23/06/2021 | Unit 2:- Carbohydrate metabolism

Sequence of reactions and pathways of glycolysis:-

→ Glycolysis involves the breakdown of glucose to pyruvate while using the free energy released in the process to synthesize ATP to ADP and Pi.

→ The 10-reaction sequences of glycolysis is divided into two stages: energy investment and the energy recovery.

In glycolysis glucose converts to two C_3 units (pyruvate). The free energy released in the process is harvested to synthesize ATP from ADP and Pi. Thus glycolysis is a pathway of chemically coupled phosphorylation reactions

Stage I:

Energy investment (Reactions 1-5). In this preparatory stage, the hexose glucose is phosphorylated and cleaved to yeild two molecules of the triose glyceraldehyde-3-phosphate. This process consumes 2 ATP.

Stage II:

Energy recovery (Reaction 6→10). The two molecules of glyceraldehyde-3-phosphate are converted to pyruvate, with concomitant generation of 4 ATP. Glycolysis therefore has a net "profit" of 2 ATP per glucose: Stage I consumes 2 ATP; Stage II produces 4 ATP.

The phosphoryl groups that are initially transfered from ATP to the hexose do not immediately result in "high-energy" compounds. However subsequent enzymatic transformations converts these 'low-energy' products to compounds with high phosphoryl group-transfer potentials, which are capable of phosphorylating ADP to ATP. The overall reaction is →

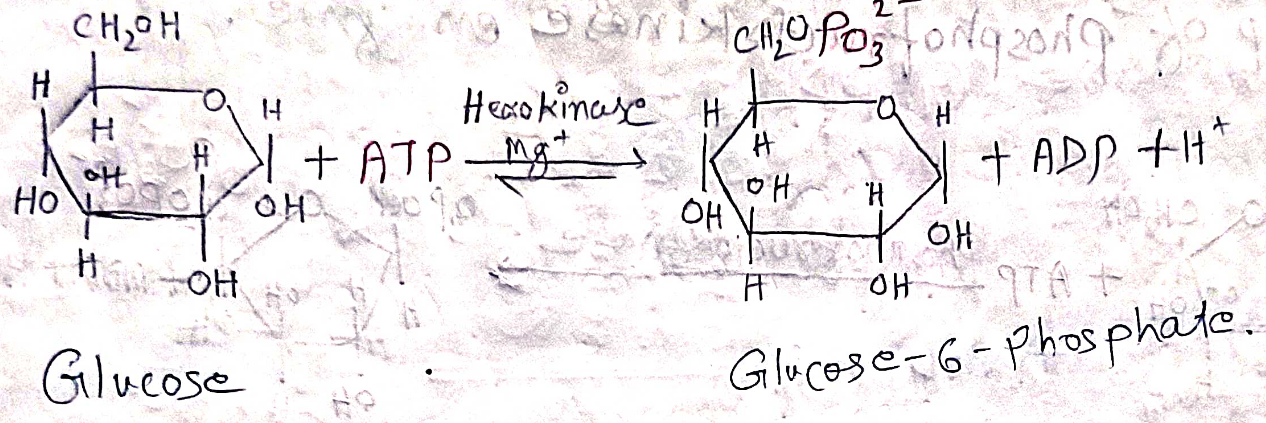
$$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P_i \rightarrow 2C_3H_3O_3 \text{ (Pyruvate)}$$

$$+ 2NADH + 2ATP + 2H_2O + 4H^+$$

Hence the NADH formed in the process must be continually reoxidized to keep the pathway supplied with its primary oxidizing agent, NAD^+.

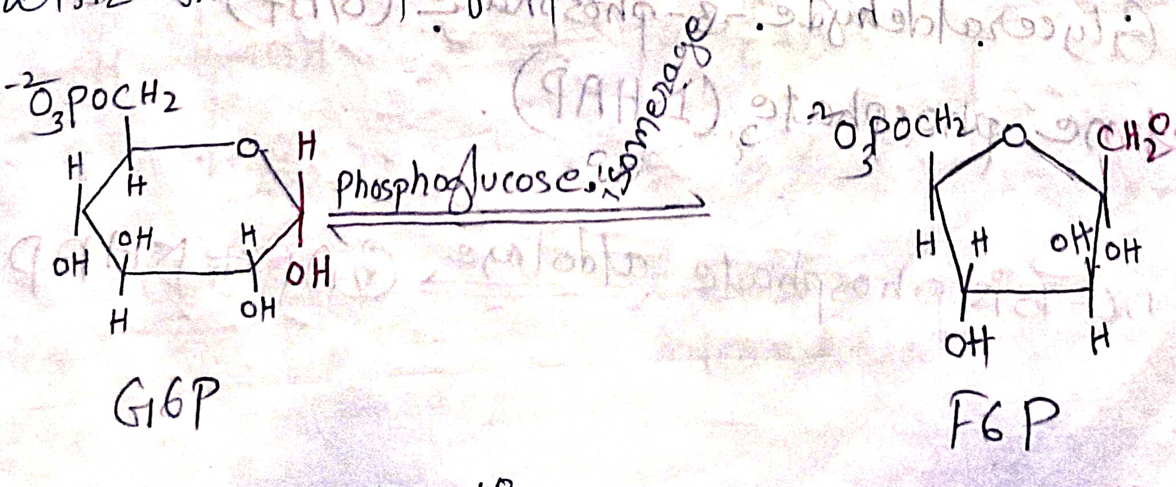
(I) Hexokinase uses the First ATP :-

In the first step of glycolysis transfer of phosphoryl group from ATP to glucose to form glucose-6-phosphate in a reaction catalyzed by hexokinase.



(II) Phosphoglucose Isomerase converts Glucose-6-phosphate to fructose-6-phosphate :-

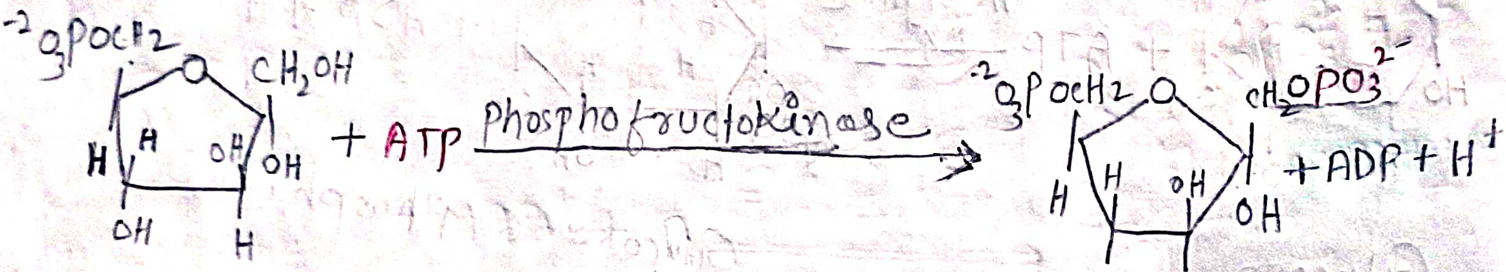
In the second step of glycolysis Glucose-6-phosphate convert to fructose-6-phosphate by moving the carbonyl group from first carbon to 2nd carbon with the help of phosphoglucose isomerase enzyme.



This is isomerization of aldose to a ketose

(iii) Phosphofructokinase uses the Second ATP:

In the 3rd Step of glycolysis fructose-6-phosphate converts to fructose-1,6-bisphosphate by adding ~~phosph~~ P_i ~~phosph~~ P_i to 1-no carbone of fructose-6-phosphate with the help of phosphofructokinase enzyme.



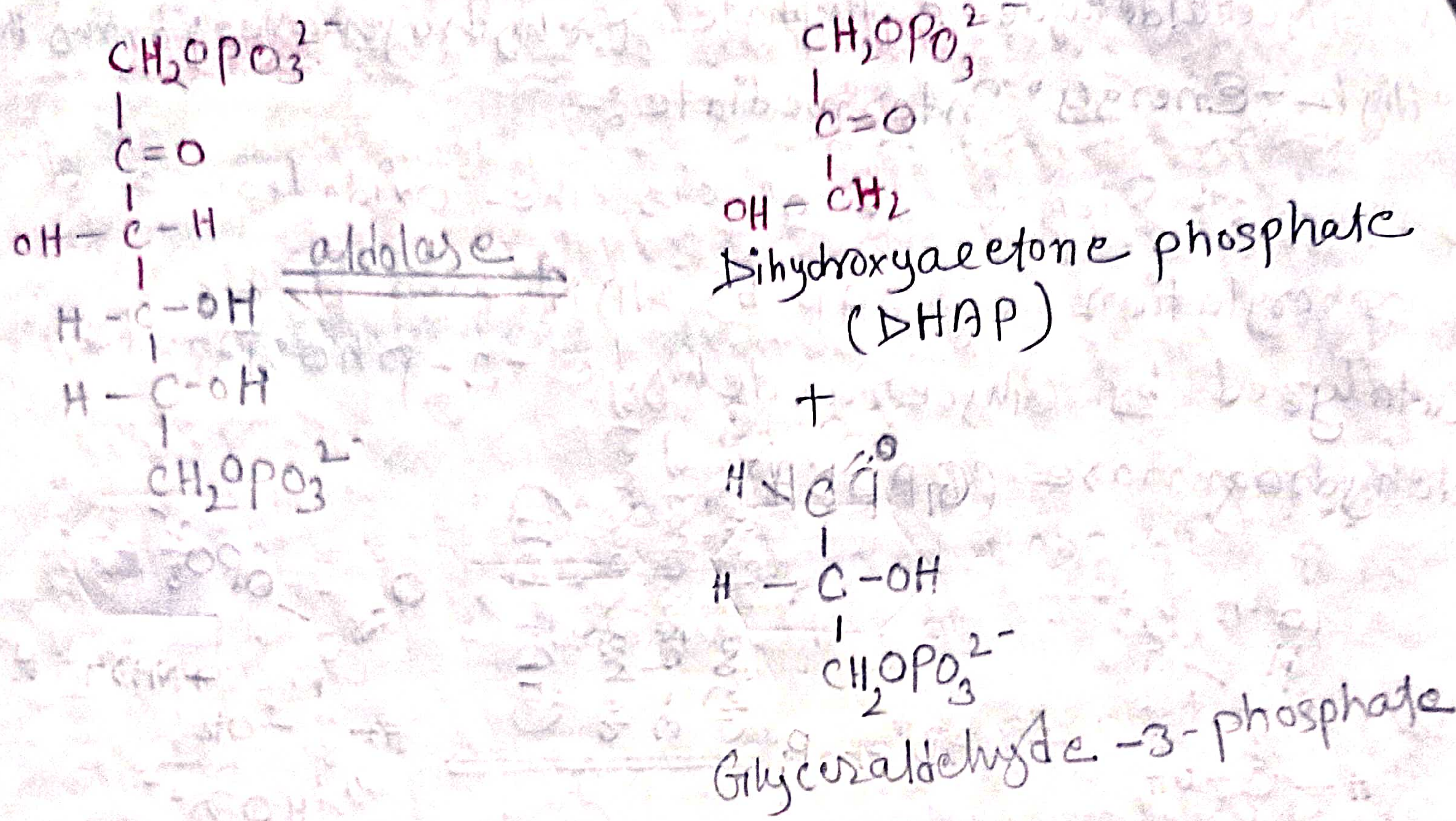
F6P

Fructose-1,6-Bisphosphate (FBP)

(iv) Aldolase converts a 6-carbon compound to two 3-carbon compound:-

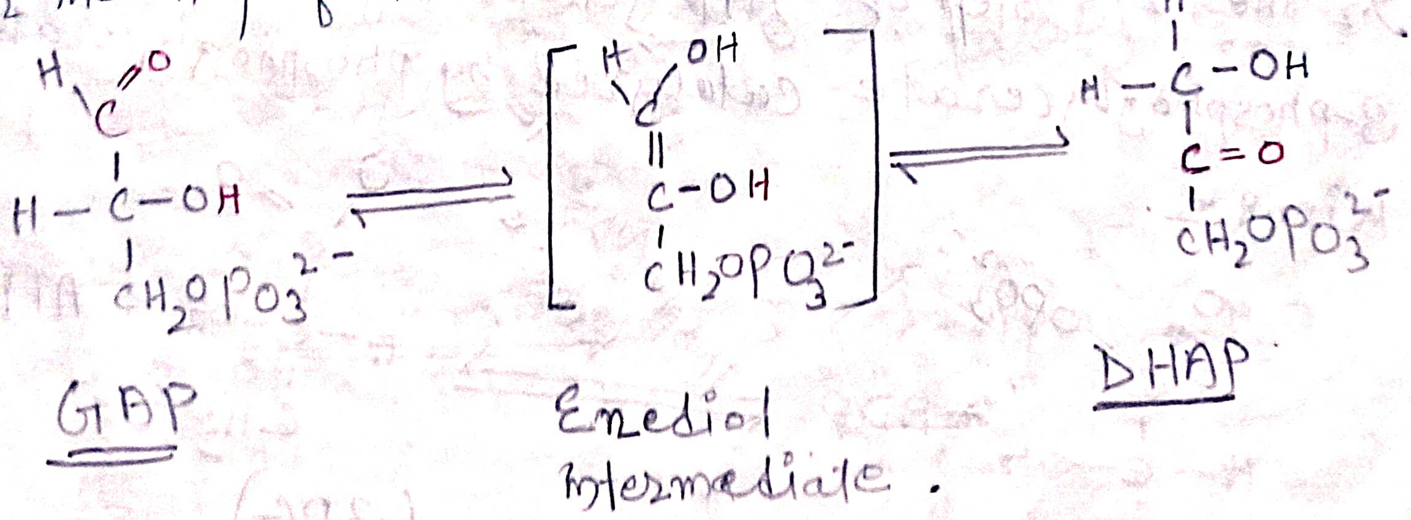
Aldose catalyzes | In the 4th Step of glycolysis aldose converts a FBP to two 3-carbon compound or two trioses Glyceraldehyde-3-phosphate (GAP) and Dihydroxyacetone phosphate (DHAP).

Fructose-1,6-Bisphosphate $\xrightleftharpoons{aldolase}$ GAP + DHAP



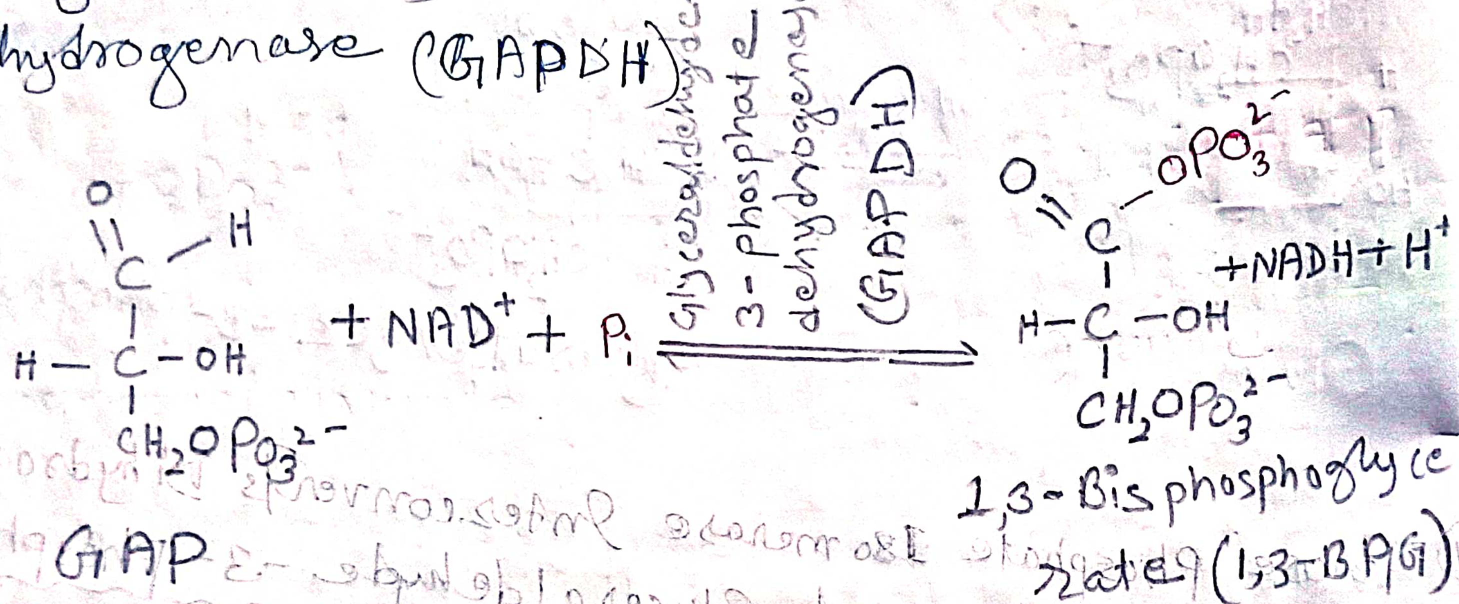
(v) Triose Phosphate Isomerase Interconverts Dihydroxy-aceton phosphate and Glyceraldehyde-3-Phosphate:

In the 5th step of glycolysis GAP and DHAP is interconverted with the help of triose phosphate isomerase enzyme.



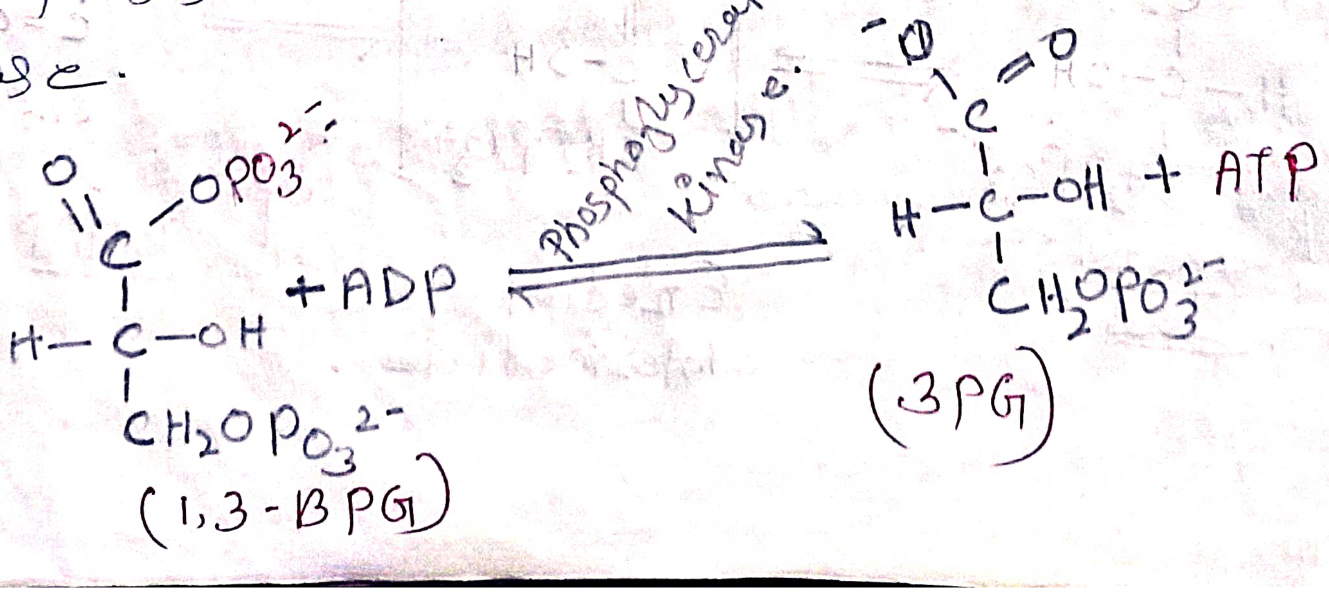
(VI) Glyceraldehyde-3-phosphate Dehydrogenase Forms the "High-Energy" Intermediate:-

In the 6th step of glycolysis the oxidation and phosphorylation of GAP by NAD^+ and P_i as catalyzed by Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)



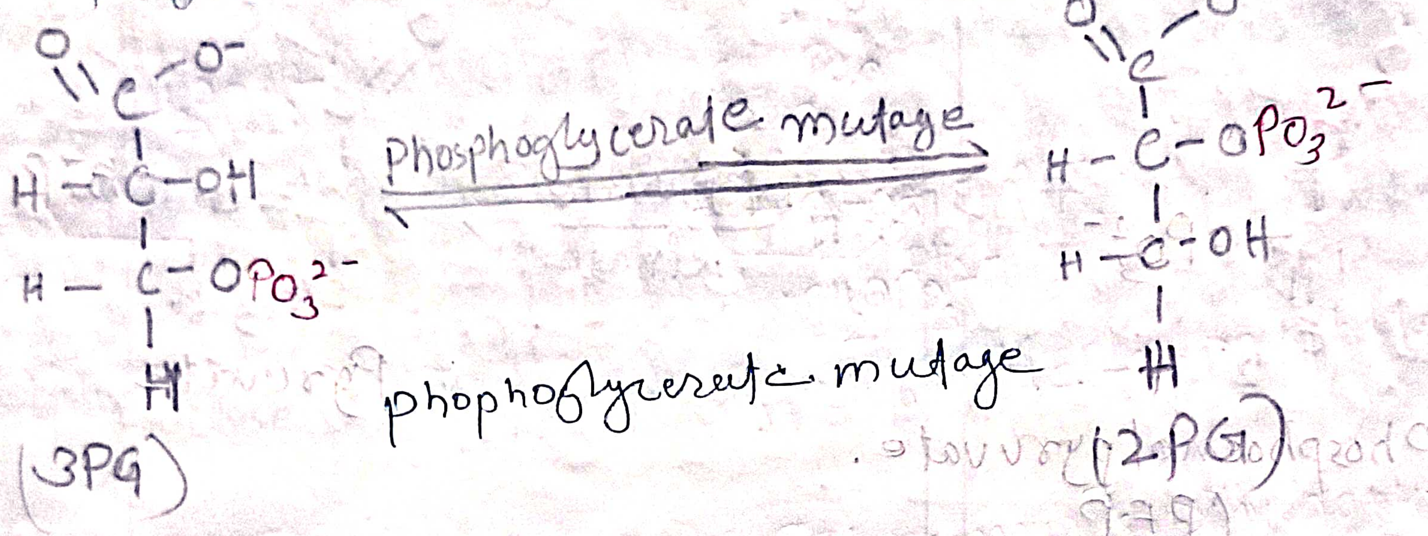
(VII) Phosphoglycerate Kinase Generates the First ATP:

In the 7th step of ATP yeilds together with 3-phosphoglycerate catalyzed by phosphoglycerate kinase.



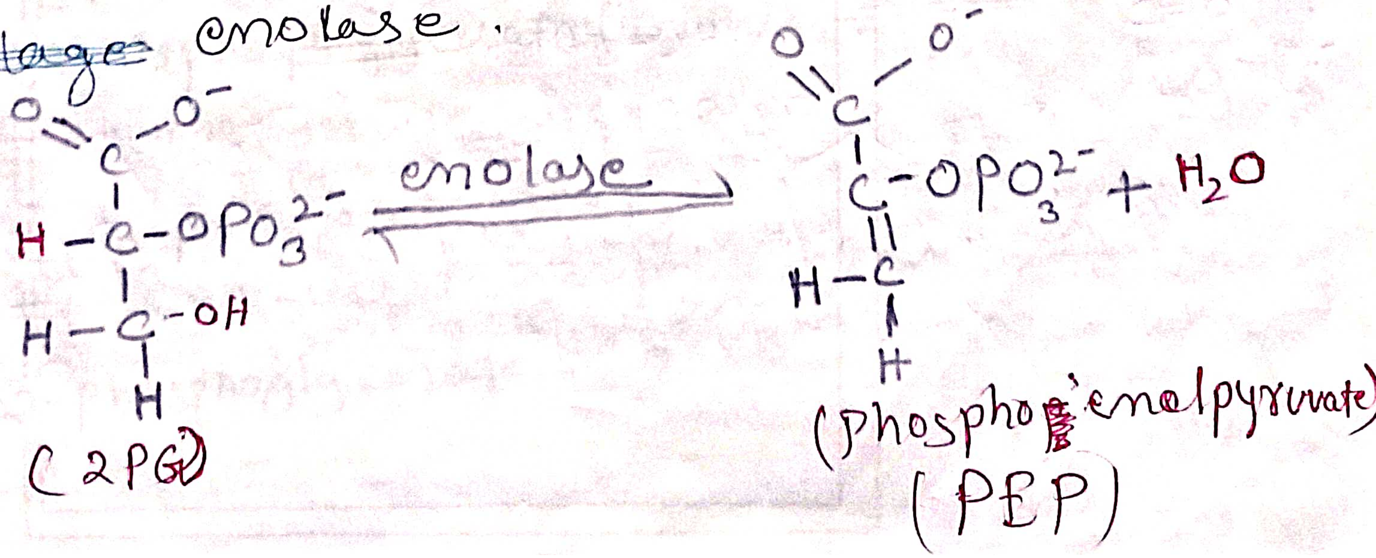
(VIII) Phosphoglycerate Mutase Interconverts 3-Phosphoglycerate and 2-phosphoglycerate:-

In the 8th reaction of glycolysis 3PG converted to 2-Phosphoglycerate (2PG) by phosphoglycerate.



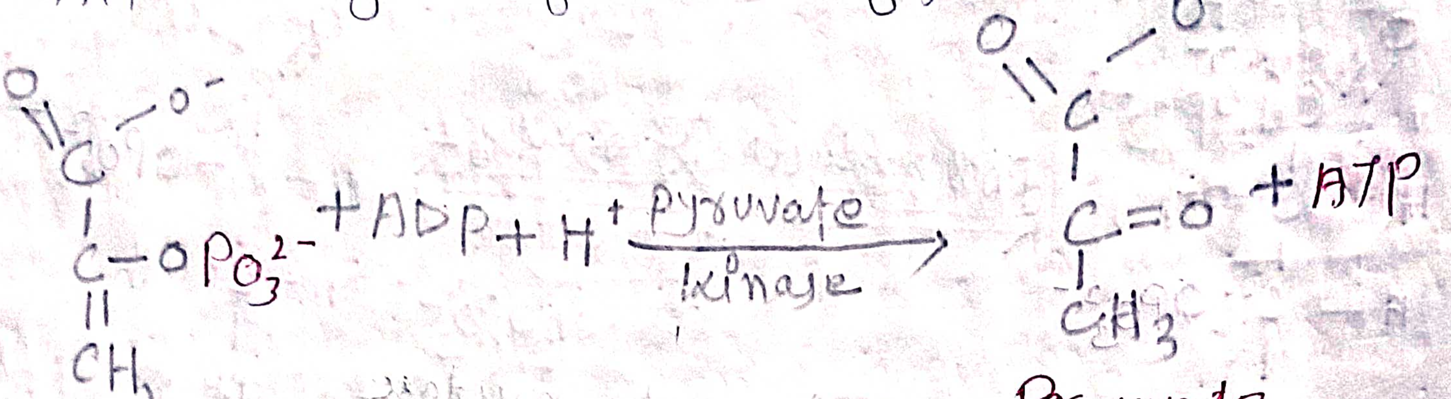
(IX) Enolase forms the Second "High-Energy" Intermadiate:-

In the 9th reaction of glycolysis 2PG is dehydrated to phosphoenolpyruvate (PEP) catalyzed by ~~enolase~~ enolase.



(x) Pyruvate kinase Generates the Second ATP:

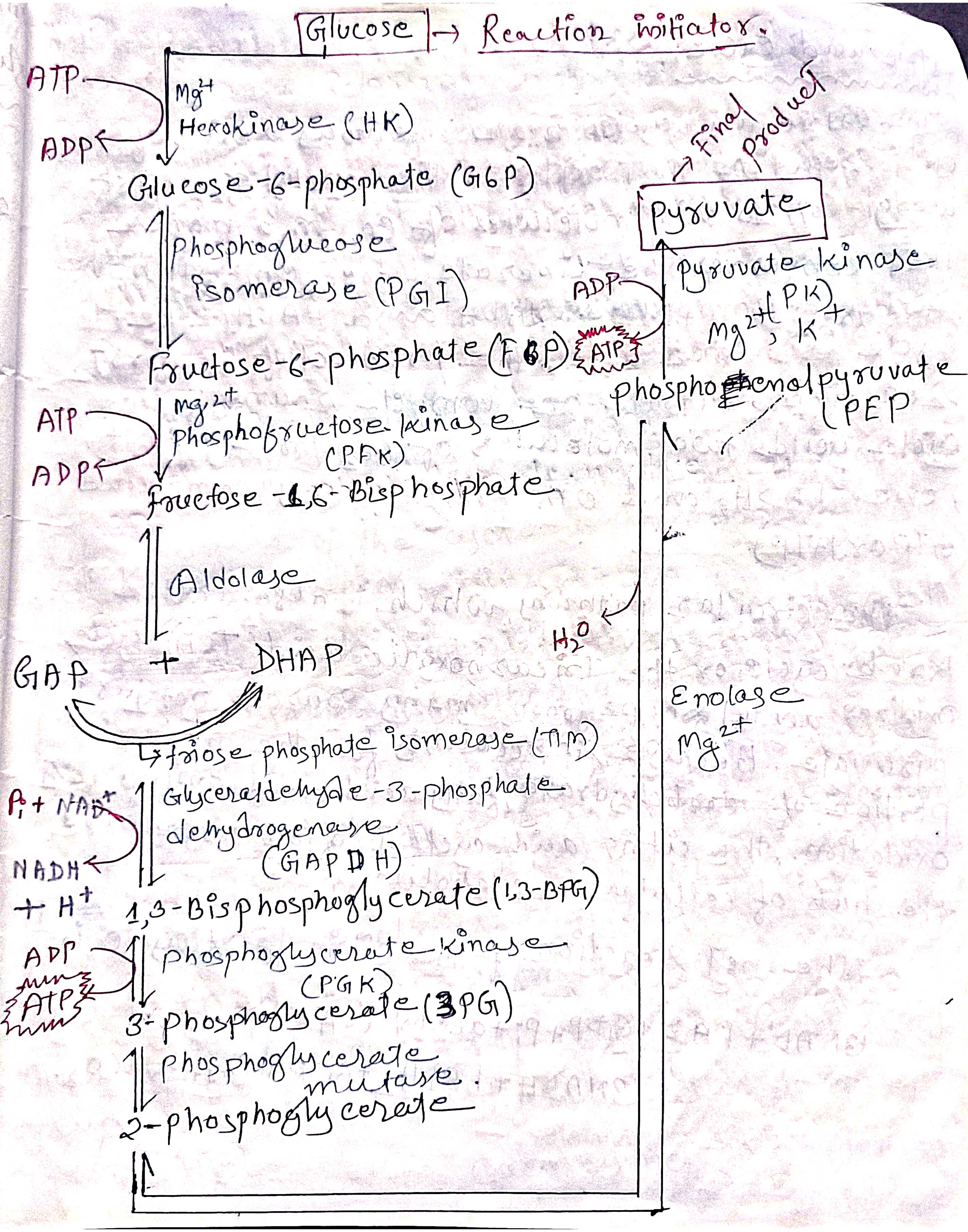
In 10th reaction of glycolysis pyruvate kinase couples the free energy of PEP cleavage to the synthesis of ATP during the formation of pyruvate.



Pyruvate

Phosphoenolpyruvate.
(PEP.

P.T.O →


Glucose → Reaction initiator.
ATP
ADP
Mg^{2+}
Hexokinase (HK)
Glucose-6-phosphate (G6P)
Phosphoglucose isomerase (PGI)
Fructose-6-phosphate (F6P)
ATP
ADP
Mg^{2+}
phosphofructose kinase (PFK)
Fructose-1,6-Bisphosphate
Aldolase
GAP + DHAP
triose phosphate isomerase (TIM)
$P_i + NAD^+$
NADH + H^+
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
1,3-Bisphosphoglycerate (1,3-BPG)
ADP
ATP
phosphoglycerate kinase (PGK)
3-phosphoglycerate (3PG)
Phosphoglycerate mutase.
2-phosphoglycerate
H_2O
Enolase
Mg^{2+}
Phosphoenolpyruvate (PEP)
ADP
ATP
Pyruvate kinase (PK)
Mg^{2+}, K^+
Pyruvate
→ Final product

Citric acid cycle:

The Citric acid cycle is an ingenious series of eight reactions that oxidizes the acetyl group of acetyl-CoA to two molecules of CO_2 in a maner that conserves the liberated free energy in the reduced compounds NADH and $FADH_2$. The cycle is named after the product of the first reaction, citrate. One complete round of the cycle yeilds two molecules of CO_2, three NADH, one $FADH_2$ and one "high-energy" compound (GTP or ATP)

The circular pathway, which is also called the Krebs cycle or the Tricarboxylic acid (TCA) cycle, oxidizes acetyl groups from many sources, not just pyruvate. Because it accounts for the major portion of carbohydrate, fatty acid and amino acid oxidation, the citric acid cycle is often considered the "hub" of cellular metabolism.

The net reaction of the citric acid cycle is

$$3NAD^+ + FAD + GDP + P_i + acetyl\text{-}CoA \longrightarrow 3NADH + FADH_2 + GTP + CoA + 2CO_2.$$

The oxaloacetate that is consumed in the first step of the citric acid cycle is regenerated in the last step cycle. Thus, the citric acid cycle ~~acet~~ acts as a multistep catalyst that can oxidize an unlimited number of acetyl groups.

The carbon atoms of the two molecules of CO_2 produced in one round of the cycle are not the two carbon of the acetyl group that began the round. These acetyl carbon atoms are lost in subsequent round of the cycle. However the net effect of each round of the cycle is the oxidation of one acetyl group of 2 CO_2.

Citric acid cycle intermediates are precursors for the bio synthesis of other compounds.

The oxidation of an acetyl group to 2 CO_2 requires the transfer of four pair of electrons. The reduction of 3 NAD^+ to 3 NADH accounts for three pairs of electrons. The reduction of ~~3NAD~~+ FAD to $FADH_2$ accounts for the fourth pair. Much of the free energy of oxidation of the acetyl group is conserved in these reduced ~~co~~ coenzymes.

Energy is also recovered as GTP.

Synthesis of Acetyl-Coenzyme A:-

Pyruvate dehydrogenase is multienzyme complex that catalyzes a five-part reaction in which Pyruvate releases CO_2 and the remaining acetyl group becomes linked to Coenzyme A.

The reaction sequence requires the cofactors TPP, lipoamide, coenzyme A, FAD and NAD^+.

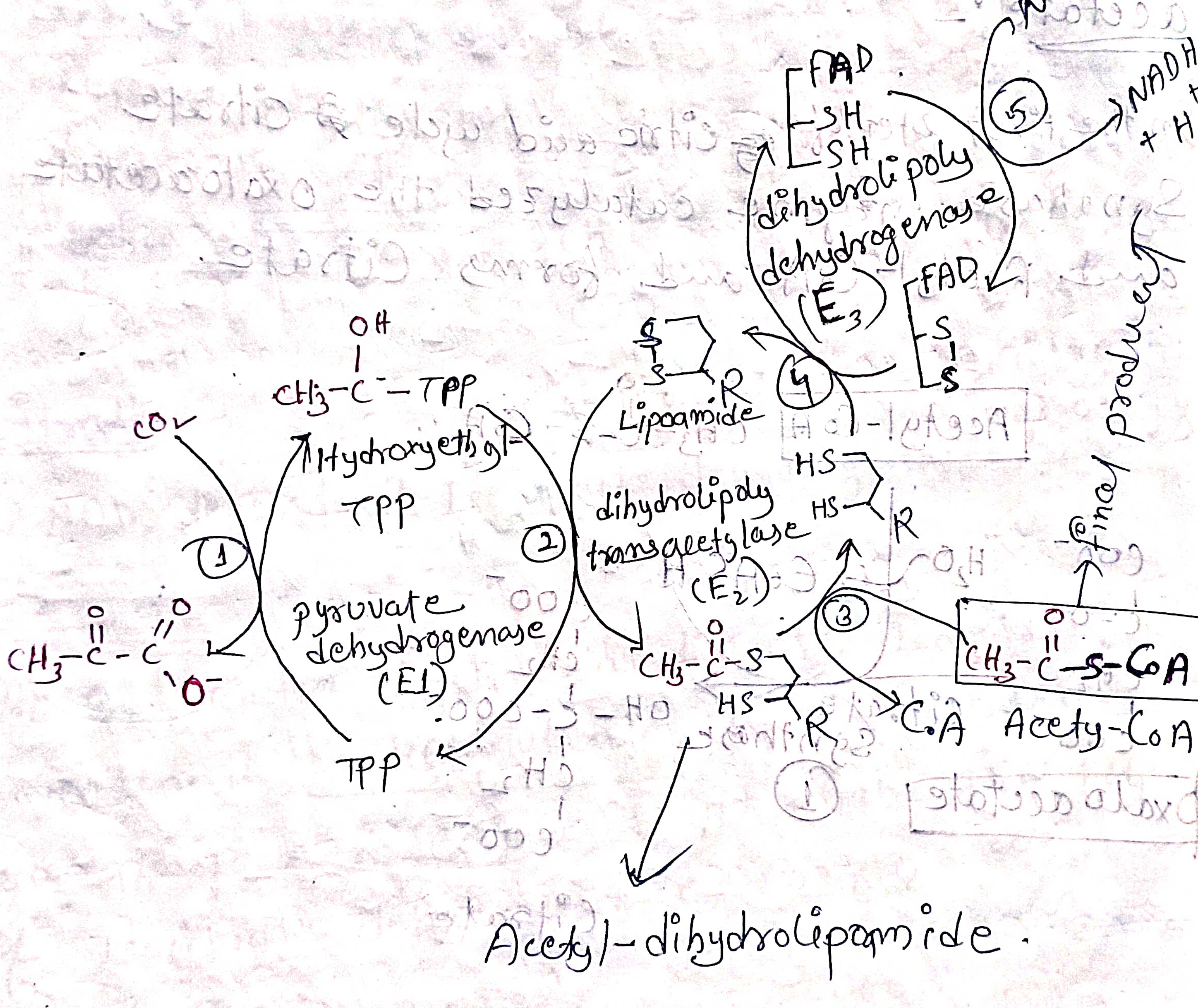
Acetyl group enter the citric acid cycle as part of "high-energy" compound acetyl-CoA.

The pyruvate Dehydrogenase complex catalyzes Five reactions:-

The pyruvate dehydrogenase complex catalyzes five Sequential reactions ~~with~~

$$Pyruvate + CoA + NAD^+ \longrightarrow acetyl\text{-}CoA + CO_2 + NADH$$

The five reactions of the pyruvate dehydrogenase multienzyme complex:-

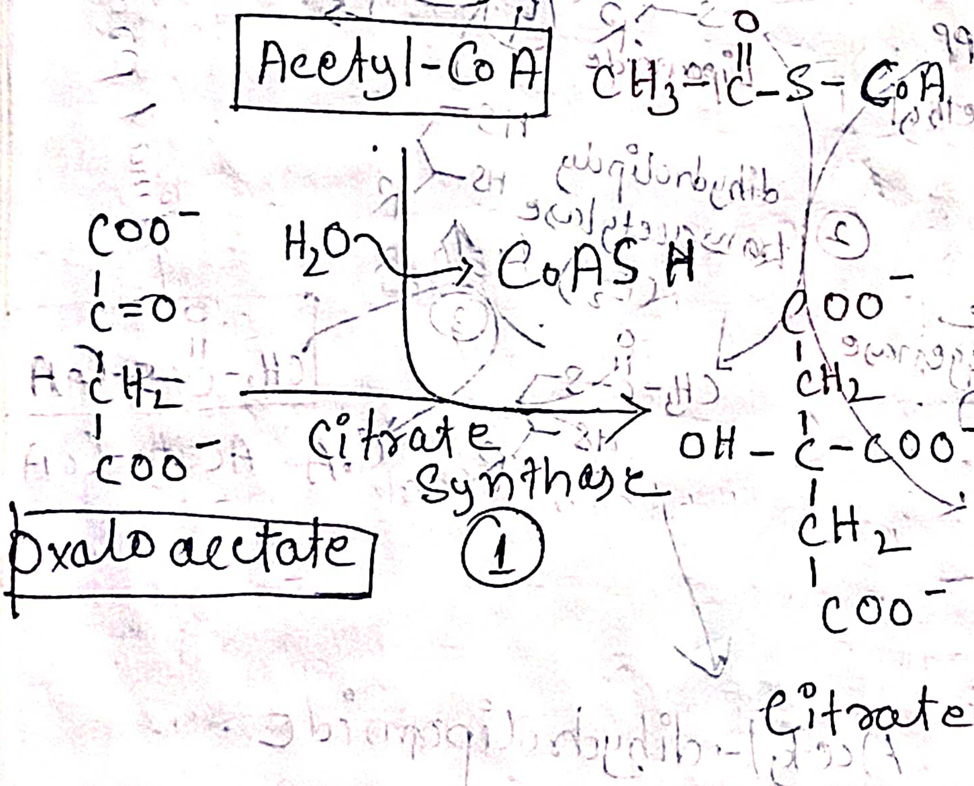


CO₂
CH₃-C-C-O⁻
pyruvate dehydrogenase (E1)
TPP
OH
CH₃-C⁻-TPP
Hydroxyethyl-TPP
Lipoamide
dihydrolipoly transacetylase (E₂)
CH₃-C-S
HS
R
Acetyl-dihydrolipoamide
HS
HS
R
CH₃-C-S-CoA
CoA
Acety-CoA
final product
dihydrolipoly dehydrogenase (E₃)
FAD
SH
SH
FAD
S
S
NAD⁺
NADH + H⁺

Reactions of Citric Acid cycle:-

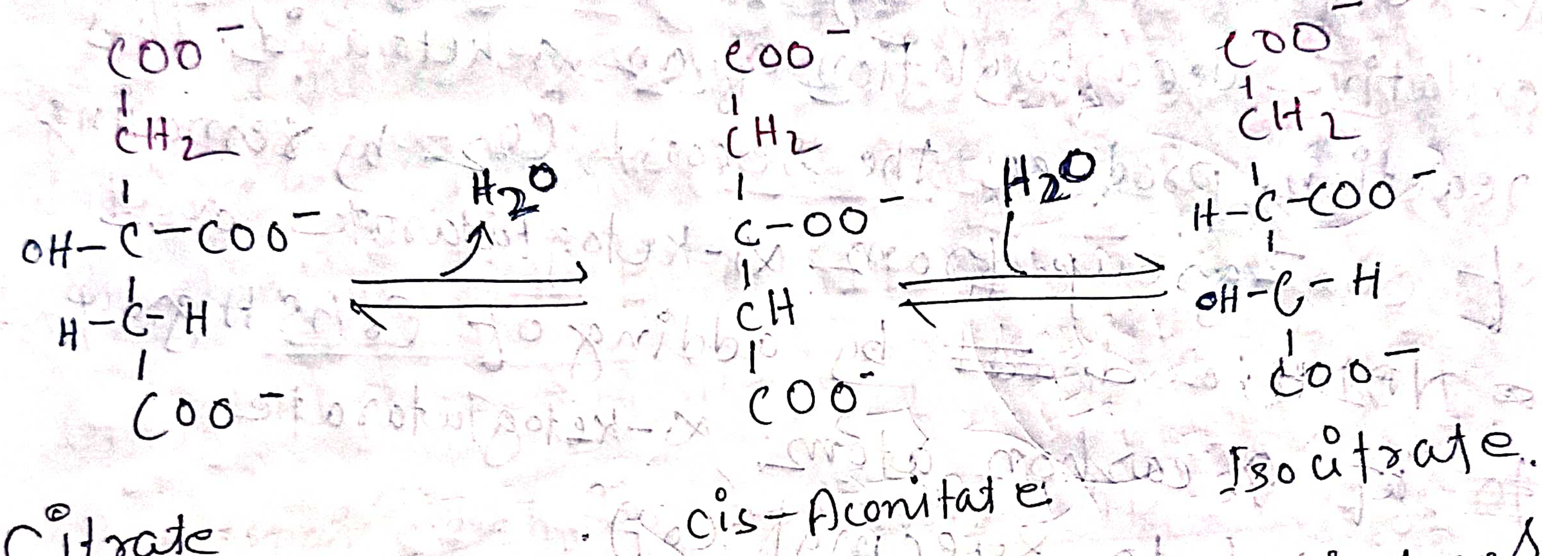
(A) Citrate Synthase joins an Acetyl group to Oxalo-acetate:-

In the first step of citric acid cycle citrate Synthase enzyme catalyzed the oxaloacetate and Acetyl-CoA and forms Citrate.



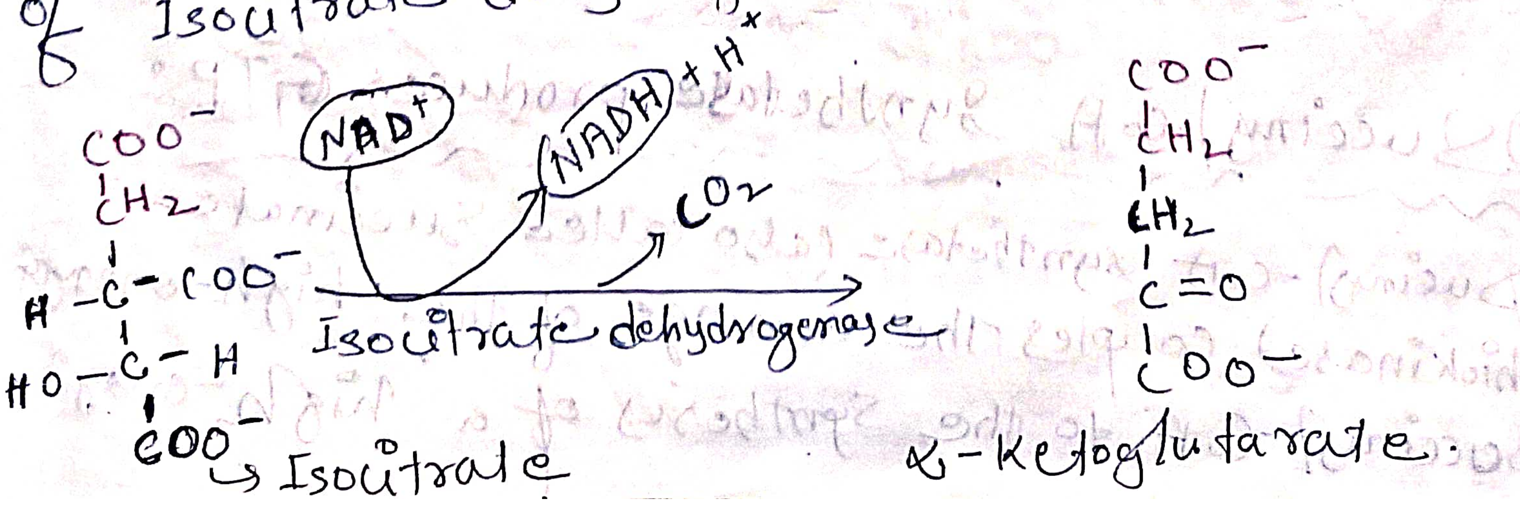
(B) Aconitase Interconverts Citrate and Isocitrate:

In the second step of citric acid cycle ~~Aconi~~ Aconitase convert Citrate to Isocitrate by transfering OH-group 3rd carbon atom to 4 carbon atom of citrate.



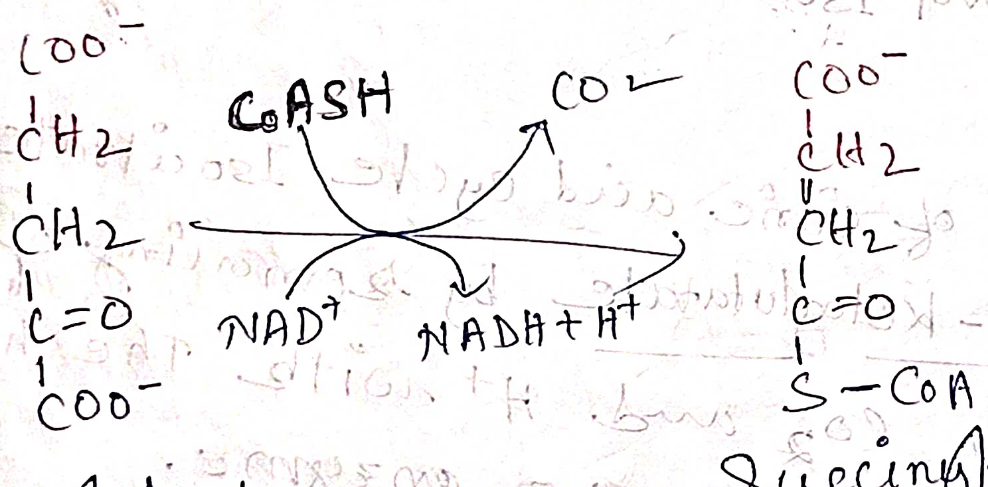
ⓒ NAD⁺ dependent Isocitrate dehydrogenase Releases CO_2:—

In 3rd reaction of citric acid cycle Isocitrate converted to α-ketoglutarate by removing of COO⁻ group as a CO_2 and H^+ with the help of Isocitrate dehydrogenase enzyme.



(4) α-ketoglutarate Dehydrogenase Resembles Pyruvate dehydrogenase:-

α-ketoglutarate dehydrogenase catalyzes the oxidative decarboxylation of an α-keto acid. This reaction produces the second CO_2 by removing of COO^- group from α-ketoglutarate and NADH; and ~~att~~ by adding of CoASH group ~~to~~ at 4 carbon atom α-ketoglutarate converted to Succinyl-CoA.



α-ketoglutarate   Succinyl-CoA.

(5) Succinyl-CoA Synthetase produces GTP:

Succinyl-CoA Synthetase (also called Succinate thiokinase) couples the cleavage of the "high-energy" succinyl-CoA to the synthesis of a "high-energy" nucleoside triphophate.

In this reaction Succinyl-CoA synthetase ~~catalyzed the~~ converts the succinyl-CoA to succinate by removing of CoASH ~~from~~. Additionaly in this step GTP is ~~from~~ formed by combining GDP + P_i ⟹ GTP.

$$GTP + ADP \rightleftharpoons GDP + ATP, \quad \Delta G^{\circ\prime} = 0$$

⑥ Succinate Dehydrogenase Generates $FADH_2$:-

In this reaction of TCA cycle succinate dehydrogenase catalyzes the stereospecific dehydrogenation of succinate to fumarate:

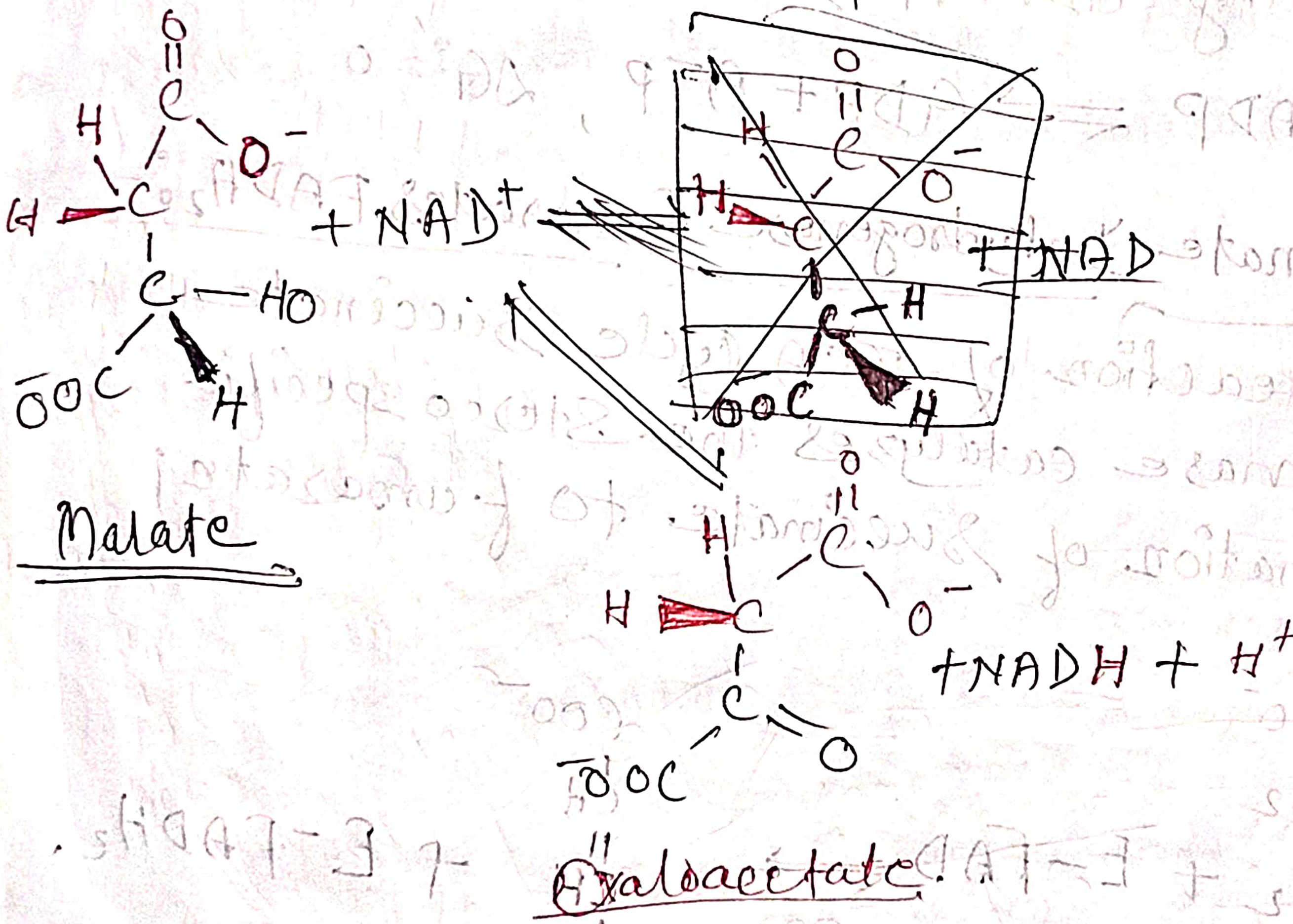
$$\begin{matrix} COO^- \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ COO^- \end{matrix} + E\text{-}FAD \rightleftharpoons \begin{matrix} COO^- \\ | \\ CH \\ \| \\ CH \\ | \\ COO^- \end{matrix} + E\text{-}FADH_2.$$

Succinate

Fumarate

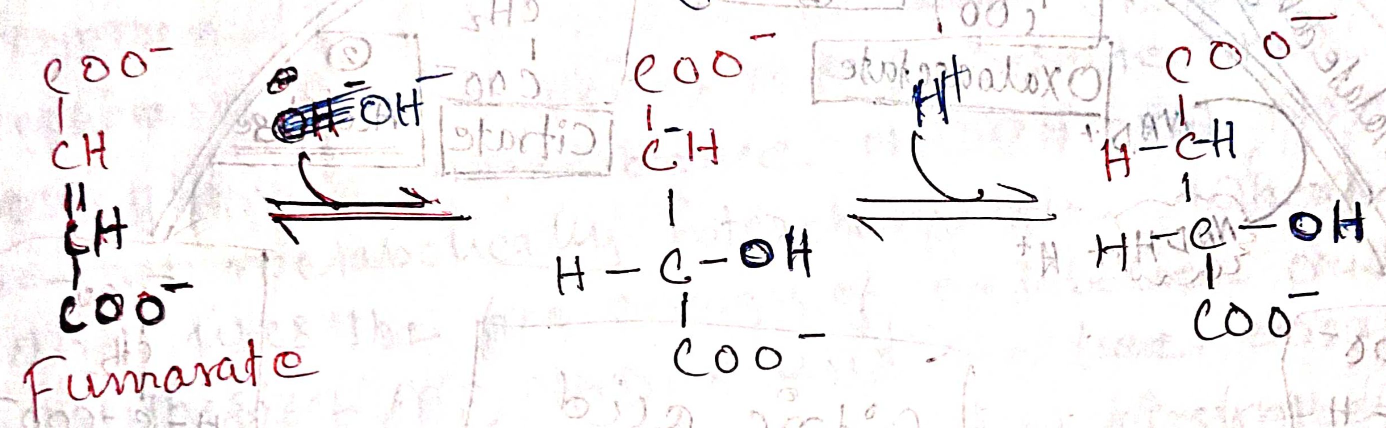
(8) Malate dehydrogenase ~~Reg.~~ regenerates oxalo-acetate:-

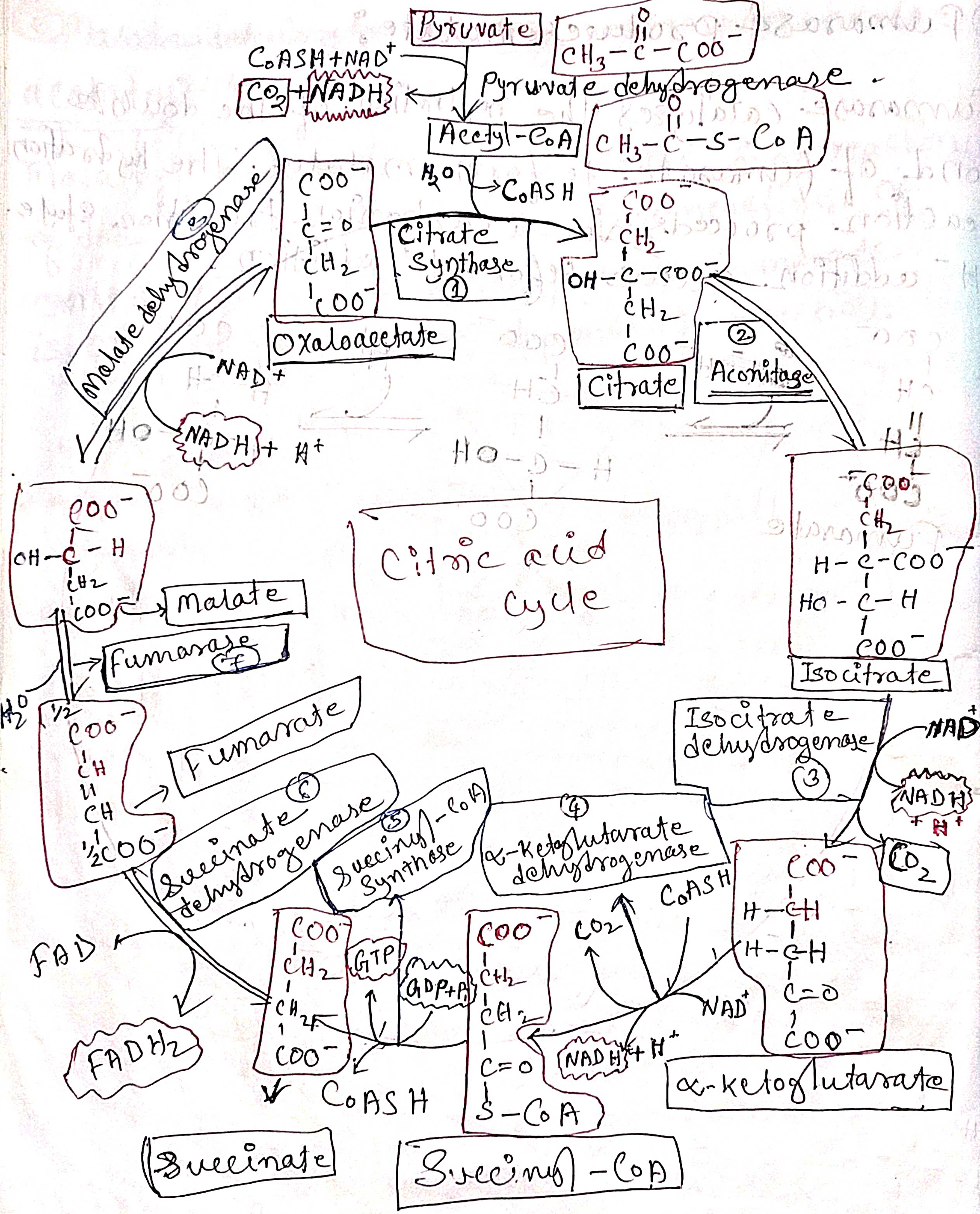
Malate dehydrogenase catalyzes the final reaction of the citric acid cycle, the regeneration of oxaloacetate. The hydroxyl group of malate is oxidized in an NAD^+ dependent reaction.



(7) Fumarase produces Malate:

Fumarase catalyzes the hydration of the double bond of fumarate to form malate. The hydration reaction proceeds via a carbanion transition state. OH^- addition occurs before H^+ addition.





Pyruvate
$CH_3-C(=O)-COO^-$
$CoASH + NAD^+$
$CO_2 + NADH$
Pyruvate dehydrogenase
Acetyl-CoA
$CH_3-C(=O)-S-CoA$
H_2O
CoASH
Citrate Synthase 1
Oxaloacetate
Citrate
2 Aconitase
Isocitrate
Isocitrate dehydrogenase 3
NAD^+
$NADH + H^+$
CO_2
α-ketoglutarate
4 α-ketoglutarate dehydrogenase
CoASH
CO_2
NAD^+
$NADH + H^+$
Succinyl-CoA
5 Succinyl-CoA Synthase
GTP
$GDP + P_i$
CoASH
Succinate
6 Succinate dehydrogenase
FAD
$FADH_2$
Fumarate
Fumarase 7
H_2O
Malate
Malate dehydrogenase 8
NAD^+
$NADH + H^+$
Citric acid cycle

Phosphate pentose pathway :-

ATP is the cell's "energy currency", its exergonic cleavage is coupled to many otherwise endergonic cell function

Cells also have a second currency, reducing power. Many endergonic reactions, notably the reductive biosynthesis of fatty acids and ~~chles~~ cholesterol, require NADPH in addition to ATP. Despite their close chemical resemblance. NADPH and NADH are not metabolically interchangeable. whereas NADH uses the free energy of metabolite oxidation to synthesize ATP, NADPH uses the free energy of metabolite oxidation for reductive biosynthesis. This differentiation is possible because the dehydrogenases involved in oxidative and reductive metabolism are highly specific for the ~~reproduct~~ ~~enzymes~~. respective coenzymes.

NADPH is generated by the oxidation of G6P via an alternative pathways to glycolysis, the pentose phosphate path way (also called the hexosemonophosphate shunt)

⇒ The pentose phosphate pathway consists of three stages, in which NADPH is produced, pentoses undergo isomerization and glycolytic intermediates are recovered.

⇒ The pathways provides NADPH for reductive biosynthesis and ribose-5-phosphate for nucleotide biosynthesis in the quantities that cell requires.

The overall reaction of the pentose phosphate pathway is

$$3G6P + 6NADP^+ + 3H_2O \rightleftharpoons$$
$$6NADPH + 6H^+ + 3CO_2 + 2F6P + GAP$$

Pathway can be considered to have three stage:-

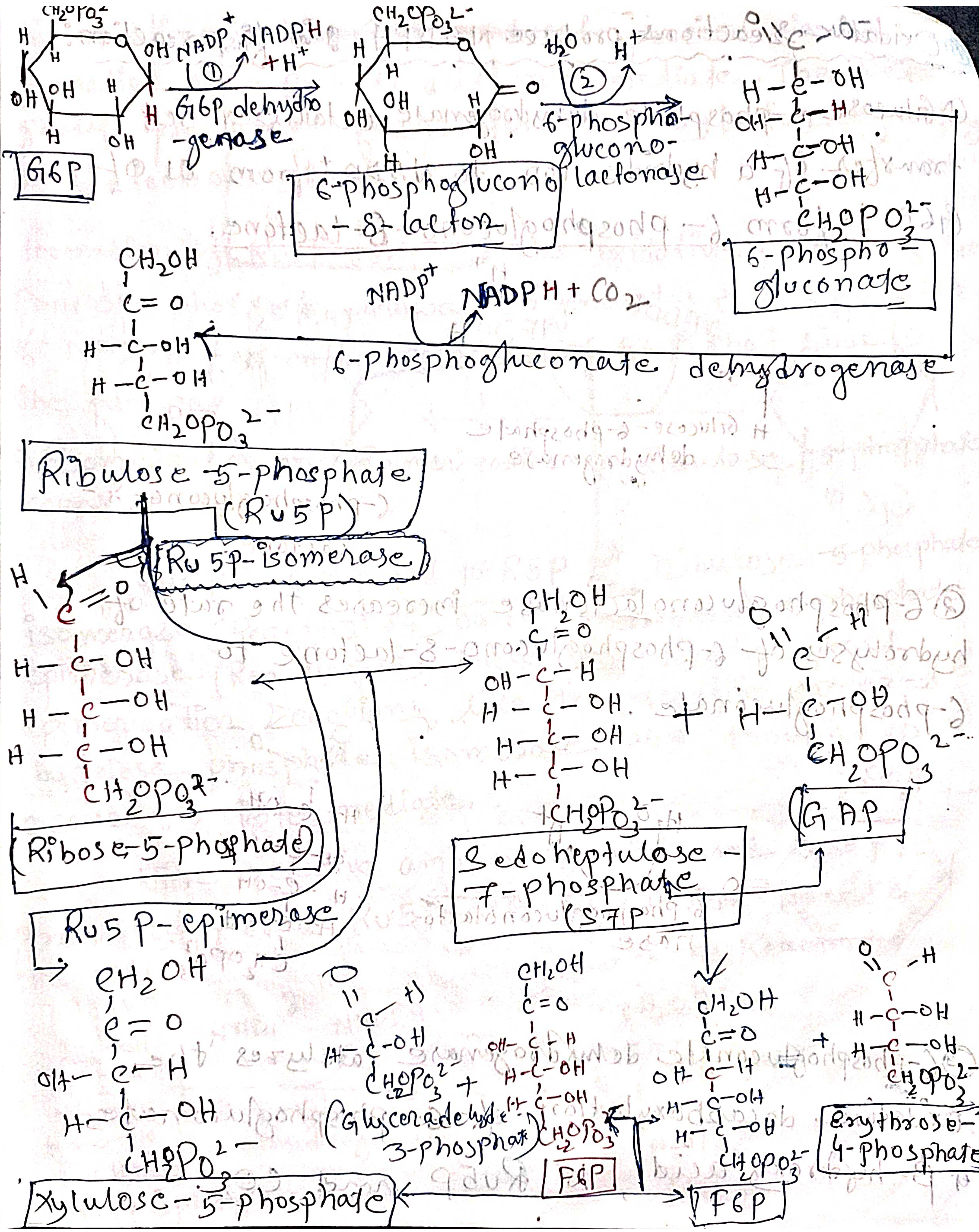
Stage I:- Oxidative reactions (Reactions 1→3) which geild NADPH and ribulose-5-phosphate.

$$3G6P + 6NADP^+ + 3H_2O \longrightarrow 6NADPH + 6H^+ + 3CO_2 + 3Ru5P$$

Stage II:- Isomerization and epimerization reactions (Reaction 4→5) which transform Ru5P either to ribose-5-phosphate or to xylulose-5-phosphate.

$$3Ru5P \rightleftharpoons R5P + 2Xu5P$$

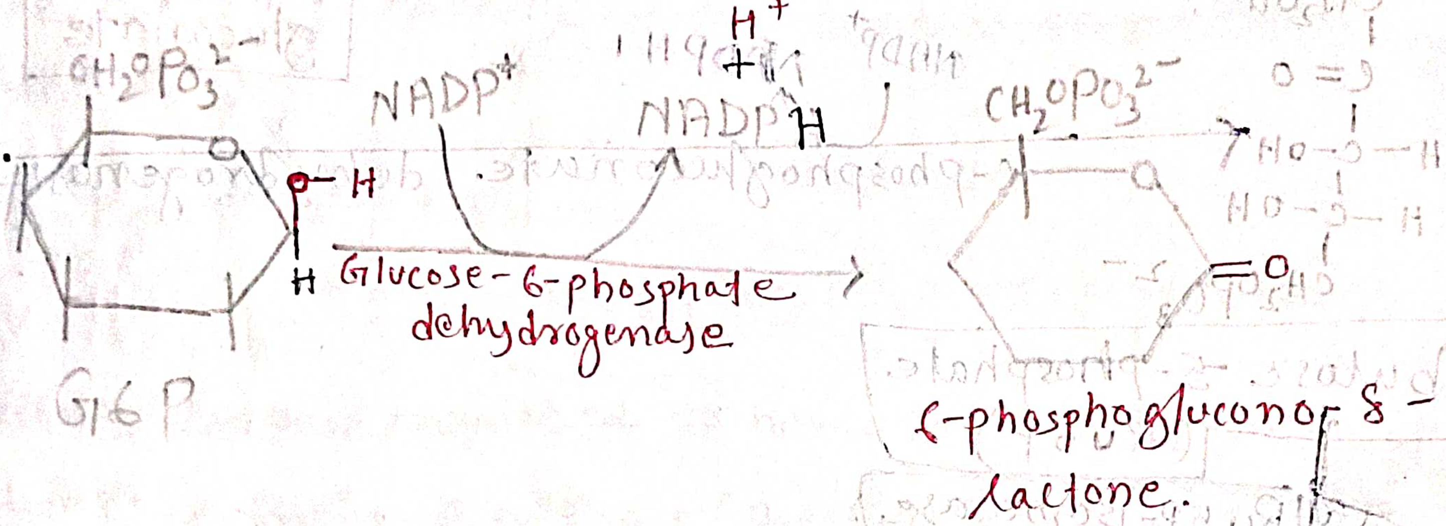
Stage III:- A series of C-C bond cleavage and formation reactions (Reaction 6→8) that convert two molecules of Xu5P and one molecule of R5P to two molecules of F6P and one molecule of Glyceraldehyde-3-phosphate. (GAP)



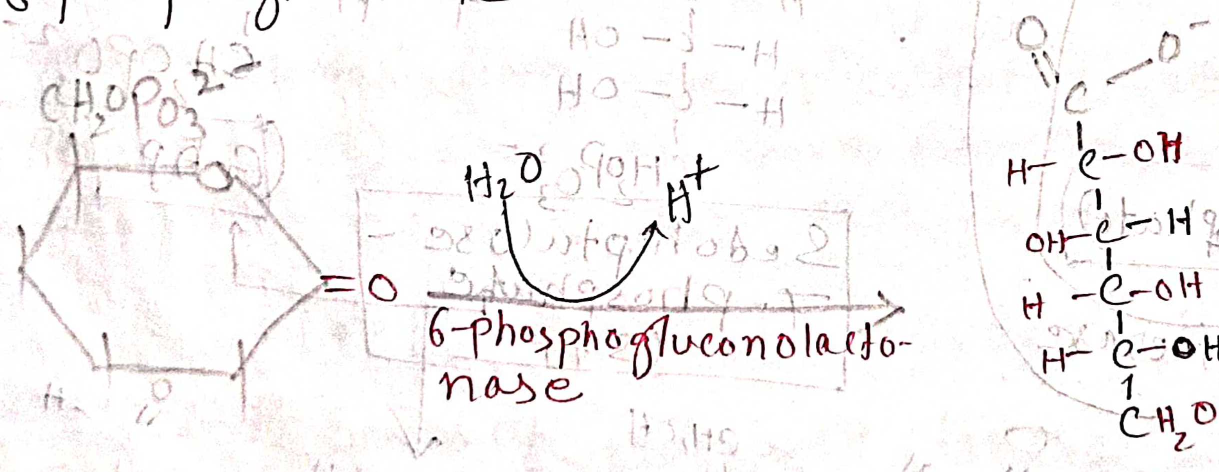
G6P
NADP+
NADPH + H+
①
G6P dehydrogenase
6-phosphoglucono-δ-lactone
H2O
H+
②
6-phosphogluconolactonase
6-phosphogluconate
NADP+
NADPH + CO2
6-phosphogluconate dehydrogenase
Ribulose-5-phosphate (Ru5P)
Ru5P-isomerase
Ribose-5-phosphate
Ru5P-epimerase
Sedoheptulose-7-phosphate (S7P)
GAP
Xylulose-5-phosphate
Glyceraldehyde-3-phosphate
F6P
Erythrose-4-phosphate

(A) Oxidative reactions produce NADPH in first reaction:

① Glucose-6-phosphate dehydrogenase catalyzes net transfer of a hydride ion to $NADP^+$ from C1 of G6P to form 6-phosphoglucono-δ-lactone.



② 6-Phosphogluconolactonase increases the rate of hydrolysis of 6-phosphoglucono-δ-lactone to 6-phosphogluconate.



③ 6-phosphogluconate dehydrogenase catalyzes the oxidative decarboxylation of 6-phosphogluconate, a β-hydroxy acid, to Ru5P and CO_2.

This reactions ~~is though to produced~~ proceed via the formation of a β-keto acid intermediate. The keto group presumably facilitates decarboxylation by acting as a electron sink.

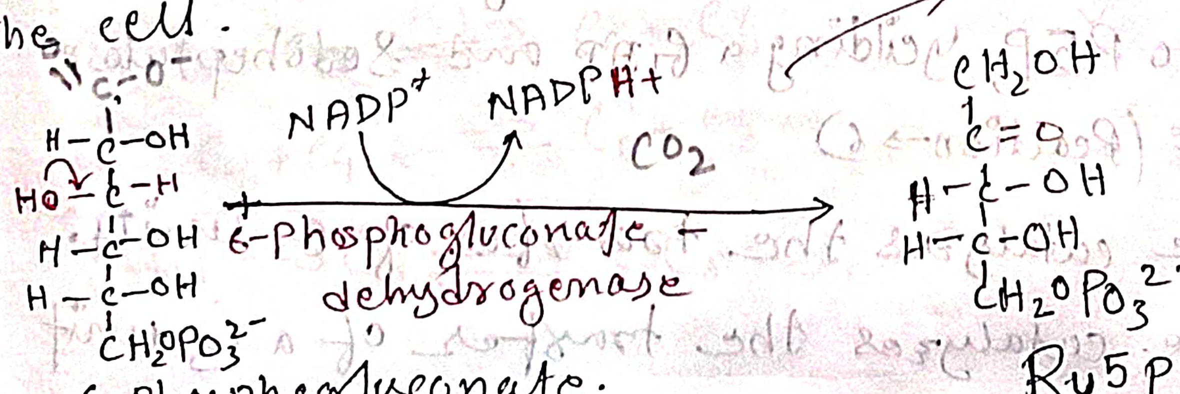
Formation of Ru5P completes the oxidative portion of the pentose phosphate pathway. It generates two molecules of NADPH for each molecule of G6P that enters the pathway.

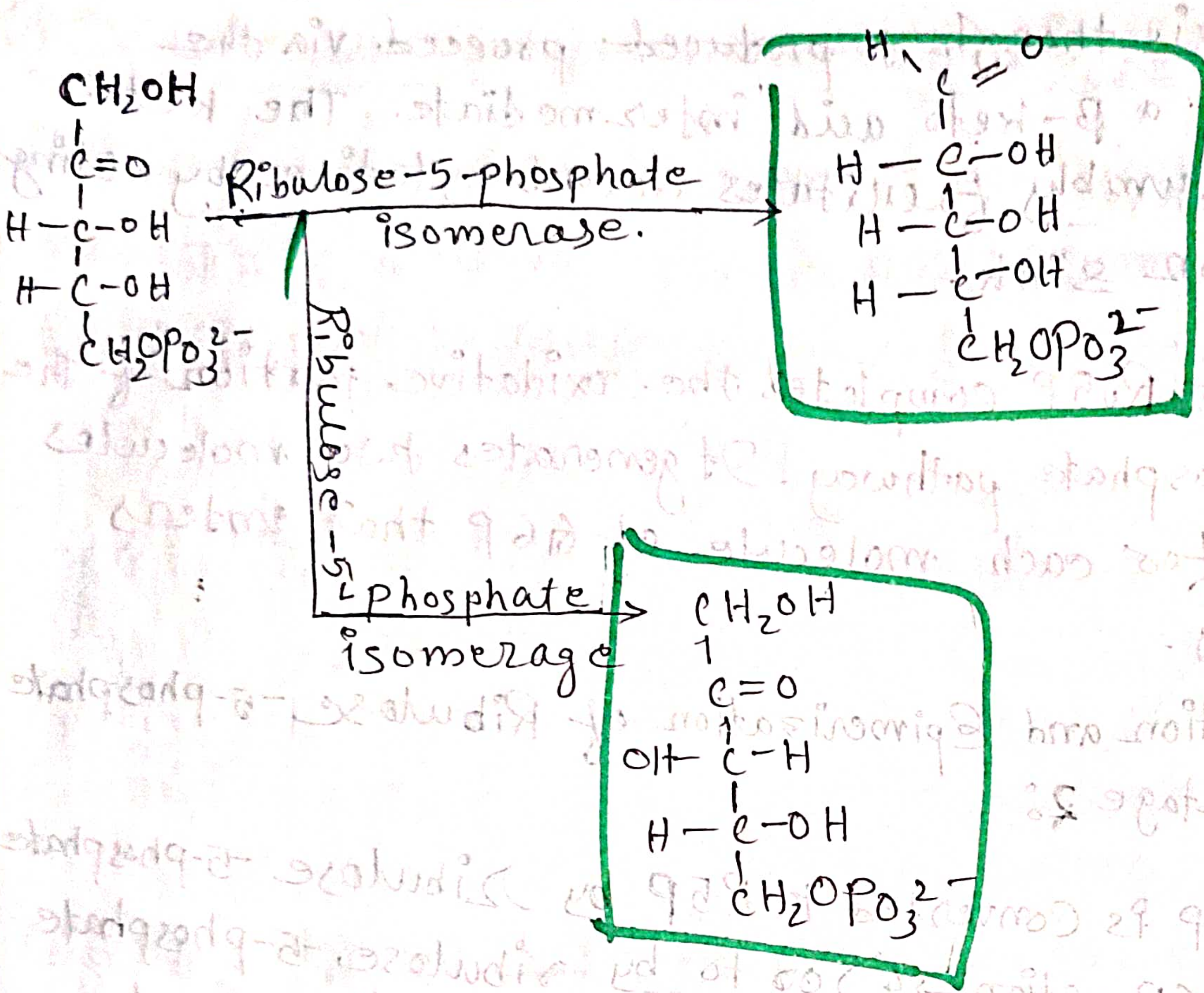
(B) Isomerization and Epimerization of Ribulose-5-phosphate occurs in Stage 2:

Ru5P is converted to R5P by ribulose-5-phosphate isomerase (Reaction → 4) or to by ribulose-5-phosphate epimerase (Reaction → 5). These isomerization and epimerization reactions, like the reaction catalyzed by triose phosphate isomerase are thought to occur via intermediate.

The relative amount of R5P and Xu5P produced form Ru5P depend on the needs of the cell.

A → ③ Reaction.





① Stage ③ involved carbon - carbon Bond Cleavage and formation:

Transketolage catalyzes the transfer of C_2 unit:

Transketose, which has a thiamine pyrophosphate cofactor, catalyzes the trasfer of a C_2 unit from Xu5P to R5P, yeilding a GAP and Sedoheptulose-7-phosphate (Reaction → 6)

Transaldolase catalyzes the transfes of C_3 unit:

Trasaldolase catalyzes the trasfer of a C_3 unit from S7P to GAP yeilding erythrose-4-phosphate (E4P) and fructose-6-phosphate (F6P).

A Second Transketolase Reaction yeilds Glyceraldehyde-3-phosphate and a Second fructose-6-phosphate molecule.

In a second transketolase reaction, a C_2 unit is transferred from a Second molecule of Xu5P to E4P to form GAP and another molecule of F6P (Reaction-8). The third Stage of the pentose phosphate pathway thus transforms two molecules of Xu5P and one of R5P to two molecule of F6P and one molecule of GAP. To summarize a Series of C-C bond formations and cleavage convert three C_5 Sugars to two C_6 Sugars and one C_3 Sugar. Here the number to the left of each reaction is keyed to the corresponding reaction.

(6) $C_5 + C_5 \rightleftharpoons C_7 + C_3$

(7) $C_7 + C_3 \rightleftharpoons C_6 + C_4$

(8) $C_5 + C_4 \rightleftharpoons C_6 + C_3$

Total = $3C_5 \rightleftharpoons 2C_6 + C_3$.

Gluconeogenesis :-

The nanocarbohydrate precursors that can be conver to glucose include the glycolysis products lactate and pyruvate, citric acid cycle intermediates, and the carbon skeletons of most amino acid.

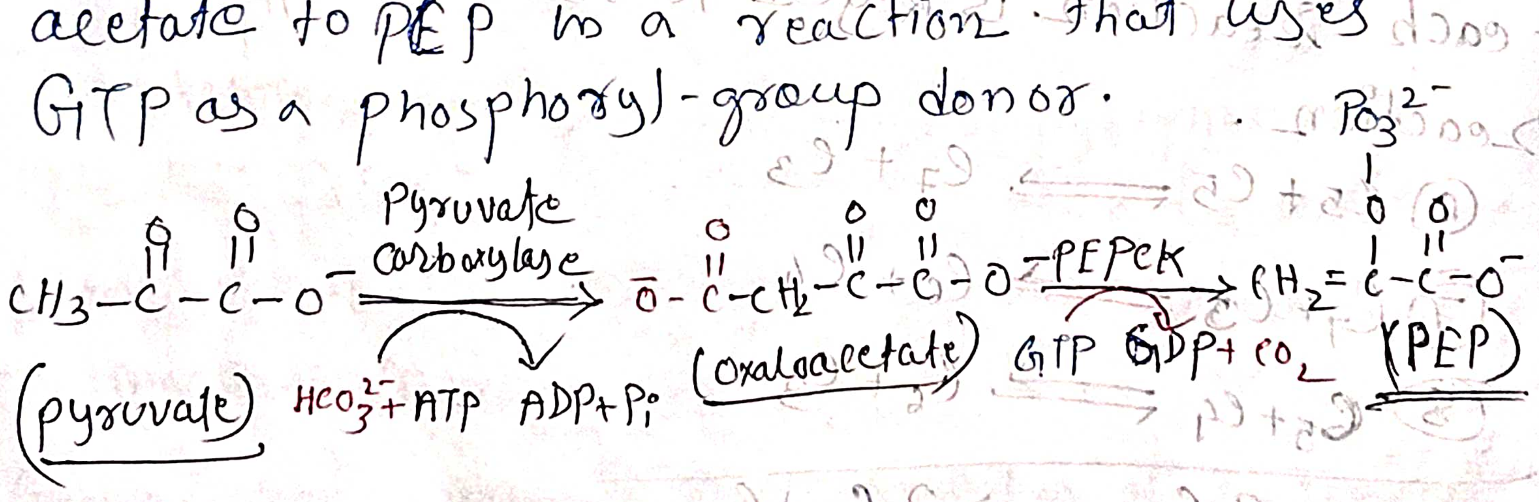
Gluconeogenesis is a pathway by which pyruvate is converted to Glucose.

(A) Pyruvate is converted to phosphoenolpyruvate in two steps.

In this reactions:-

(1) Pyruvate carboxylase catalyzes the ATP-driven formation of oxaloacetate from pyruvate and HCO_3^-.

(2) PEP carboxykinase (PEPCK) converts oxaloacetate to PEP in a reaction that uses GTP as a phosphoryl-group donor.



(B) A Series of Reversible reaction considered as a Second step of gluconeogenesis:-

(i) Phosphoenolpyruvate converted to 2-phosphoglycerate with the help of enolase enzyme.

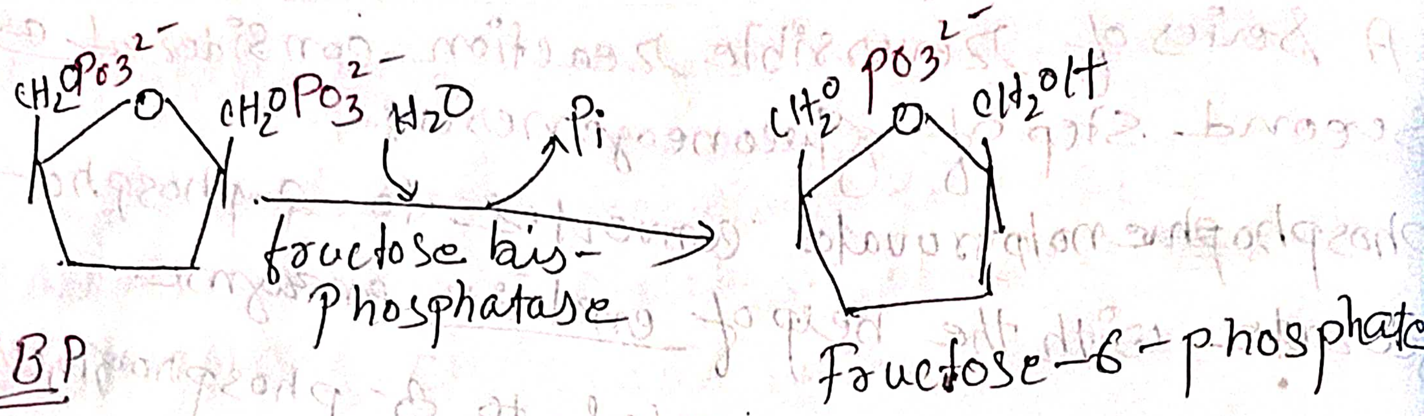
(ii) 2-phosphoglycerate converted to 3-phosphoglycerate with the help of phosphoglycerate mutase enzyme.

(iii) Phosphoglycerate kinase catalyzes the 3-phosphoglycerate into 1,3-bisphosphoglycerate by ~~withdrawing~~ withdrawing one P_i from ATP.

(iv) Glyceraldehyde-3-phosphate dehydrogenase catalyzes 1,3-bisphosphoglycerate in GAP and DHAP (which are interchangable with the help of triose phosphate isomerase enzyme) by withdrawing one P_i and H^+.

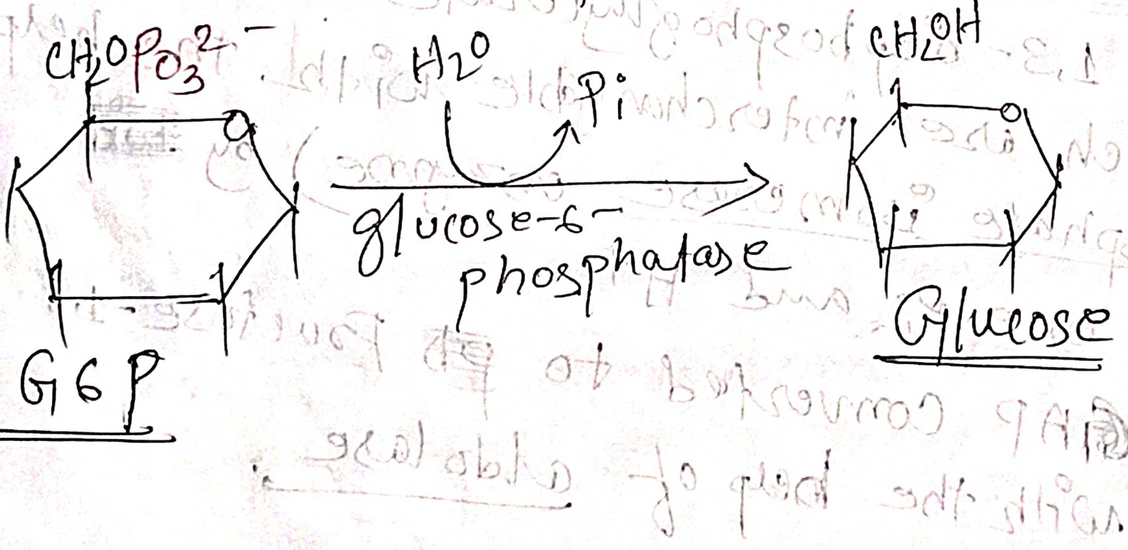
(v) DHAP or GAP converted to Fructose-1,6-bisphosphate with the help of aldolase.

(C) Fructose bis-phosphatase catalyze Fructose-1,6-bisphosphate into Fructose-6-phosphate by withdrawing one P_i with presence of water.



(D) Phosphoglucose isomerase catalyze the Fructose-6-phosphate to glucose-6-phosphate.

(E) Glucose-6-phosphatase converted G6P to Glucose by removing of Pi group from C_6 atom with presence of water.



Glycogenolysis:-

The breakdown of glycogen into glucose-6-phosphate is called glycogenolysis.

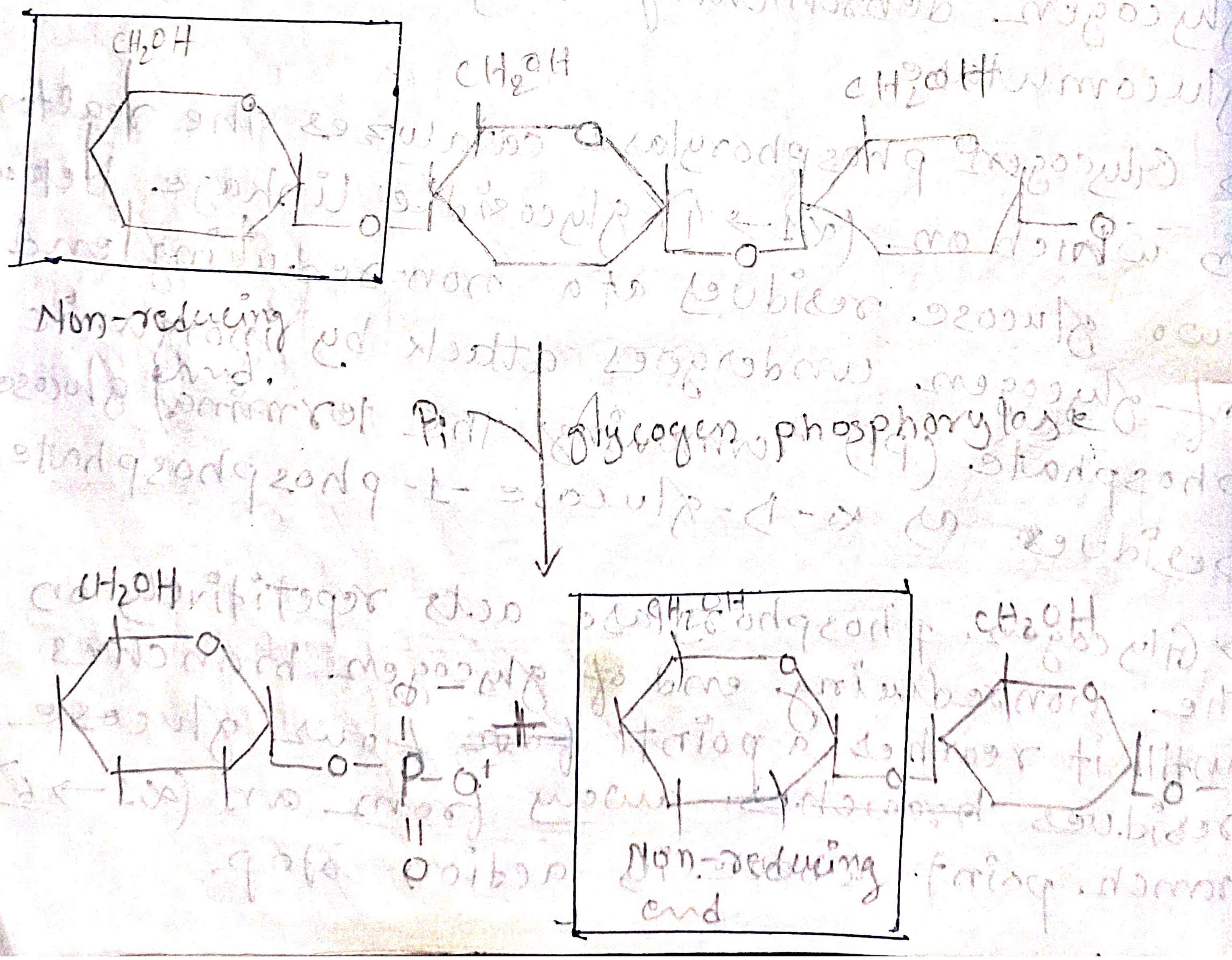
→ In skeletal muscle or liver the glucose units of the outer branches of glycogen enter the glycolytic pathway through the action of three enzymes: glycogen phosphorylase, glycogen debranching enzyme and phosphoglucomutase.

→ Glycogen phosphorylase catalyzes the reaction in which an (α1→4) glycosidic linkage between two glucose residues at a non-reducing end of glycogen undergoes attack by inorganic phosphate (Pi) removing the terminal glucose residue as α-D-glucose-1-phosphophosphate.

→ Glycogen phosphorylase acts repetitively on the nonreducing end of glycogen branches untill it reaches a point ~~four~~ four glucose residues ~~branches~~ away from an (α1→6) branch point, where its action stop.

→ Further degradation by glycogen phosphorylase can occur only after the debranching enzyme formally known as oligo (α1→6) to (α1→4) glucantransferase, catalyzes two successive reactions that transfer branches.

→ Once these branches are transferred and glucosyl residue at C-6 is hydrolyzed glycogen phosphorylase activity can continue.



In other words:

⇒ Glycogen breakdown near an i.e four glucose residues away from an ($\alpha 1 \rightarrow 6$) branch point.

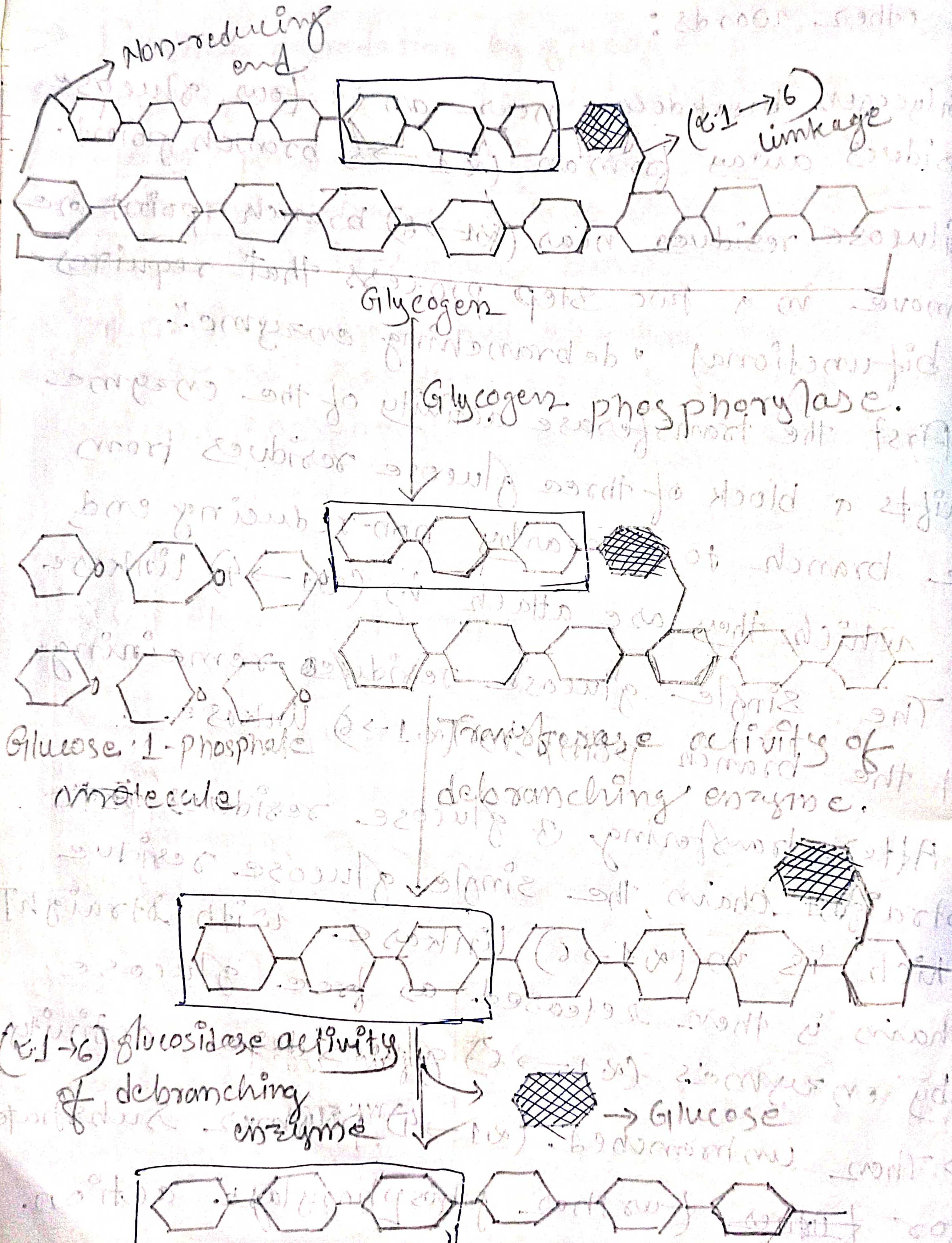
⇒ Glucose residues near ($\alpha 1 \rightarrow 6$) branch point are remove in a two step process that requires a bifunctional "debranching enzyme".

⇒ First the transferase activity of the enzyme shifts a block of three glucose residues from the branch to a nearby non-reducing end, to which they are attach in ($\alpha 1 \rightarrow 4$) linkase.

⇒ The single glucose residues remaining at the branch point, in ($\alpha 1 \rightarrow 6$) linkase.

⇒ After transfering 3 glucose residues into straight chain, the single glucose residue which is in ($\alpha 1 \rightarrow 6$) linkase with straight chain is then released as free glucose by enzyme's ($\alpha 1 \rightarrow 6$) glucosidase activity.

⇒ Then unbranches ($\alpha 1 \rightarrow 4$) polymer substrate for ~~futher~~ further phosphorylase action.


Non-reducing end
(α1→6) linkage
Glycogen
Glycogen phosphorylase.
Glucose 1-phosphate molecule
Transferase activity of debranching enzyme.
(α1→6) glucosidase activity of debranching enzyme
Glucose

(8) How glucose-1-Phosphate converted into Glucose-6-phosphate?

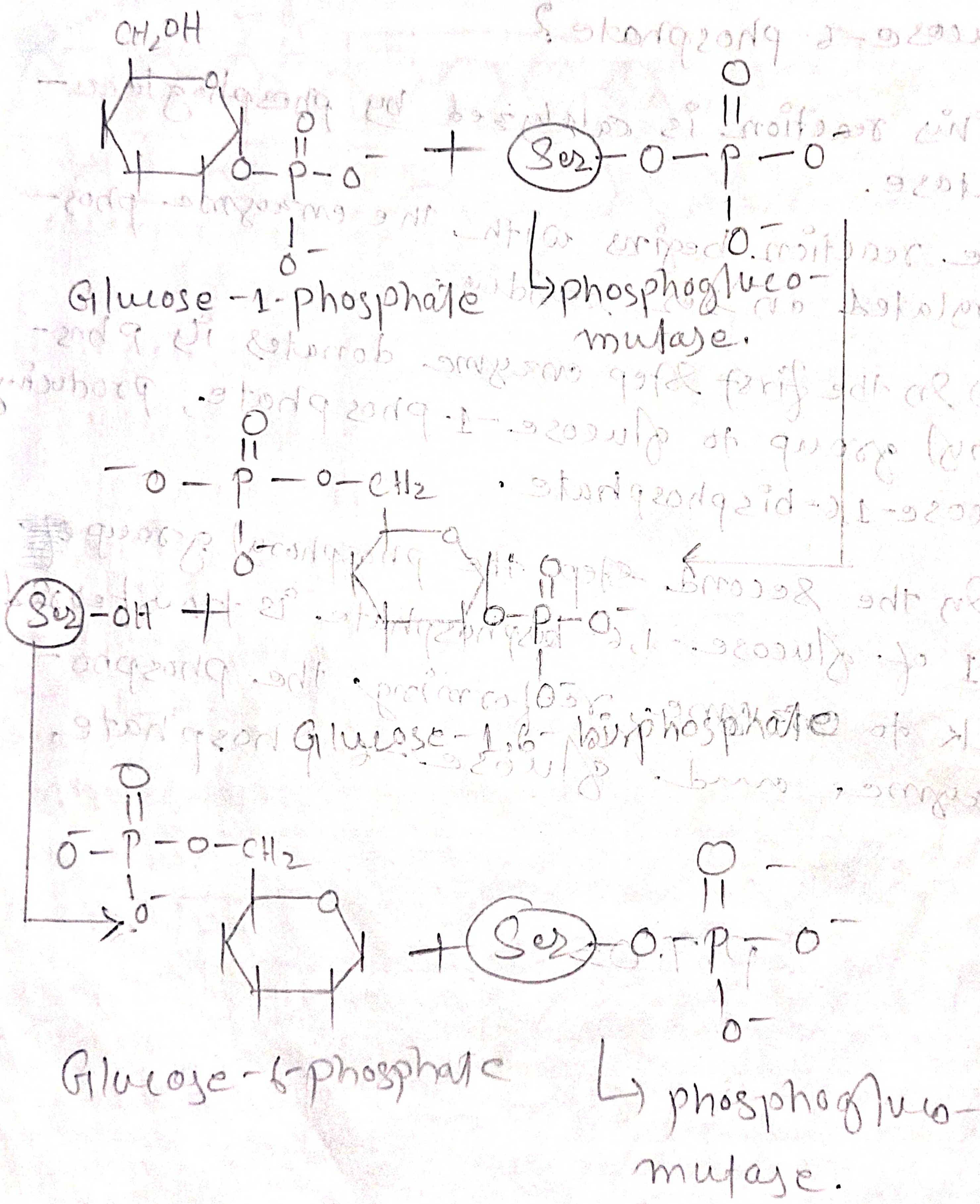
⇒ This reaction is catalyzed by phosphoglucomutase.

The reaction begins with the enzyme phosphorylated on ser residue.

(i) In the first step enzyme donates its phosphoryl group to glucose-1-phosphate, producing glucose-1,6-bisphosphate.

(ii) In the second step the phosphoryl group at C-1 of glucose-1,6-bisphosphate is transferred back to enzyme, re-forming the phospho-enzyme and glucose-6-phosphate.

P.T.O →


CH2OH
Ser
Glucose-1-phosphate
phosphoglucomutase.
Ser
Glucose-1,6-bisphosphate
Ser
Glucose-6-phosphate
phosphoglucomutase.