

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 in two categories: purines and pyrimidines. Their construction and degradation takes place in a progression of single steps, while different enzymes and cofactors are involved in the process. Beside degradation, there is also a form of reprocessing purine and pyrimidine nucleotides, defined as Salvage-pathways.



Nucleotide Construction

The four nucleotides serve as DNA (**Deoxyribonucleic acid**) components. A nucleotide is, in turn, composed of a **deoxyribose** (sugar), a **phosphate group**, and a **nitrogen basis**.

There are four different nitrogen bases, and, therefore, four sorts of nucleotides. The four bases are:

- Adenine
- Guanine
- Cytosine
- Thymine

The resulting nucleotide is named accordingly. If the base is e.g., adenosine, it is named **deoxyadenosine-5'-monophosphate**. The respective nucleotides are therefore

indicated, in short form, with the initial of their base, i.e. A, G, C, and T.

Nitrogen bases are alike two by two since their construction, and are grouped into two categories. Thus, adenine and guanine constitute the **purine** category, while cytosine and thymine form the **pyrimidine** class.

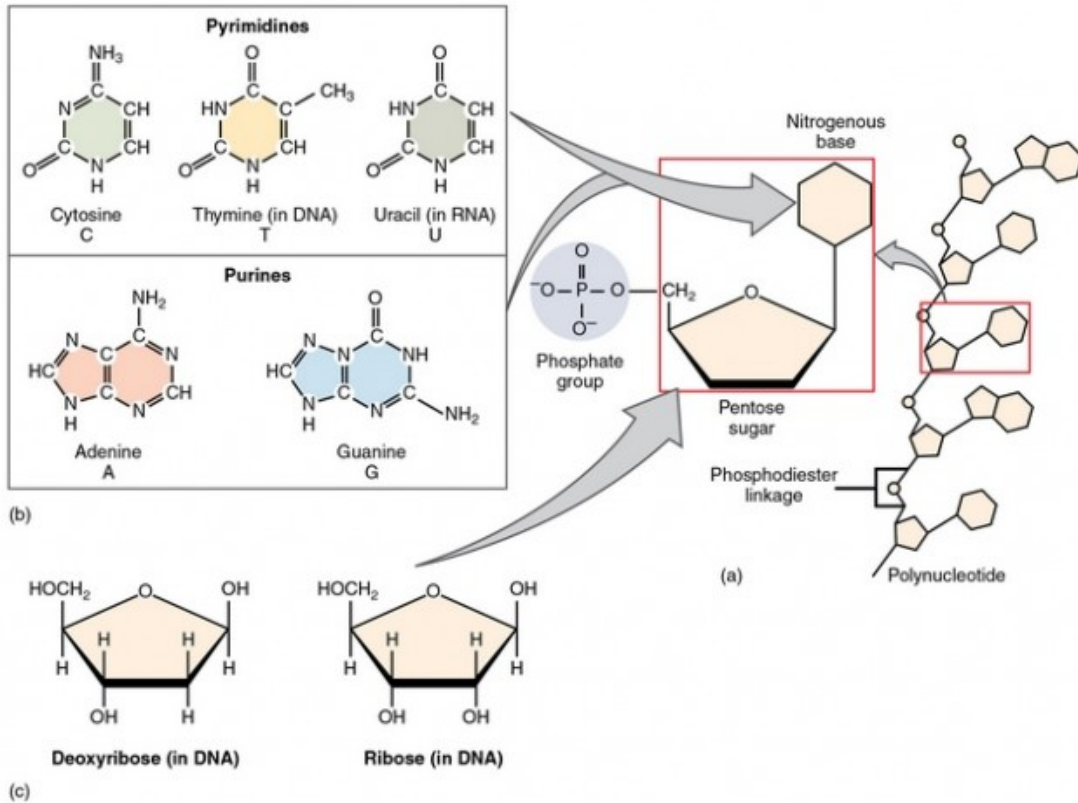


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Nucleoside Construction

A nucleoside is composed of **deoxyribose** and a **nitrogen basis**, i.e. contrary to a nucleotide, it doesn't have a phosphate group anymore. This means that, a nucleoside can come from a nucleotide through the removal of a phosphate group.

Purine Nucleotide de Novo Synthesis Process

Purine nucleotides are synthesized directly at the nucleotide's **ribose** within their synthesis process. This represents an important difference from **pyrimidine synthesis** (see below), in which the ribose is only added subsequently to the complete formation of the pyrimidine ring.

The purine nucleotide synthesis takes place in multiple individual steps. During the first step, **Ribose-5-Phosphate** becomes phosphoribosylpyrophosphate (**PRPP**) – converting ATP to AMP. The enzyme involved is called PRPP-synthetase, in accordance with its final product.

Meanwhile, the rate-limiting reaction, the second step of the purine nucleotide synthesis, takes place. During this reaction, the combination of **PRPP** and **glutamine** with the separation of **pyrophosphate** (PP) results in **5-Phosphoribosylamine**. The

configuration on the C-1 atom of the ribose is converted from an α -structure to a β -structure.

The enzyme involved in this rate-limiting reaction is **glutamine-phosphoribosylpyrophosphate-amidotransferase**. Substances like IMP, GMP and AMP hinder the enzyme and the reaction with it. The reaction can be re-driven through **pyrophosphate hydrolysis**.

During the third step of the purine nucleotide synthesis, which is represented by an ATP-dependent reaction, **5-phosphoribosylamine** becomes a **glycinamide-ribonucleotide**. The **glycinamide-kinosynthase** enzyme mediates this reaction.

Through formylation, the **glycinamide-ribonucleotide** becomes a **formyl-glycinamide-ribonucleotide**. The necessary residual formyl comes from a **N10-formyltetrahydrofolate**.

In the following step, an N-atom with glutamine serves as a donor and a closed 5-membered ring is formed through a H₂O separation. Glutamine thus becomes **glutamate**.

A CO₂ molecule is formed in the closed 5-membered ring. Another N-atom is subsequently added through an ATP-dependent reaction, which, in this case, is provided by **aspartate** (similarly to the urea cycle).

Aspartate becomes **fumarate** through the loss of the N-atom. Alongside this process, another C-1 fragment is incorporated in the 5-member ring, which becomes a 6-member ring through the separation of H₂O. The C-1 fragment is obtained from the **N10-formyltetrahydrofolate**.

The resulting product is called **inosine monophosphate** (IMP). It serves as a pre-amplifier to adenosine **monophosphate** (AMP) and **guanosine monophosphate** (GMP) synthesis.

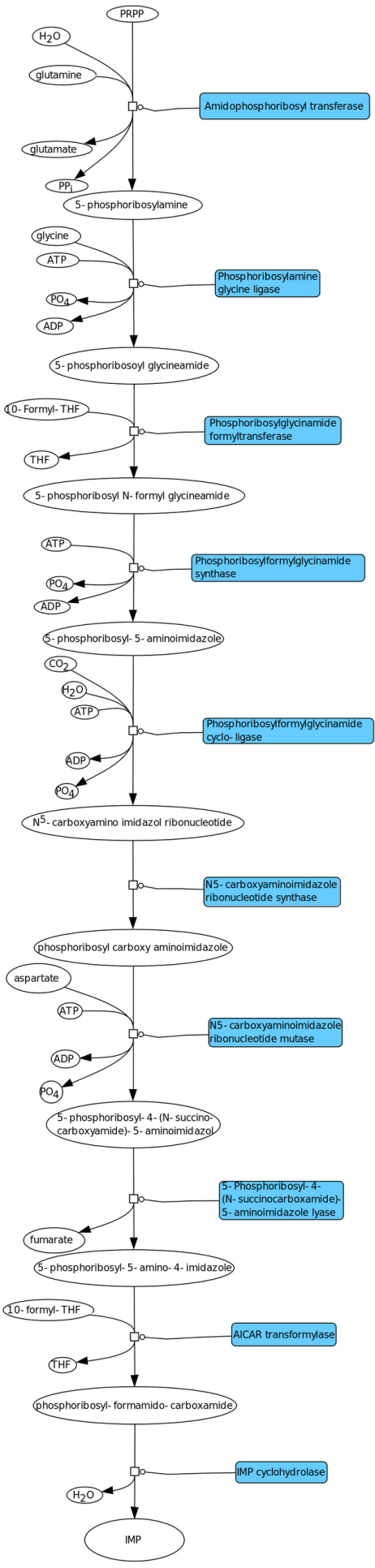
The synthesis to AMP is also GTP-dependent and aspartate-dependent. Within this reaction, the keto group is replaced with a NH₂ group in position C6.

The synthesis of GTP from IMP is, on the contrary, composed of two steps. In the first step, IMP is oxidized to xanthine monophosphate in a NAD⁺-dependent reaction. **Xanthine monophosphate**, however, is later aminated, and the amine group (NH₂) is obtained from the glutamine. The second step is both glutamine and ATP-dependent.

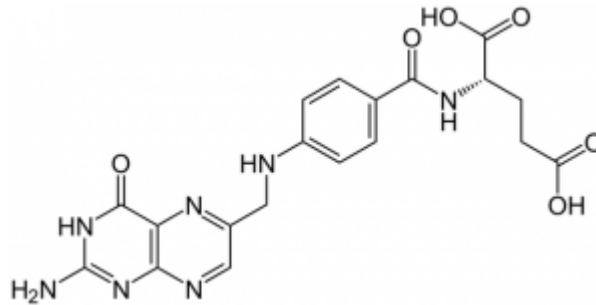
Note: Glutamine and aspartate belong to the amino acids involved in the purine nucleotide synthesis, where they serve as donors of N atoms and thus become glutamate or fumarate.







Role of Folic Acids



Folic acid is a very important component of an efficient purine nucleotide synthesis. A lack of folic acids leads to a strongly reduced purine nucleotide synthesis.

This reduction becomes particularly clear in processes with high cell turnover, e.g., erythropoiesis.

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

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

Further symptoms of folic acid deficiency are, among others, **gastritis** or **dermatitis**. The lack of folic acid during pregnancy can somewhat increase the risk of the baby being born with **spina bifida**.

Folic Acid Metabolism Process

Folic acids are composed of a **p-aminobenzoic acid** rest, a **glutamine** rest and a **pteridine** rest. Folic acid is available in its biologically active form as **tetrahydrofolic acid** (TH-4), in which it is involved, among other things, in the construction of the purine nucleotide (see above).

The active form of folic acid serves all in all as a coenzyme in the C-1 transmission, in which the rest of the C-1 position is bound to the N atoms of position 5 and 10 of the pteridine or 4-aminobenzoic acid rest. Possible rests that could be transferred in this context are methyl, hydroxyl, formic, and formyl rests.

The conversion to the active form takes place, among others, in a reaction which is dependent on **NADP+** and **vitamin C**. In its first step **7, 8-dihydrofolic acid** is created from folic acid - with the investment of **NADPH + H+** and is mediated by **folate reductase**.

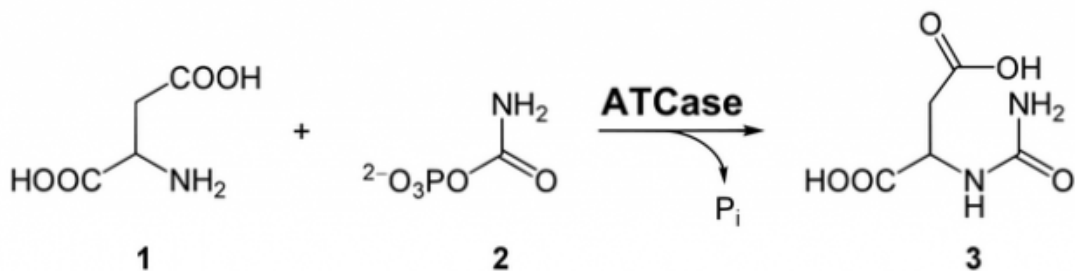
In a **NADPH + H+**-dependent reaction, **5, 6, 7, 8-tetrahydrofolate** is also created by this process. **Tetrahydrofolate** synthesis, in its active form, takes place thanks to the dihydrofolate reductase enzyme. This enzyme can be inhibited through a series of substances like e.g., **trimethoprim**.

Pyrimidine Nucleotide De Novo Synthesis Process

The temporary product of the pyrimidine synthesis is initially a **ribonucleotide**. During the process, the ribose is reduced to a **2'-deoxyribose**, so it can be incorporated in the DNA as such.

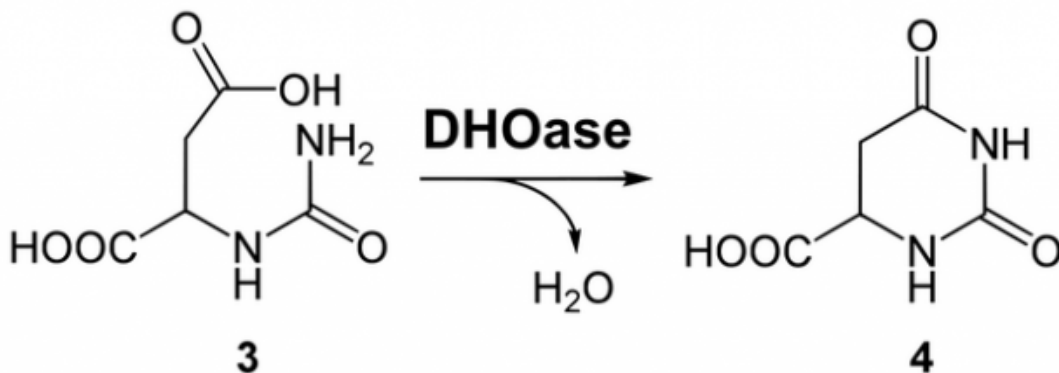
The entire pyrimidine nucleotide biosynthesis takes place in multiple individual steps which involve different enzymes. Here, the **pyrimidine ring** is synthesized first, and **ribose** is subsequently added to it.

Within the first step of pyrimidine synthesis, **carbamoyl phosphate** and **aspartate** react and produce **carbamoyl aspartate** through a phosphate release. The involved enzyme is called aspartate **transcarbamoylase**.

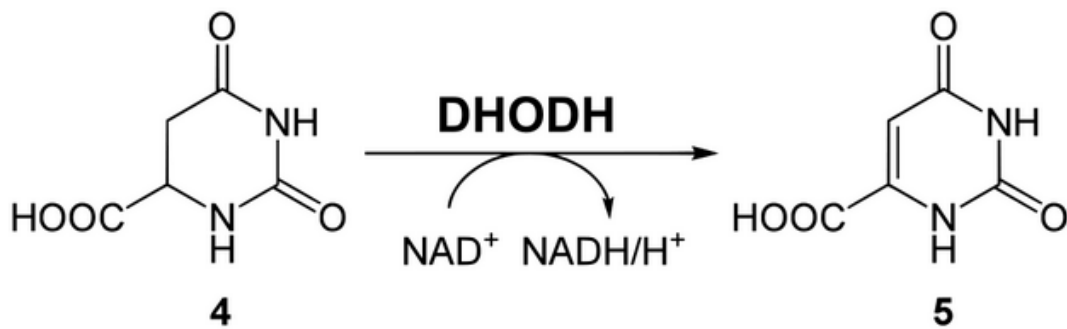


Note: The pyrimidine synthesis key reaction represents the reaction between carbamoyl phosphate and aspartate to carbamoylaspartate.

Dihydroorotic acid develops from carbamoyl aspartate through a water (H₂O) separation. This second step of the pyrimidine synthesis includes a carbamoyl aspartate cyclisation and involves the **dihydroorotase** enzyme.

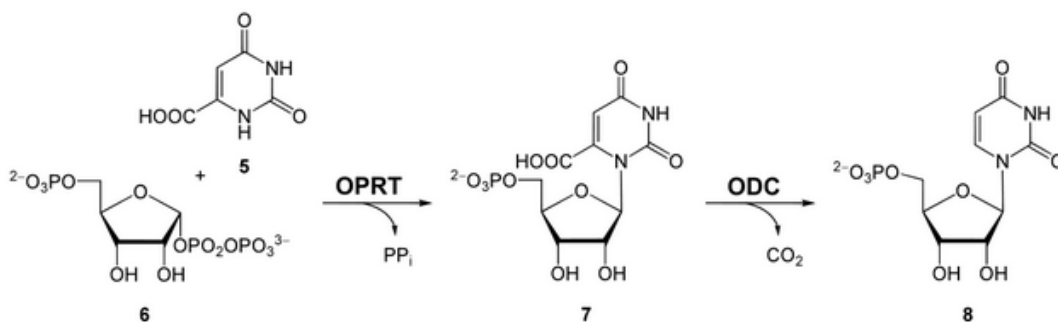


The following step is an oxidation of **dihydroorotic** acid to **orotic acid** through the **orotic-acid-dehydrogenase** enzyme. This enzyme has NAD⁺ as coenzyme, which comes out as NADH + H⁺ at the end of this reaction.



Orotic acids form **orotidine-5-phosphate** with phosphoribosyl pyrophosphate (PRPP) through the separation of pyrophosphate (PP). This reaction is mediated by the **orotate-phosphoribosyltransferase**.

From **orotidine-5-phosphate**, **uridine-5-phosphate** (UMP) develops within a **decarboxylation** process (through a separation of CO₂) through the **orotidine-5-phosphate-decarboxylase** enzyme.



The **uridine-5-phosphate** constitutes the primary product of a series of further reactions. Firstly, **UDP** results from **UMP** via phosphorylation, which can be converted to a **UTP** through further phosphorylation. The phosphate group required for this is obtained through an ATP-to ADP-reaction.

In turn, through an ATP and glutamine-dependent reaction, CTP (**cytidine triphosphate**) can be obtained from UTP. This reaction is mediated by the **CTP-synthetase** enzyme.

On the other hand, uridine-5-phosphate can be reduced to d-UMP through d-TMP-synthetase (**thymidylate-synthase**). This reduction is NADPH + H⁺-mediated. The following step of the d-TMP-synthetase consists of a **methylation** of d-UMP to **d-TMP**. The necessary methyl group is obtained through the **N5-N10-Methylene-H4-Folate**, which subsequently emerges as H₂-Folate.

Deoxy-forms Synthesis of Purine and Pyrimidine Nucleotides

The final product of both the purine and the pyrimidine nucleotide synthesis (see above) is the **ribonucleotide**, which must be reduced further into the **2'-deoxy-form** in order to be incorporated in the DNA.

The enzyme involved in this is **ribonucleotide-reductase**, which has **thioredoxin** as a cofactor. Thioredoxin, in turn, contains two **SH groups**, which are converted to a disulfide form in the reduction process. Thioredoxin in its disulfide form is reconverted to

its original form through NADP⁺-dependent **thioredoxin reductase**.

Degradation of Purine Nucleotide or Purine Bases

Like the purine nucleotide construction, their degradation also takes place in multiple individual steps. In this process, each step's differentiation from the others is partly based on their dependence from their respective purine base (adenosine or guanosine).

However, the first step of the degradation is a conversion from nucleotide to nucleoside. This takes place through a hydrolytic separation through the **nucleotidase** enzyme. Additionally, a phosphorylic separation in a free base (purine or pyrimidine) and in ribose-1-phosphate takes place. The nucleoside phosphorylase enzyme mediates this step. The degradation of the purine bases adenosine and guanosine takes place afterwards.

In a first step, adenosine is converted into inosine through NH₃ separation (**deamination**), involving the adenosine deaminase enzyme. The second step is identical for both inosine and guanosine. Both are converted to hypoxanthine (**inosine**) or guanine (**guanosine**) through an ATP-dependent ribose separation. The enzyme involved in this is nucleoside phosphorylase.

During the next step, **xanthine** is obtained from inosine and **hypoxanthine**. However, this step is different for both cases. Guanine is deaminated to xanthine, while hypoxanthine is oxidized to xanthine through xanthine oxidase.

Note: Xanthine oxidase is an iron-bearing flavoprotein, which contains a molybdate atom in its active centrum.

A further step which is mediated by xanthine oxidase is the conversion of xanthine to uric acid. This step also represents an oxidation, where molecular oxygen serves as a means for oxidation. Uric acid will be pruned over urine.

Enzyme Defects That Can Lead to an Altered Uric Acid Level

The uric acid level plays a clinical role since, during a solubility products exceedance (ca. 7 mg/100 ml), a **urate crystal** deficit in the tissue can occur. Moreover, this can cause local inflammation – the corresponding clinically manifest disease is called **gout**.

Urate crystals fail particularly in badly capillarized tissues, probably because a low temperature promotes a urate crystal deficit. That is why e.g., the metatarsophalangeal joint (**podagra**), but also the cornea or the lens, are particularly affected by this.

The gout-affected joint appears flushed, overheated and swollen, besides causing a very strong pain. These clinical signs are the cardinal ones of an inflammation (**rubor, calor, tumor, and dolor**).

Along with purine metabolism, enzyme defects can lead to either an increased or a diminished uric acid level. Among others, enzyme defects that can cause an increased uric acid level are a partial and a complete lack of **hypoxanthine-guanine-phosphoribosyltransferase**. A partial lack prevents the recycling of IMP to GMP, thus increasing the de novo purine synthesis. The respective disease is called **Kelley Seegmiller syndrome**.

A complete hypoxanthine-guanine-phosphoribosyltransferase lack is called **Lesch Nyhan syndrome**. Clinically, affected children present a trio of hyperuricemia, progressive kidney insufficiency and neurologic symptoms, for example the tendency to self-mutilate.

One enzyme defect, which, on the other hand, can lead to a diminished uric acid level, is a reduced xanthine oxidase activity (**xanthinurie**).

Note: Xanthine oxidase activity can be intentionally diminished through the **allopurinol** agent within a gout therapy.

Besides enzyme defects, other factors, like the renal function (uric acid secretion), an increased cell turnover (e.g., in diseases like leukemia) or a strongly purine-based diet (e.g., flesh), can also influence the uric acid level.

Pyrimidine Nucleotide and Bases Degradation

The first step of pyrimidine nucleotide degradation is the conversion of nucleotides to nucleosides, similar to the purine nucleotide degradation process (see above). Here, the respective steps are identical.

The degradation of cytosine and thymine, which has been obtained during the first step of the pyrimidine bases degradation, takes place in the liver. Here, the **pyrimidine ring** is broken down through multiple steps and pyrimidine bases are consequently degraded.

Note: The ring is degraded during the pyrimidine nucleotide degradation, while the purine ring is preserved during the pyrimidine nucleotide degradation.

The two bases go through two independent degradation ways in which the reaction steps are identical, except for the first step regarding cytosine degradation.

In the first step, cytosine is degraded to **uracil** through a separation of the amino group. Uracil and thymine are, in turn, reduced to **dihydrouracil** and **dihydrothymine** through a NADPH + H⁺-dependent reaction.

Through a separation of CO₂ and NH₃, **β-alanine** develops from dihydrouracil, while **β-aminobutyrate** results from dihydrothymine.

β-alanine and β-aminobutyrate are partially further degraded into respectively **acetate** and **propionate**, NH₃ and CO₂ via multiple intermediate steps. The nitrogen atoms that were obtained by this become a part of the urea cycle.

Recycle of Purine and Pyrimidine Nucleotides (Salvage-Pathways)

Since purine nucleotide degradation doesn't result in any energy gain and pyrimidine nucleotide degradation only provides a small one, while the synthesis of both needs a great amount of energy, recycling is energetically more convenient.

However, the exact steps of recycling are only known for purine bases, which is why only those are covered here.

First, during the purine bases recycling, they are **phosphoribosylized** to nucleotides through PRPP. The transmission of each purine base to PRPP takes place for adenine through **adenine-phosphoribosyltransferase** and for hypoxanthine and guanine through **hypoxanthine-guanine-phosphoribosyltransferase**.

Through the synthesized final product, both enzymes are inhibited. The final product is **AMP** for adenine, while hypoxanthine results in **IMP**. The end product of guanine is **GMP**.

Review Questions

The answers can be found below the references.

1. Which of the following substances does not belong to nucleotides?

- A. Adenine
- B. Guanine
- C. Thymine
- D. Cytosine
- E. Glutamine

2. Which statement about purine metabolism is not correct?

- A. The final product of purine degradation in humans is uric acid.
- B. Adenine-Phosphoribosyltransferase is responsible for adenine recycling.
- C. The rate-limiting reaction in purine nucleotide synthesis is mediated by glutamine-phosphoribosylpyrophosphate-amidotransferase.
- D. The AMP synthesis is ATP-dependent.
- E. The GMP synthesis is NAD⁺-dependent and ATP-dependent.

3. Which statement about pyrimidine metabolism is not correct?

- A. The key reaction in pyrimidine nucleotide synthesis includes the reaction of carbamoyl phosphate and aspartate to carbamoyl aspartate.
- B. Uridine Monophosphate (UMP) is produced through a decarboxylation from orotidine-5-phosphate.
- C. The first step of pyrimidine nucleotide degradation is the conversion from nucleosides to nucleotides.
- D. The nitrogen atoms that were obtained during degradation are introduced in the urea cycle.
- E. Cytosine is degraded through the separation of NH₃ to uracil.

4. Which sentence about folic acids is not correct?

- A. Folic acids consist of a pteridine rest, a p-aminobenzoic acid rest and a cytosine rest.
- B. The biologically active form is tetrahydrofolic acid (TH₄).
- C. Folic acid activation is ATP-dependent.
- D. The product of dihydrofolate reductase is 7, 8-dihydrofolic acid.
- E. The function of the active form is to transmit NH₃-groups.

References

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Correct answers: 1E, 2D, 3C, 4B

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Notes