GENETICS

What is PCR ? Mention it's Steps & Applications

PCR :

PCR is a Test tube method For amplifying a selected DNA sequences that does not relay on biological Cloning method permits synthesis of Million of Copies of a specific nucleotides sequence in Few hours.

STEPS :

(i) Primer construction

(ii) Denature the DNA

(iii) Anealing of primers to single standard

(iv) Chain Extension

Applications :

(i) Comparison of a normal Gene with mutant Form Gen

(ii) Detection of low Abandance nucleic Acid.

(iii) Fronseic analysis of DNA Sample.

(iv) Prenatal diagnosis and Carrier detection of cystic Fibrosa.

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Post transcriptional changes of mRNA:

(i) Endonuclear Cleavage

(ii) 5' Terminal end is capped by 7-methyl guanosine

(iii) 3' Terminal end contain Polymer of Adenylate residue. → which is poly A Tail

(iv) Methylation

(v) Removal of introns

(vi) Splicing of Axon.

Post transcriptional changes in tRNA:

(i) 16 nucleotide are remove from 5' end

(ii) 14 nucleotide are remove from Anticodon end

(iii) UUA is replace by CCA at 3' end

(iv) PHOSPHate group is added to 5' end

(v) Modification of bases

Steps of DNA replication in eukaryotes:

(i) identification of origion of replication

(ii) Denaturation of double bond strand DNA to form Single strand DNA Tempelate.

(iii) Formation of replication fork & Synthesis of RNA Primer

(iv) Initiation of DNA Synthesis & Elongation

(v) formation of replication bubble & ligation of newly Synthesis DNA segment.

(vi) Recontition of Chromatin material.

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(b) **Transition:** In this case A Purine / Pyramidine replaced by another.

Consequences:

Silent mutation: The codon Containing change base pair may code for same aminoacid

UCA ⟶ Serine

UCU ⟶ serine

Misense Mutatation:

The codon containing change base pair may code for different amino acid.

UCU ⟶ Serine

ACU ⟶ Threonine

Non-sense Mutation: The codon containing change base pair may code for terminating codon.

UCUA ⟶ serine

UAA ⟶ Terminating codon e.g. Sickle ce Anemi

2. Frame shift Mutation

When one or more Base pair inserted or deleted.

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What are mutations and mutagenic agent Mention Different Types of Mutation?

Mutation: An Alteration in genetic material result in mutation. It is defined as

"Changes in nucleotides sequence of DNA"

Mutation refer to a change in DNA structure of Gene.

Mutagenic Agent:

The substance which can induce mutation collectively caued Mutagens.

Types:

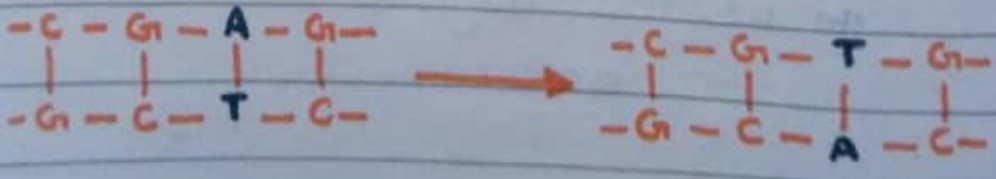
(i) Point Mutation

(ii) Frame Shift Mutation

1. **Point Mutation:** "Changes in a Single Nucleotide" Replacement of one Base Pair by another result in Point mutation.

Sub-Types:

(a) **Transversions:** These are characterized by replacement of a Purine by a Pyramidine.



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inhibitors

Reversible	Irreversible
Tetracyclin	Streptomycin
inhibit binding of aminoacyl tRNA to A site	inhibit initiation Complex formation

Chloramphnicol inhibit Peptidyl L Transferase and interfere peptide Chain elongation

Puromycin: Release of incomplete peptide.

Recombinant DNA Technology: It is a Transfer of information b/w Two Segments of DNA.

(i) Selection of Desire Peice of DNA

(ii) insertion of selected DNA into vactor i.e Plasmid to foom recombinant DNA & Chimeric DNA.

(iii) Introducing the recombinent vactor into host.

(iv) Multiplication and selection of clone Containing recombinent molecule.

(v) Expression of gene to form desire product.

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Role of Kidney in maintaining PH of Blood:

1) Excretion of H^+ ion
2) Reabsorption of bicarbonate ion
3) Excretion of tetrable acid
4) Excretion of ammonia

Role of lung in maintaining PH of Blood:

Respiratory system provide rapid but Short Term mechanism for regulating blood PH.

In acidosis (↓PH) there will be hyper ventilation to excrete more CO_2 & ↓ H_2CO_3

Role of Hb maintaining PH:

Hb serves as transport of CO_2 from tissue to lungs with minimum change in PH

Carbonic anhydrase is responsible for below rxn

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

$$H_2CO_3 + \longrightarrow H^+ + HCO_3^-$$

$$H^+ + Hb \longrightarrow HHb$$

$$HHb + O_2 \longrightarrow HbO_2 + H^+$$

$$HCO_3^- + H^+ \longrightarrow H_2CO_3$$

$$H_2CO_3 \longrightarrow H_2O + CO_2$$

In this way CO_2 eliminated through lungs.

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Significance:

1) Manufacturing of Protein, Hormone, interferance Blood Clotting Factors.

2) Diagnosis of Molecular Diseases i.e Sickle cell Anemia, cystica Fibrosica, thalesemia.

3) Prenatal Diagnosis

4) Aids test has become rapid & simple

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Write a note on Gout?

→ Gout is an abnormality of uric acid metabolism that result in Deposition of Sodium urate crystal in joints, Soft tissue and urinary tract.

Normal uric acid level: Male → 3.4–7.0 mg/dl
Female → 2.4–6.0 mg/dl

→ This disorder Characterized by Hyperuricemia.

→ In Gout, the Hyperuricemia result Primarily From underexcretion of uric acid.

overproduction of uric acid is less comon and known causes involve certain/ inborn error of metabolism or ↑ availibility of Purines.

intense joint pain, Limit range motion

→ Crystal Deposition may be seen in Soft tissues and in kidney. (urolithiasis)

inflammation & redness

Treatment:

→ Reduce Dietry purines intake and restrict Alcohol.

→ **Drugs:**

① Allopurinol: Block Formation of uric acid

② Uricase: Break down uric acid

③ probenecid: ↑ excretion of uric acid in urine.

④ Colchicine: Anti-Inflammatry agent, very useful to arrest in Arthritis in

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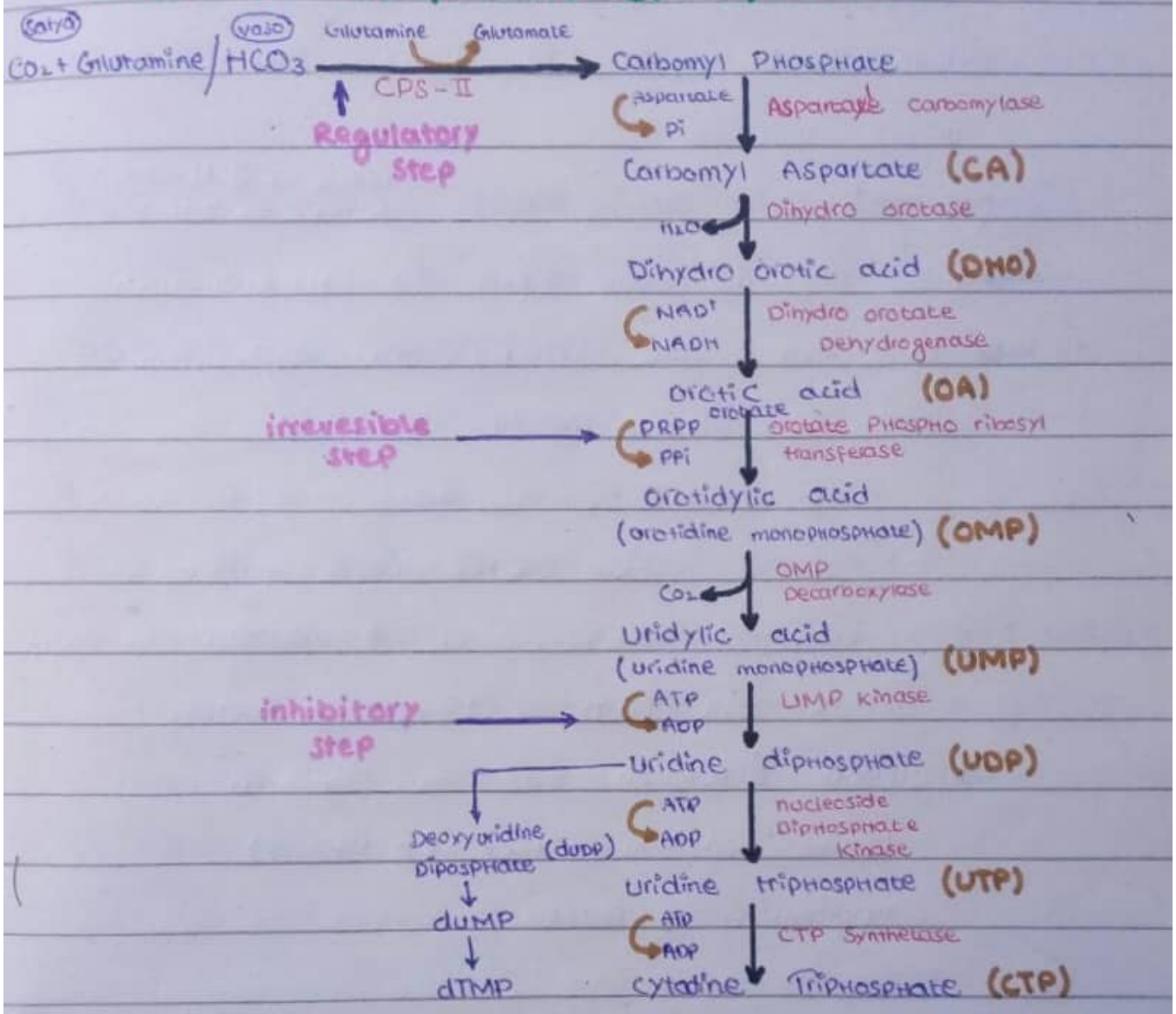
NUCLEOTIDE

Steps of Biosynthesis of Pyrimidine



Regulation:

(i) **In mamalian cell:** Carbomyl Phosphate Synthetase - II Domain of Trifunctional CAD is inhibited by UTP and Activated by PRPP. 5 Phosphoribosyl - 1 pyrophosphate.

(ii) **In prokaryotic cell:** Aspartate Transcarbomylase is inhibited by CTP and is regulated.

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Write down differences b/w CPSI & CPSII ?

variable	CPS-I	CPS-II
• Cellular location	Mitochondria	Cytosol
• pathway involve	Urea cycle	Pyrimidine Synthesis
• Source of Nitrogen	Ammonia	γ- amide group of Glutamine
• Regulators	Activator: N-acetyl-Glutamate	Activator: PRPP inhibitor: UTP

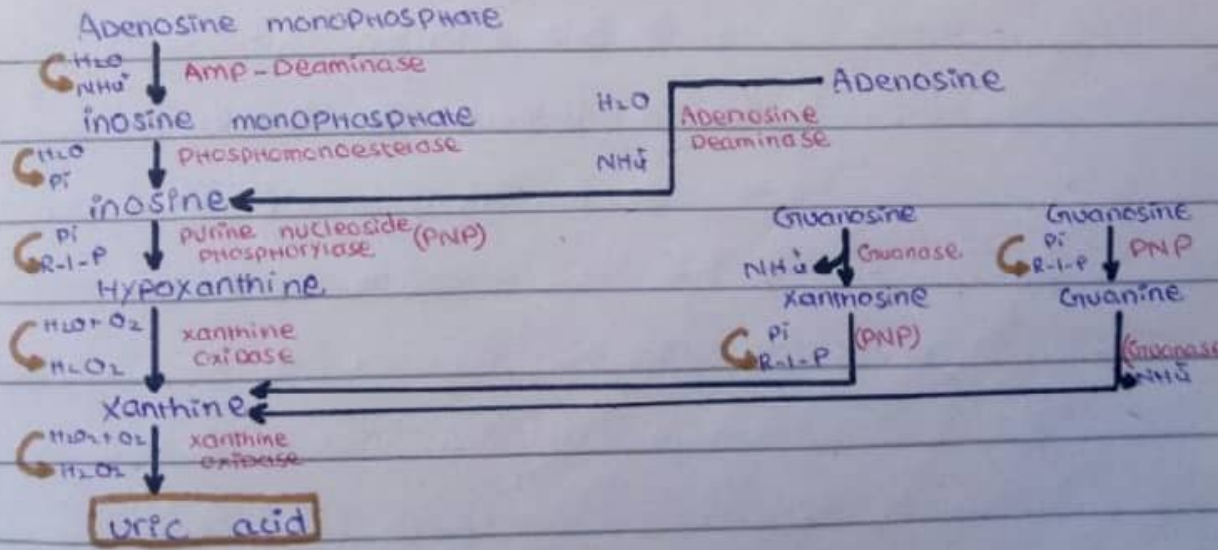
How uric acid formed from Purine ?

Write down steps of catabolism of Purine bases ?

The end product of purine bases catabolism is uric acid.

occurence: mainly in Liver.

Pathway:



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What is Lesch-Nyhan Syndrome?

This is a rare X-linked inherited disorder of Purine metabolism.

Deficient Enzyme: HGPRTase

The deficiency result in an inability to salvage.

Firstly ① Decreased utilization of Hypoxanthine or guanine, from which excessive amount of uric acid, the end product of Purine Degarodation.

Second In Addition lack of Salvage pathway causes ↑ PRPP level & ↓ IMP & GMP level.

Consequences:

① Mental Retardation
② Excessive Uric acid
③ Nephrolithiasis
④ Motor dysfunction
⑤ Self mutilation
⑥ Behavioral Disturbance
⑦ involuntary muscle movement

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Difference b/w Nucleaside & Nucleotide ?

Nucleaside: Nitrogenous bases + Pentose Sugar

THE Pentose Sugar react with base through a Glycosidic bond resulting in formation of Nucleasides.

- Adenine + Sugar = Adenosine
- Guanine + Sugar = Guanosine
- Thymine + Sugar = Thymidine
- Cytosine + Sugar = Cytidine

Nucleotides: Nucleaside + PHOSPHATE

Sugar is Pentose monosaccharide

PHOSPHATE group can be 1, 2 / 3.

Nucleotides are monomers joined by PHOSPHODIASTER bond Form nucleic acid

- Adenosine + PHOSPHATE = Adenosine monoPHOSPHATE (MP)
- THymidine + PHOSPHATE = THymidine MP
- Cytidine + PHOSPHATE = Cytidine MP
- Guanidine + PHOSPHATE = Guanidine MP
- Uridine + PHOSPHATE = Uridine MP

Sugar is Pentose in both. oxidized form → Ribose; Reduced form → Deoxy

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Write a note on Orotic Aciduria ?

The condition result from absence of either one or both Enzyme, orotatate phosphoribosyl transferase & OMP decarboxylase

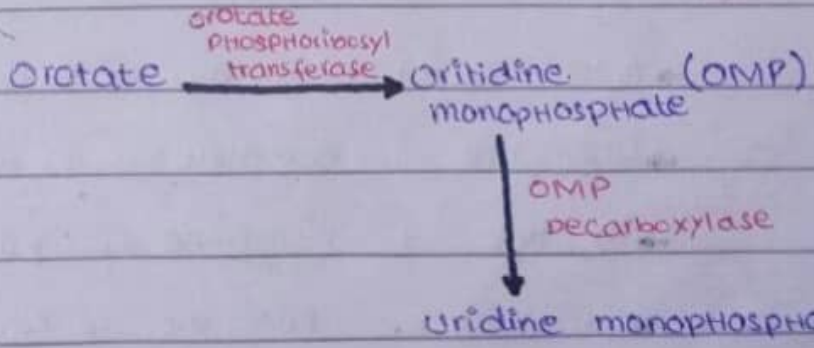
Causes :

- Retarted Growth
- Megaloblastic Anemia
- Urinary Tract obstruction.

Treatment :

Due to lack of feedback inhibition orotic acid production is excessive and this condition treated by feeding cytidine or uridine

locate metabolic step involved :



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What is Hyperuricemia mention it's Causes?

Hyperuricemia: is an abnormal High level of uric acid in Blood.

Uric acid passes through Liver enter Bloodstream

Causes:

(i) Burns
(ii) Crush injuries
(iii) Severe hemolytic Anemia
(iv) Plasma cell myeloma
(v) Myoproliferation disorder

Uric acid level:

Male → 3.4 - 7.0 mg/dl

Female → 2.4 - 6.0 mg/dl

Write a note on Nitrogenous Bases?

There are Two group of Nitrogenous Bases.

(i) **Purines** ① Adenine ② Guanine

(ii) **Pyramidine** ① cytosine ② Thymine ③ uracil

DNA	RNA
Thymine is present	Thymine is absent
Uracil is absent	Uracil is Present.

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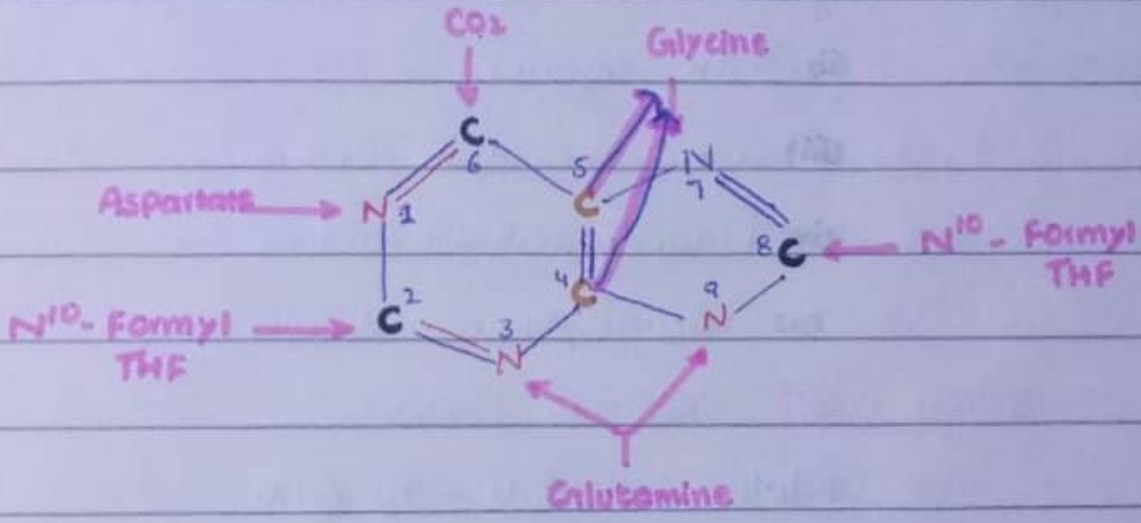
What are two main pathways of Purine Synthesis in Human body?

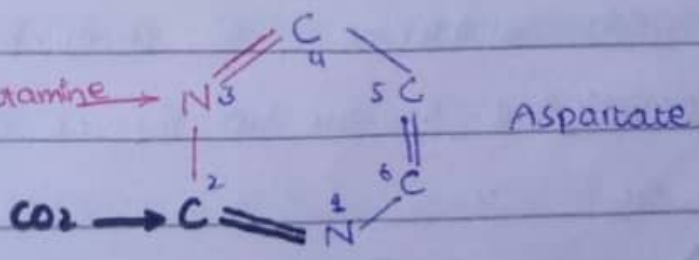
(i) Denova Synthesis

(ii) Salvage Pathway

Draw Structure of Purine ring to show it's Sources of Carbon and Nitrogen?



Draw Structures of Pyramidine



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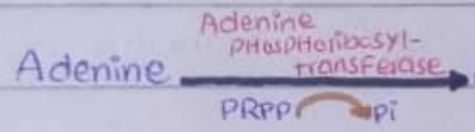
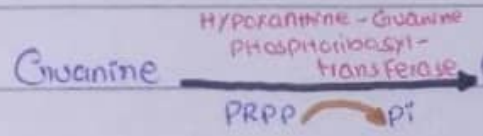
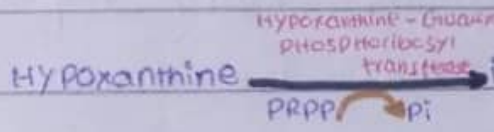
Write a note on Salvage Pathway of Purines?

Salvage Pathway:

Purines can be converted directly to the corresponding nucleotides, this process known as salvage pathway.







The salvage pathway is particularly important in certain tissue such as Erythrocyte and brain where De-novo synthesis is not operat

Write a note on Formation of Deoxyribo-nucleotides from ribonucleotides?

The synthesis of Purine & Pyramidine Deoxyribonucleotide occus from Ribonucleotide. By reduction of at C_2 of Ribose moiety. This reaction is catalyzed by the multisubunit (B_1 & B_2) by ribonuclotide reductase.

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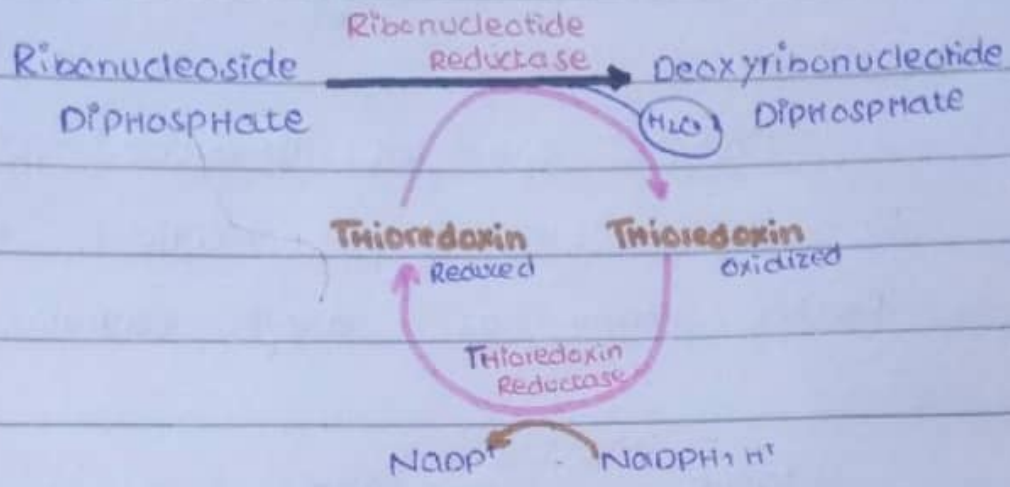

Regulation:

Deoxyribonucleotide mostly required for synthesis of DNA. The activity of enzyme ribonucleotide reductase maintain Adequate supply of Deoxyribonucleotides.

Drug: Hydroxyurea

↓

inhibit ribonucleotide reductase by destroying free radicls required by this enzym

Hydroxyurea used in treatment of cancer Chronic myelogenous leukemia.

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