Biochemistry
Purine and Pyrimidine Metabolism

University Of Fallujah
College Of Medicine

Lecture : 1

Stage : Second 2nd

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Department: Chemistry and Biochemistry

Date:

- **Learning Objectives**
- Study the constituents, chemistry & types of Nucleic acids .
- Learn the biologically important nucleotides and their functions.
- Study the structure of two major purines and three major pyrimidines .
- Correlate clinically with hyperuricemia & gout.

Learning outcomes

Knowledge

- Core definitions
- Pathway steps

Scientific basis

- Regulation
- Energy use

Clinical link

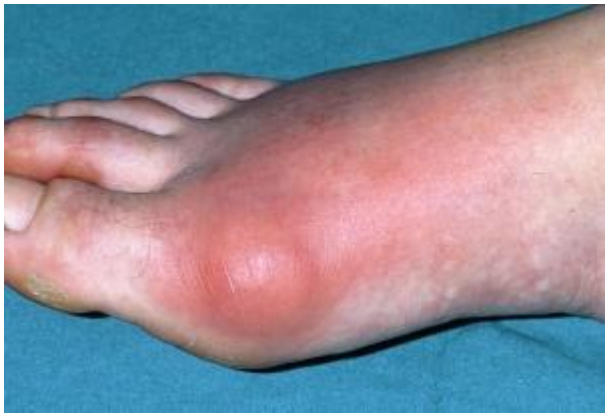
- HGPRT
- Hyperuricemia
- Gout

Professionalism

- Self-review
- Basic-clinical integration

Case Presentation

- A 45-year-old man presents with:
- Severe pain in the **big toe (1st MTP joint)** ((First Metatarsophalangeal Joint)) ,Sudden onset at night Redness, swelling, and tenderness



- History reveals:

✓ High intake of red meat and seafood

✓ Frequent alcohol consumption

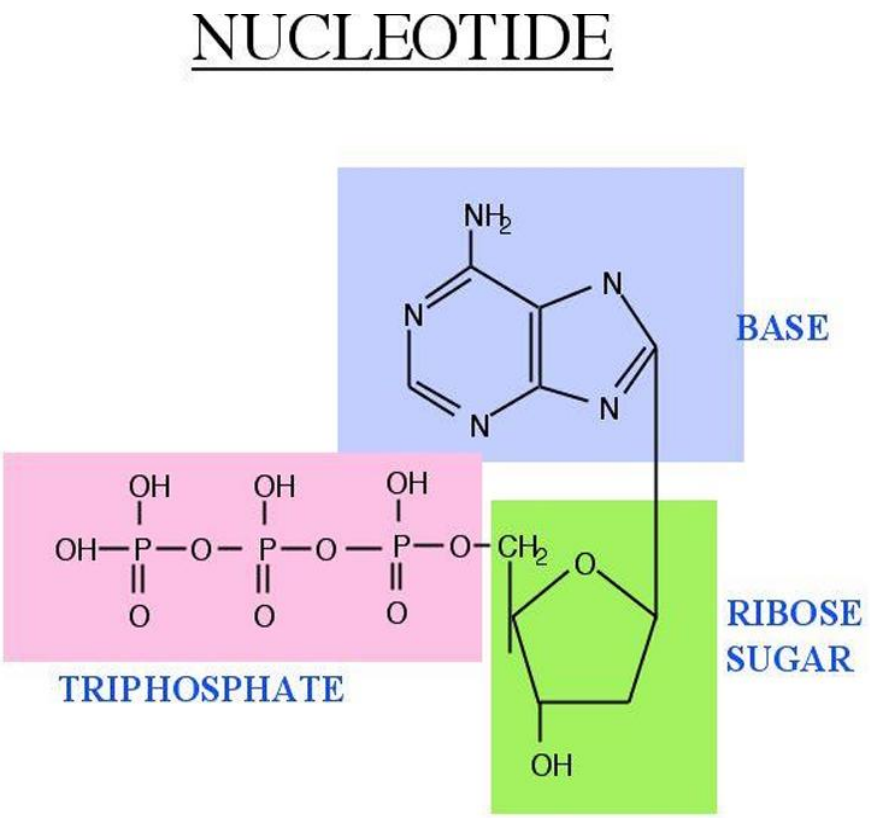
- ☐ **Laboratory Findings**
- Serum uric acid: **9.5 mg/dL (↑)**
- Urine pH: **Acidic**
- Renal function: Normal

Questions for Students

- What is the most likely diagnosis?
- What is the biochemical cause of this condition?
- Which pathway is mainly involved?
- Name the key enzyme in uric acid production
- Why does alcohol increase uric acid?

❑Nucleic acid Metabolism

- Nucleotides are organic molecules that serve as the monomers of nucleic acids like DNA and RNA. The building blocks of nucleotides are composed of a nitrogenous base, a five-carbon sugar (ribose or deoxyribose and phosphate group).



- **Nucleotides** are essential for the synthesis of DNA and RNA, playing a critical role in **DNA replication and transcription.**

- They are also involved in **cellular energy transfer**, where nucleoside triphosphates such as **ATP and GTP** provide the energy required for biochemical reactions that would otherwise be energetically unfavorable.

- The precursor for nucleotide synthesis, **ribose-5-phosphate,** is generated through the **hexose monophosphate (HMP) shunt.**

- Ribose-5-phosphate is subsequently activated by the addition of pyrophosphate from ATP, forming **phosphoribosyl pyrophosphate (PRPP)** in a reaction catalyzed by the enzyme **PRPP synthetase.**

❑ Cells synthesize nucleotides in 2 ways:

1. de novo synthesis
2. salvage pathways.

❑ De Novo Synthesis

- Nucleotides are synthesized from small precursor molecules (amino acids, CO_2, formate)
- Occurs mainly in the liver
- Requires high energy (ATP consumption)
- PRPP acts as an essential activated intermediate
- Relatively slow and complex process

❑ Salvage Pathway

✓ Recycles preformed purine and pyrimidine bases

✓ Converts bases → nucleotides using specific enzymes

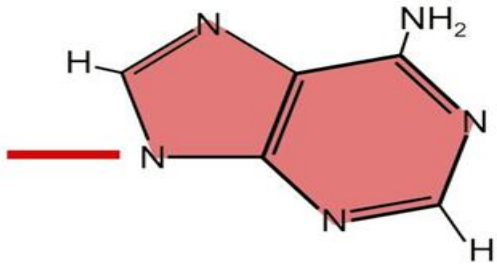
✓ Energy-efficient (requires less ATP)

✓ Occurs in most tissues

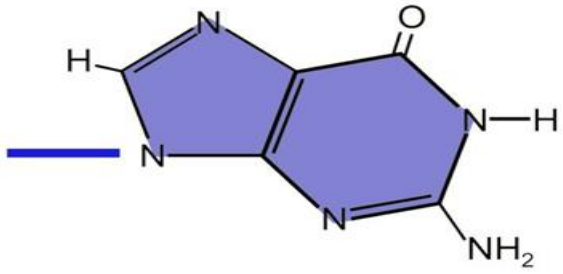
✓ Faster and more economical

- There are two kinds of nitrogen-containing bases - purines and pyrimidines.
- **Purines** consist of a six-membered and a five-membered nitrogen-containing ring, fused together.
- **Pyridmidines** have only a six membered nitrogen-containing ring. There are 2 purines and 3 pyrimidines nitrogen-containing bases.

Adenine

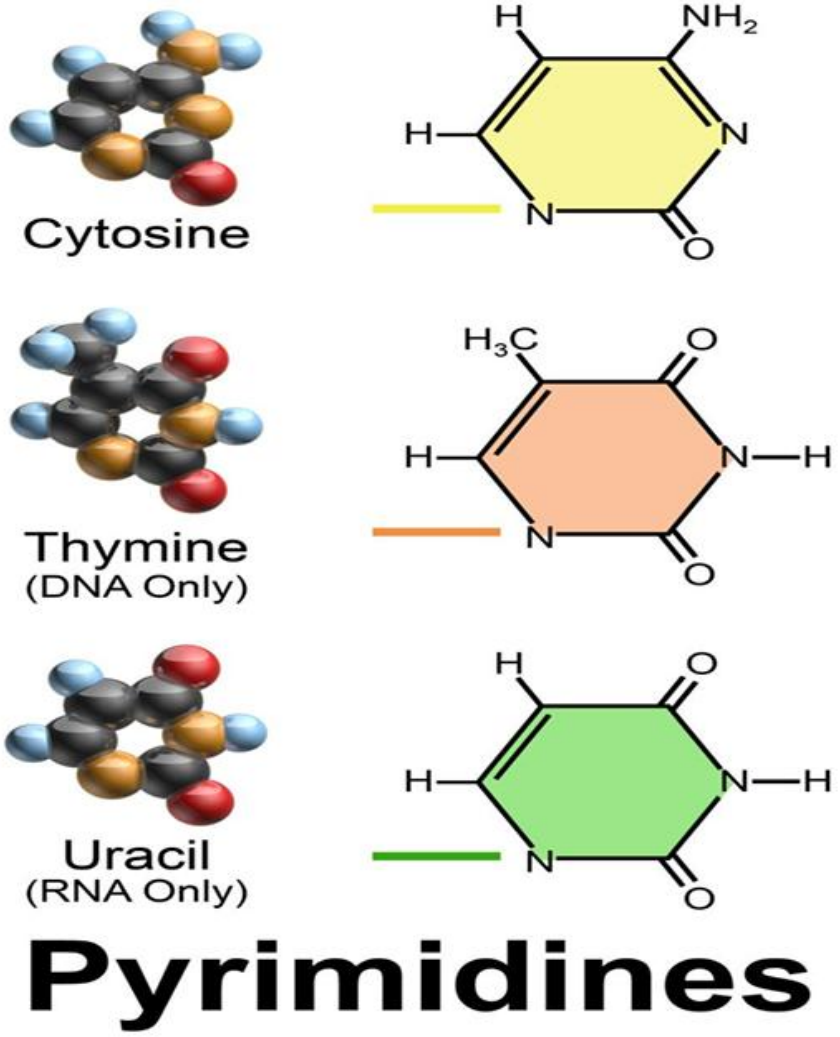


Guanine



Purines

- Three nucleobases found in nucleic acids - cytosine (C), thymine (T), and uracil(U), are pyrimidine derivatives:



- In DNA, the purines adenine (A) and guanine (G) pair up with the pyrimidines thymine (T) and cytosine (C), respectively.

❑ Biomedical importance:

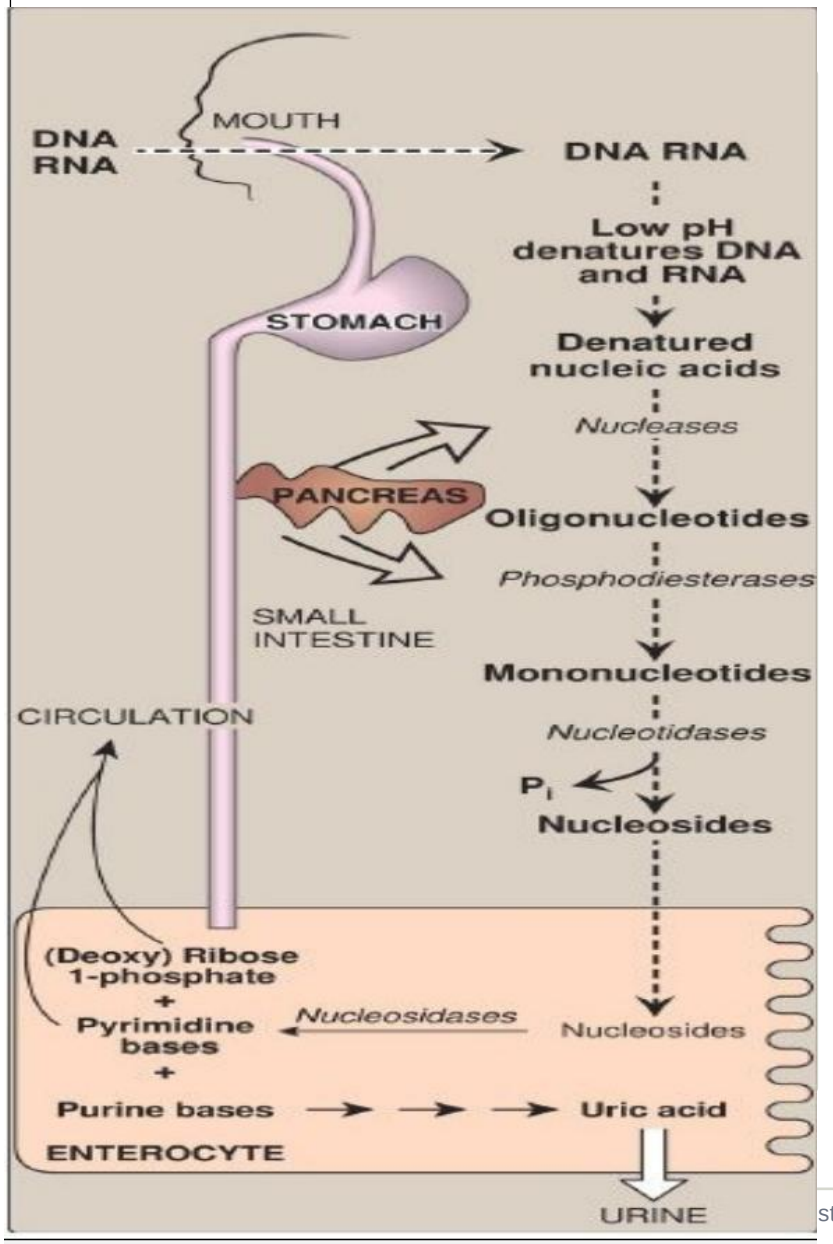
1. Purines and pyrimidines are heterocyclic bases that form the fundamental components of nucleotides, present in all cells.
2. DNA, located in the nucleus, forms chromosomes and is responsible for the transmission of genetic information and regulation of protein synthesis.
3. RNA, found in both the nucleus and cytoplasm, plays a central role in protein synthesis.
4. Most viruses are composed primarily of nucleoproteins (nucleic acids + proteins).
5. Synthetic nucleotide analogs are used in the treatment of cancer and viral infections by:

- Acting as enzyme inhibitors, or Incorporating into nucleic acids, thereby inhibiting replication of cancer cells and viruses

❑ Digestion of nucleoproteins:

- ✓ The protein is successively removed from the nucleoproteins by pepsin and trypsin.
- ✓ The nucleic acids are split by a series of enzymes of the pancreatic juice (**the nucleases: DNA-ase and RNA-ase**).
- ✓ Nucleases (DNA-ase і RNA ase) decompose nucleic acids into **oligonucletides.**
- ✓ **Phosphodiesterases** decompose oligonucleotides to mononucleotides
- ✓ **Phosphatases** – enzymes that split off phosphoric acid and formed – nucleosides
- ✓ **Nucleosidases** – enzymes, that catalyse decomposition nucleosides to nitrogenous bases and pentoses

Digestion, absorption, and salvage overview



☑ **Dietary nucleoproteins are progressively broken down in the gastrointestinal tract.**

- **Pepsin and trypsin remove the protein component from nucleoproteins.**

- **Pancreatic nucleases form oligonucleotides; phosphodiesterases and phosphatases generate nucleosides and bases.**

- **Nucleotides and nucleosides are absorbed more readily than free bases.**

- **Salvage pathways recycle preformed bases and conserve cellular energy.**

Clinical relevance: increased turnover or impaired salvage increases the load on purine degradation.

DESTINY OF NITROGENOUS BASES, PENTOSES AND PHOSPHORIC ACIDS IN THE ORGANISM

Nitrogenous bases → *oxidation to the end products*

Pentoses → *oxidation with energy formation;*
synthesis of nucleotided;
synthesis of hexoses;
synthesis of coenzymes

Phosphoric acid → *phosphorilation;*
ATP synthesis;
synthesis of phospholipids;
buffer systems;
constituent of bones, cartilages

☐Absorption:

1. Nucleotides and Nucleosides

- Efficiently absorbed from the small intestine
- After absorption, they undergo:
- Salvage pathway → incorporation into tissue nucleic acids
- Hepatic catabolism in the liver

2. Purine and Pyrimidine Bases

- Poorly absorbed from the small intestine
- Primarily undergo catabolism in the liver
- Limited reutilization via salvage pathways:
- Adenine may be partially incorporated into nucleic acids

☐ Take-Home Message:

✓ Nucleosides > Bases in absorption efficiency

✓ Liver plays a central role in nucleotide metabolism

❑The Biosynthetic origins of purine ring atoms

- ✓ N1 - arises from the amino group of Aspartate
- ✓ C2 and C8 - originate from folate (vitamin B10)
- ✓ N3 and N9 - are contributed by the amide group of Glutamine
- ✓ C4, C5 and N7 - are derived from Glycine
- ✓ C6 - comes from HCO_3^- (CO_2)

❑PURINE SYNTHESIS :

- Purines are synthesized de novo beginning with PRPP **phosphoribosyl pyrophosphate**.
- The most important enzyme is PRPP amidotransferase, which catalyzes the first and rate limiting reaction of the pathway.
- It is inhibited by the 3 purine nucleotide end products AMP, GMP, and IMP.

- **"Salvage Reactions" Convert Purines & Their Nucleosides To Mononucleotides**
- Conversion of purines, their ribonucleo**sides**, and their deoxyribonucleo**sides** to mononucleo**tides** involves so called "salvage reactions" that require far less energy than de novo synthesis. The more important mechanism involves:
- 1- phosphoribosylation by PRPP(5-phosphoribosyl -1-pyrophosphate) of a free purine (Pu) to form a purine 5′-mononucleotide.
- **2- Synthesis of 5-phosphoribosylamine:** Synthesis of 5'-phosphoribosylamine from PRPP and glutamine**.**

- **Synthesis of inosine monophosphate (IMP),** the "parent" **purine nucleotide** :
- The next nine steps in purine nucleotide biosynthesis leading to the synthesis of IMP, (whose base is hypoxanthine) are illustrated in source.

❑Purine de novo synthesis :

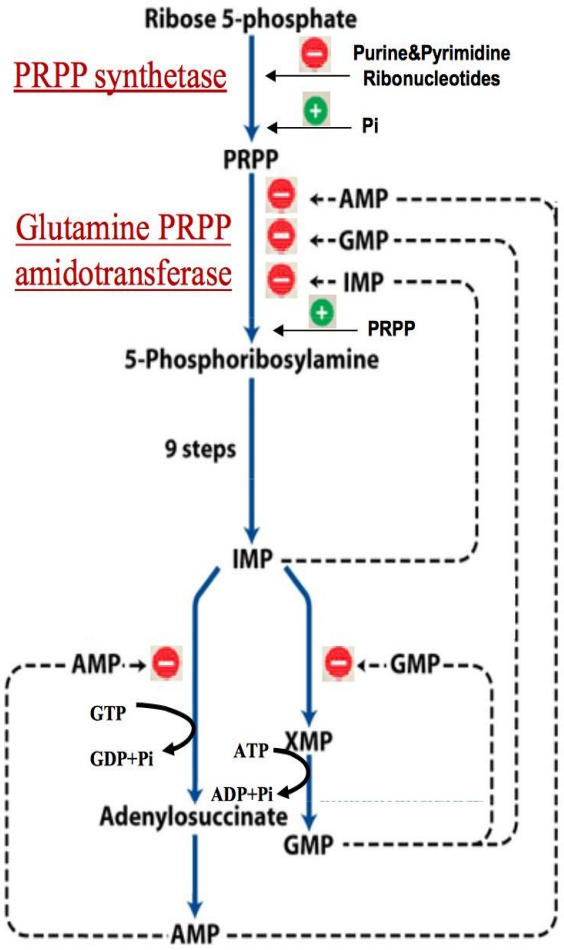
De novo synthesis of purine nucleotides

■ 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



1. Formation of PRPP
Ribose-5-phosphate ⟶ PRPP
Enzyme: PRPP synthetase
☐Rate-limiting step.

2. Committed Step
PRPP ⟶ 5-phosphoribosylamine
Enzyme: Glutamine:PRPP amidotransferase
☐First committed step in purine synthesis.

3. Formation of IMP
Multi-step process (~9 steps)
Product: IMP (Inosine Monophosphate)
☐Central intermediate.

4. Branch Point
IMP ⟶ AMP
Requires: GTP
Inhibited by: AMP
IMP ⟶ GMP

IMP is the branch point that later generates AMP and GMP.

Conversion of IMP to AMP and GMP

Pathway 1: IMP → AMP

IMP + Aspartate + GTP

- ✓ Enzyme: **Adenylosuccinate synthetase**
- ✓ Produces: **Adenylosuccinate**

GTP → GDP + Pi

2. Adenylosuccinate → AMP

Enzyme: **Adenylosuccinase**

Releases: **Fumarate**

Pathway 2: IMP → GMP

IMP → XMP (Xanthosine Monophosphate)

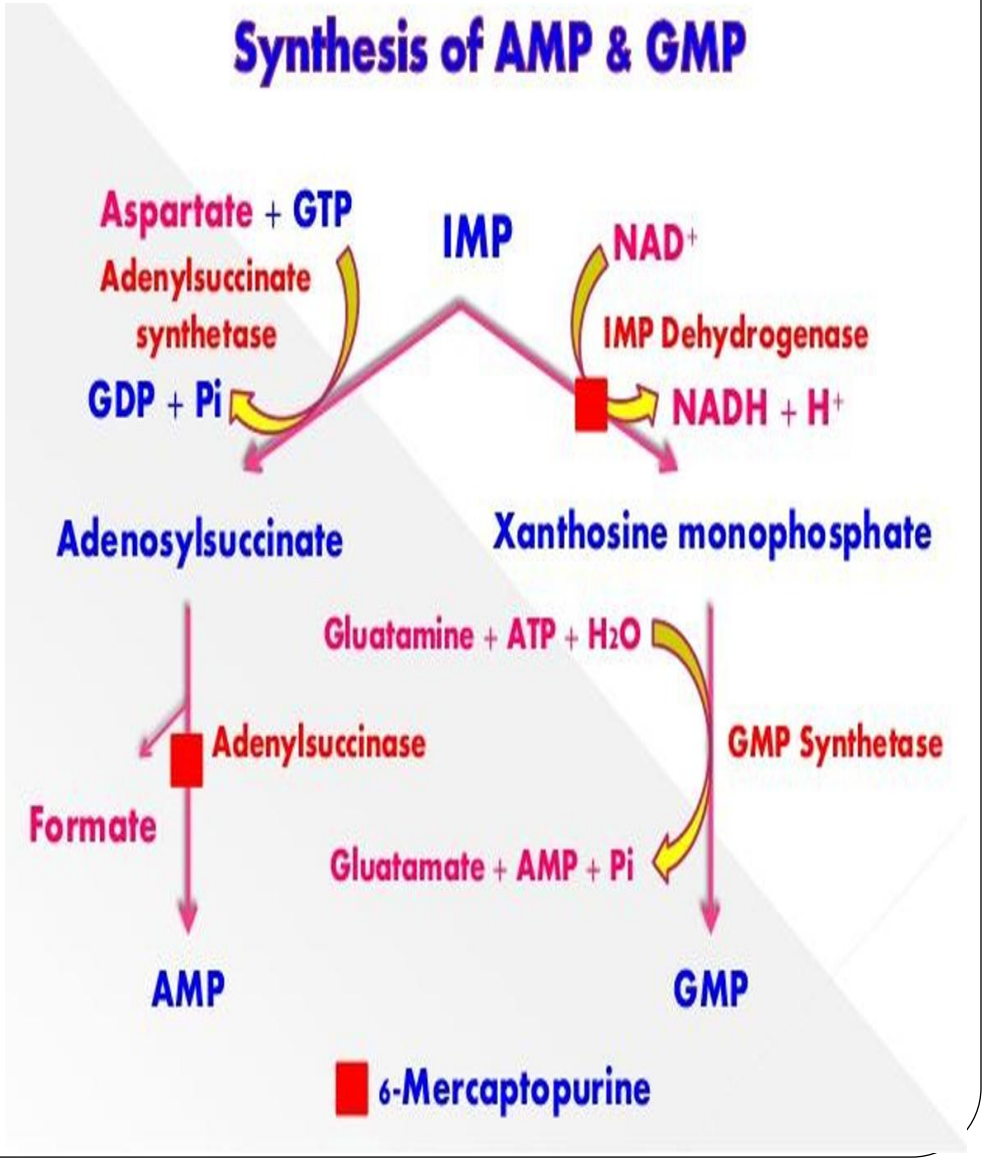
- ✓ Enzyme: **IMP dehydrogenase**
- ✓ Requires: **$NAD^+ \rightarrow NADH + H^+$**

XMP → GMP

- ✓ Enzyme: **GMP synthetase**
- ✓ Requires: **ATP Glutamine**

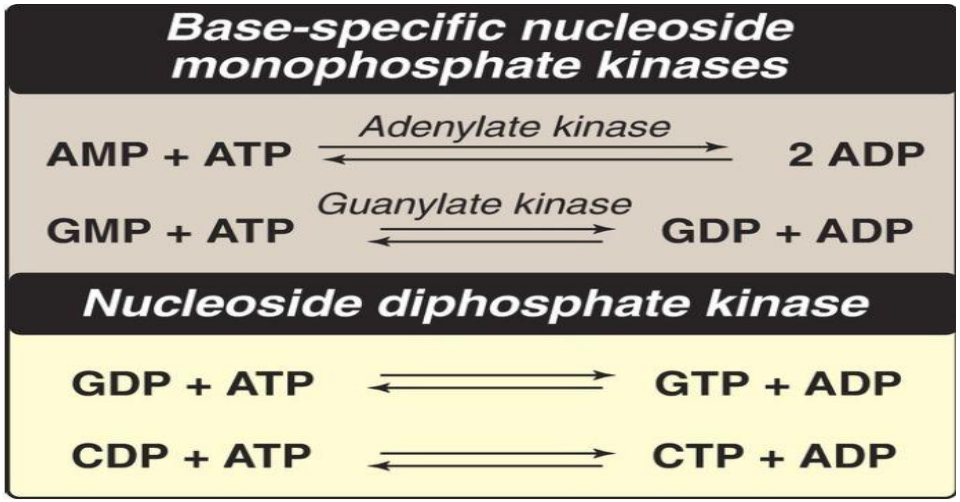
Produces:

GMP + Glutamate



Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates:

- Nucleoside diphosphates (NDP) are synthesized from the corresponding nucleoside monophosphates (NMP) by **base-specific nucleoside monophosphate kinases** (Figure).
- b



- **(Note):** These kinases do not discriminate between ribose or deoxyribose in the substrate.]
- ATP is generally the source of the transferred phosphate, because it is present in higher concentrations than the other nucleoside triphosphates.

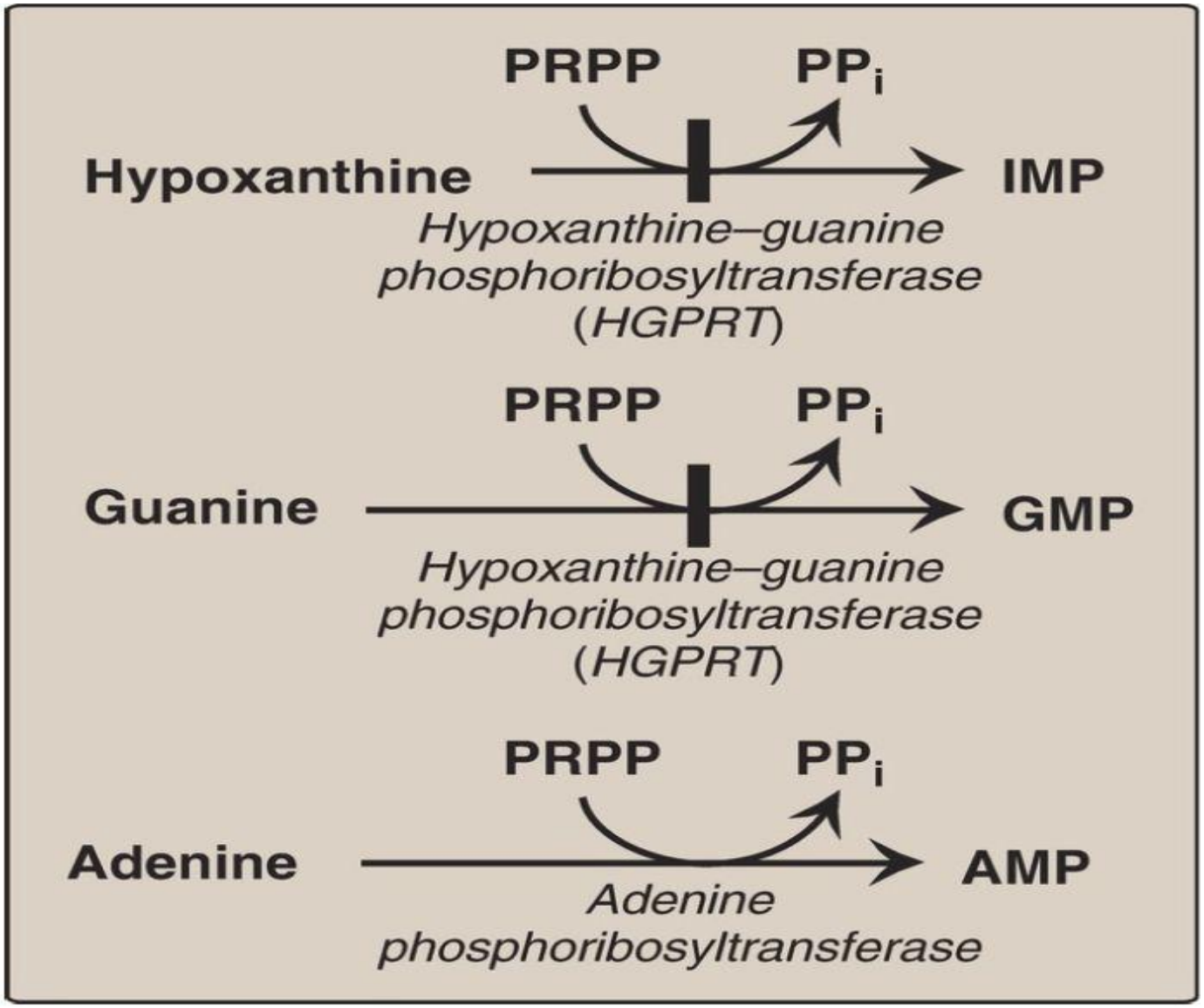
❑Salvage pathway for purines

- Purines that result from the normal turnover of cellular nucleic acids, or that are obtained from the diet and not degraded, can be reconverted into nucleoside triphosphates and used by the body.

✓ **This is referred to as the "salvage pathway" for purines.**

- **1. Conversion of purine bases to nucleotides:** Two enzymes are involved:
  - **Adenine phosphoribosyltransferase**
  - **Hypoxanthine-guanine phosphoribosyltransferase .**

- Both enzymes use **PRPP** as the source of the ribose 5-phosphate group.
- The release of pyrophosphate makes these **Reactions irreversible**



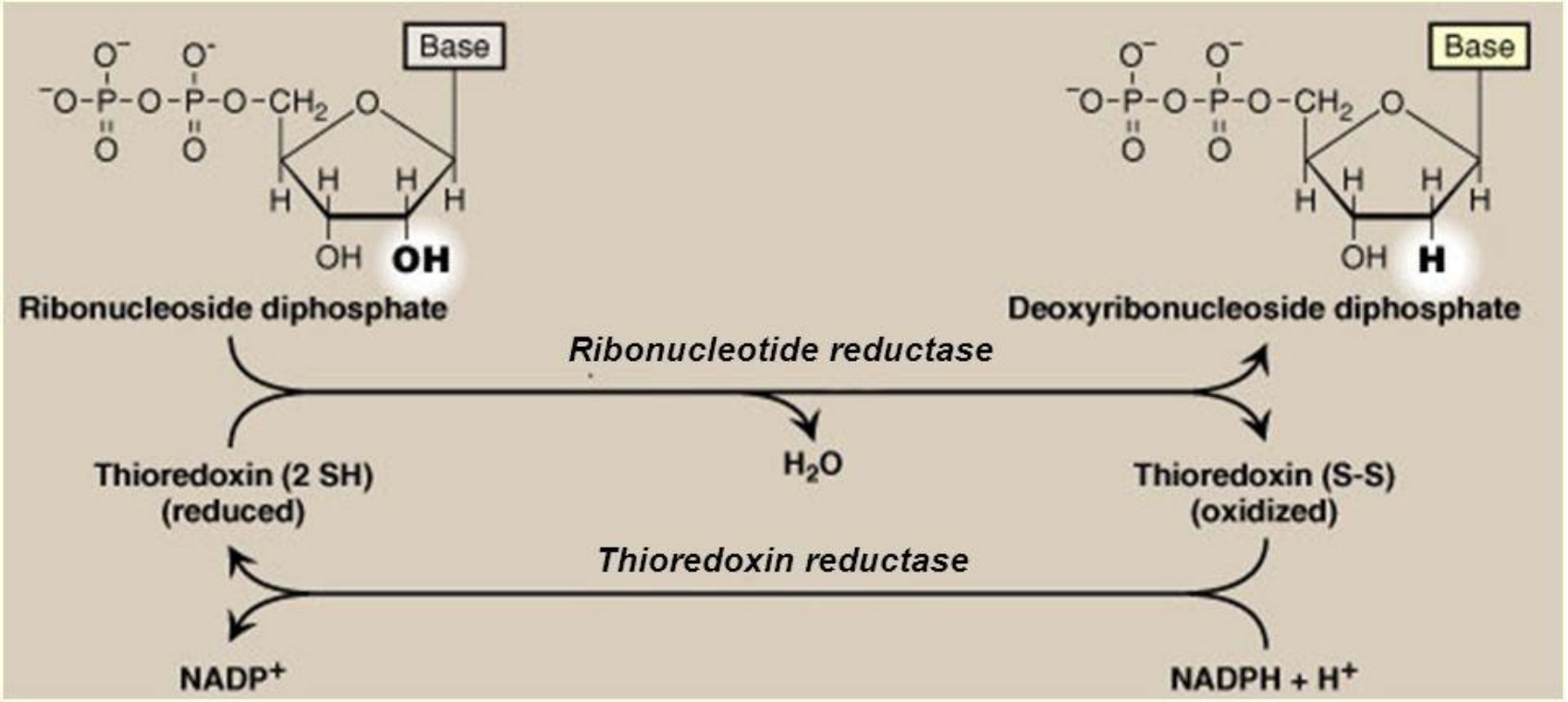
Purine Salvage Pathway

- **Synthesis of deoxy ribonucleotides :**
- The nucleotides required for DNA synthesis, however, are 2` Deoxyribonucleotides which are produced from ribonucleoside diphosphates by the enzyme ***ribonucleotide reductase***.

❑ A. Ribonucleotide reductase

- ***Ribonucleotide reductase {ribonucleoside diphosphate reductase)*** that is specific for the reduction of nucleoside diphosphates (**ADP, GDP, CDP, UDP**) and to their deoxy-forms (**dADP,dGDP, dCDP, and dUDP**).
- The immediate donors of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond.

Ribonucleotides to Deoxyribonucleotides



Inhibited by **dATP**; Activated by **ATP**

❑Degradation Of Purine Nucleotides

- Degradation of dietary nucleic acids occurs in the small intestine, where a family of pancreatic enzymes hydrolyzes the nucleotides to nucleosides and free bases.
- Inside cells, purine nucleotides are sequentially degraded by specific enzymes, with **uric acid** as the end product of this pathway.

- [Note: Mammals other than primates oxidize uric acid further to allantoin, which, in some animals other than mammals, may be further degraded to urea or ammonia.]

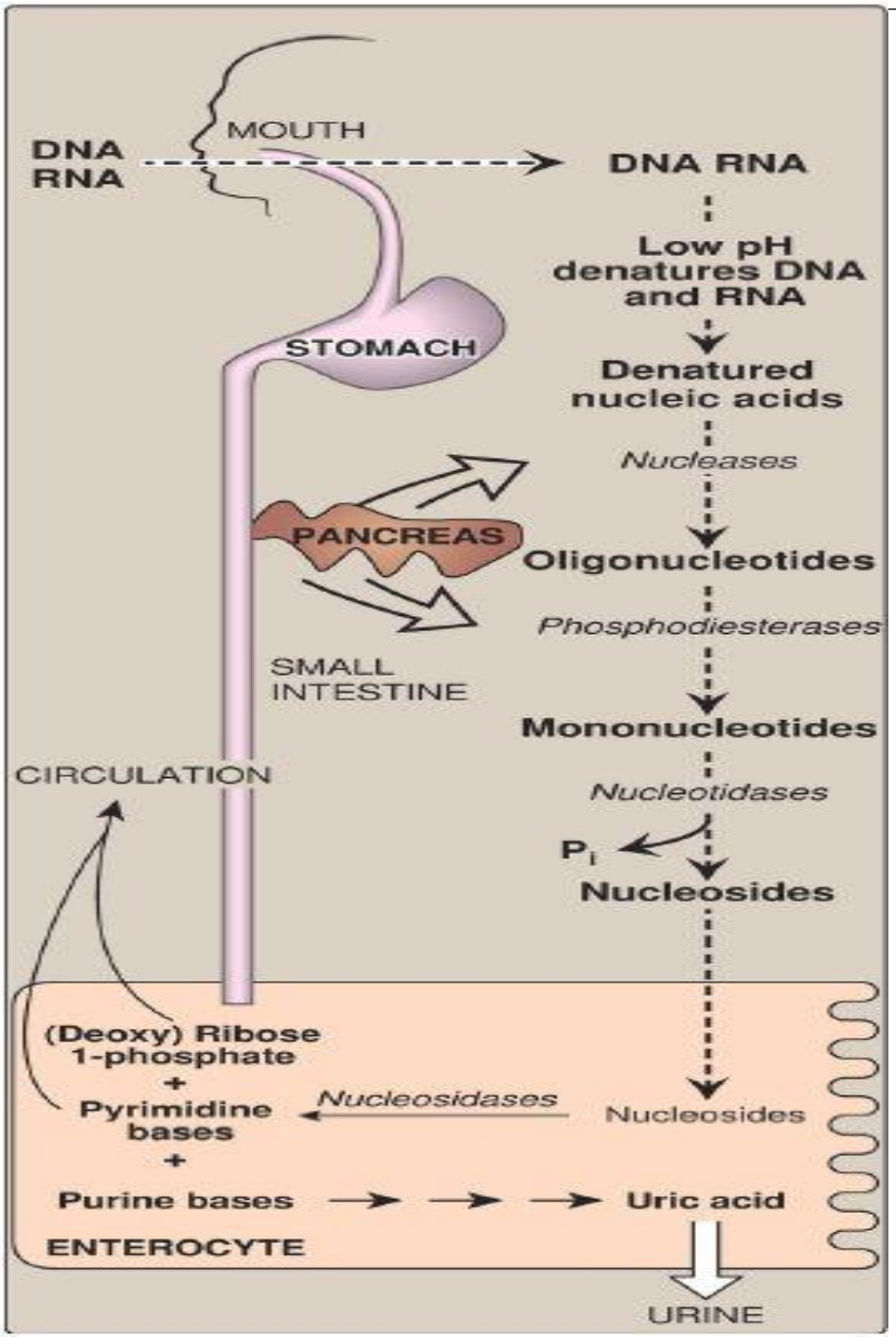
A. Degradation of dietary nucleic acids in the small intestine

- **Ribonucleases** and d**eoxyribonucleases**, secreted by the pancreas, hydrolyze RNA and DNA primarily to oligonucleotides.
- **Oligonucleotides** are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides.
- A family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that may be absorbed by the **intestinal mucosal cells**, or be further degraded to **free bases before uptake.**
- [Note: Dietary purines and pyrimidines contribute minimally to the synthesis of tissue nucleic acids. Instead, most dietary purines are metabolized in the intestinal mucosal cells and converted into uric acid.

- .Most of the uric acid enters the blood, and is eventually excreted in the urine.

- For this reason, individuals with a tendency toward gout should be careful about consuming foods such as organ meats, anchovies, sardines, or dried beans, which contain high amounts of nucleic acids.

- The remainder of the dietary purines are metabolized by the intestinal flora.] A summary of this pathway is shown in Figure


MOUTH
DNA
RNA
DNA RNA
Low pH
denatures DNA
and RNA
STOMACH
Denatured
nucleic acids
Nucleases
PANCREAS
Oligonucleotides
Phosphodiesterases
SMALL
INTESTINE
Mononucleotides
CIRCULATION
Nucleotidases
P_i
Nucleosides
(Deoxy) Ribose
1-phosphate
+
Pyrimidine
bases
+
Nucleosidases
Nucleosides
Purine bases
Uric acid
ENTEROCYTE
URINE

❑ B. Formation of uric acid

- A summary of the steps in the production of uric acid and genetic diseases associated with deficiencies of specific degradative enzymes

1. An amino group is removed from AMP to produce IMP by AMP (adenylate) deaminase or from adenosine to produce inosine (hypoxanthine-ribose) by **adenosine deaminase.**

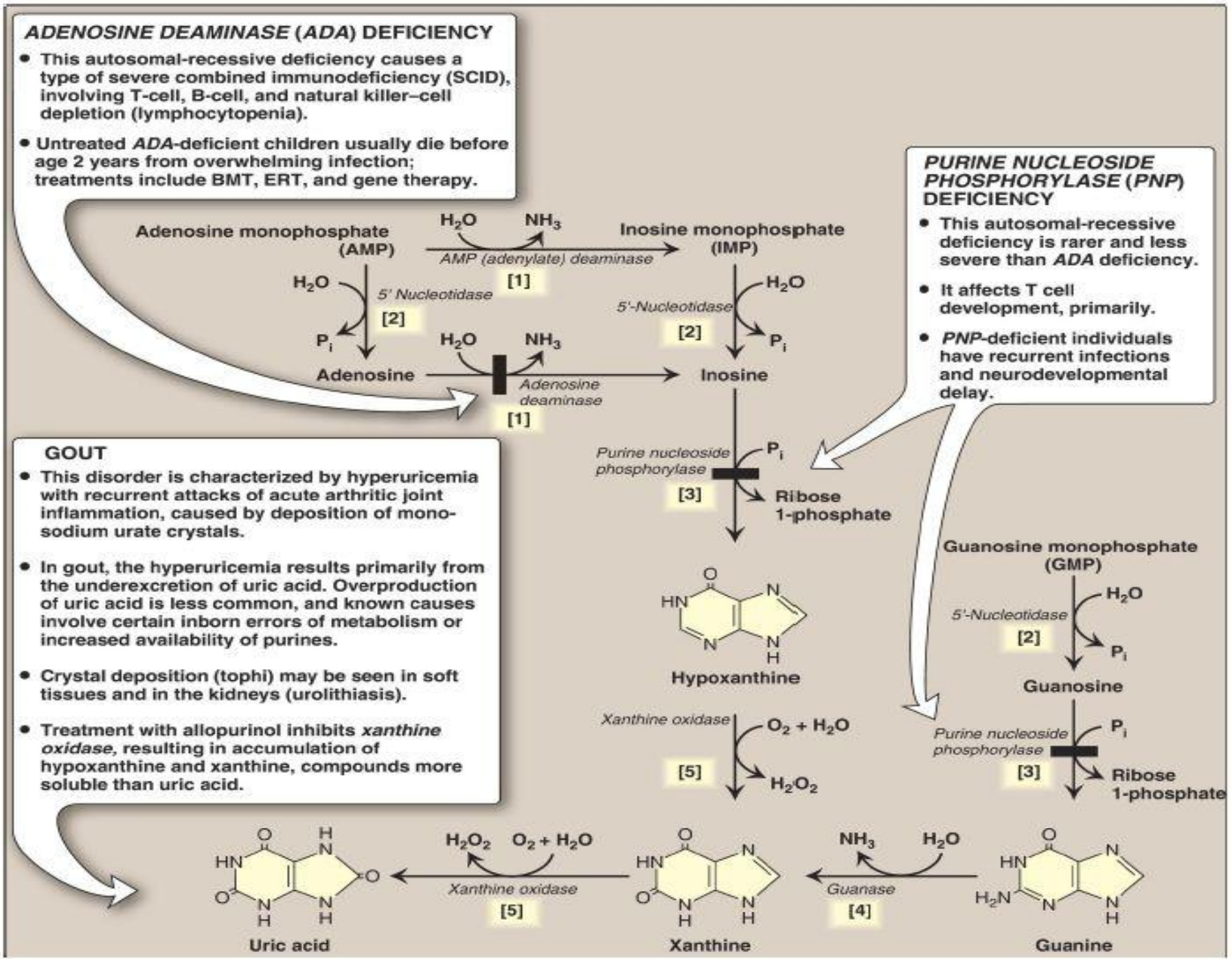
2. IMP and GMP are converted into their respective nucleoside forms, inosine and guanosine, by the action of **5′-nucleotidase**.

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 molybdenum-containing xanthine oxidase (XO) to xanthine, which is further oxidized by XO to uric acid, the final product of human purine degradation. Uric acid is excreted primarily in the urine.


ADENOSINE DEAMINASE (ADA) DEFICIENCY
This autosomal-recessive deficiency causes a type of severe combined immunodeficiency (SCID), involving T-cell, B-cell, and natural killer–cell depletion (lymphocytopenia).
Untreated ADA-deficient children usually die before age 2 years from overwhelming infection; treatments include BMT, ERT, and gene therapy.
PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFICIENCY
This autosomal-recessive deficiency is rarer and less severe than ADA deficiency.
It affects T cell development, primarily.
PNP-deficient individuals have recurrent infections and neurodevelopmental delay.
GOUT
This disorder is characterized by hyperuricemia with recurrent attacks of acute arthritic joint inflammation, caused by deposition of mono-sodium urate crystals.
In gout, the hyperuricemia results primarily from the underexcretion of uric acid. Overproduction of uric acid is less common, and known causes involve certain inborn errors of metabolism or increased availability of purines.
Crystal deposition (tophi) may be seen in soft tissues and in the kidneys (urolithiasis).
Treatment with allopurinol inhibits xanthine oxidase, resulting in accumulation of hypoxanthine and xanthine, compounds more soluble than uric acid.
Adenosine monophosphate (AMP)
H2O
NH3
AMP (adenylate) deaminase
[1]
Inosine monophosphate (IMP)
H2O
5' Nucleotidase
[2]
Pi
5'-Nucleotidase
[2]
H2O
Pi
Adenosine
H2O
NH3
Adenosine deaminase
[1]
Inosine
Purine nucleoside phosphorylase
[3]
Pi
Ribose 1-phosphate
Guanosine monophosphate (GMP)
5'-Nucleotidase
[2]
H2O
Pi
Guanosine
Purine nucleoside phosphorylase
[3]
Pi
Ribose 1-phosphate
Hypoxanthine
Xanthine oxidase
[5]
O2 + H2O
H2O2
Uric acid
H2O2
O2 + H2O
Xanthine oxidase
[5]
Xanthine
NH3
H2O
Guanase
[4]
Guanine

Pyrimidine Synthesis and Degradation

- Unlike the synthesis of the purine ring, which is constructed on a pre-existing ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP.
- The sources of the atoms in the pyrimidine ring are glutamine, CO2, and aspartate (Fig).
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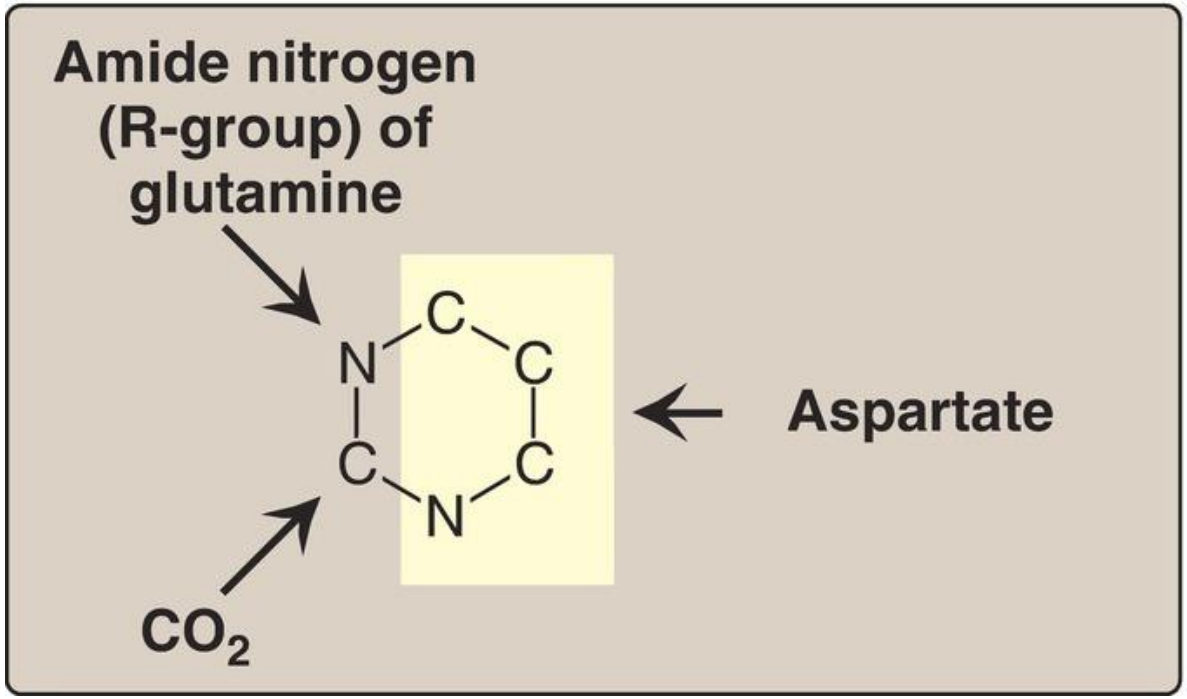


Sources of the individual atoms in the pyrimidine ring. CO2 = carbon dioxide

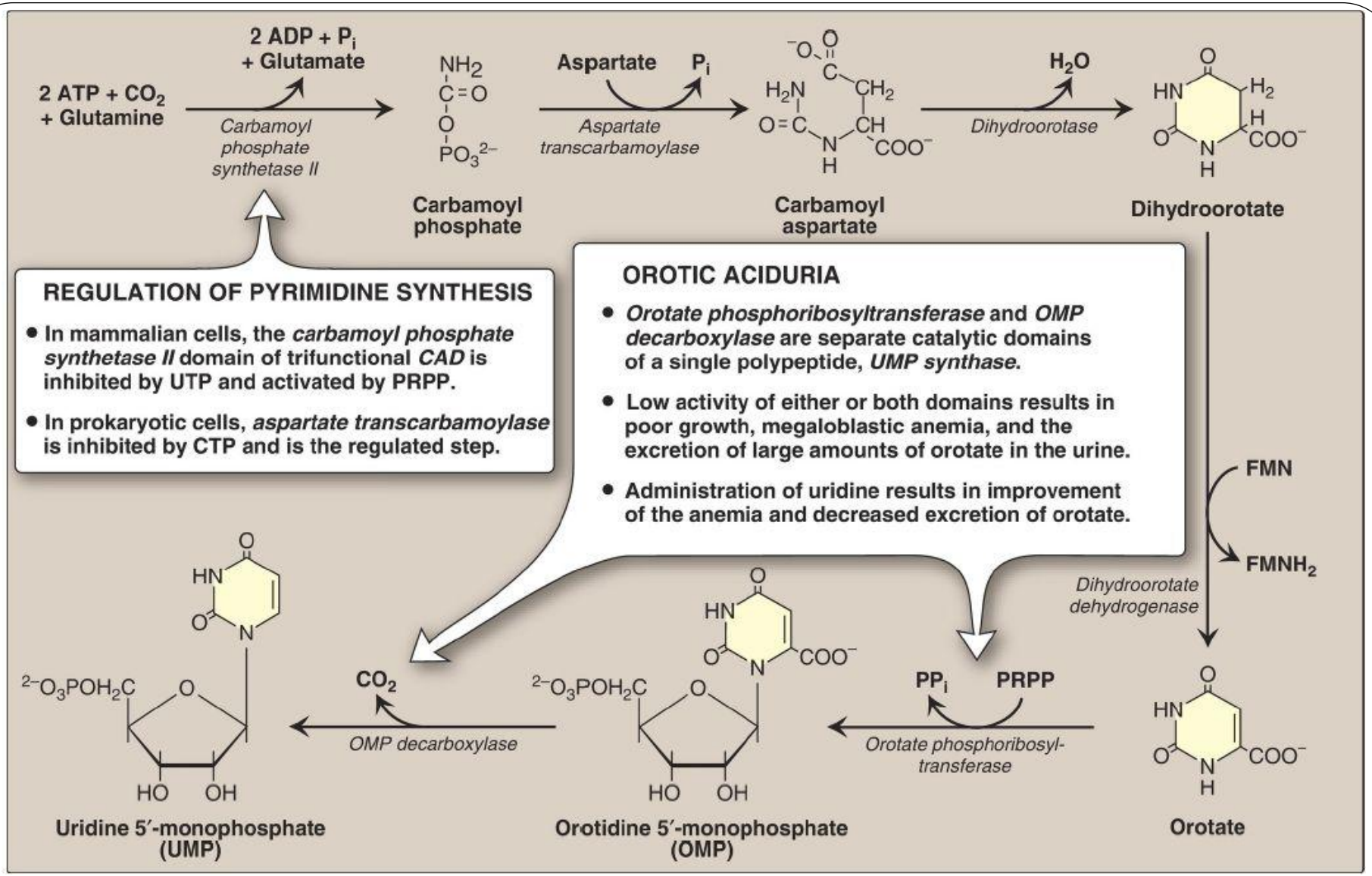
A. Carbamoyl phosphate synthesis

The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO2, catalyzed by carbamoyl phosphate synthetase (CPS) II.

- CPS II is inhibited by uridine triphosphate (the end product of this pathway, which can be converted into the other pyrimidine nucleotides) and is activated by PRPP.
- [Note: Carbamoyl phosphate, synthesized by CPS I, is also a precursor of urea .
- Defects in ornithine trans carbamylase of the urea cycle promote pyrimidine synthesis because of increased availability of carbamoyl phosphate.
- A comparison of the two enzymes is presented in Figure

Variable	*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

Summary of the differences between carbamoyl phosphate synthetase (CPS) I and II. PRPP = 5-phosphoribosyl-1-pyrophosphate; UTP = uridine triphosphate.



De novo pyrimidine synthesis. ADP = adenosine diphosphate; Pi = inorganic phosphate; FMN(H2) = flavin mononucleotide; CTP = cytidine triphosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; PPi = pyrophosphate.

❑ Salvage of pyrimidines

- Pyrimidine salvage occurs mainly via nucleosides using specific kinases → forming UMP, CMP, dCMP, and TMP:

➢ **Clinical Note (High-Yield)**

- **Thymidine kinase** is highly active in:
  - **Herpes simplex virus (HSV)**
- ➡☐ This is important because:
- Drugs like **Acyclovir** are activated by viral thymidine kinase
- Leads to **selective toxicity for infected cells**

Degradation of pyrimidine nucleotides

- Unlike the purine rings, which are not cleaved in human cells, the pyrimidine ring can be opened and degraded to highly soluble structures, such as β-alanine, and β-aminoisobutyrate, which can serve as precursors acetyl CoA and succinyl CoA, respectively.

Hyperuricaemia

☐ Clinical Importance

- **Hyperuricaemia is important due to its ability to cause:**
  - ✓ **Gout** → inflammatory arthritis caused by deposition of **monosodium urate crystals** in joints
  - ✓ **Renal stones (uric acid calculi)**

Pathophysiology

- Acidic urine (**low pH**) increases the risk of:
  - ✓ **Uric acid crystal formation** in kidneys

☐ Critical Level

- Risk of crystal precipitation increases when:
  - **Serum urate > 0.38 mmol/L** .

☐ Important Note

- The relationship between:
  - ✓ Serum urate level
  - ✓ Development of gout or kidney stones
    is **not purely dependent on solubility**
    ➡☐ Other factors (e.g., local conditions, pH, inflammation) also play a role .

❑Dietary factors

1. ***High purine diets***: A high meat diet or one rich in seafood increases the purine load. The protein content per se does not appear to be responsible.

2. • ***Alcohol excess***: Nutritional surveys have established a strong link between hyperuricaemia and alcohol intake.

☑ ↑ **ATP degradation.**

☑ ↑ **Lactate production (lactic acidosis) .**

☑ Especially with **beer/**

3. ***Fructose-containing beverages*:**

- Fructose metabolism (via **fructokinase**) leads to:
  - Rapid **ATP consumption**
  - ↑ **ADP → AMP (via adenylate kinase)**

➡☐ AMP is degraded to **uric acid**

❑ Endogenous overproduction of urate

- A number of mechanisms are possible. For example:

1. • Unspecified over activity of the pathways of nucleotide metabolism, as opposed to nucleic acid synthesis, leading to urate formation ('endogenous overproduction').

2. • Decreased activity of the 'salvage' pathway so that purine bases are metabolised to urate rather than re-incorporated into nucleotides and nucleic acids.

3. Increased nucleic acid breakdown when cell turnover or destruction is increased.

❑ Defective elimination of urate

- Renal excretion of urate is a complex process.
- Except for a small fraction bound to plasma proteins, urate is completely filtered at the glomerulus; this is then mostly reabsorbed in the proximal tubule.
- In the distal tubule, there is both active secretion and post secretory reabsorption at a more distal site. These processes can all be affected by disease or drugs:

1. ***GFR:*** When the GFR becomes reduced for any reason
2. ***Tubular reabsorption:*** Around 90% of the filtered urate load is reabsorbed in the proximal nephron via specific anion transporters. The specific transport called **URAT1 (**Proximal renal tubule (apical membrane)) is a target for some drugs .

3. *Distal tubular secretion*:

- Urate excretion also depends upon distal tubular secretion. This process is competed for by other organic acid anions such as lactate, 3-hydroxybutyrate. Any condition that gives rise to lactic acidosis or ketosis tends to be associated with hyperuricaemia.
- Drugs may also compete with urate for this excretory mechanism (e.g. thiazide diuretics) and thereby increasen serum urate concentration.

❑ Gout

- Hyperuricaemia is associated with ***gout***, a condition characterised by recurrent attacks of mono articular arthritis.
- Typically this involves the 1st metatarsophalangeal joint but the ankle, knees or other joints may be involved.

- Articular gout may be preceded by an asymptomatic phase of hyperuricaemia, followed by acute attacks with symptom-free periods and eventually leading to chronic, gouty arthritis.

- Patients with chronic, primary gout often show deposition of urate as tophi in soft tissues.

- Some also develop renal stones, mainly composed of uric acid, increasing the risk of renal dysfunction.
- The incidence of renal stones varies widely, largely depending on the presence of other contributory factors such as dehydration or a low urinary pH.

❑ Primary gout,

☐ Definition

- Primary gout occurs **in the absence of identifiable acquired or monogenic causes**

☑ ☐ Genetic Contribution

- Strong genetic influence:
  - **~60% of serum urate variability** is genetically determined

☑ Pathophysiology

- Main mechanism:
  - **Reduced renal excretion of urate**
- Less commonly:
  - **Increased urate production**

☑ Associated Conditions

- Commonly associated with:
  - **Hyperlipidaemia**
  - **Ischaemic heart disease**
  - **Metabolic syndrome**

☑ ☐☐ Important Note

- These associations may reflect:
  - **Shared genetic predisposition**
  - ± Environmental factors (diet, lifestyle)

❑ Secondary Gout

☐ Definition

- Secondary gout occurs **as a result of underlying conditions** that:
  - ✓ **↑ Urate production**
  - ✓ or **↓ Urate excretion**

☐ Causes

- **Increased Uric Acid Production**
- Conditions with **high cell turnover**:
  - e.g., **myeloproliferative disorders**
- Rare **metabolic disorders**
- **Decreased Uric Acid Excretion**
  - Renal impairment / renal failure
  - Drugs or other factors affecting renal handling

.

- Classification of hyperuricemia Either renal (under execretion, 90%) and metabolic (over production, 10%)

- (Each is primary or secondary…)
- **• Primary defect**
- Inherited metabolic disorders,
- e.g. **Lesch-Nyhan syndrome**

- **Secondary causes**
  - Excess dietary intake
  - Increased nucleic acid turn over, e.g.malignancy, psoriasis
- **Decreased Excretion**
  - Primary (idiopathic) causes
  - Secondary causes

1. Chronic renal failure
2. Lactic acidosis
3. Thiazide diuretics therapy

Thank you for listening