# Nucleotide Metabolism – Purines and Pyrimidines

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**Nucleotide metabolism results in the synthesis of the four nucleotides that form DNA. These are further divided into two categories, namely, purines and pyrimidines. Their synthesis and degradation occur over several steps in the presence of different enzymes and cofactors. In salvage pathways, the nucleosides and free bases resulting from DNA breakdown are re-converted to nucleotide monophosphates.**

## Composition of Nucleotides

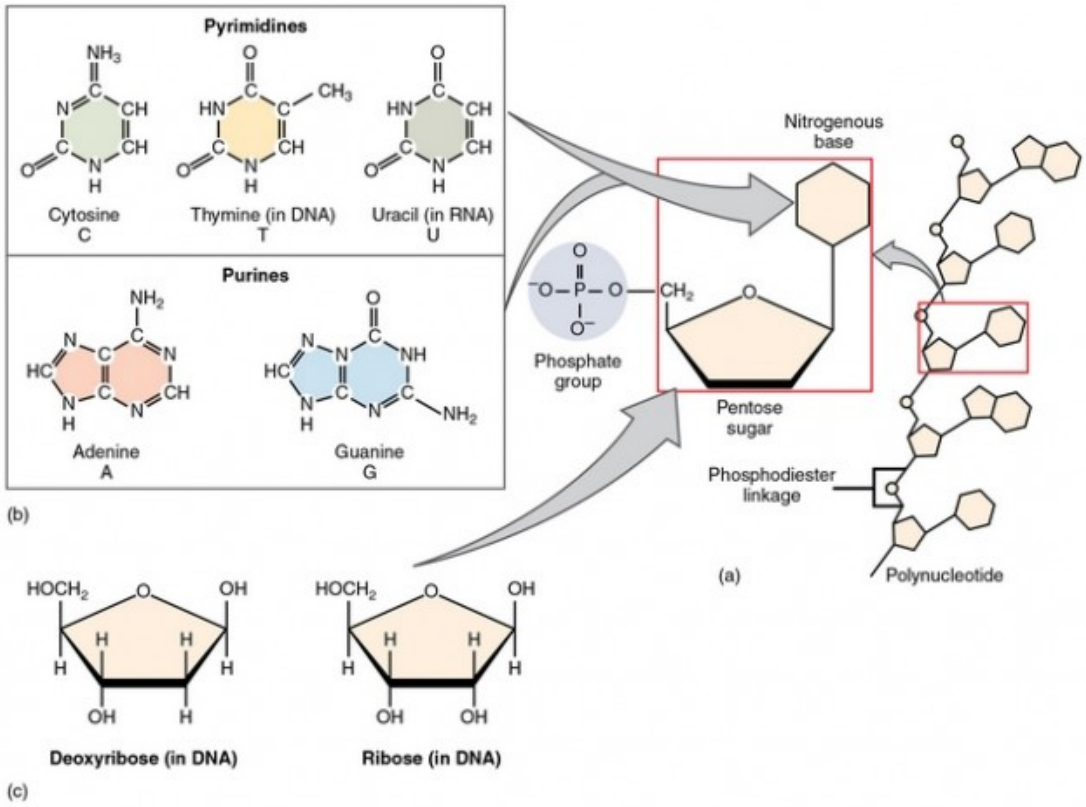
Nucleotides, comprising a **deoxyribose** (sugar), a **phosphate group,** and a **nitrogen base,** constitute the **deoxyribonucleic acid (DNA)** backbone.

There are four nitrogen bases, and therefore, four types of nucleotides. These four nitrogen-containing bases are as follows:

- Adenine
- Guanine
- Cytosine
- Thymine

The resulting nucleotide is named accordingly. For example, if the base is adenosine, the nucleotide is known as **deoxyadenosine-5’-monophosphate**. The nucleotides are abbreviated with the initials of their base, i.e. A, G, C, and T.

Nitrogen bases are grouped into two categories; adenine and guanine constitute the **purine** category, whereas cytosine and thymine form the **pyrimidine** class.

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## Nucleoside Construction

A nucleoside is composed of a **deoxyribose sugar** and a **nitrogen base.** As opposed to a nucleotide, a nucleoside lacks a phosphate group and can be derived from a nucleotide through the removal of the phosphate group.

## De Novo Synthesis of Purine Nucleotides

**Purine nucleotides** are synthesized directly by the addition of a pyrophosphate at C-1 of the **ribose** sugar. This represents an important difference from **pyrimidine synthesis** (discussed later in this article), in which the ribose is added subsequently after the complete formation of the pyrimidine ring.

The synthesis of purine nucleotides occurs over multiple steps. During the first step, **ribose-5-phosphate** is converted to phosphoribosyl pyrophosphate (**PRPP**), followed by the conversion of ATP to AMP. The enzyme involved in this step is PRPP-synthetase, which is named in accordance with the final product it catalyzes.

The next step is the rate-limiting reaction in the synthesis of purine nucleotides. During this reaction, **PRPP** and **glutamine** combine**,** resulting in the formation of **5-phosphoribosylamine along** with the release of **pyrophosphate** (PP). The configuration on the C-1 atom of the ribose is converted from an α-structure to a β-structure.

**Glutamine phosphoribosyl pyrophosphate amidotransferase** is the enzyme involved in this rate-limiting reaction. IMP, GMP, and AMP inhibit the enzyme, and consequently, the reaction it catalyzes. The reaction can be re-driven through **pyrophosphate hydrolysis**.

The third step in the synthesis of purine nucleotides is represented by an ATP-dependent reaction, in which **5-phosphoribosylamine** is converted to **glycinamide ribonucleotide.** The reaction is catalyzed by **glycinamide kinosynthase**.

The **glycinamide ribonucleotide** undergoes formylation and is converted to **formylglycinamide ribonucleotide**. The formyl group is obtained from **$N^{10}$-formyltetrahydrofolate**.

In the next step, the nitrogen atom of glutamine serves as an electron donor and a five-membered ring is formed after the removal of a water molecule. Glutamine is thus converted to **glutamate**.

A CO2 molecule is formed during ring closure. Another nitrogen atom is subsequently added through an ATP-dependent reaction, which, in this case, is provided by **aspartate** (similar to that in the urea cycle).

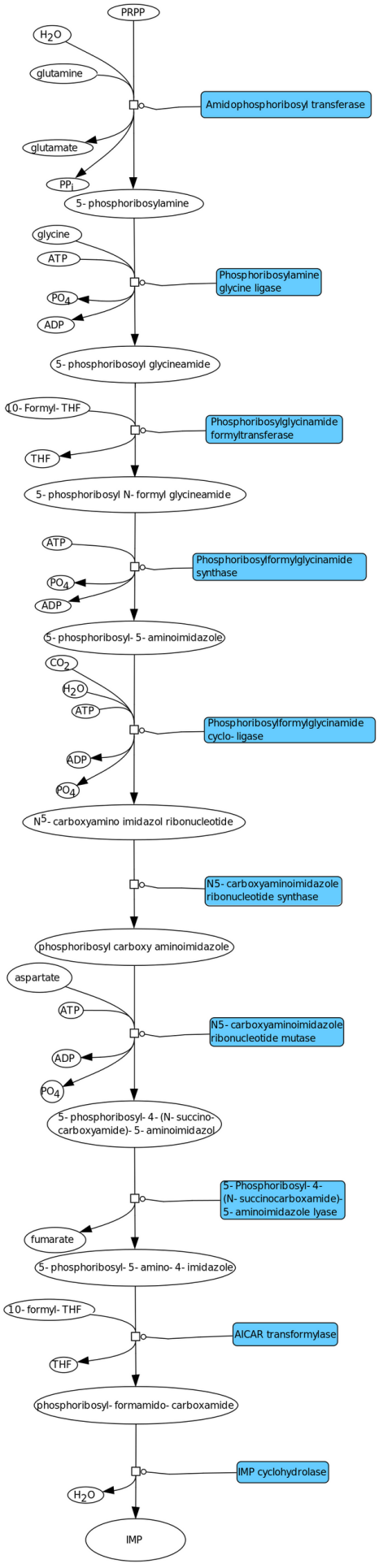
Aspartate is converted to **fumarate** through the loss of ammonia. Alongside this process, another C-1 fragment is incorporated in the 5-membered ring, converting it into a 6-membered ring after the removal of a water molecule. The C-1 fragment is obtained from **$N^{10}$-formyltetrahydrofolate**.

The resulting product, **inosine monophosphate** (IMP), serves as a precursor to **adenosine monophosphate** (AMP) and **guanosine monophosphate** (GMP) synthesis.

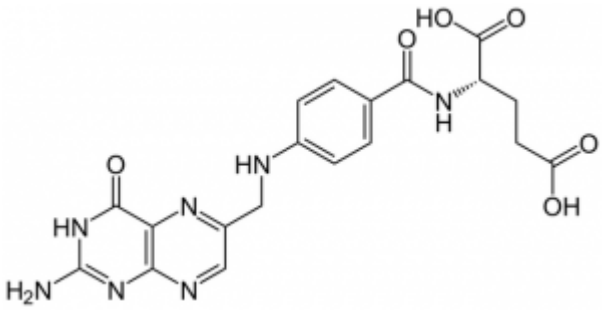
The synthesis of AMP is GTP and aspartate dependent. In this reaction, the keto group is replaced with an amino group at position C-6.

The synthesis of GTP from IMP involves two steps. In the first step, IMP is oxidized to xanthine monophosphate in an NAD+-dependent reaction. **Xanthine monophosphate** undergoes amination, where the amino group is provided by glutamine. The second step is both glutamine and ATP dependent.

**Note**: Glutamine and aspartate are amino acids involved in the synthesis of purine nucleotides; they serve as N donors and get converted to glutamate and fumarate, respectively.


PRPP
$H_2O$
glutamine
Amidophosphoribosyl transferase
glutamate
$PP_i$
5- phosphoribosylamine
glycine
ATP
Phosphoribosylamine glycine ligase
$PO_4$
ADP
5- phosphoribosoyl glycineamide
10- Formyl- THF
Phosphoribosylglycinamide formyltransferase
THF
5- phosphoribosyl N- formyl glycineamide
ATP
Phosphoribosylformylglycinamide synthase
$PO_4$
ADP
5- phosphoribosyl- 5- aminoimidazole
$CO_2$
$H_2O$
ATP
Phosphoribosylformylglycinamide cyclo- ligase
ADP
$PO_4$
$N^5$- carboxyamino imidazol ribonucleotide
N5- carboxyaminoimidazole ribonucleotide synthase
phosphoribosyl carboxy aminoimidazole
aspartate
ATP
N5- carboxyaminoimidazole ribonucleotide mutase
ADP
$PO_4$
5- phosphoribosyl- 4- (N- succino- carboxyamide)- 5- aminoimidazol
5- Phosphoribosyl- 4- (N- succinocarboxamide)- 5- aminoimidazole lyase
fumarate
5- phosphoribosyl- 5- amino- 4- imidazole
10- formyl- THF
AICAR transformylase
THF
phosphoribosyl- formamido- carboxamide
IMP cyclohydrolase
$H_2O$
IMP

## Role of folic acid




Folic acid is an important component in the synthesis of purine nucleotides. A lack of folic acid leads to the reduced synthesis of purine nucleotides.

This deficiency is particularly apparent in processes with high cell turnover, e.g., erythropoiesis.

A consequence of folic acid deficiency, in this context, is megaloblastic **anemia.** In this condition, a malfunction occurs both in DNA synthesis as well as in the nuclear maturation during myelopoiesis, which leads to the appearance of megaloblasts.

Besides folic acid deficiency, megaloblastic anemia can also occur due to a lack of vitamin B-12, which, overall, is a more common cause of megaloblastic anemia than the former.

Other symptoms of folic acid deficiency include **gastritis** and **dermatitis**. Folic acid deficiency during pregnancy increases the risk of the baby being born with **spina bifida**.

## Folic acid metabolism

Folic acid is composed of **p-aminobenzoic acid**, **glutamine,** and **pteridine** molecules. Folic acid is available in its biologically active form as **tetrahydrofolic acid** (TH-4), which plays a role in the synthesis of purine nucleotides.

The active form of folic acid functions as a coenzyme in C-1 transmission, in which the groups on the C-1 position are bound to the N atoms at positions 5 and 10 of the pteridine or 4-aminobenzoic acid moiety. The possible groups that can be transferred in this context are methyl, hydroxyl, and formyl groups.

The conversion to the active form occurs via a reaction that is dependent on **NADP+** and **vitamin C**. In the first step, folic acid is converted to **7,8-dihydrofolic acid** in the presence of **folate reductase**; **NADPH** and **H+** are the byproducts.

In an NADPH + H+-dependent reaction, **5,6,7,8-tetrahydrofolate** is also generated by a similar process. **Tetrahydrofolate** synthesis occurs in the presence of dihydrofolate reductase. This enzyme can be inhibited by several drugs including **trimethoprim**.

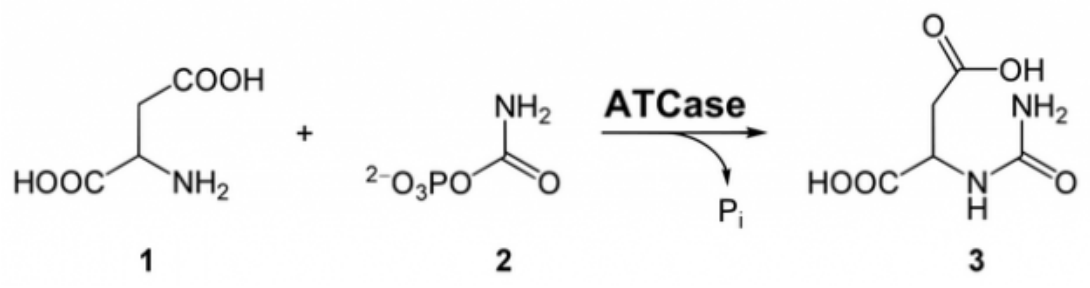
## De Novo Synthesis of Pyrimidine Nucleotides

The intermediate product of pyrimidine synthesis is initially a **ribonucleotide**. During this process, the ribose is reduced to **2’-deoxyribose,** which can be incorporated into the DNA.

The biosynthesis of pyrimidine nucleotides occurs over multiple steps involving different

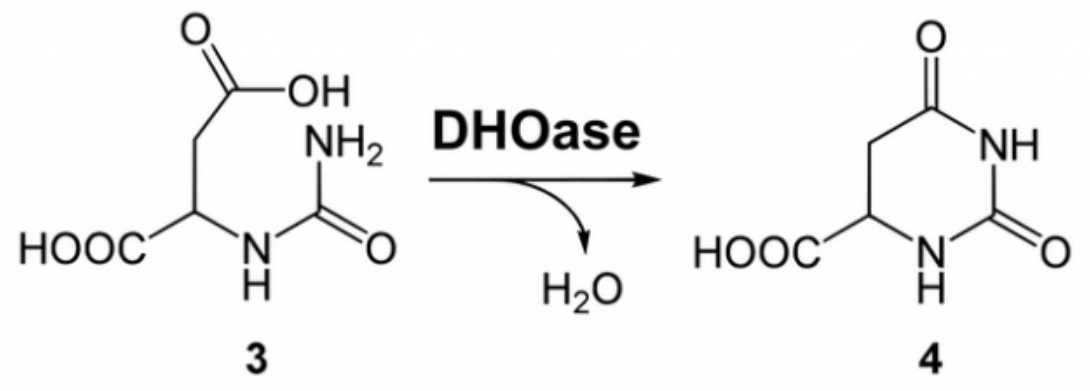
enzymes. The **pyrimidine ring** is synthesized first and the **ribose** sugar is subsequently added to it.

In the first step of pyrimidine synthesis, the **carbamoyl phosphate** and **aspartate** react to produce **carbamoyl aspartate** along with the release of a phosphate moiety. This reaction is catalyzed by aspartate **transcarbamoylase**.

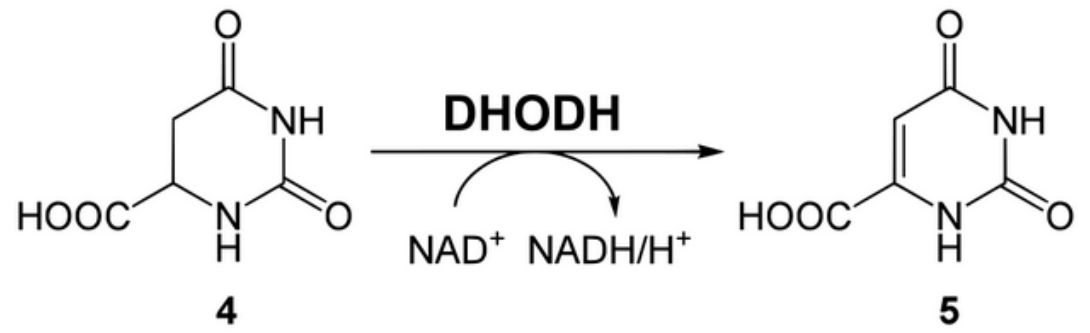



**Note**: The key reaction in pyrimidine synthesis represents the reaction between carbamoyl phosphate and aspartate to form carbamoyl aspartate.

Carbamoyl aspartate loses a water molecule to form **dihydroorotic** acid. This second step involves the cyclization of carbamoyl aspartate in the presence of the enzyme, **dihydroorotase**.



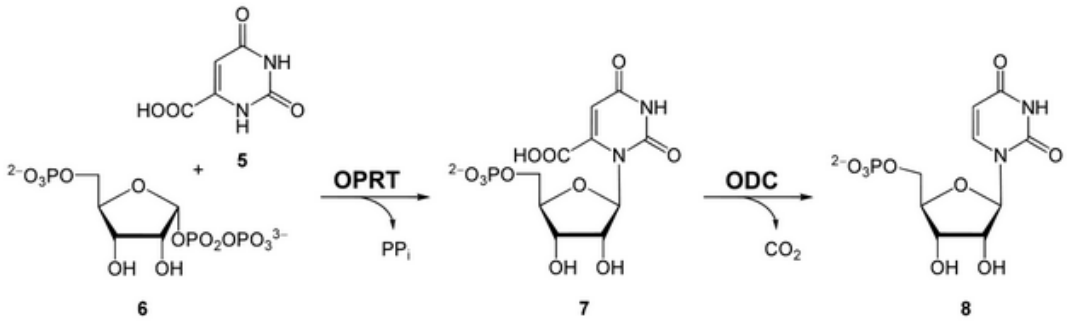

Next, **dihydroorotic** acid is oxidized to **orotic acid** in the presence of **orotic acid dehydrogenase**. This enzyme uses NAD+ as a coenzyme yielding NADH and H+ as the byproducts.




Orotic acid reacts with phosphoribosyl pyrophosphate (PRPP) to form **orotidine-5-phosphate** with the release of pyrophosphate. This reaction is mediated by **orotate phosphoribosyltransferase**.

**Orotidine-5-phosphate** undergoes **decarboxylation** to form **uridine-5-phosphate**

(UMP) in the presence of **orotidine-5-phosphate decarboxylase**.




**Uridine-5-phosphate** constitutes the building block of the subsequent reactions. First, **UMP** is phosphorylated to form **UDP**, which can be further phosphorylated to **UTP**. The phosphate group required for this reaction is obtained from the conversion of ATP to ADP.

Following an ATP- and glutamine-dependent reaction, UTP is converted to **cytidine triphosphate (CTP)** in the presence of **CTP-synthetase**.

On the other hand, uridine-5-phosphate can be reduced to d-UMP by the action of d-TMP-synthetase (**thymidylate-synthase**). This reduction is NADPH + H+-mediated. The subsequent reaction catalyzed by d-TMP-synthetase is the **methylation** of d-UMP to **d-TMP**. The methyl group that is required for this conversion is obtained from **$N^5$,$N^{10}$-methylene tetrahydrofolate**, which in turn is converted to dihydrofolate.

## Synthesis of the Deoxy Forms of Purine and Pyrimidine Nucleotides

The final product of the synthesis of both purine and pyrimidine nucleotides is **ribonucleotide**, which must be reduced further to the **2'-deoxy-form** to be incorporated into the DNA.

**Ribonucleotide reductase** catalyzes this reaction in the presence of **thioredoxin** as a cofactor. Thioredoxin, in turn, contains two **SH groups**, which are converted to the disulfide form after reduction. Thioredoxin, in its disulfide form, is reconverted to its original form through NADP+-dependent **thioredoxin reductase**.

## Degradation of Purine Nucleotides and Bases

Similar to the stepwise synthesis of purine nucleotides, their degradation also occurs via multiple steps. The steps involved in degradation depends on the purine bases (adenosine or guanosine) that are present.

The first step in the degradation reaction is the conversion of the nucleotide to the nucleoside. This occurs through a hydrolysis reaction mediated by **nucleotidase**. Additionally, a phosphate molecule is lost, which leads to the formation of a free base (purine or pyrimidine) and ribose-1-phosphate. This step is mediated by nucleoside phosphorylase. The degradation of the purine bases, adenosine and guanosine, occurs subsequently.

First, adenosine undergoes **deamination** in the presence of adenosine deaminase and is

converted to inosine. The second step is identical for both inosine and guanosine, in which they are converted to hypoxanthine and guanine, respectively, through an ATP-dependent removal of ribose. Similar to the previous step, this reaction is mediated by nucleoside phosphorylase.

In the next step, inosine and hypoxanthine are converted to **xanthine**. However, this step proceeds differently for each nucleoside. Guanine is deaminated to xanthine, whereas hypoxanthine is oxidized to xanthine in the presence of xanthine oxidase.

**Note**: Xanthine oxidase is an iron-bearing flavoprotein that contains a molybdenum atom in its active center.

Another reaction mediated by xanthine oxidase is the conversion of xanthine to uric acid. This step also proceeds via oxidation, where molecular oxygen serves as a means for oxidation. Eventually, the uric acid that is generated is excreted in the urine.

## Enzyme defects leading to altered uric acid levels

The normal blood uric acid level ranges from 2–7 mg/100 mL. When this level is exceeded, urate crystals are formed, which accumulate in tissues and joints leading to local inflammation or **gout**.

Poor vascularization and low temperatures promote the crystallization of uric acid, which likely explains why the metatarsophalangeal joint (**podagra**), cornea, and the lens of the eye are potential sites for uric-acid deposition.

Gout-affected joints appear flushed, overheated, and swollen, and are very painful. These presentations represent the cardinal signs of inflammation (**rubor, calor, tumor,** and **dolor**).

Enzyme defects can also lead to increased or diminished uric acid levels. A partial or complete lack of **hypoxanthine-guanine phosphoribosyltransferase** results in increased uric acid levels. A deficiency of this enzyme results in a condition known as **Kelley-Seegmiller syndrome**, which is associated with high purine levels.

**Lesch-Nyhan syndrome** is a condition resulting from the complete lack of hypoxanthine-guanine phosphoribosyltransferase. The affected children present a trio of hyperuricemia, progressive kidney insufficiency, and neurological symptoms, for example, a tendency to self-mutilate.

Conversely, reduced xanthine oxidase activity can lead to diminished uric acid levels and the accumulation of xanthine (**xanthinuria**).

**Note**: Xanthine oxidase activity can be inhibited using **allopurinol** during the management of gout.

Some other factors influencing uric acid levels include renal function (uric acid secretion), an increased cell turnover (diseases including leukemia), or high-purine foods (beer, fish, and certain meats).

# Degradation of Pyrimidine Nucleotides and Bases

The first step in the degradation of pyrimidine nucleotides is their conversion to nucleosides, similar to that discussed in the degradation of purine nucleotides.

The degradation of cytosine and thymine, produced in the first step of the degradation of

pyrimidine bases, occurs in the liver.

**Note**: The pyrimidine ring is broken down during nucleotide degradation; however, the purine ring is preserved during the degradation process.

Cytosine and thymine undergo independent degradation pathways in which the reaction steps are identical except for the first step in the degradation of cytosine.

In this first step, cytosine is degraded to **uracil** by the removal of an amino group. Uracil and thymine are, in turn, reduced to **dihydrouracil** and **dihydrothymine,** respectively, via an NADPH + H+-dependent reaction.

Dihydrouracil undergoes decarboxylation and deamination to form **β-alanine;** dihydrothymine undergoes a similar conversion to **β-aminobutyrate**.

β-alanine and β-aminobutyrate are partially further degraded to **acetate** and **propionate**, respectively, after the loss of ammonia and carbon dioxide across multiple intermediate steps. The nitrogen atoms resulting from the breakdown are utilized in the urea cycle.

## Recycling of Purine and Pyrimidine Nucleotides (Salvage Pathway)

The degradation of purine nucleotides does not result in any energy gain, whereas the breakdown of pyrimidine nucleotides results in only marginal energy generation. Since the synthesis of both purine and pyrimidine nucleotides requires significant energy, recycling is an energetically viable option. This occurs via the salvage pathway.

The exact steps involved in recycling are only known for purine bases and are discussed below.

First, the purine bases are **phosphoribosylized** to nucleotides by PRPP. The transmission of each purine base to PRPP in adenine is mediated by **adenine phosphoribosyltransferase, while hypoxanthine-guanine phosphoribosyltransferase** catalyzes this reaction for hypoxanthine and guanine.

The end product of adenine salvage is **AMP**, while that for hypoxanthine is **IMP**. The end product of guanine salvage is **GMP**. The synthesized final products inhibit the corresponding enzymes.

## References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Notes