mechanistic and regulatory characteristics. The thymine nucleotides are derived from dCDP and dUMP.

■ Uric acid and urea are the end products of purine and pyrimidine degradation.

■ Free purines can be salvaged and rebuilt into nucleotides. Genetic deficiencies in certain salvage enzymes cause serious disorders, such as Lesch-Nyhan syndrome and ADA deficiency.

■ The accumulation of uric acid crystals in the joints, possibly caused by another genetic deficiency, results in gout.

■ Enzymes of the nucleotide biosynthetic pathways are targets for an array of chemotherapeutic agents used to treat cancer and other diseases.