Purine and Pyrimidine metabolism

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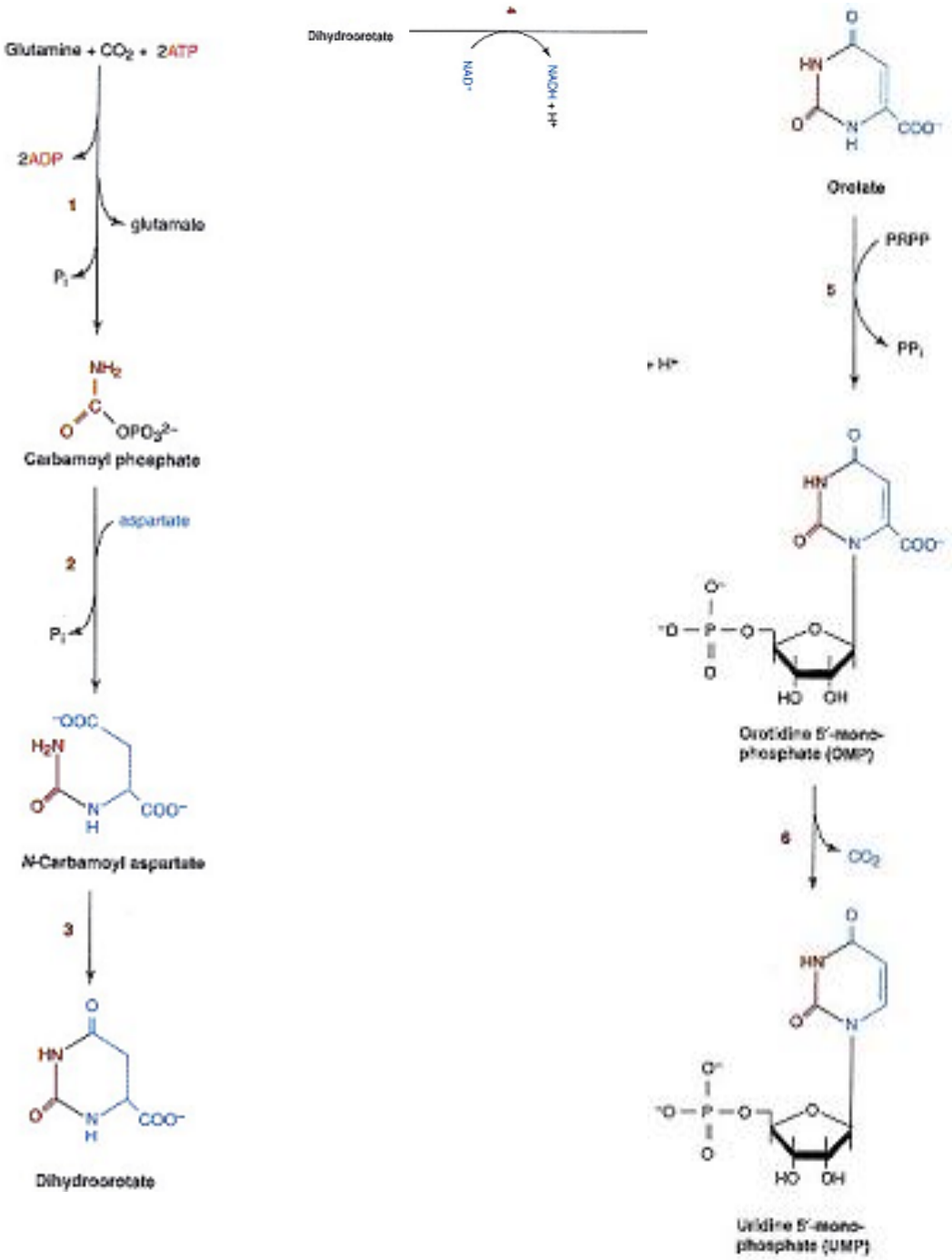
Lecture 2

Metabolism of pyrimidine nucleotides

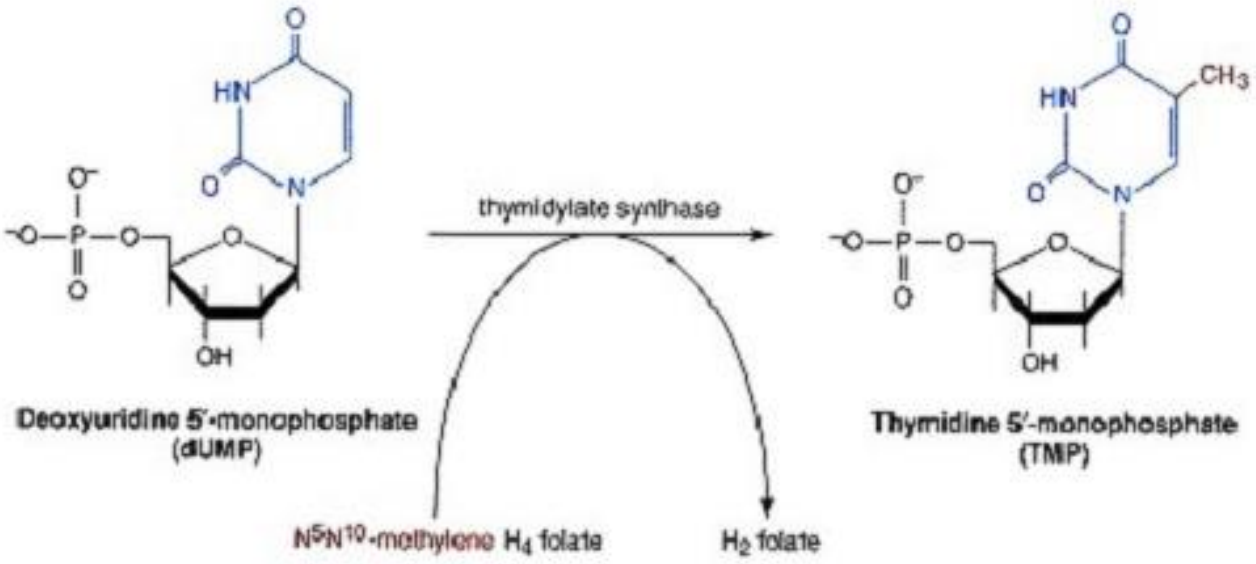
- The pyrimidine ring is synthesized *de novo* in mammalian cells using amino acids as carbon and nitrogen source and CO_2 as carbon donor.
- *De novo* synthesis of pyrimidine nucleotide leads to uridine 5'-monophosphate (UMP).
- In contrast to *de novo* purine nucleotide synthesis, all enzymes of *de novo* pyrimidine synthesis are not found in the cytosol of the cell.
- The pyrimidine ring is formed first and then ribose 5-phosphate is added via PRPP.
- The enzyme catalyzing the formation of carbamoyl phosphate,carbamoyl phosphate synthetase II, is cy tosolic and is distinctly different from carbamoyl phosphate synthetase I found in the mitochondria as part of urea cycle.



1= carbamoyl phosphate synthetase II
2= aspartate carbamoyl transferase
3= dihydroorotase,
4= dihydroorotate dehydrogenase
5= orotate phosphoribosyltransferase
6= OMP decarboxylase.

Deoxythymidylate Synthesis

- Deoxythymidylate (dTMP) is formed from 2 deoxyuridine 5 -monophosphate (dUMP).
- Thymidylate synthase catalyzes the reaction in which a one-carbon unit from N^5,N^{10}-
  methylene tetrahydrofolate is transferred to dUMP and simultaneously reduced to a methyl group.



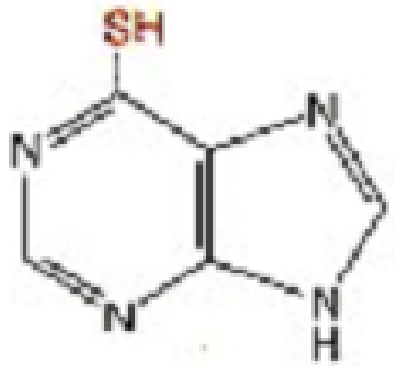
Purine and pyrimidine synthesis in Cancer

- Levels of ribonucleotide reductase, thymidylate synthase and IMP dehydrogenase increase as a function of tumor growth rate.
- While some enzymes are increased in both fast-growing normal tissue (e.g., embryonic and regenerating liver) and tu mors, the total quantitative and qualitative patterns for normal and tumor tissue can be distinguished.
- Compounds that are specific inhibitors of enzymes involved in nucleotide synthesis or interconversions have proved to be useful chemotherapeutic agents.
- These include antimetabolites, antifolates and glutamine antagonists.

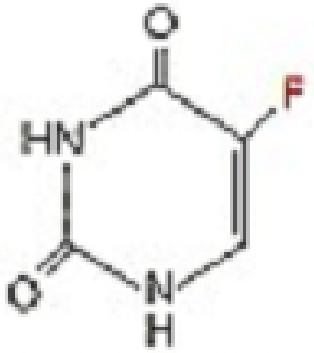
Antimetabolites

- Antimetabolites are structural analogs of purine and pyrimidine bases or nucleosides that interfere with very specific metabolic sites.
- They include 6-mercaptopurine and 6-thioguanine for treatment of acute leukemia.
- **6Mercaptopurine** (6-MP) is a useful antitumor drug in humans. The cytotoxic activity of this agent is related to formation of 6-mercaptopurine ribonucleotide by the tumor cell.
- Utilizing PRPP and HGPRTase, 6mercaptopurine ribonucleoside 5-monophosphate accumulates in the cell and is a negative effector of PRPP amidotransferase the committed step in *de novo* pathway.
- Since 6mercaptopurine is a substrate for xanthine oxidase and is oxidized to 6-thiouric acid, allopurinol is generally administered to inhibit degradation of 6-

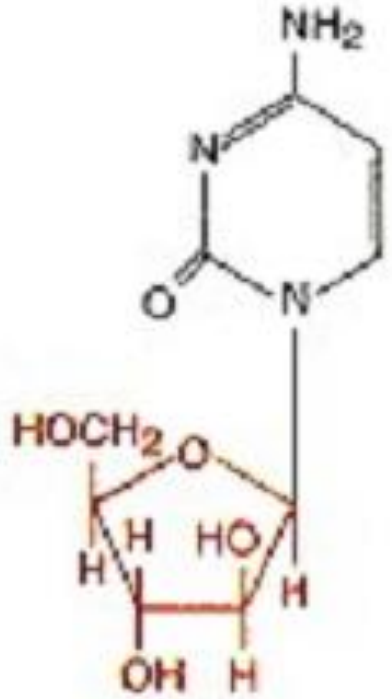
Structures of 6mercaptopurine, 5-fluorouracil, and cytosine arabinoside.



6-Mercaptopurine



5-Fluorouracil



Cytosine arabinoside

5Fluorouracil

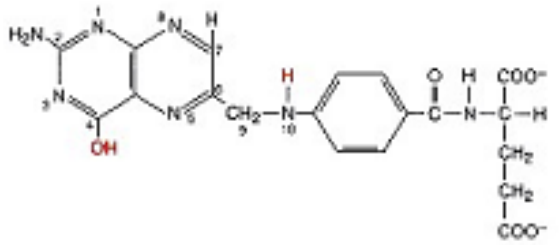
- 5Fluorouracil is a pyrimidine analog of uracil. 5Fluorouracil is inactive on itself and requires activation. It must be converted by cellular enzymes to active metabolites 5-Fluorouridine 5-triphosphate (FUTP) and 5-Fluoro-2’-deoxyuridine-5’-monophosphate (FdUMP).
- FUTP is efficiently incorporated into RNA and once incorporated into RNA inhibits maturation of 45S precursor rRNA into 28S and 18S species and alters splicing of pre-mRNA into functional mRNA.
- FdUMP is a potent and specific inhibitor of thymidylate synthase. The covalent bonding of FdUMP to thymidylate synthase results in inhibition of dTMP synthesis and leads to death of cells.

Cytosine arabinoside (araC)

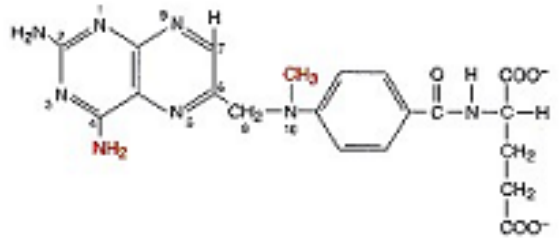
- Cytosine arabinoside is used in treatment of several forms of human cancer.
- AraC must be metabolized by cellular enzymes to cytosine arabinoside 5’-triphosphate (AraCTP) to exert its cytotoxic effects.
- AraCTP competes with dCTP in DNA polymerase reaction and araCMP is incorporated into DNA. This results in inhibition of synthesis of the growing DNA strand.
- Clinically, the efficacy of araC as an antileukemic drug correlates with the concentration of araCTP that is achieved in the tumor cell.

Methotrexate

- Methotrexate a close structural analog of folic acid, is used as an antitumor agent in treatment of human cancers.



Folic acid



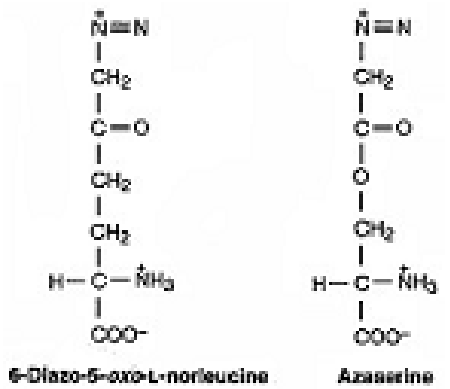
Methotrexate

- Methotrexate inhibits the formation of tetrahydrofolate by inhibition of dihydrofolate reductase (DHFR). Methotrexate causes depletion of thymidine and purine nucleotides.

- In thymidylate synthase reaction dihydrofolate is generated and unless it is reduced back to tetrahydrofolate via DHFR, cells would not be capable of *de novo* synthesis of purine nucleotides or thymidylate synthesis due to depletion of tetrahydrofolate.
- In human cancer treatment , normal cells can be rescued from the toxic effects of high dose methotrexate by leucovorin (N^5-formyl-tetrahydrofolate).

Glutamine Antagonists

- Azaserine (O-diazoacetyl-L-serine) and 6-diazo-5-oxo-l-norleucine (DON) are very effective inhibitors of enzymes that utilize glutamine as the amino donor. However these are extremely toxic and not of clinical use.



References

1. Lehninger, Nelson and Cox (2008). Principles of Biochemistry, 5th Edition
2. Jeremy M Berg, John L Tymoczko, and Lubert Stryer (2002). Biochemistry 6th Edition, New York: W H Freeman
3. Thomas M. Devlin. Textbook of Biochemistry with clinical correlations, 4th Edition

Example questions

1. Consider the three amino acid auxotrophic bacteria (that are unable to synthesize) for glycine, glutamine, and aspartate, respectively. For each mutant, what nitrogenous products other than proteins would the cell fail to synthesize?
2. Allopurinol an inhibitor of xanthine oxidase, is used to treat chronic gout. Explain the biochemical basis for this treatment. Patients treated with allopurinol sometimes develop xanthine stones in the kidneys, although the incidence of kidney damage is much lower than in untreated gout. Explain this observation.
3. Azaserine is a powerful inhibitor of glutamine amidotransferases. If growing cells are treated with azaserine, what intermediates of nucleotide biosvnthesis will accumulate? Explain.
4. Lesch-Nyhan syndrome is virtually limited to males. Explain.

MCQs

1. Nucleotides serve all of the following roles EXCEPT:
A. monomeric units of nucleic acids.
B. physiological mediators.
C. sources of chemical energy.
D. structural components of membranes.
E. structural components of coenzymes

2. The amide nitrogen of glutamine is a source of nitrogen for the:
A. *De novo* synthesis of purine nucleotides
B. *De novo* synthesis of pyrimidine nucleotides
C. Synthesis of GMP from IMP
D. All of the above
E. None o f the above

3.The type of enzyme known as a phosphoribosyltransferase is involved in all of the following EXCEPT:

A. salvage of pyrimidine bases.

B. the de novo synthesis of pyrimidine nucleotides.

C. the de novo synthesis of purine nucleotides.

D. salvage of purine bases

4. Uric acid is:

A. formed from xanthine in the presence of O_2.

B. a degradation product of cytidine.

C. deficient in the condition known as gout.

D. a competitive inhibitor of xanthine oxidase.

E. oxidized, in humans, before it is excreted in urine

5. Which of the following antitumor agents works by impairing de novo purine synthesis?

A. Acyclovir (acycloguanosine)

B. 5-Fluorouracil (antimetabolite)

C. Methotrexate (antifolate)

D. Hydroxyurea

E. Allopurinol