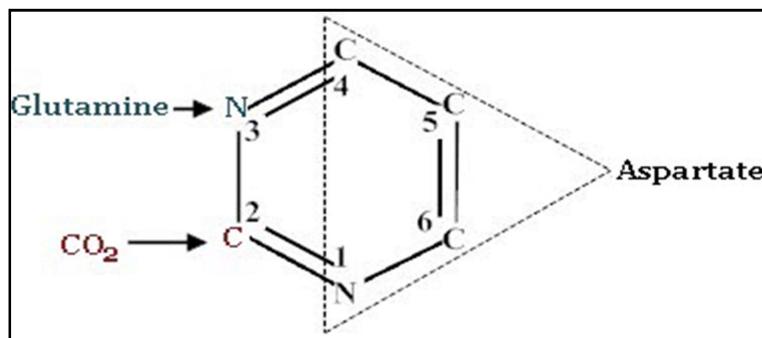


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PYRIMIDINE METABOLISM

- The nitrogenous bases i.e. Purines and Pyrimidines found in nucleotides (and, therefore, nucleic acids) are aromatic heterocyclic compounds.
- The pyrimidine **cytosine (C)** is found in both DNA and RNA. However, the nucleic acids differ with respect to the second pyrimidine base. **DNA** contains **thymine (T)** whereas **RNA** contains **uracil (U)**.
- Thymine and uracil differ in structure by the presence (in T) or absence (in U) of a methyl group.
- Pyrimidine bases are not synthesized as such, but they are formed as ribonucleotides/ deoxyribonucleotides.
- Purines and pyrimidines are **Non-essential** in the diet as dietary nucleic acids are degraded by pancreatic ribonuclease and deoxyribonuclease to mononucleotides. They are then converted to nucleosides by mononucleotidase and finally to free bases by nucleosidases. The phosphate and sugar produced by the digestion of nucleic acids are reused. Very little dietary purines and pyrimidines are incorporated into Nucleic acids.
- Most of the pyrimidine bases are catabolized and excreted. Pyrimidine degradation leads to formation of urea.

Sources for pyrimidine ring



- Glutamine provides N_3
- Aspartic acid/ aspartate furnishes C_4 , C_5 , C_6 & N_1
- CO_2 provides C_2

PYRIMIDINE SYNTHESIS

- Purine and pyrimidine nucleotides are synthesized by two pathways:

De novo synthesis:

- In de novo synthesis, pyrimidines are synthesized from the smaller precursor molecules such as **aspartic acid/ aspartate, glutamine and CO_2** . This pathway is expensive; several reactions require ATP.

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PYRIMIDINE METABOLISM

Salvage pathway:

- In salvage pathway the free bases and nucleosides released during the nucleic acid breakdown are reused.
- Both types of pathways are important.

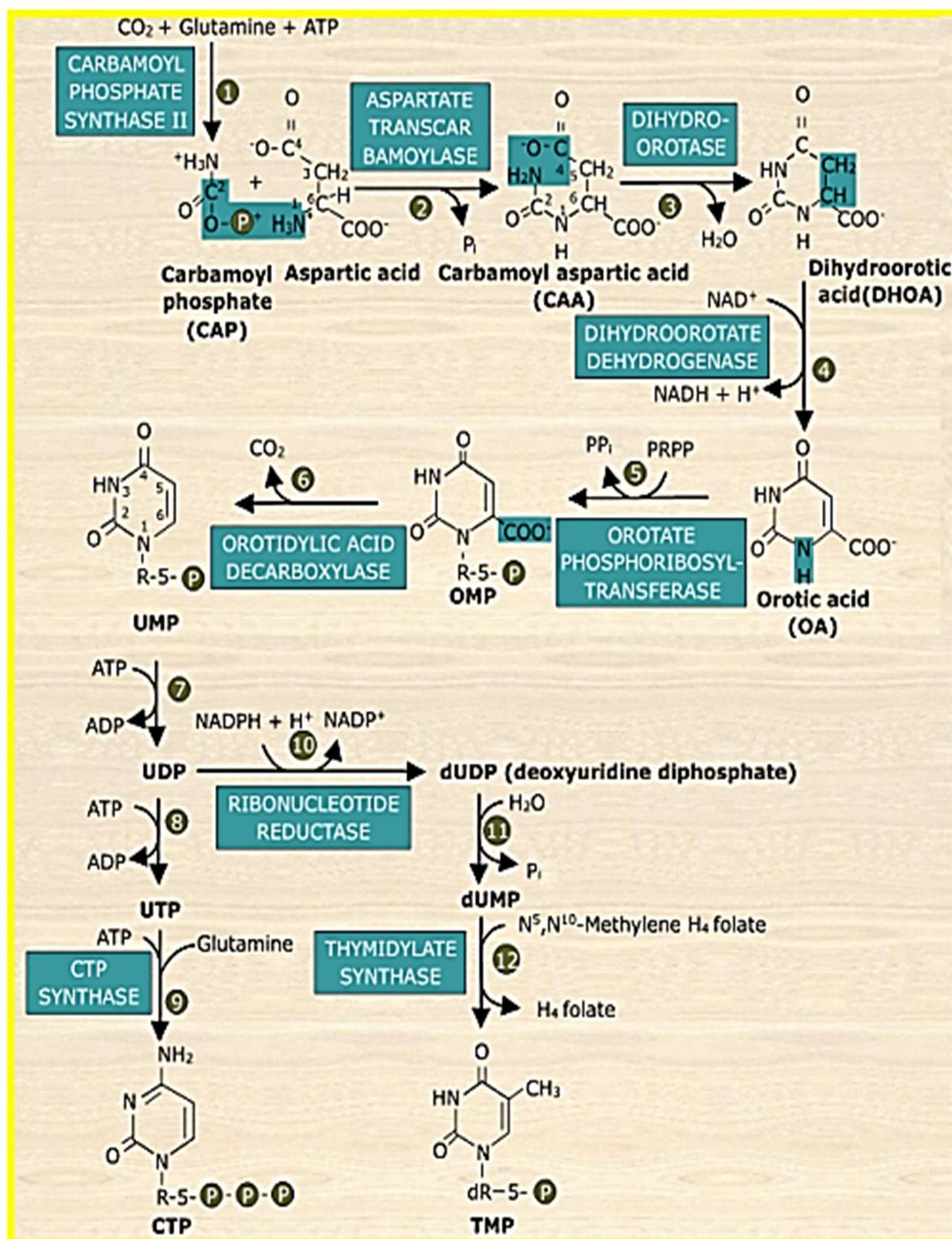
De novo Pathway of Pyrimidine Synthesis

- The common pyrimidine ribonucleotides are cytidine 5'- monophosphate (CMP; cytidylate) and uridine 5'- monophosphate (UMP; uridylate), which contain the pyrimidines cytosine and uracil. De novo pyrimidine nucleotide biosynthesis proceeds in a somewhat different manner from purine nucleotide synthesis; the six-membered pyrimidine ring is made first and then attached to ribose 5-phosphate. (This is in contrast to purine nucleotide synthesis wherein purine ring is built upon a pre-existing ribose 5-phosphate). Required in this process is **carbamoyl phosphate**, also an intermediate in the urea cycle.
- Therefore, Carbamoyl phosphate and aspartate are the precursors of pyrimidines ring.
- The **first step** is the **formation of carbamoyl phosphate**. Glutamine transfers its amido nitrogen to CO₂ to produce carbamoyl phosphate in the presence of 2 ATP. This reaction is ATP-dependent and is catalysed by cytosomal enzyme **carbamoyl phosphate synthetase II (CPS II)**, present in the **cytosol**. (CPS-I present in mitochondria which synthesizes carbamoyl phosphate from ammonia NH₄ and CO₂ and, in turn urea synthesis).
- Carbamoyl phosphate is combined with **aspartic acid/aspartate** to form **carbamoyl aspartate** catalyzed by the enzyme **aspartate transcarbamoylase (ATCase)**.
- Carbamoyl aspartate cyclizes to form a six membered ring, **dihydroorotate** by the enzyme **dihydroorotase**.
- Two hydrogen atoms are removed from the ring by the enzyme **dihydroorotate dehydrogenase** to form **orotic acid**. This **dihydroorotate (DHOA) dehydrogenase** enzyme is the only enzyme which is present in **Mitochondria**.
- The next step is the **addition of PRPP (Ribose 5-phosphate) to orotate** to produce **orotidine monophosphate (OMP)**. This reaction is catalysed by **orotate phosphoribosyltransferase**, an enzyme comparable with HGPRT in its function.
- The OMP is then decarboxylated to form **uridine mono-phosphate (UMP)** mediated by the enzyme **OMP decarboxylase**.
- UMP is converted to UDP by the enzyme **nucleoside monophosphate kinase**. It is again phosphorylated to form UTP by **nucleoside diphosphate kinase**.
- UTP reacts with **glutamine** in an ATP dependant reaction to form **cytidine triphosphate (CTP)**, which has an amino group on C-4. This conversion utilizes the amide nitrogen of glutamine.
- Similarly, from UDP, by the action of ribonucleotide reductase, **dUDP** is formed, which is converted to **dUMP** by phosphorylase.

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PYRIMIDINE METABOLISM

- Then, thymidylate synthase methylates dUMP into **TMP**. The methyl donor is **N^5,N^{10} - methylene H₄ folate**. This reaction is inhibited by **Methotrexate** (anticancer drug).



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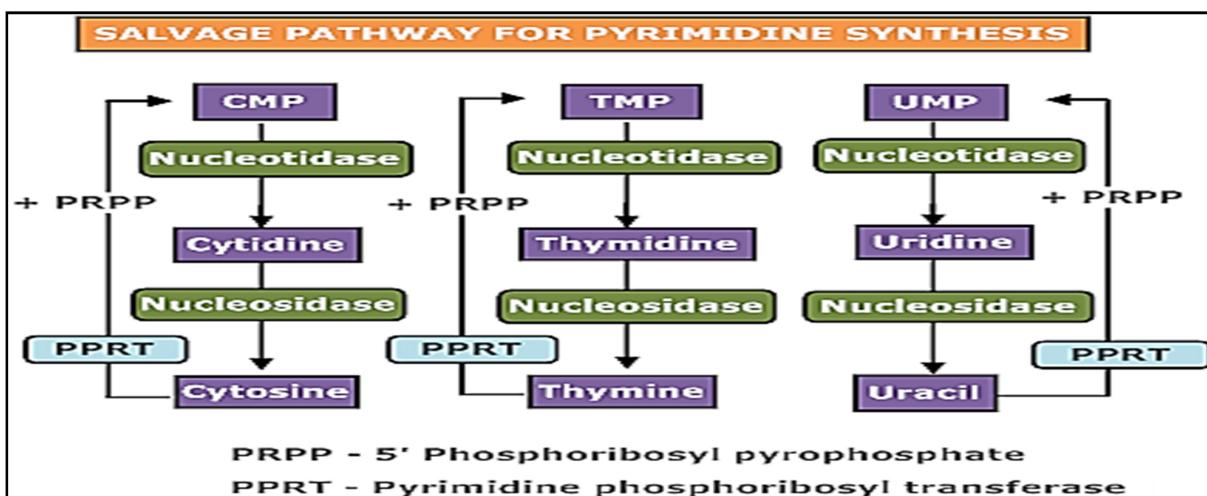
PYRIMIDINE METABOLISM

Regulation of pyrimidine synthesis

- **Carbamoyl phosphate synthetase II (CPS II)** is the regulatory enzyme of pyrimidine synthesis in animals. It is activated by PRPP and ATP and inhibited by UDP and UTP.
- In bacteria, **aspartate transcarbamoylase (ATCase)** catalyses a committed step in pyrimidine biosynthesis. ATCase is a good example of an enzyme controlled by **feedback mechanism** through CTP i.e. end product. In certain bacteria, UTP also inhibits ATCase. ATP, however, stimulates ATCase activity.
- **OMP decarboxylase**, inhibited by UMP and CMP, also controls pyrimidine formation.
- In eukaryotes, the first three enzymes in this pathway—carbamoyl phosphate synthetase II, aspartate transcarbamoylase, and dihydroorotate—are part of a single trifunctional protein. The protein, known by the acronym **CAD**, contains three identical polypeptide chains (each of Mr 230,000), each with active sites for all three reactions. This suggests that large, multienzyme/ multifunctional complexes may be the rule in this pathway.
- Orotate phosphoribosyltransferase and OMP decarboxylase are domains of a single protein. A defect in this bifunctional enzyme causes **orotic aciduria**

Salvage Pathway

- It is a metabolic pathway that utilizes compounds formed in catabolism for biosynthetic reactions.
- During cellular metabolism and during digestion in animals nucleic acids are degraded to mononucleotides, nucleosides and finally to free bases. Some of the purines and pyrimidines formed in this way are further degraded. But considerable amount is salvaged by reacting with PRPP to reform nucleotides.
- The recycling of bases conserves cellular energy.
- Pyrimidines are converted to nucleotides by the enzyme **pyrimidine phosphoribosyltransferase (PPRT)** utilizing PRPP as the source of ribose.

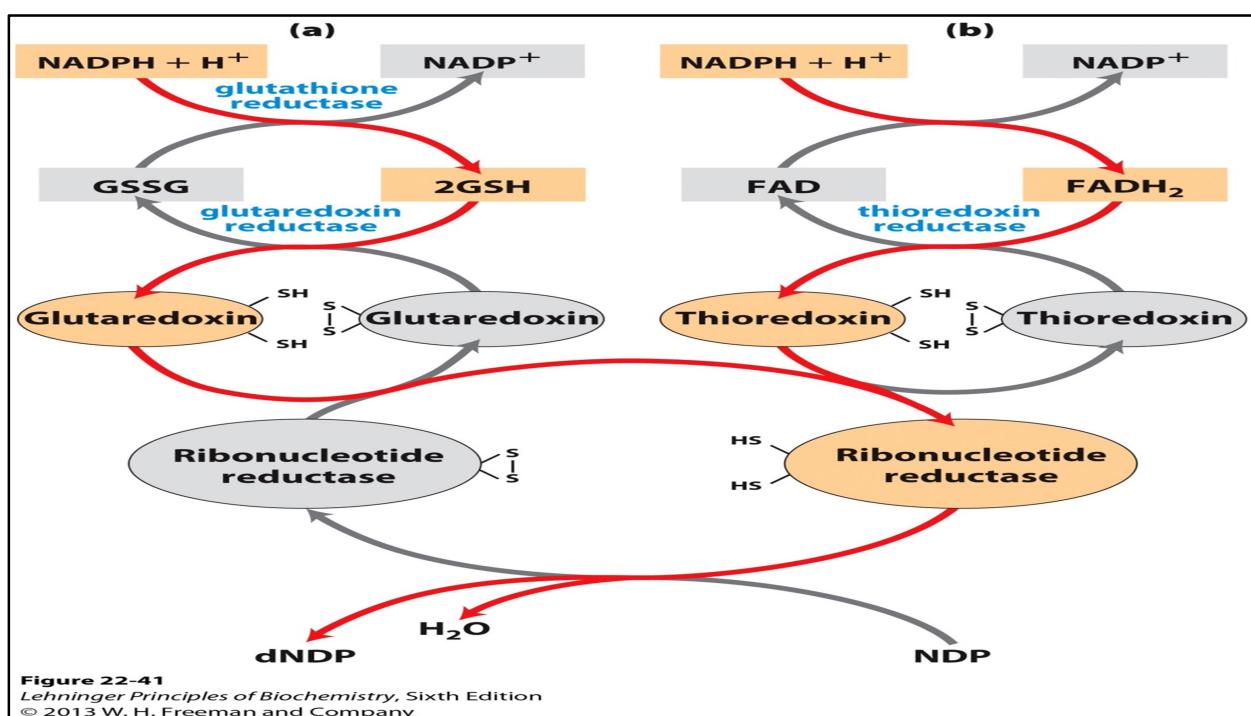
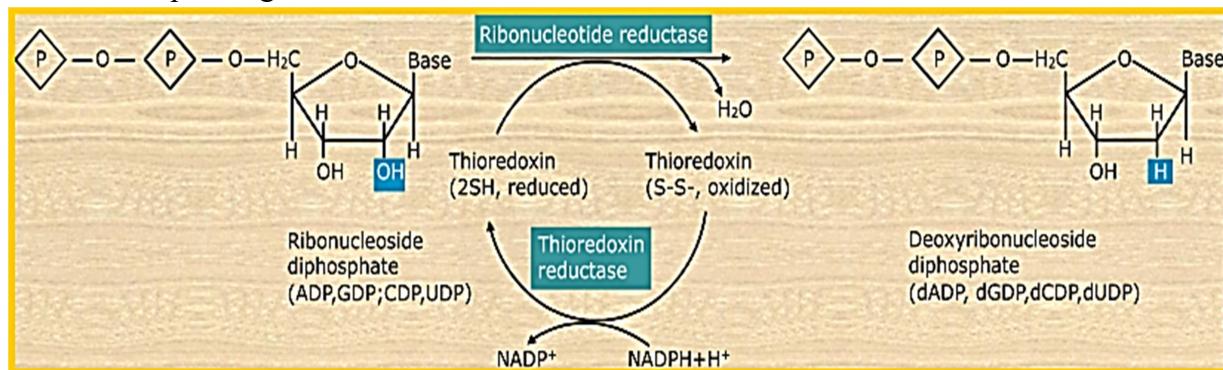


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PYRIMIDINE METABOLISM

FORMATION OF DEOXYRIBONUCLEOTIDES

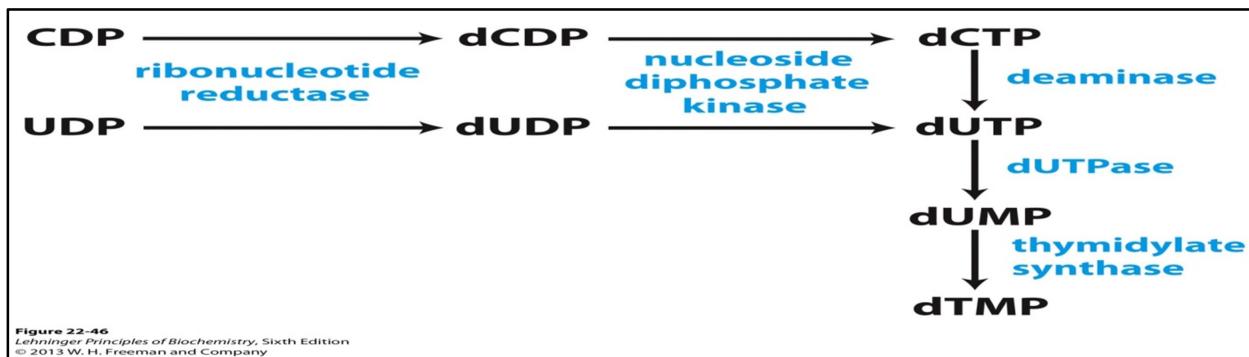
- Ribonucleotides are converted to deoxyribonucleotides by the replacement of the 2'-hydroxyl group by hydrogen. The substrates are ribonucleoside diphosphates. The enzyme **ribonucleotide reductase** complex catalyzes this reaction. In this way nucleotide diphosphates such as ADP, GDP, CDP and UDP are converted to their corresponding dADP, dGDP, dCDP and dUDP.



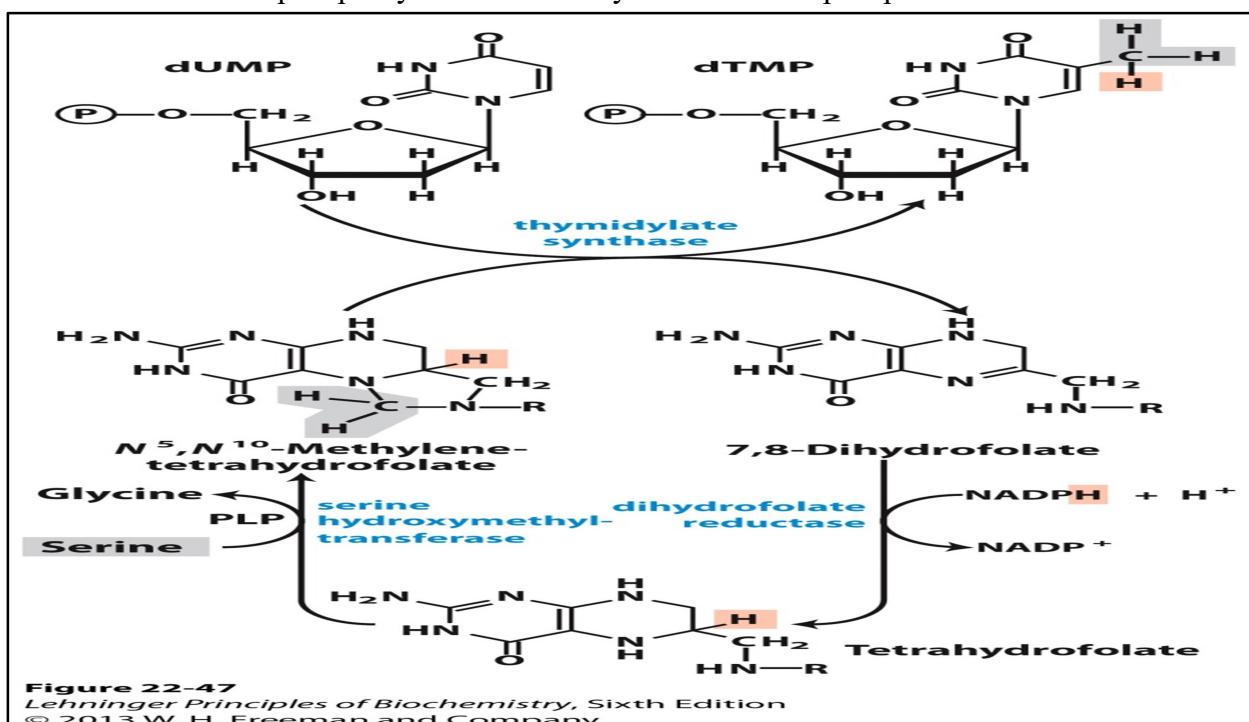
Reduction of ribonucleotides to deoxyribonucleotides by ribonucleotide reductase. Electrons are transmitted to the enzyme from NADPH by (a) glutaredoxin or (b) thioredoxin. The sulfide groups in glutaredoxin reductase are contributed by two molecules of bound glutathione (GSH; GSSG indicates oxidized glutathione). Thioredoxin reductase is a flavoenzyme, with FAD as prosthetic group. dNDP: deoxynucleoside diphosphates.

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PYRIMIDINE METABOLISM



- DNA contains thymine rather than uracil. The immediate precursor is dUMP.
- First the dUTP is hydrolyzed to dUMP.
- $dUTP + H_2O \rightarrow dUMP + PP_i$.
- The dUMP is converted to dTMP by **thymidylate synthase**, which utilizes N^5, N^{10} -methylene tetrahydrofolate as the source of methylene group. The immediate donor of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group is given by a protein called thioredoxin (a protein with two cysteine residues), which after donating hydrogen atoms forms disulfide bonds. **Thioredoxin reductase** catalyzes the formation of reduced thioredoxin using NADPH as coenzyme.
- The dTMP is phosphorylated to dTTP by the transfer of phosphate from ATP.



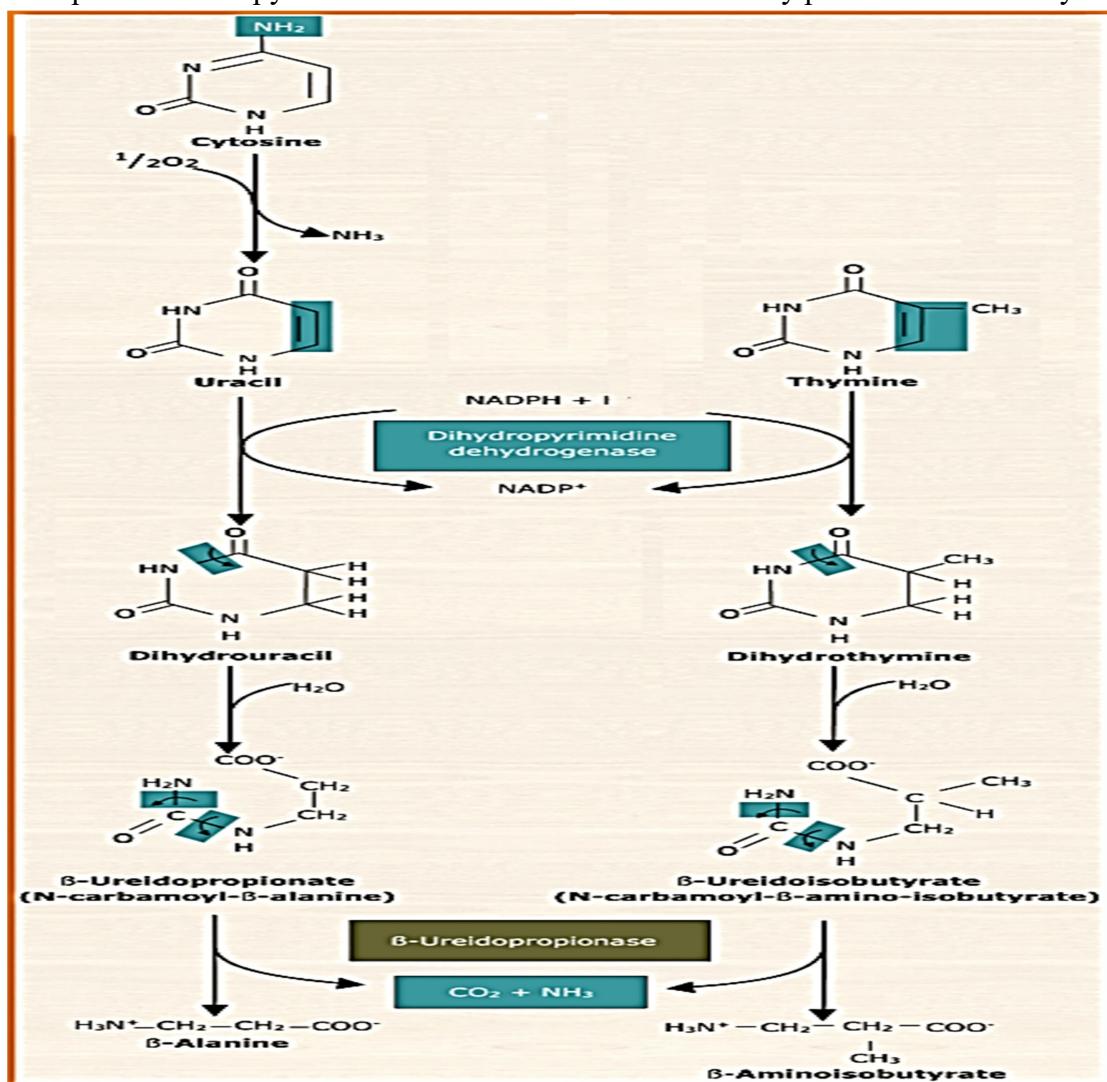
Conversion of dUMP to dTMP by thymidylate synthase and dihydrofolate reductase. Serine hydroxymethyltransferase is required for regeneration of the N^5, N^{10} -methylene form of tetrahydrofolate. In the synthesis of dTMP, all three hydrogens of the added methyl group are derived from N^5, N^{10} -methylenetetrahydrofolate

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PYRIMIDINE METABOLISM

PYRIMIDINE CATABOLISM

- Pyrimidine nucleotides are hydrolyzed to the corresponding nucleosides and Pi by nucleotidases. Then ribose is removed to give the free bases cytosine, uracil and thymine.
- **Cytosine** is deaminated to produce uracil, which can be converted to dihydrouracil by the enzyme uracil dehydrogenase. The ring is then opened by hydrolysis to yield N-carbamoyl β -alanine. This compound is hydrolyzed to β -alanine, ammonia and CO₂. β -alanine is converted to **acetyl Co A**.
- **Thymine** is also metabolized in a series of parallel reactions forming CO₂, ammonia and β -aminoisobutyrate, which is converted to **succinyl Co A**. The compounds formed are highly water-soluble.
- Ammonia and CO₂ will be used in the synthesis of **urea** and excreted.
- Overproduction of pyrimidine metabolites does not cause any problem for the body.



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PYRIMIDINE METABOLISM

Disorders of pyrimidine metabolism

- **Orotic aciduria:** This is a rare metabolic disorder characterized by the excretion of orotic acid in urine, severe anemia and retarded growth. It is due to the deficiency of the enzymes Orotate phosphoribosyl transferase and OMP decarboxylase of pyrimidine synthesis. Both these enzyme activities are present on a single protein as domains (bifunctional enzyme). Feeding diet rich in uridine and/or cytidine is an effective treatment for orotic aciduria. These compounds provide (through phosphorylation) pyrimidine nucleotides required for DNA and RNA synthesis. Besides this, UTP inhibits carbamoyl phosphate synthetase II and blocks synthesis of orotic acid.
- **Reye's syndrome:** This is considered as a secondary orotic aciduria. It is believed that a defect in ornithine transcarbamoylase (of urea cycle) causes the accumulation mitochondrial of carbamoyl phosphate. This is then diverted for the increased synthesis and excretion of orotic acid.

ANTIMETABOLITES

These are synthetic analogue of Nucleosides. Several drugs that are used in the treatment of bacterial/ viral infections or in cancer therapy act by inhibiting directly or indirectly the nucleotide (purine/ pyridimine) metabolism or either by inhibiting their synthesis or by competing with them in DNA or RNA synthesis. Their maximal cytotoxic effects are in S-phase (and are, therefore, cell-cycle specific).

- **5-fluorouracil:** It is used in the treatment of cancer; it interferes with the **thymidylate** synthesis. In the cells salvage pathways convert dUMP to FdUMP, which then binds to the enzyme thymidylate synthase thereby inactivates the enzyme. Inhibition of the enzyme decreases cellular level of thymidine nucleotides. Cells lacking thymine will die.
- **6-mercaptopurine:** It is a **hypoxanthine analog**, which inhibits purine nucleotide biosynthesis.
- **Azaserine and acivicin:** They are **glutamine analogues**. Glutamine acts as a nitrogen donor in a number of reactions in the synthesis of nucleotides. The reaction is catalyzed by the enzyme glutamine amidotransferase. The reaction is inhibited by the presence of glutamine analog.
- **Sulfonamide antibiotics:** They are used in the treatment of various infectious diseases. These are structural analog of p-amino benzoic acid, a component of folate. Sulfonamides inhibit competitively the incorporation of p-amino benzoate into folate. Humans are resistant to the action of sulfonamides because folate synthesis does not occur in human cells and preformed folate, a vitamin is required.
- **Trimethoprim:** is an antibiotic that inhibits dihydrofolate reductase in sensitive bacteria. Human dihydrofolate reductase is resistant to the action of trimethoprim.
- **Azathiopurine:** is an immunosuppressive agent used in the treatment of tissue rejection in individuals who have organ transplant. This compound is converted to mercaptapurine by glutathione.

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PYRIMIDINE METABOLISM

- **Methotrexate:** it is used in the cancer therapy; it is a **folate analog**, which competitively inhibits the enzyme dihydrofolate reductase. For coenzyme activity, dihydrofolate should be reduced to tetrahydrofolate catalyzed by the enzyme dihydrofolate reductase. Methotrexate prevents the regeneration of tetrahydrofolate.
- **Aminopterin:** also inhibits the activity of dihydrofolate reductase.
- **Allopurinol:** It (**anti-gout drug**) was developed by Gertrude Elion and George Hitchings, who also developed acyclovir, used in treating people with AIDS, and other **purine analogs** used in cancer chemotherapy.
- **Purine antagonist** used for the treatment of malignant diseases (mercaptopurine, thioguanine), but also for immunosuppression (azathioprine) and antiviral chemotherapy (**acyclovir**, ganciclovir, vidarabine, and zidovudine).

Antimetabolites

Folate antagonist:	Methotrexate (Mtx).
Purine antagonist:	6-Mercaptopurine (6-MP), 6-Thioguanine (6-TG), Azathioprine, Fludarabine.
Pyrimidine antagonist:	5-Fluorouracil (5-FU), Capecitabine Cytarabine (cytosine arabinoside).