15/05/2023
Monday

NUCLEOTIDE CHEMISTRY

- Dr. Gayathri Madam

Chemistry

- Base
- Nucleoside
- Nucleotide
- Diagram
- Functions
- Digestion
- Preface to synthesis

$S + NB \Rightarrow NS$

$NS + P \Rightarrow NT$

Purines :- Adenine, Guanine
"Pure As Gold"

• Structure of purines ?

Pyrimide :- Thymine, Cytosine, Uracil

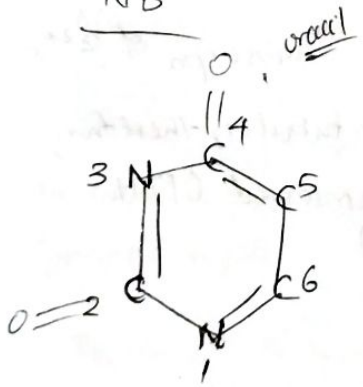
[minor purines & pyrimidines]
[biological imp.]

NB: purines or pyrimidine

Pentose sugar : Ribose/deoxy ribose

Phosphate groups

NB



uracil

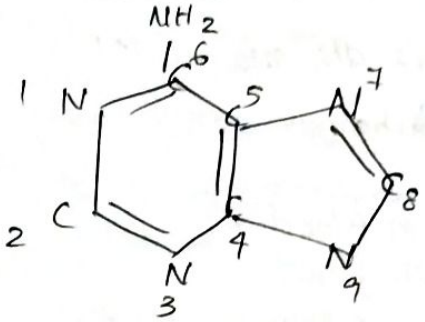
cytosine
Uracil
Thymine

Pyrimidine

time

clockwise

Purine



6- amino purine

2- amino - 6- oxy purine

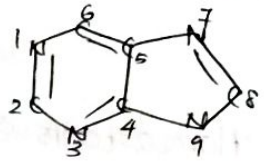
N9 of purine of C1 of ribose sugar
N1 of pyramidine } attached to sugar

to form nucleoside

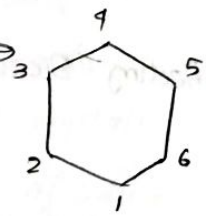
@ 5th C → phosphate } NT

Nitrogenous base

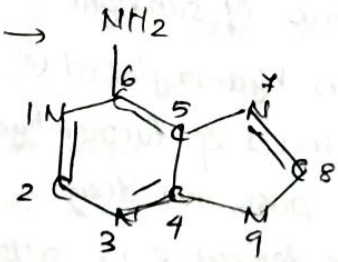
Purine →



Pyramidine →



Adenine →



Minor purines

- Bases may be found in small amounts in nucleic a & hence called minor bases

6-oxo-purine
↳ Hypoxanthine

2,6-dioxopurine → xanthine

2,6,8-trioxopurine → uric a

[formed as end pdt of catabolism of other purine bases]

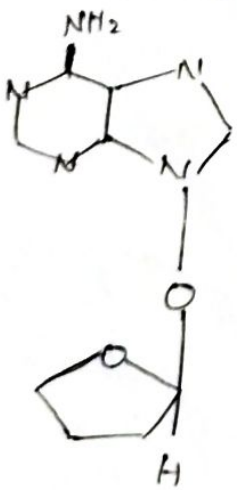
Modified pyrimidines

- 5 methyl cytosine
- Dihydro uracil [tRNA]

Nucleoside

- Base + sugar
- β-N-glycosidic linkage b/w 1C of pentose sugar N19 of purine.

Adenosine



Adenine → Adenosine
Guanosine

Nomenclature

- Nucleoside with purine bases : -sine
- Adenosine guanosine
- d-Adenosine, d-guanosine
- Nucleoside with pyramidine base : -dine
- Uridine, cytidine
- d-cytidine, d-thymidine

Nucleotide

- Phosphate esters of nucleoside
- Base + sugar + phosphate (3' or 5')

Eg: AMP d-AMP
GMP d-GMP FAD
UMP d-CMP NAD
CMP d-TMP ATP

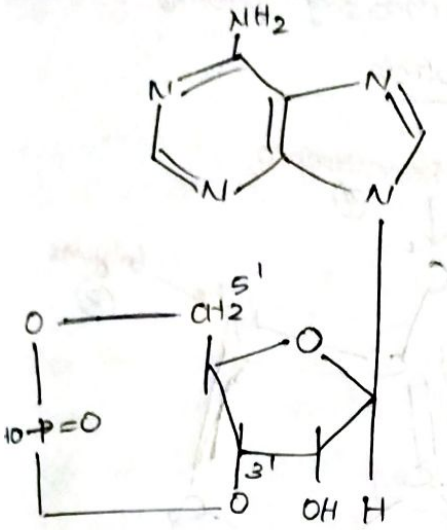
AMP

- High energy compounds containing AMP
- Active methionine (S-Adenosyl met)
- Amino acid adenylates
- Active sulfate

[Fn of nucleotide ⇒ 2mark ∴]

c-AMP

- Phospho diester linkage formed b/w 3' & 5'
- Major metabolic regulator
- cGMP
- second messenger



Structure is also imp draw it mnemonic

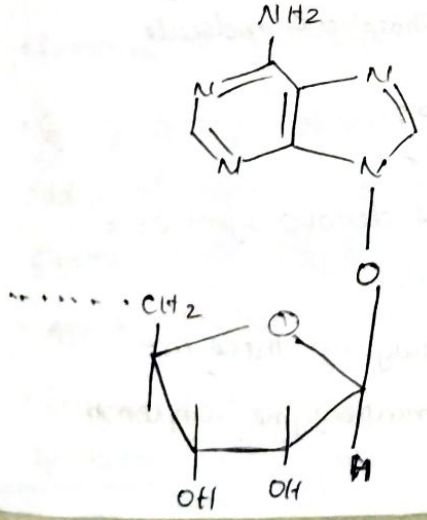
Nucleoside triphosphate

- Nucleoside + 2 Phosphate = Nucleoside di phosphate
- Nucleoside + 3 phosphate = Nucleoside tri phosphate
- NTP or d-NTP
  ↓
  2 high energy bond
- NDP has 1 high energy bond
- NTP " 2 "

Smarik

ATP

- Universal energy currency
- Formed during oxidative proces by trapping energy as high energy phosphate bond.



- Deoxy ribonucleotide → syn. of DNA
- Ribonucleotide → syn of RNA

Function

- Nucleotide precursor of nucleic a
- Nucleic a : Storage & transfer of genetic informa
- Universal currency of energy ATP is a nucleotide
- Components of coenzymes:
- NAD FAD
- Components of metabolic regulator
- cAMP cGMP.

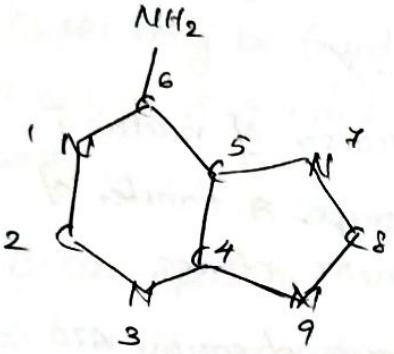
Purine and pyrimidines are dietary non-essential components

- Dietary nucleic a & nucleotides donot provide for the biosynthesis of endogenous n.a
- Humans can synthesize purine & pyrimidine nucleotide de-novo i.e, from amphibolic intermediates
- Humans are therefore said to be prototrophs.

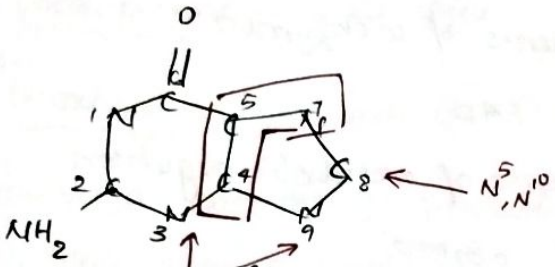
Digesn of dietary n.a

- Ingesn of n.a
  ↓
  Liberan of n.a in intestine — Ribonuclease / deoxyribonuclease, polynucleotidases
  ↓
  Nucleotides — Nucleotidases & phosphatases
  ↓
  Nucleosides — Nucleosidases → Free bases & pentose sugar
  ↓
  Absorbed directly — Uric a (purine)

Purine

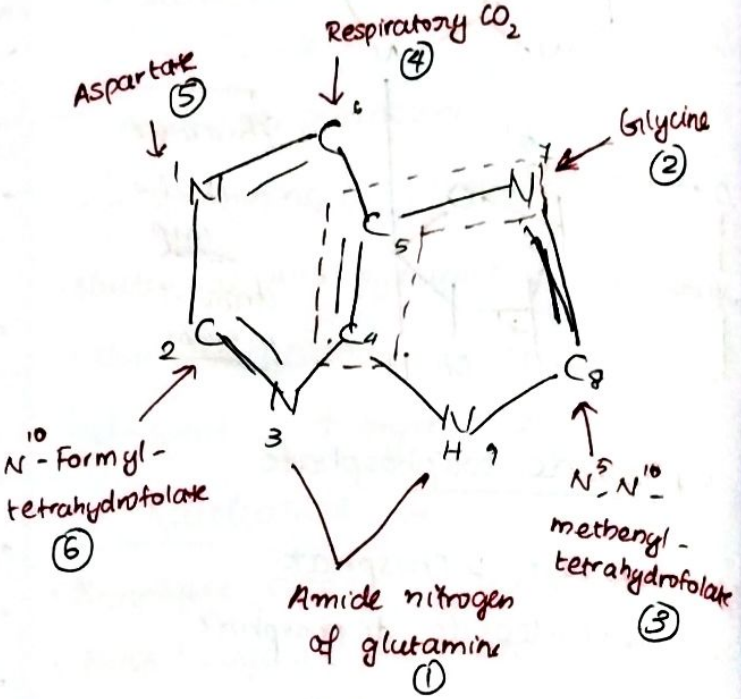


Adenine



17/05/2023 wednesday

De-novo synthesis of purine nucleotide



** Long essay **

Biosynthesis of purine nucleotides

- Site: most tissues synthesize purine nucleotide
- Major site: liver
- Intracellular site: cytosolic pathway
- Two parent nucleotides of nucleic acids are:
- adenosine monophosphate: AMP
- Guanosine monophosphate: GMP

Two pathways

- De-novo synthesis → New synthesis from amphibolic intermediates
- Salvage pathway →
  1. Phosphorylation of free purine ~~base~~ nucleosides [P + BNs]
  2. Phosphoribosylation of free purine bases [P + Ns + B]

* N_3, N_9 ⟶ Glutamine

* C_4, C_5, N^7 ⟶ Glycine

* C_8 ⟶ N^5, N^{10} - methenyl THFA

* C_6 ⟶ Respiratory CO_2

* N_1 ⟶ Aspartate

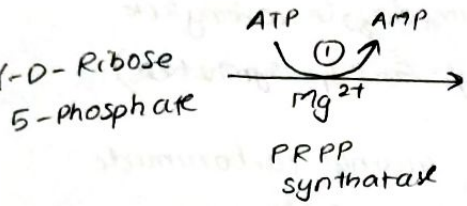
* C_2 ⟶ N^{10} - Formyl - THFA

- Denovo synthesis purine ring built on a ribose-5-Phosphate molecule

Major steps

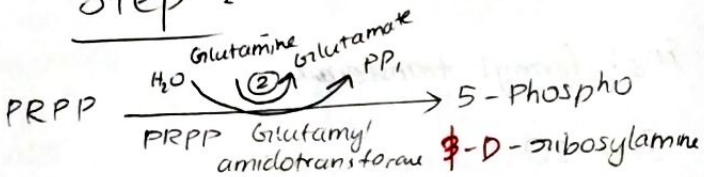
- 10 steps in the denovo synthesis pathway
- The enzyme catalysing these are existing as a multienzyme complex in eukaryotic cells

Step 0 or Preparatory step



- the biosynthesis of purine begins with ribose-5-phosphate (derived from HMP (pentose pathway) converted to PRPP by transfer of pyrophosphate group from ATP to C_1 of the ribose
- Enzyme PRPP synthetase
- Intermediate in purine salvage pathway & in biosynthesis of NAD+ & NADP+
- Purine ring is later assembled on ribose 5-phosphate

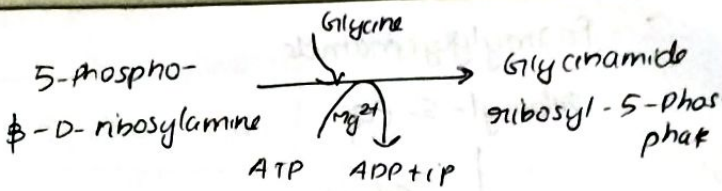
Step 1



- Enzyme is PRPP AMIDOTRANSFERASE
- 1st rate limiting step in the formation of IMP
- Provide N9 of purine ring.

Step 2

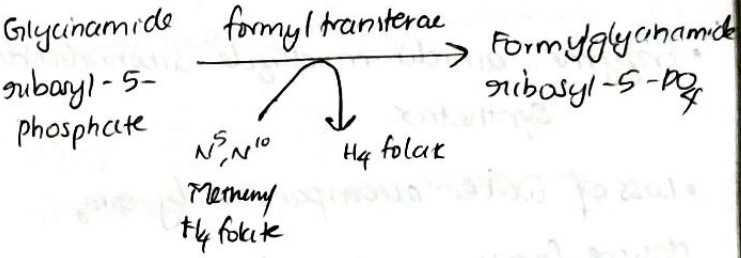
- Formation of glycinamide ribonucleotide
- C_4, C_5 & N_7 are next provided by the addition of amino & glycine to form glycine amide ribosyl 5-phosphate
- ATP is consumed in this rxn
- Enzyme is phosphoribosyl glycinamide synthetase



Step 3

Adding of C moiety → Formyl transferase

- Carbon added to Glycinamide Ribosyl 5-phosphate to form formyl glycinamide ribosyl 5 phosphate or FGAR.



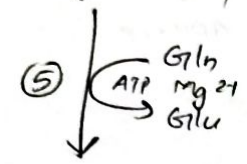
PRPP
ARSPO4 - Amido ribosyl-5-PO4
GARSPO4 - Glycineamide RSPO4
FGARPO4 - formyl

- Enzyme: Glycinamide ribosyl 5-PO4 formyl transferase.
- Carbon that will become C8 of purine ring
- N^5, N^{10} methenyl H_4 ā serves as je pool

Step-4

- Formation of formyl glycinamide ribonucleotide
- The N3 of purine structure is introduced by another amination using glutamine & ATP to form FGAM.
- Enzyme: formylglycinamidine-5-PO4 synthetase

Formylglycinamide
ribosyl-5-PO_4



Formylglycinamidine
ribosyl-5-PO_4

Step-5

- Formation of amido imidazole ribonucleotide
- Enzyme: amido imidazole ribonucleotide Synthetase
- Loss of water accompanied by ring closure forms amino imidazole ribosyl-5-PO_4 or [AIRx]
- ~~The enzyme is~~

Step-6

- Formation of aminoimidazole 4-carboxylate ribosyl-5-PO_4
- Addition of CO_2 to aminoimidazole ribosyl PO_4 adds C_6 of the purine structure and forms aminoimidazole 4-carboxylate ribosyl 5-PO_4
- Enzyme is carboxylase

Step-7 (Don't refer text)

- Formation of N-Succinyl 5-amino imidazole carboxamide ribonucleotide
- Condensation of aspartate with amino imidazole carboxylate ribosyl-5-PO_4
- ~~Enz: Aminoimdi~~
- Enz: (Aminoimidazole carboxylate ribosyl-5-PO_4 synthetase)
- catalysed by succinyl carboxamide ribosyl 5-PO_4 synthetase

Step-8

- Liberation of succinyl grp as fumarate
- catalysed by amino imidazole carboxamide ribosyl phosphate

Step 9

- C_2 of purine is added by derivative of THFA
- formyl aminoimidazole carboxyamide ribonucleotide
- Enz: formyl transferase

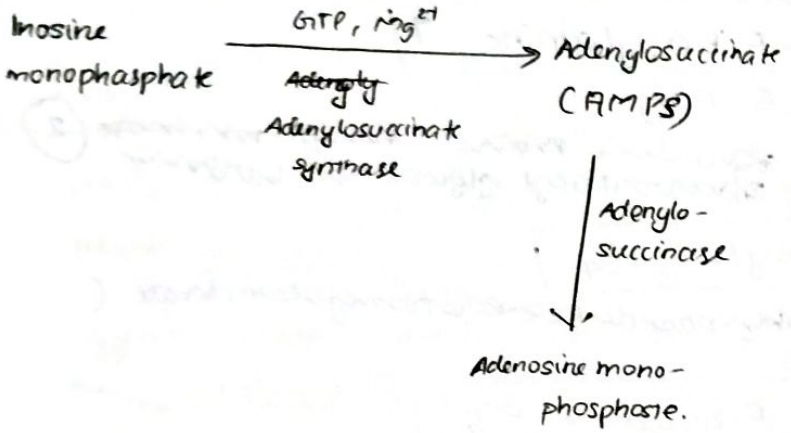
Step 10

- Formation of IMP
- formyl aminoimidazol carboxyamide ribonucleotide ring closure
- catalysed by IMP cyclohydrolase
- Adenosine and guanosine are produced from IMP using nitrogens of aspartate & glutamine respectively.

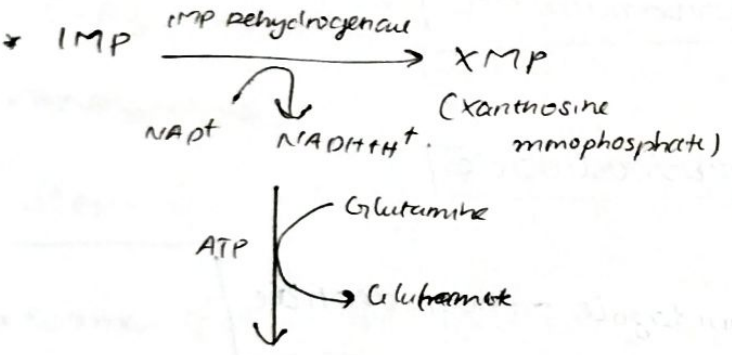
19/05/2023
Fryday

Formation of AMP

- Addition of aspartate to IMP forms adenylosuccinate
- Enz: adenylosuccinate synthetase
- GTP



- Adenylosuccinase in turn catalyses the ....



* IMP first oxidised to xanthosine monophosphate NAD^+ linked enz IMP dehydrogenase. XMP then accepts an amino gp from glutamine presence of ATP

- Enzyme guanosine phosphate synthetase

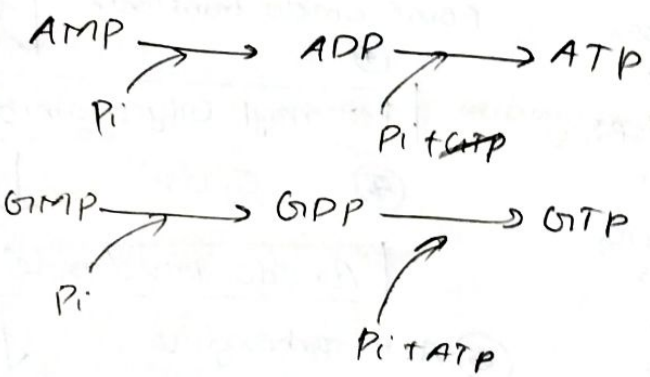
[8 marks AMP & GMP]

... Start from IMP but write final 2 steps...

- AMP & GMP converted to nucleoside diphosphate by phosphorylation
- catalysed by nucleoside monophosphate kinase.
- ADP formed by AMP is then phosphorylated to ATP

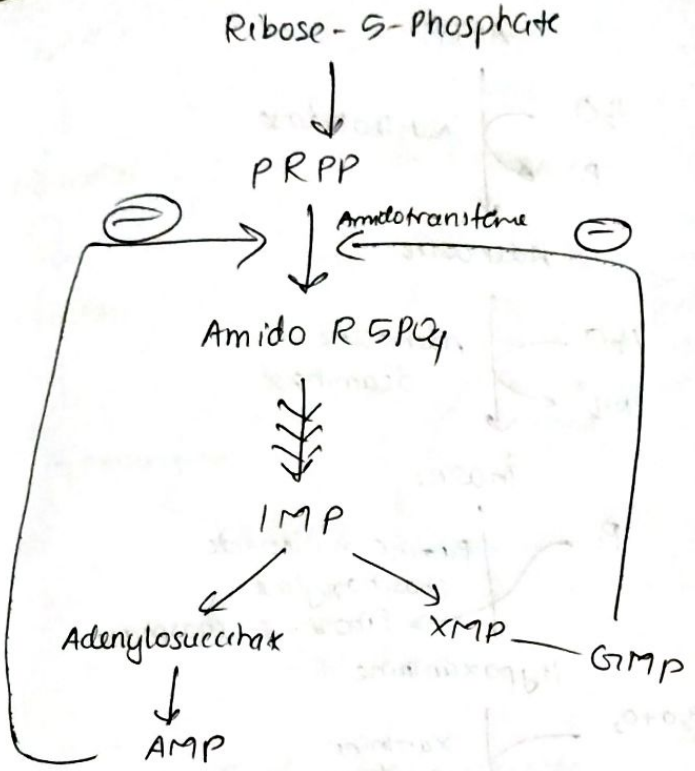
or
- In of glycolysis &
- TCA cycle or
- respiratory chain: oxidative phosphorylation

- GDP is converted to GTP by nucleoside diphosphorylate kinase at the expense of another ATP.

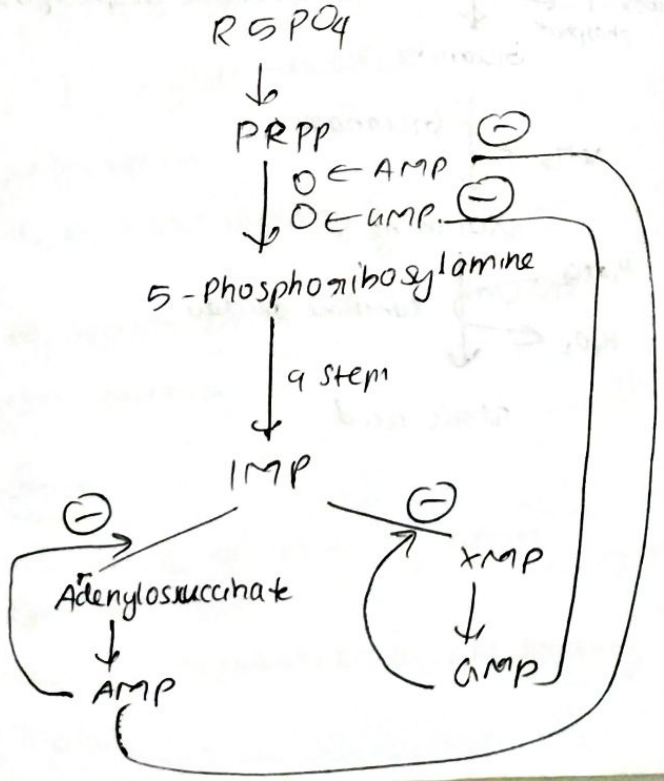


Regulation of denovo synthesis

- The committed step in denovo synthesis is the reaction catalysed by amidotransferase (step 1)
- Inhibited by AMP & GMP
- Act as allosteric modifiers
- Dimeric inactive form
- AMP & GMP can bind to the same enzyme molecule at diff. site they act synergistically



- Both AMP & GMP inhibit their own formation by feed back inhibition of adenylosuccinate synthetase & IMP dehydrogenase
- The formation of AMP from IMP required GTP similarly formation of GMP required ATP.
- Hence both GTP & ATP are made available in sufficient quantities



- The available of PRPP is another imp regulatory factor
- The activity of PRPP synthetase is regulated by negative modifiers: purine & pyrimidine synthesis
- PRPP is inhibited by AMP, GMP, IMP

Regulation

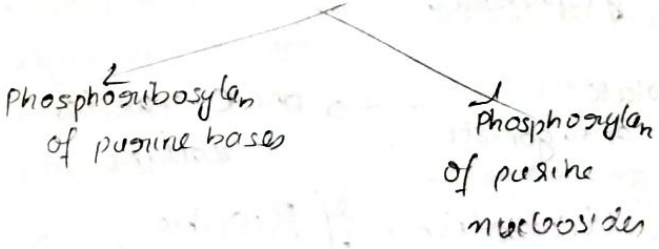
1. PRPP: Induced
2. AMP GMP → amido transferase
   → PRPP synthetase
3. ~~→ PRPP sy~~

22/05/2023 Monday

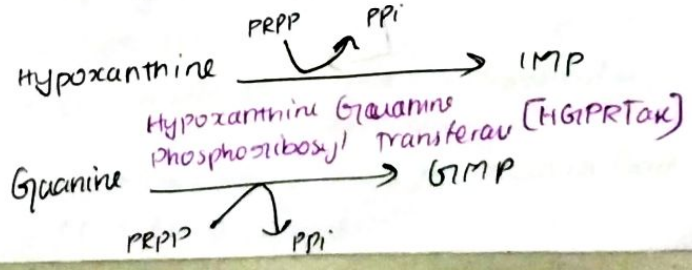
Purine salvage pathways

- Site: mainly in tissues like brain (→ low level of PRPP amido transferase)
- Erythrocyte and polymorphonuclear leukocytes.
- This pathway: de novo pathway is not operating.
- Definition: the pathway involved in the conversion of purines/purine (d) nucleoside to mononucleotides is called salvage pathway

Purine salvage pathways



Phosphoribosylation of purine bases



Phosphoribosylan of purine bases

Adenine + PRPP → (Adenine Phosphoribosyl Transferase) → AMP + PPi

In this type of salvage an Ribose phosphate moiety of PRPP is transferred to the purine to form corresponding nucleotide

Phosphorylan of purine nucleosides

Adenosine + ATP → (Adenosine Kinase, Mg^{2+}) → AMP + ADP

This an involves direct phosphorylan of purine nucleoside by ATP to purine nucleotide.

INHIBITORS

* They act as competitive inhibitors of the naturally occurring nucleotides that are used to synthesis DNA. The DNA becomes functionally inactive

- Azaserine → Glutamine antagonist
- Mercaptopurine Mycophenolic → IMP dehydrogenase
- Folate antagonists → one C transfer reactions

Catabolism of Purine

AMP
↓ H_2O, P_i — Nucleotidase
Adenosine
↓ H_2O, NH_4^+ — Adenosine deaminase
Inosine
↓ P_i — Purine nucleoside phosphorylase → Ribose-1-phosphate
Hypoxanthine
↓ $H_2O + O_2$, H_2O_2 — xanthine oxidase
Xanthine
↓ $H_2O + O_2$, H_2O_2 — xanthine oxidase
Uric acid

GMP
↓ H_2O, P_i — Nucleotidase
Guanosine
↓ P_i — ~~Guanosine deaminase~~ Purine nucleoside phosphorylase → Ribose-1-phosphate
Guanine
↓ NH_3 — Guanase
Xanthine
↓ $H_2O + O_2$, H_2O_2 — xanthine oxidase
Uric acid

AMP → Adenosine → Inosine → Hypoxanthine → Xanthine → Uric acid

GMP → Guanosine → Guanine → Xanthine → Uric acid

- The end pdt of purine nucleotide catabolism is uric acid (urate)
- This degradation is taking place mainly in lives
- The xanthine oxidase is a metalloflavoprotein

Uricotelic | Uricotelic | Allantoin

Uric acid

- 2-5 mg/dl → female
- 3-7 mg/dl → male
- Excretion
  - 500-700 mg/day

-Hyperuricemia >6 or 7 mg/dl (F / M)

- uricosuria

Gout

- accumulation of urate crystals in synovial fluid
- Lowered temperature lowers solubility
- Tophi

- calculi

Gout

8 marks viva

1° → Metabolic defect
2° → ↑ sed turnover, ↓ sed excretion

- PRPP synthetase: ↑ sed abnormal activity
- Amido transferase: Abnormal
  - No allosteric control
  - X linked recessive

(X feedback inhibition X)

- Salvage pathway enzyme: deficient (HGPRTase X) → PRPP increases
- G-6-phosphatase def.
- $G6PO_4$ enter HMP
- $R5PO_4 \longrightarrow PRPP$
- Glutathione reductase variant: ↑ sed activity.
  - NADPH used
  - HMP Pathway induced

25/05/2023 Thursday

Secondary Gout

- ↑ sed production:
  - Malignancy
  - cytotoxic drugs
  - Trauma
  - Starvation
- ↓ sed excretion
  - Renal failure
  - Thiazides (drugs)
  - Lactic/keto acidosis
  - alcohol consumption

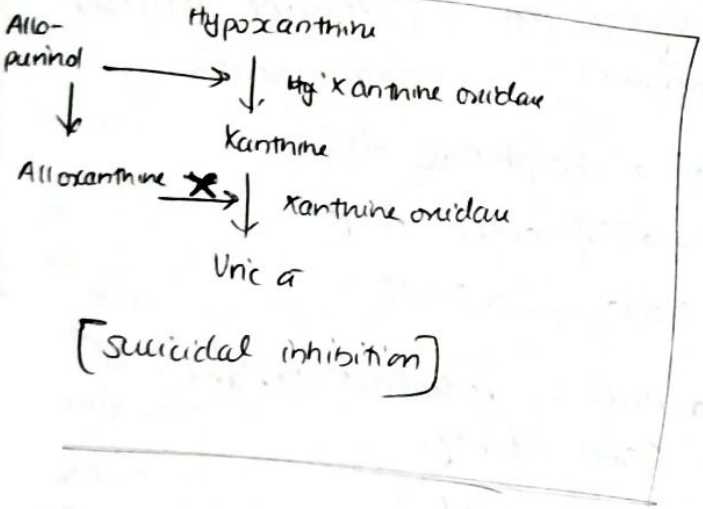
Gout

- At physiological pH
- Urate ion
- form monosodium urate → deposited in synovium
- This inflammatory condition is called as Gout
- CF (Clinical features)

- Excruciating joint pains
- first metatarsophalangeal jt.
- Synovial fluid - birefringent
- Renal medulla
- Urolithiasis

TREATMENT

- Reduced purine intake
- Alcohol restriction
- Probenecid: Uricosuric drug
- uricase: degrade uric acid



- Allopurinol: Hypoxanthine analogue
  xanthine oxidase inhibitor
- competitor & suicide inhibitor
- Alloxanthine
- colchicine - antiinflammatory

LESCH NYHAN SYNDROME

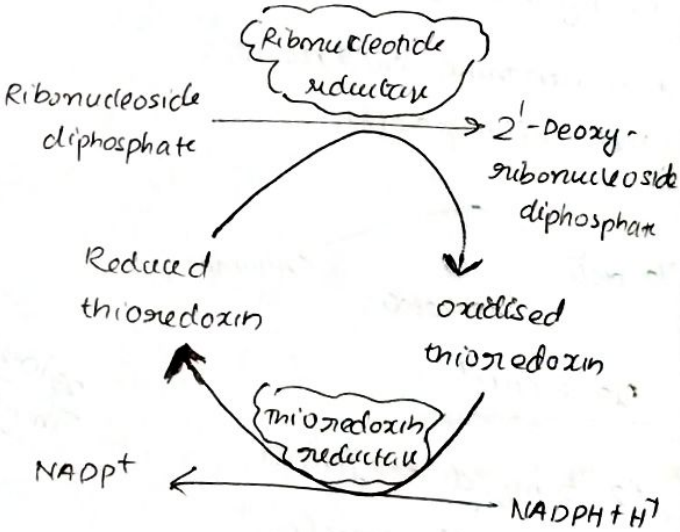
- X linked
- 1 : 10000 males
- HGPRTase deficiency
- Salvage pathway decreased
- PRPP Accumulated
- Purine increased: Inhibition ineffective
- Hyperuricemia
- Clinical features:
- Signs & symptoms of gout:
- Neurological abnormalities
- compulsive self destructive behaviour
- Irresistible desire to bite their fingers
- lips
- Self mutilation
- Behave aggressively

Bubble boy.

26/05/2023
Friday

ADA deficiency

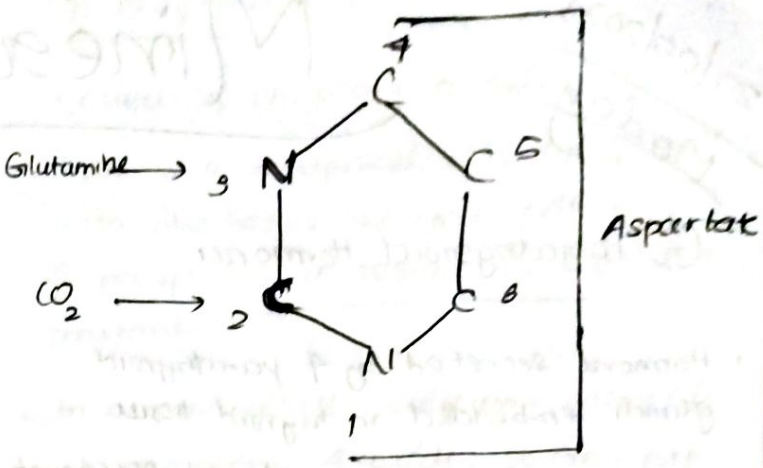
- SCID: severe combine immunodef. d/s
- Lymphocytes dysfunctional



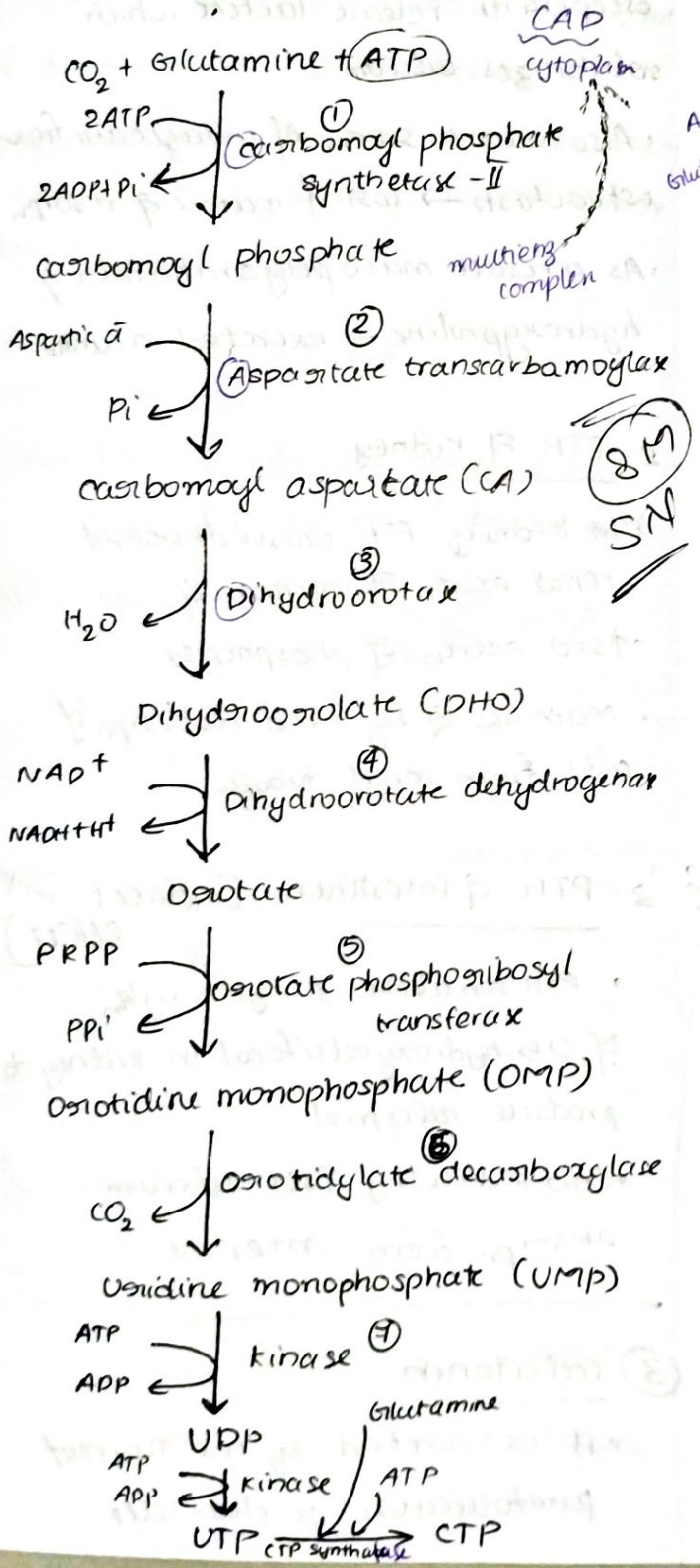
Synthesis of dNDP

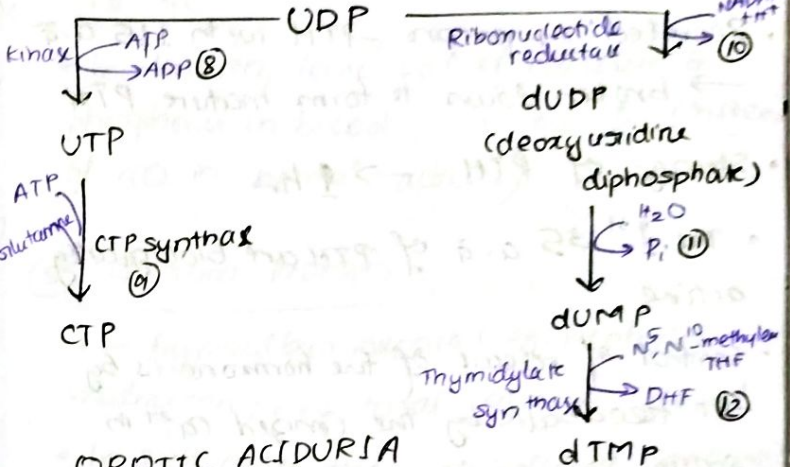
- The enz. is functional: cells actively synthesizing DNA/DNA repair
- Enz: ribonucleotide reductase
- Reduced thioredoxin
- NADPH
- Products: dADP, dGDP, dCDP, TDP

- severe combined immunodef.
- Both T-cell & B-cell affected
- levels of dATP are elevated
- causes feed back inhibition of ribonucleotide reductase
- DNA synthesis and replican affected
- First gene therapy as successful cure is by transfecting ADA gene.



Denovo Synthesis of Pyrimidine



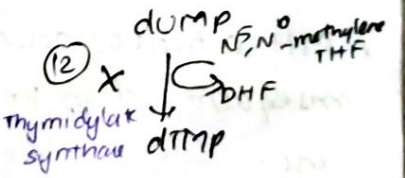


OROTIC ACIDURIA

- Autosomal recessive
- Defect in
  - orotate phosphoribosyl transferase (or)
  - orotidylate decarboxylase
- results in accumulation of orotic ā ... stones (crystals)
- Growth affected
- megaloblastic anemia

Drugs

- Methotrexate
- dihydrofolate reductase
- 5-fluorouracil
- 5-iodouracil
- 3-deoxyuridine
- 6 aza uridine,
- cytosine arabinoside



- thymidylate synthase