Chapter 8

Carbohydrate Metabolism

Overview of Metabolism

Metabolism

- The sum total of **chemical changes/reactions** in chemically complex biological system
- Generally include processes by which cells:-
  - Convert **simpler molecules** (Lactate, ethanol, H_2O, CO_2, NH_3 etc…) into biological **macromolecules**
    - ✓To serve as ***structural/functional components***; ***cell growth***, ***cell replication*** etc…
    - ✓ Process is called **anabolism** (or **biosynthesis**)
      - Mostly require input of energy (as ATP or reduced electron carriers)
  - Degrade **macromolecules** to obtain **energy** or to **recycle/salvage** their building blocks for future construction
    - ✓ Process is called catabolism

Overview of Metabolism

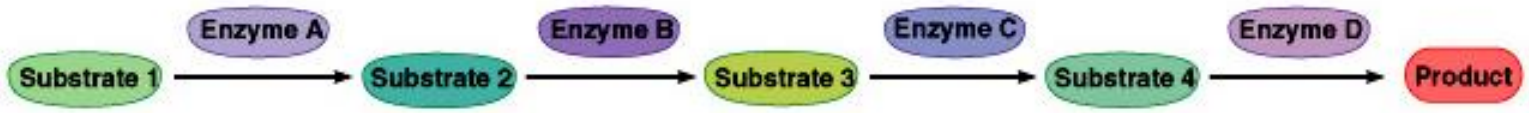
Why to deal with metabolism

- It is a means by which living things acquire and use energy from the environment.
- It accompany such cell changes (reproduction, aging and death), disease conditions etc…
- Basic understanding of the process is helpful to control reactions in a living cell.

Overview of Metabolism

Metabolic pathways

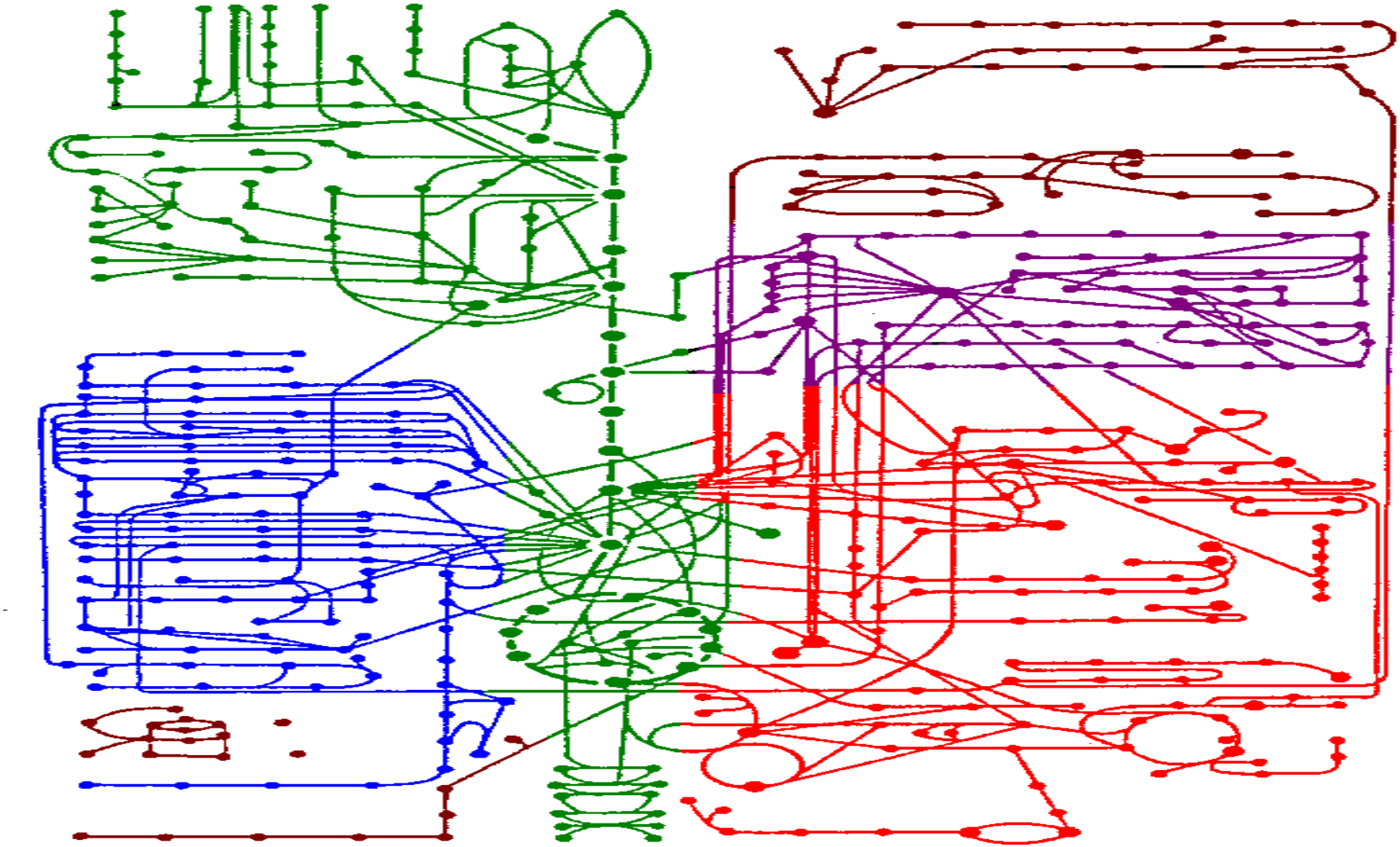
- Series of enzyme-controlled reactions leading to formation of a product
  - Each involve new substrate, enzyme and a product (s)



- Metabolic pathways can be
  - **Linear**-product of rxns are substrates for subsequent rxns
  - **Branched**- yield multiple useful end products from a single precursor or converting several starting materials into a single product
  - **Cyclic**-starting materials are regenerated in a series of reactions or intermediates are recycled
  - **Spiral** - same set of enzymes used repeatedly

Metabolism Overview of

Metabolic pathways



Overview of Metabolism

- Energy is extracted, channeled, and consumed in living system.

- Quantitative study of energy transformations in biological systems is termed as **bioenergetics**

- Biological processes usually take place at constant temperature and pressure, thus only **free energy** is available to do work.
  - So life obeys the **laws of thermodynamics**

- The flow of electrons in **oxidation-reduction reactions** underlies energy transduction in living cells.

- Living organisms extract energy from either **fuels/dietary/** or **sunlight.**

Overview of Metabolism

Gibbs free energy (G)

– Is energy available to do useful work

– Is related to the change in enthalpy and the change in entropy:

$$G = H - TS$$

So change in G (ΔG) = $\Delta H - T\Delta S$

❖ **Sign of ΔG determines reaction direction**

➢ **ΔG < 0 (*negative ΔG*) *"favorable",*** "spontaneous", **exergonic** process
i.e Process goes **left to right, just as described**

➢ **ΔG > 0 (*positive ΔG*) *"unfavorable", "non*-**spontaneous", **endergonic** process
i.e Process goes **right to left, reverse direction** from what is written

➢ **ΔG = 0 Process is *AT EQUILIBRIUM; no net* reaction in either direction**

❖**For the Process:** $H_2O_{solid} \rightleftharpoons H_2O_{liquid}$

Temp.	ΔH (kJ/mol)	TΔS (kJ/mol)	ΔG = ΔH - TΔS (kJ/mol)	Direction toward equilib?
+10 °C	+6.4	+6.6	–0.2	**to right**
0 °C	+6.0	+6.0	0	**Neither (at equilib.)**
–10 °C	+5.6	+5.4	+0.2	**to left**

Overview of Metabolism

Free energy and chemical reactions

- **For the reaction of type** $aA + bB \rightleftharpoons cC + dD$
- **The overall free energy change (ΔG) is given by** $\Delta G' = \Delta G^{\circ\prime} + RT \ln \frac{[C]^c[D]^d}{[A]^a[B]^b}$
- Many biological processes are **endergonic (**ΔG’ > 0)
  - ➢also with physiological mass action ratio > equilib. m.a. ratio
- But a reaction that "wants" to go in reverse are seen "driven” forwards.

How????? – **(mostly by free energy coupling)**

Reaction	ΔGo'	Reaction direction
1. Condensation of glucose (alcohol) with inorganic phosphate ion (acid) **Glucose + Pi <==> Glucose-6-P + H2O**	**+ 13.8 kJ/mol**	Reverse
2. **Hydrolysis of ATP, a phosphoanhydride** **ATP + H2O <==> ADP + Pi**	**– 30.5 kJ/mol**	Forward
Coupling these reactions (add the two reactions) **ATP + glucose <==> glucose-6-P + ADP**	**–16.7 kJ/mol**	Forward

Overview of Carbohydrate Metabolism

- Carbohydrates account for ca 60% of daily food intake
  - Supply about 45% of the body energy requirements
- They are obtained from various diets such as ***milk, meat, vegetables, grains*** and ***grain products***
- Many metabolic processes are associated with carbohydrates including:-
  - **Glycolysis**:- oxidation of glucose (other monosaccharides) in to pyruvate
  - **Citric acid cycle (Kreb's cycle)-** transformation of pyruvate (acetyl CoA) in to reduced electron carriers (FADH2, NADH)

    -But also related with metabolism of other biomolecules
  - **Electron transport chain & phosphorylation:-** transformation of redued electron carriers (FADH2, N NADH) in to O2 and H2O

    - But also related with metabolism of other biomolecules
  - **Glycogenolysis**:- hydrolysis of glycogen stored in liver & muscle
  - **Glycogenesis**:- biosynthesis of glycogen in liver & muscle
  - **Gluconeogenesis**:- biosynthesis of glucose from 2-5 carbon molecules
  - **Pentose phosphate pathway**:- biosynthesis of ribose sugar & NADPH from glucose

Glycolysis

Glycolysis

- Glycolysis is a process/pathway that cleaves glucose ($C_6H_{12}O_6$) into two molecules of pyruvate ($C_3H_3O_3$).

Overall reaction

$$\text{Glucose} + 2NAD^+ + 2ADP + 2\ Pi \rightarrow 2\ \text{pyruvate} + 2NADH + 2H^+ + 2ATP + 2H_2O$$

$$\Delta G^{o'} = -35.5\ kJ/mol$$

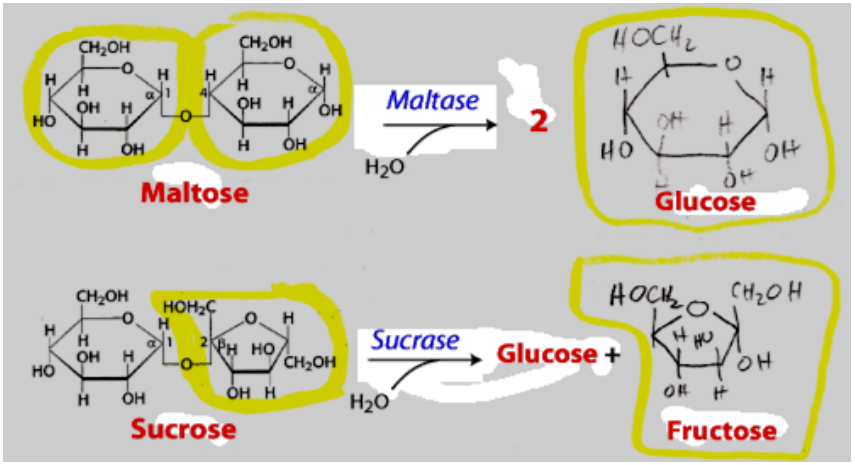
- Glycolysis essentially occur in all cells and organisms (in cytosol)

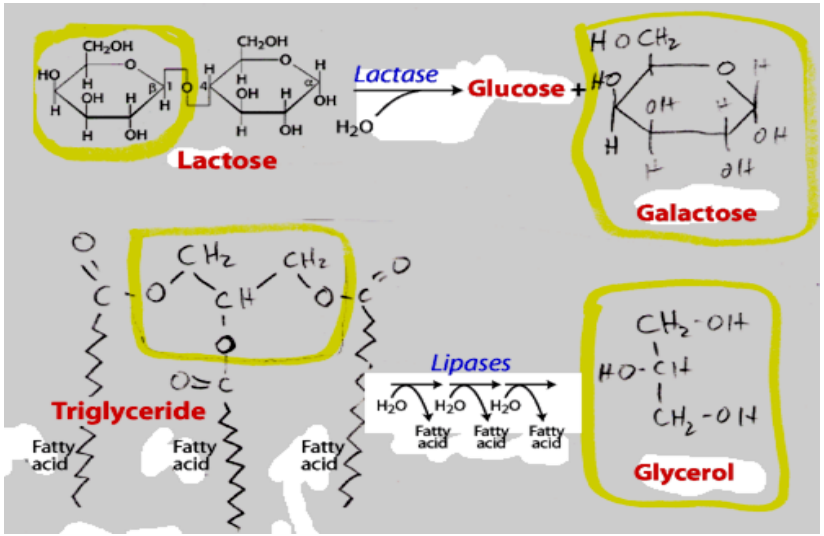
- **Role**
  - Generates a small amount of ATP which is critical under anaerobic conditions.
    - Eg. during heavy exercise and fermentation
    - The only ATP generating pathway for certain cells and tissues (brain, eye, RBC, renal medulla and sperm cells)
  - Generates pyruvate (a precursor to acetyl CoA), lactate, and ethanol (in yeast).
  - Versatile source of a large variety of metabolic intermediates in synthesis of
    - Amino acid
    - Nucleic acid
    - Triacylglycerides etc

Glycolysis

Source of glucose & other monosaccharides

- **Digestion of starch rich foods**
  - Ptyalin/salivary amylase/:- randomly cleave α-1-4-glycosidic bonds of starch to yield limited amounts of smaller oligosaccharides
  - Pancreatic amylase(in alkaline):- partially degrade disaccharides are in to monosaccharides
- **Digestion of disaccharides** (maltose ,sucrose, lactose, trehalose etc)
- **Digestion of triglycerides** by hydrolytic enzymes





- **Glucose** and **monosaccharides** rich foods
- **Some other metabolic processess**
  - **Glycogenolysis**:- hydrolysis of glycogen stored in liver & muscle
  - **Gluconeogenesis**:- biosynthesis of glucose from 2-5 carbon molecules

Glycolysis

Absorption of monosaccharides

- Absorption involves passage of glucose and other monosaccharides across the lumen of digestive tract (mainly small intestine) Into blood or lymph
  - Mainly through portal vein
  - Usually occurs along with vitamins and electrolytes
- Absorption of glucose ***increases plasma sugar level***
  - Different foods differ in their property to increase blood sugar level
- After absorption, ***glucose will be oxidized*** in the cells to produce energy
  - Through Glycolysis, Citric acid cycle, Oxidative phosphorylation and Electron transport chain (ETC)

Glycolysis

Glucose transport

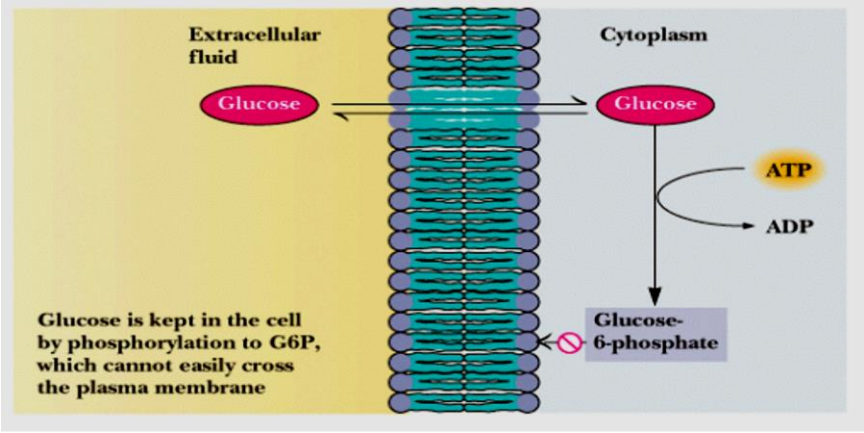
❖The first steps in glucose metabolism in any cell is transport across membranes

❖ Glucose entry into most cells is concentration driven and dependent on sodium

- Normal glucose concentration in peripheral blood is 4-8 mM (70-140 mg/dL)

❖ Transport involve five glucose transporters (GLUT1-5)

- That differ in their affinities for glucose and dominant site of existance



❖ Once inside the cell each glucose units must be phosphoryled by kinases to prevent transporter mediated efflux

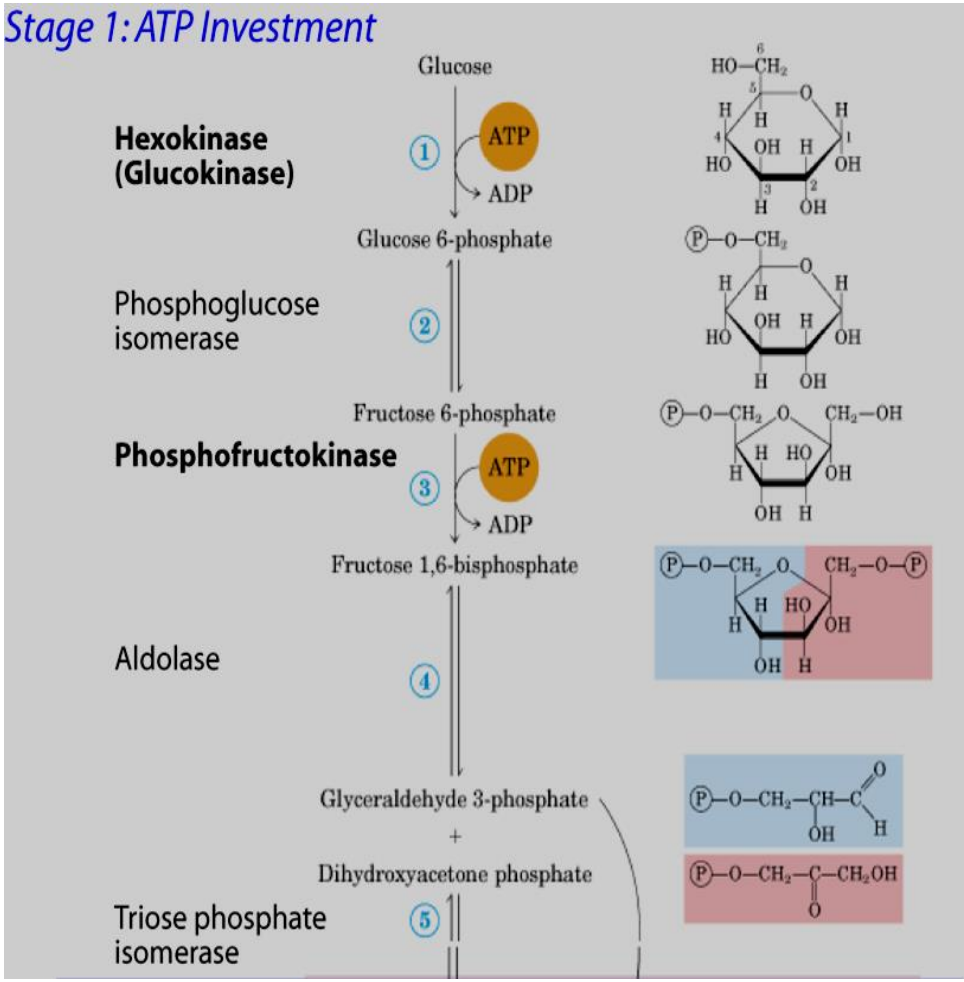
Glycolysis

Steps in glycolysis

- Glycolytic pathway consists of ten reaction steps
  - Each catalyzed by specific enzymes
  - Organized into two stages (Preparatory and pay off phases)

Stage 1

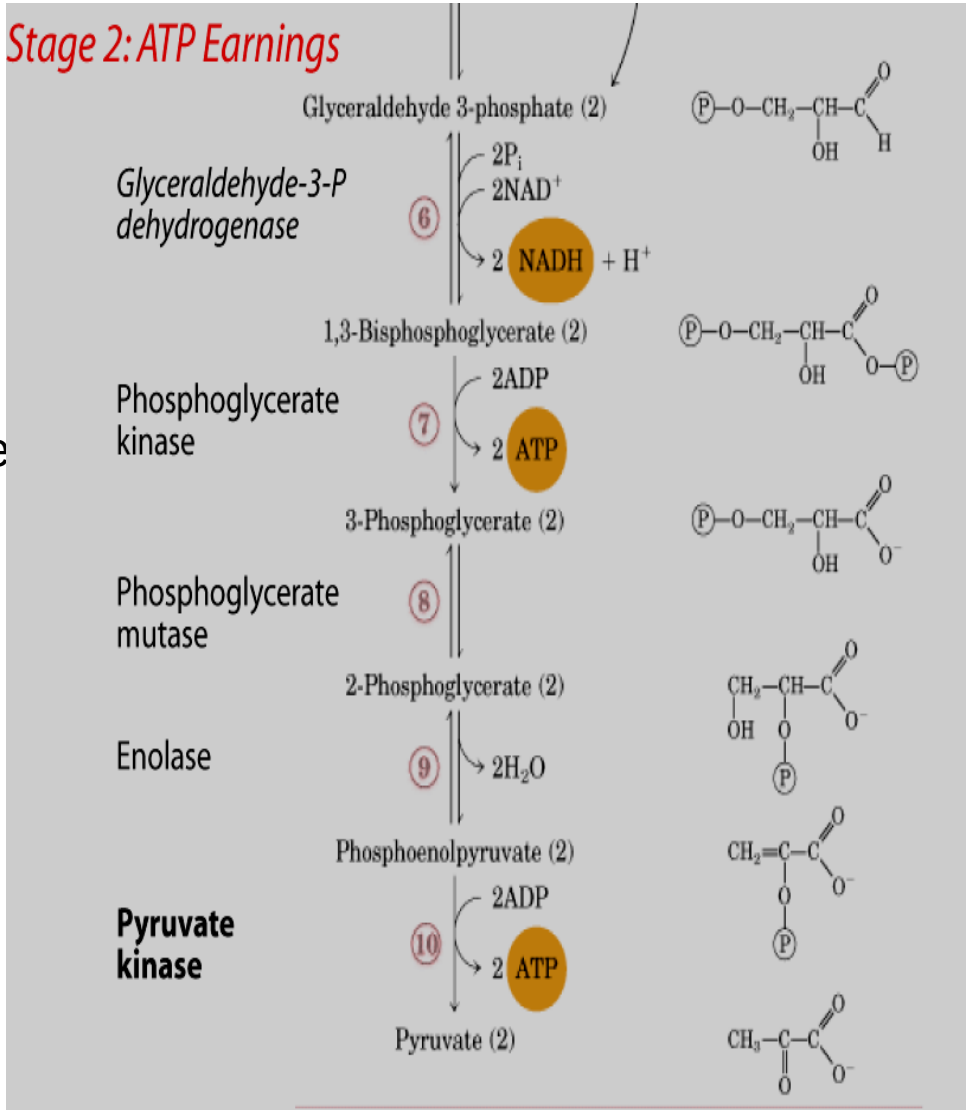
- Involve five steps
- Occur with investment of two ATP
- Yield two Glyceraldehyde-3-P (GAP)
- Engage two highly regulated enzymes
  - Hexokinase &
  - Phosphofructokinase (PFK- 1)



Glycolysis

Stage 2

- An oxidation reaction catalyzed by glyceraldehyde-3-P dehydrogenase generates 2 NADH molecules
- Two substrate level phosphorylation reactions catalyzed by Phosphoglycerate kinase and Pyruvate kinase
  -yielding 4 ATP (2 net ATP)
- Two molecules of pyruvate are produced at last



Glycolysis

- Summary of features of reactions & Free energy changes for the ten glycolytic reactions

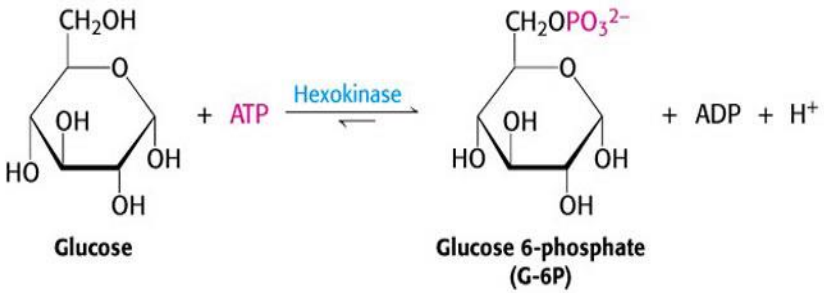
Enzyme	Reaction type	$\Delta G^{\circ\prime}$ in kcal mol^{-1} (kJ mol^{-1})	ΔG in kcal mol^{-1} (kJ mol^{-1})
1. Hexokinase	Phosphoryl transfer	−4.0 (−16.7)	−8.0 (−33.5)
2. Phosphoglucose isomerase	Isomerization	+0.4 (+1.7)	−0.6 (−2.5)
3. Phosphofructokinase	Phosphoryl transfer	−3.4 (−14.2)	−5.3 (−22.2)
4. Aldolase	Aldol cleavage	+5.7 (+23.8)	−0.3 (−1.3)
5. Triose phosphate isomerase	Isomerization	+1.8 (+7.5)	+0.6 (+2.5)
6. Glyceraldehyde 3-phosphate dehydrogenase	Phosphorylation coupled to oxidation	+1.5 (+6.3)	+0.6 (+2.5)
7. Phosphoglycerate kinase	Phosphoryl transfer	−4.5 (−18.8)	+0.3 (+1.3)
8. Phosphoglycerate mutase	Phosphoryl shift	+1.1 (+4.6)	+0.2 (+0.8)
9. Enolase	Dehydration	+0.4 (+1.7)	−0.8 (−3.3)
10. Pyruvate kinase	Phosphoryl transfer	−7.5 (−31.4)	−4.0 (−16.7)

$\Delta G^{\circ\prime}$ = -35.5 kJ/mol ΔG = -72.4 kJ/mol

Glycolysis

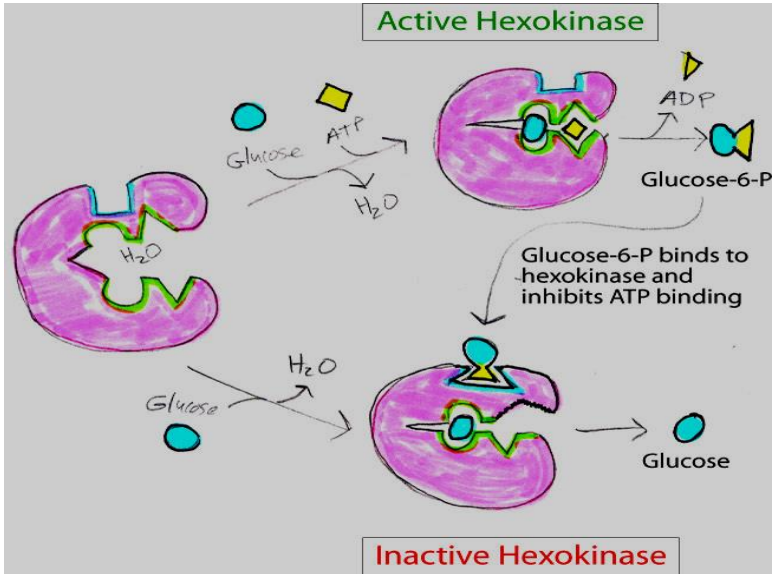
Reaction 1

- Involve phosphorylation of glucose by **hexokinase** (in all cells) or **glucokinase** (primarily in liver and pancreatic cells)



- **Hexokinase** binds glucose through an induced fit mechanism that excludes H_2O from the enzyme active site and brings the phosphoryl group of ATP into close proximity with the C-6 carbon of glucose

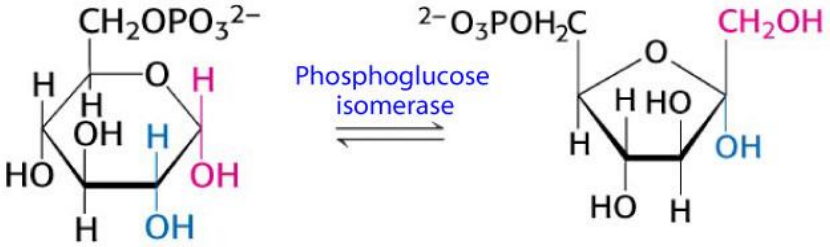
- Hexokinase is **feedback inhibited** by glucose-6-P which binds to a regulatory site in the amino terminus of the enzyme



Glycolysis

Reaction 2

- Isomerization of glucose-6-P to fructose-6-P occur by phosphoglucose isomerase



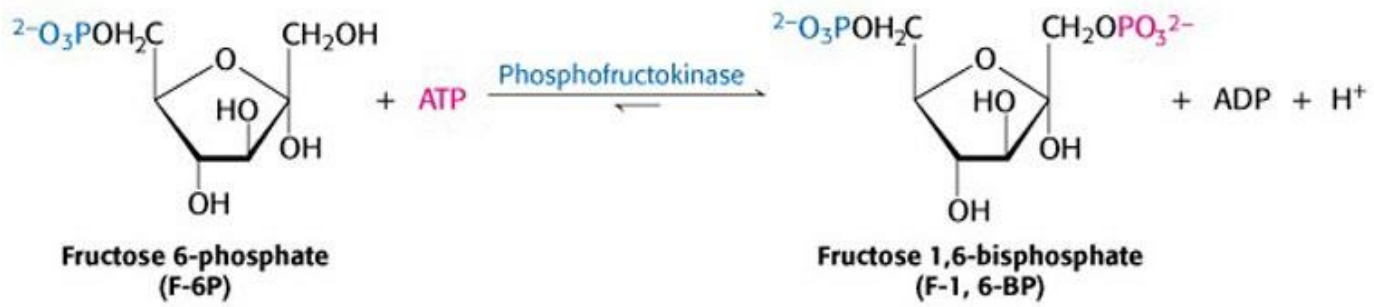
Glucose 6-phosphate (G-6P) **Fructose 6-phosphate (F-6P)**

- Phosphoglucose isomerase (phosphohexose isomerase) interconverts an aldose (glucose-6-P) and a ketose (fructose-6-P) through a complex reaction mechanism that involves opening and closing of the ring structure.

Glycolysis

Reaction 3

❖ Phosphorylation of fructose-6-P to fructose-1,6-BP by phosphofructokinase 1



Fructose 6-phosphate (F-6P)

Fructose 1,6-bisphosphate (F-1, 6-BP)

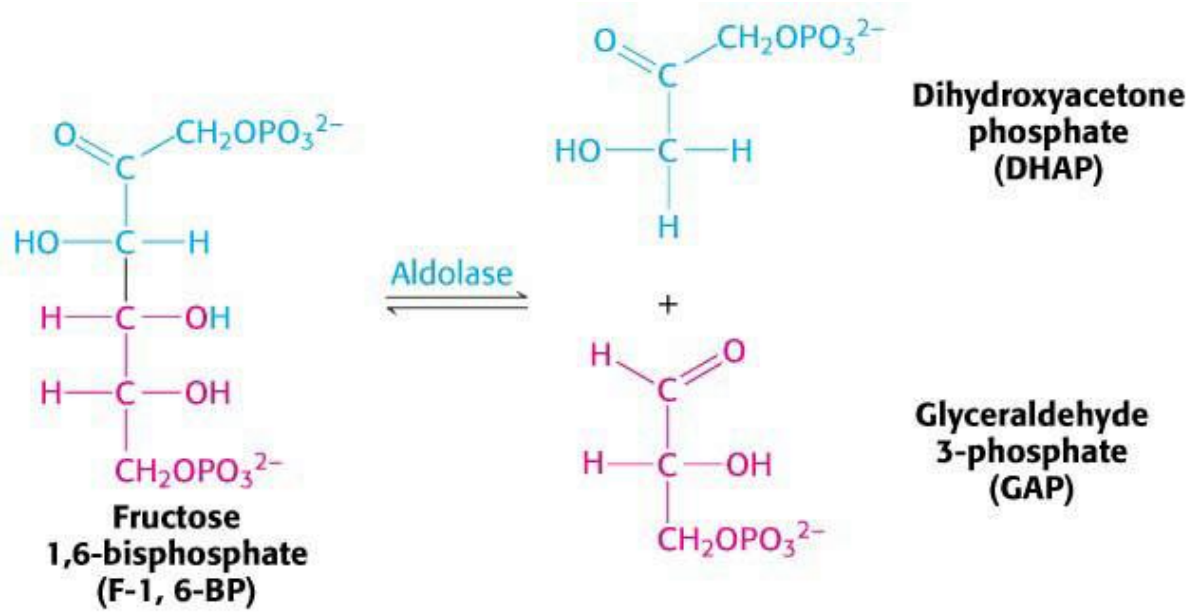
❖ Provide the second ATP investment reaction in glycolysis and involves the coupling of an ATP phosphoryl transfer reaction catalyzed by the enzyme phosphofructokinase 1 (PFK-1).

❖ This is a key regulated step in the glycolytic pathway because the activity of PFK-1 is controlled by numerous allosteric effectors (positive and negative).

Glycolysis

Reaction 4

- Cleavage of fructose-1,6-BP by aldolase to generate ***glyceraldehyde-3-P (GAP)*** and ***dihydroxyacetone-P (DHAP)***

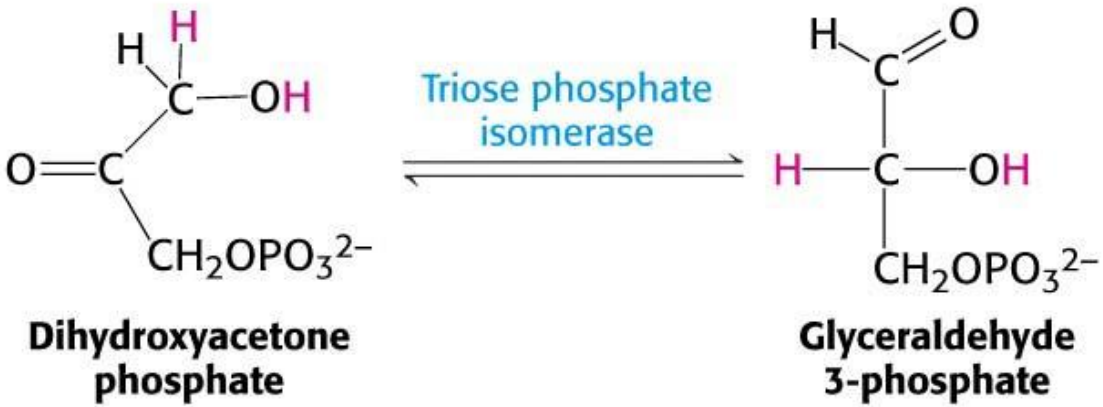


- The splitting of fructose-1,6-BP into the triose phosphates glyceraldehyde-3-P and dihydroxyacetone-P is the reaction that puts the lysis in the term “Glyco**lysis**”.

Glycolysis

Reaction 5

- Isomerization of dihydroxyacetone-P to glyceraldehyde-3-P by triose phosphate isomerase

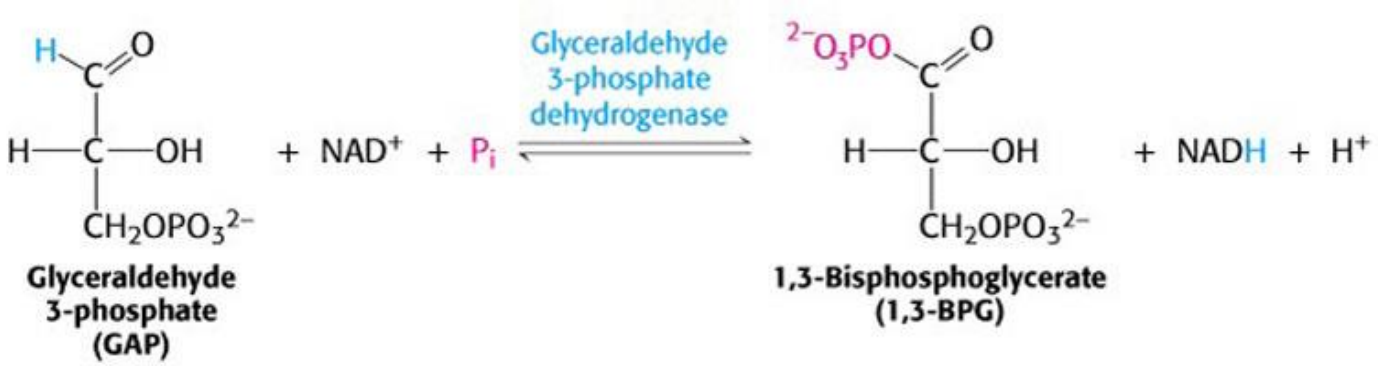


- Glyceraldehyde-3-P, rather than dihydroxyacetone-P, is the substrate for reaction 6 in the glycolytic pathway, making this isomerization necessary.

Glycolysis

Reaction 6

❖ Oxidation and phosphorylation of glyceraldehyde-3-P by glyceraldehyde-3-P dehydrogenase to form 1,3-bisphosphoglycerate

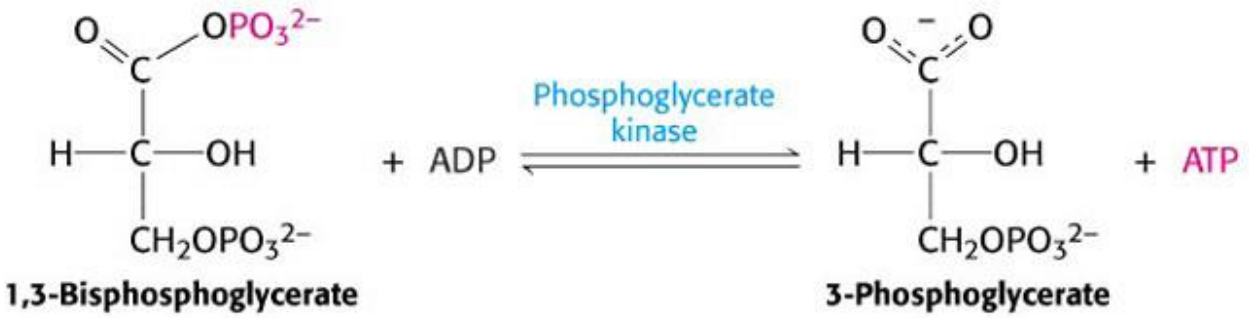


❖ The glyceraldehyde-3-P dehydrogenase reaction is a critical step in glycolysis because it uses the energy released from oxidation of glyceradehyde-3-P to drive a phosphoryl group transfer reaction using inorganic phosphate (P_i) to produce 1,3-bisphosphoglycerate.

Glycolysis

Reaction 7

- Generate ATP during conversion of 1,3-bisphosphoglycerate to 3-phosphoglycerate by phosphoglycerate kinase



1,3-Bisphosphoglycerate → 3-Phosphoglycerate

- Phosphoglycerate kinase catalyzes the payback reaction in glycolysis because it replaces the 2 ATP that were used in stage 1 to prime the glycolytic pathway.

- Remember, this occurs twice for every glucose that entered glycolysis. This is an example of a **substrate level [ADP] phosphorylation** reaction, i.e., ATP synthesis that is not the result of aerobic respiration or photophosphorylation.

Glycolysis

Reactions 6 and 7 are coupled reactions!

Rxn 6

Glyceraldehyde-3-P + Pi + NAD^+ → 1,3-bisphosphoglycerate + NADH + H^+

$\Delta G^{o'}$ = +6.3 kJ/mol *ΔG = -1.3 kJ/mol*

Rxn 7

1,3-bisphosphoglycerate + ADP → 3-phosphoglycerate + ATP

$\Delta G^{o'}$ = -18.9 kJ/mol *ΔG = +0.1 kJ/mol*

Coupled Reactions (add $\Delta G^{o'}$ values)

Glyceraldehyde-3-P + Pi + ADP + NAD^+ → 3-phosphoglycerate + ATP + NADH + H^+

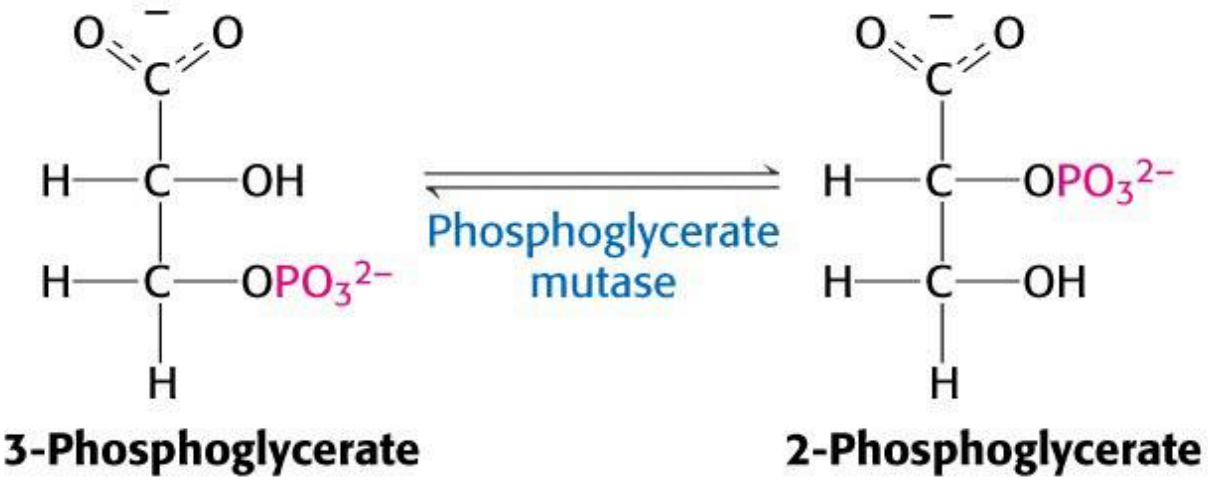
$\Delta G^{o'}$ = -12.6 kJ/mol *ΔG = -1.2 kJ/mol*

Actual change in free energy (ΔG) for each of these two reactions is very close to zero, and therefore both reactions are in fact reversible inside the cell. This is important for controlling flux through glycolysis and gluconeogenesis.

Glycolysis

Reaction 8

- Phosphoryl shift by phosphoglycerate mutase to convert 3-phosphyglycerate to 2-phosphoglycerate



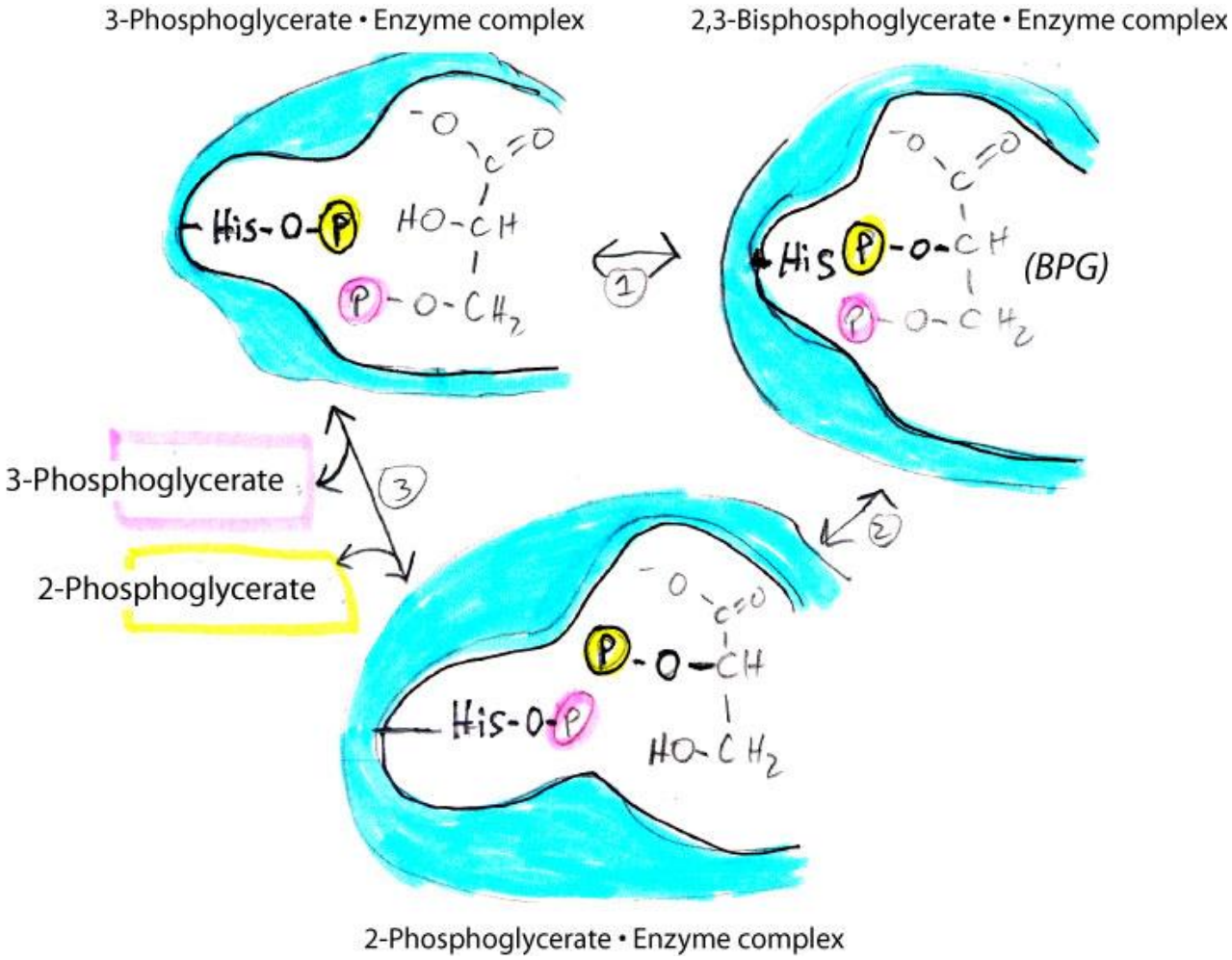
- The purpose of reaction 8 is to generate a compound, 2-phosphoglycerate, that can be converted to phosphoenolpyruvate in the next reaction, in preparation for a second substrate level phosphorylation to generate ATP.

Glycolysis

❖ The mechanism of this **highly reversible reaction** requires a phosphoryl transfer from a phosphorylated histidine residue (His-P) located in the enzyme active site

The metabolic intermediate 2,3-BPG can diffuse out of active site before it is converted to 2-phosphoglycerate.

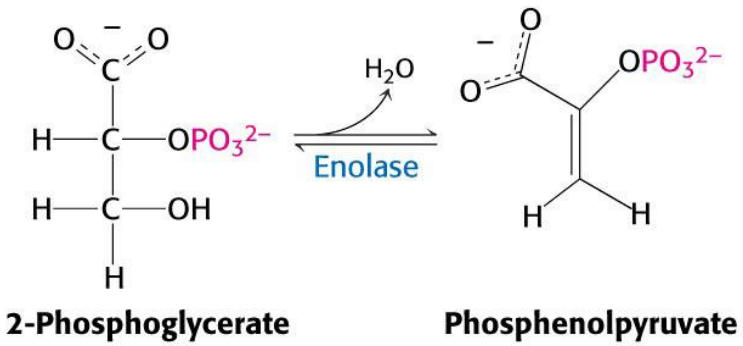
Remember that 2,3-BPG is important in the regulation of oxygen binding by hemoglobin.



Glycolysis

Reaction 9

❖Dehydration of 2-phosphoglycerate by enolase to form phosphoenolpyruvate (PEP)



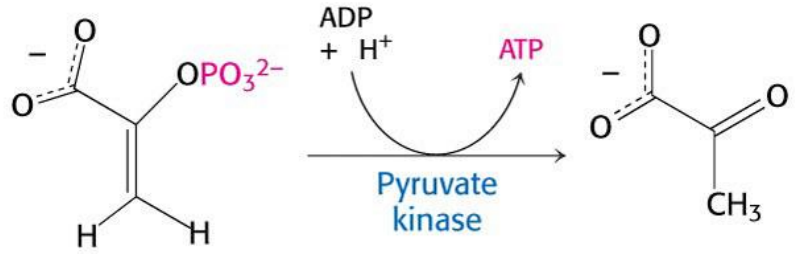
❖ The standard free energy for this reaction is relatively small ($\Delta G^{\circ\prime}$ = +1.7 kJ/mol) but it traps the phosphate group in an unstable enol form, resulting in a dramatic increase in the phosphoryl transfer potential of the triose sugar.

❖ Standard free energy change for phosphate hydrolysis in 2-phosphoglycerate is $\Delta G^{\circ\prime}$ = -16 kJ/mol, whereas the standard free energy change for phosphate hydrolysis of phosphoenolpyruvate it is an incredible $\Delta G^{\circ\prime}$ = -62 kJ/mol !

Glycolysis

Reaction 10

❖ Generation of ATP by pyruvate kinase when phosphoenolpyruvate is converted to pyruvate

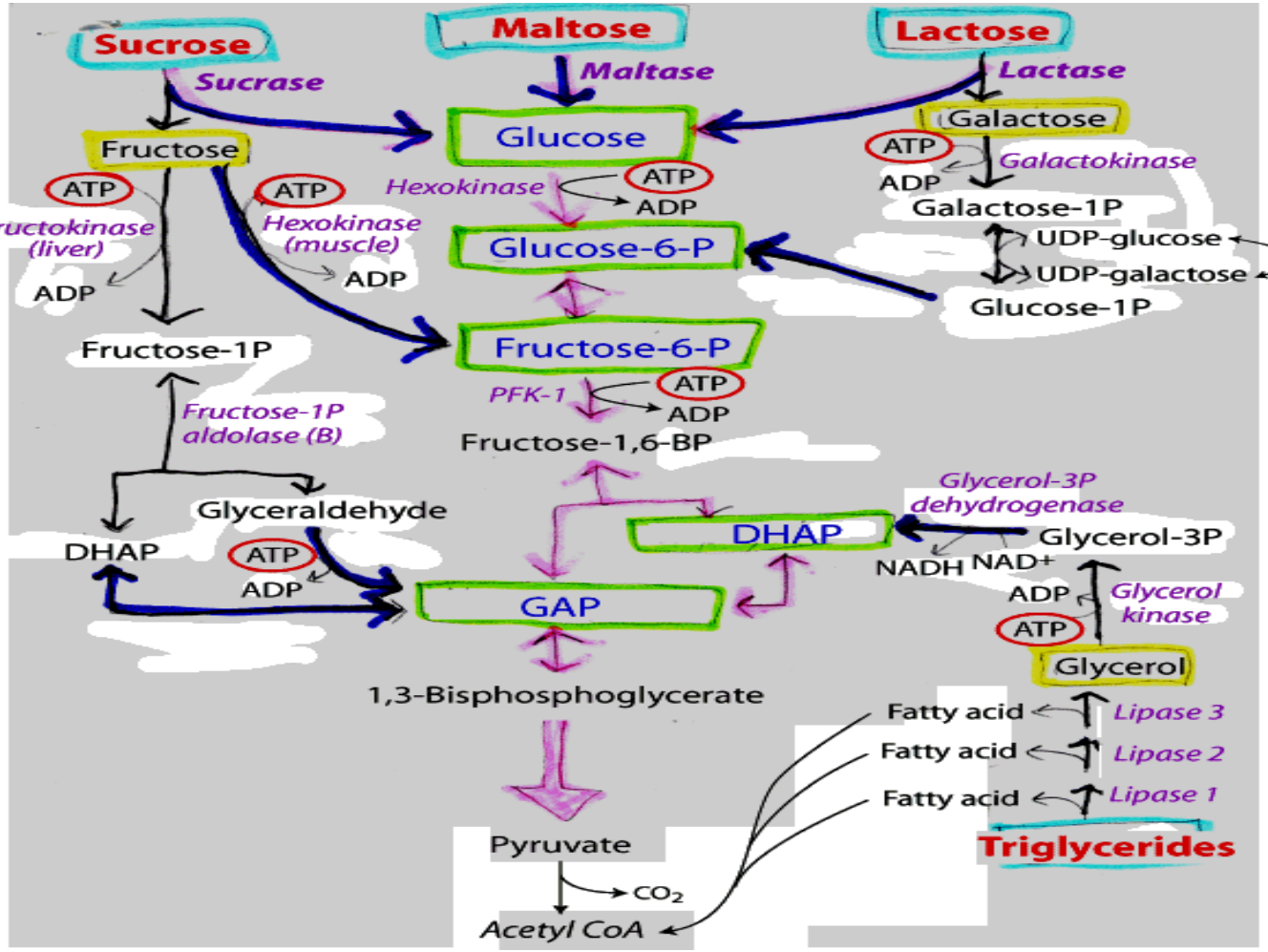


❖ The second of two substrate level phosphorylation reactions in glycolysis that couples energy released from phosphate hydrolysis ($\Delta G^{o'}$ = -62 kJ/mol) to that of ATP synthesis ($\Delta G^{o'}$ = +30.5 kJ/mol). Unlike phosphoenolpyruvate, pyruvate is a stable compound in cells that is utilized by many other metabolic pathways.

Glycolysis

Metabolism of other monosaccharides

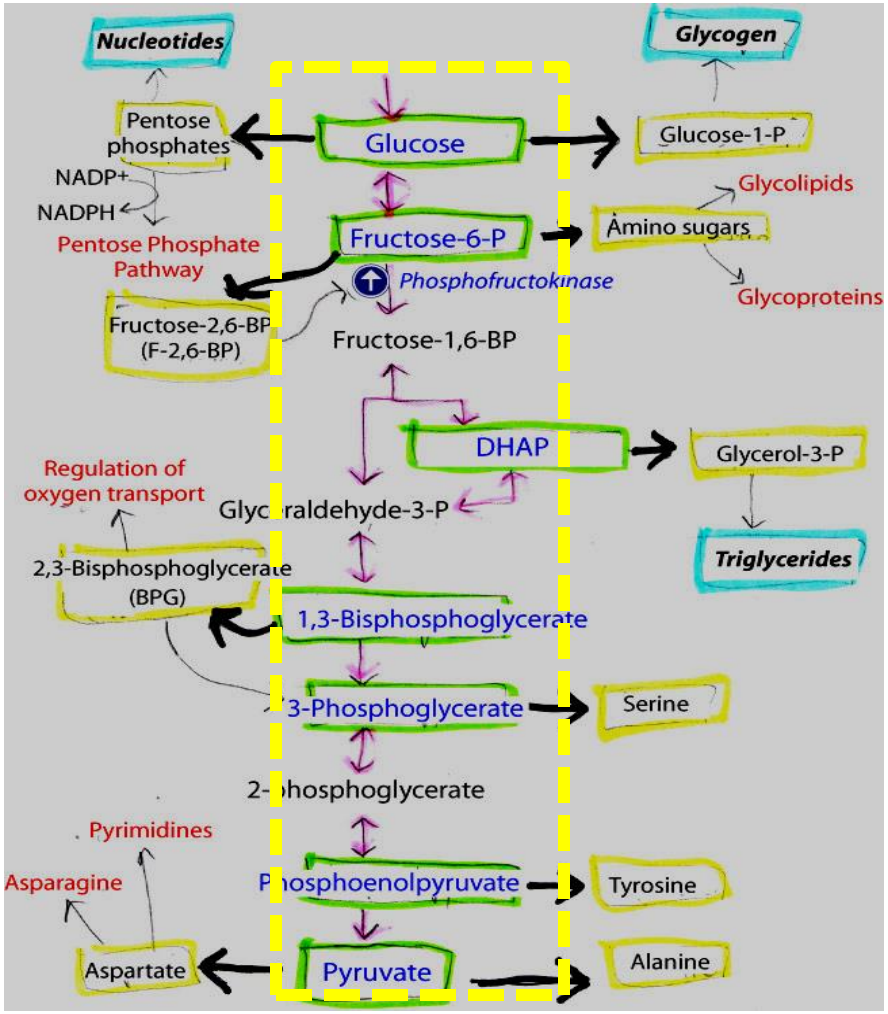
- In most organisms, monosaccharides other than glucose can undergo glycolysis
- These molecules enter the glycolytic pathway at several points after being converted into a phosphorylated derivative



Glycolysis

Demand for Glycolytic Intermediates

- Intermediates in glycolytic pathways are source in biosynthesis of a large variety of compounds such as:-
  - Amino acid (provide carbon skeletons)
  - Nucleic acid (ribose-5-P) synthesis
    - -Pentose phosphate pathway
  - Triacylglycerides (glycerol) synthesis.
  - Glucose (gluconeogenic pathway)
  - Glycogen synthesis



Glycolysis

Metabolic Fate of Pyruvate

❖ Three fates

i) Under **aerobic conditions**

- ➢ Majority of pyruvate is metabolized in the mitochondria to acetyl CoA and ultimately to CO_2 and H_2O
  - ✓ through the citrate cycle and electron transport chain

ii) Under **anaerobic conditions**

- ➢ In muscle cells during strenuous exercise or in erythrocytes which lack mitochondria pyruvate is converted into :-
  - ✓ Lactate by the enzyme lactate dehydrogenase or
  - ✓ Alanine by the enzyme alanine transaminase

- ➢ In microorganisms such as yeast pyruvate can be utilized for alcoholic fermentation to convert pyruvate to CO_2 and ethanol using the enzymes pyruvate decarboxylase and alcohol dehydrogenase respectively.

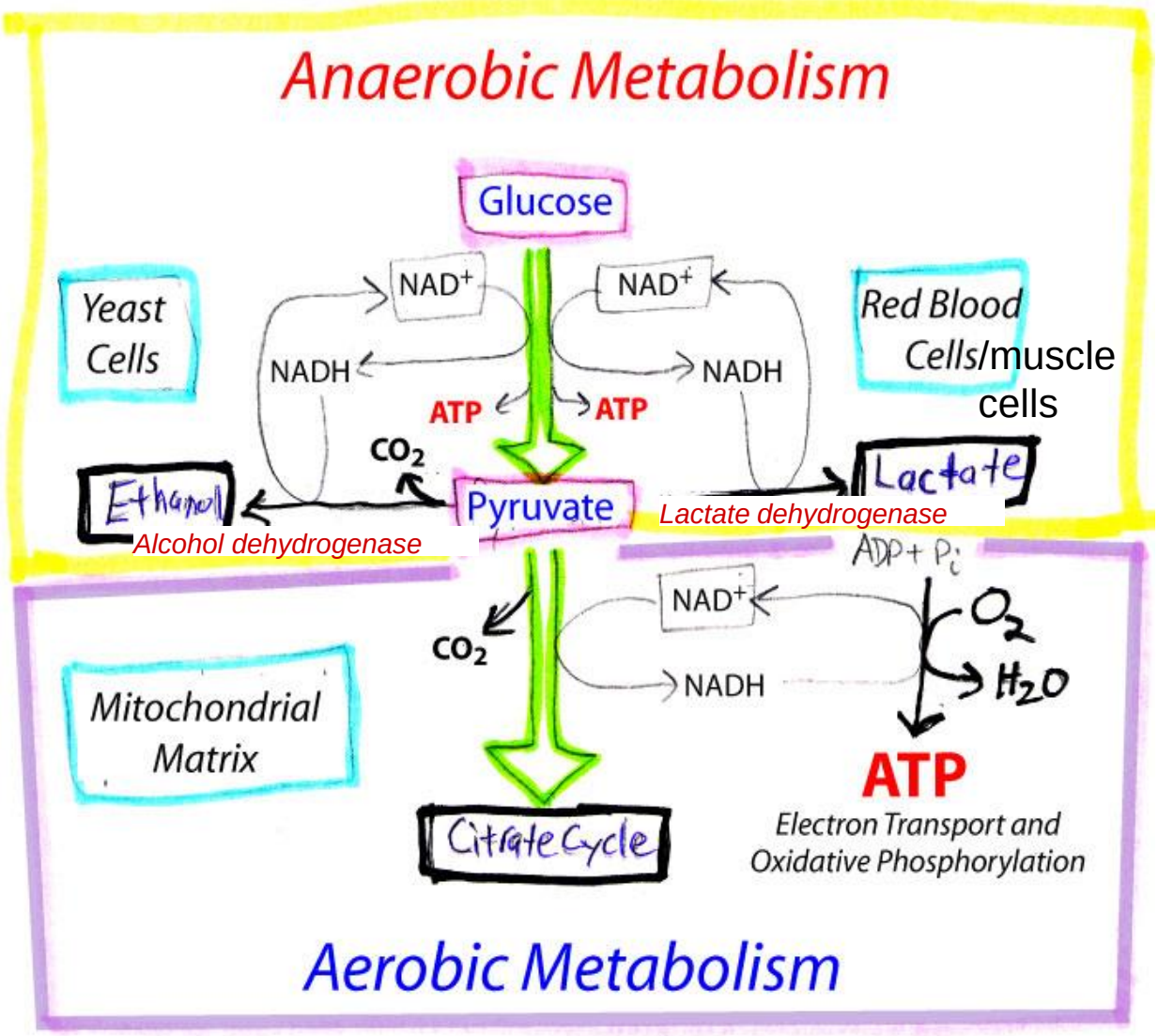
Anaerobic respiration

NAD^+ must be regenerated to maintain glycolytic flux

The glyceraldehyde-3-P dehydrogenase reaction requires a steady supply of NAD^+ which functions as a coenzyme in this oxidation reaction.

Anaerobic respiration replenishes the NAD^+ through a reduction reaction leading to lactate or ethanol production.

Aerobic respiration replaces the NAD^+ through a metabolite shuttle system since NAD/H cannot cross the mitochondrial membrane.



Citric acid cycle
(Tricarboxtlic acid cycle/ Kreb's cycle)

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

- Was first described by **Hans Kreb**, a biochemist who fled Nazi in 1937

- The pathway involve eight reactions which
  - Oxidize acetyl-CoA to generate 2 CO_2, and in the process, reduce 3 NAD^+ and 1 FAD.
  - Also produce GTP by substrate level phosphorylation which is can be converted into ATP by nucleotide kinase.
- **Process occurs in mitochondria which also involve** conversion of pyruvate to acetyl-CoA by the enzyme pyruvate dehydrogenase which reside in mitochondrial matrix

Net reaction

$$Acetyl\text{-}CoA + 3\ NAD^+ + FAD + GDP + Pi + 2\ H_2O \rightarrow CoA + 2\ CO_2 + 3\ NADH + 2\ H^+ + FADH_2 + GTP$$

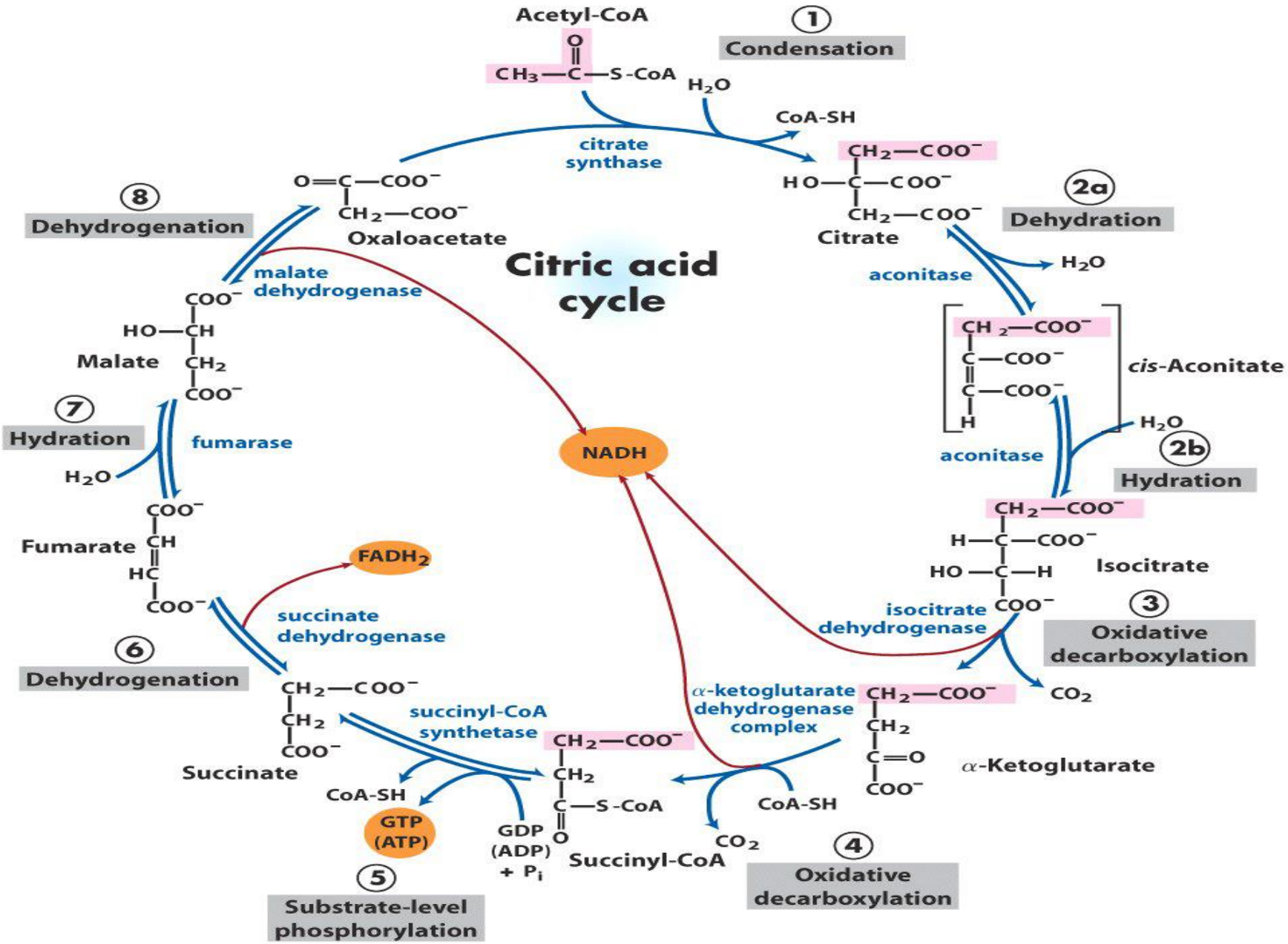
$$\Delta G^{o\prime} = -57.3\ kJ/mol$$

- The cycle is considered the "hub" of cellular metabolism because it
  - Links oxidation of metabolic fuels (carbohydrate, fatty acids and proteins) to ATP synthesis,
  - Supply metabolites for numerous other metabolic pathways.

Role:-

- Transfers 8 electrons from acetyl-CoA to the coenzymes NAD^+ and FAD to form 3 NADH and 1 $FADH_2$
  - which are then re-oxidized by the electron transport chain to produce ATP by the process of oxidative phosphorylation.
- Generates 2 CO_2 as "waste product"
- Generate 1 GTP (by substrate level phosphorylation).
- Generate metabolic intermediates for amino acid and porphyrin biosynthesis.

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)



Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Bioenergetics of the citrate cycle

TABLE 17.2 Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	$\Delta G^{\circ\prime}$ kcal mol^{-1}	$\Delta G^{\circ\prime}$ kJ mol^{-1}
1	Acetyl CoA + oxaloacetate + H_2O $\longrightarrow$ citrate + CoA + H^+	Citrate synthase		a	−7.5	−31.4
2a	Citrate $\rightleftharpoons$ *cis*-aconitate + H_2O	Aconitase	Fe–S	b	+2.0	+8.4
2b	*cis*-Aconitate + H_2O $\rightleftharpoons$ isocitrate	Aconitase	Fe–S	c	−0.5	−2.1
3	Isocitrate + NAD^+ $\rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d + e	−2.0	−8.4
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ succinyl CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	d + e	−7.2	−30.1
5	Succinyl CoA + P_i + GDP $\rightleftharpoons$ succinate + GTP + CoA	Succinyl CoA synthetase		f	−0.8	−3.3
6	Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe–S	e	~0	0
7	Fumarate + H_2O $\rightleftharpoons$ L-malate	Fumarase		c	−0.9	−3.8
8	L-Malate + NAD^+ $\rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase		e	+7.1	+29.7

*Reaction type: (a) condensation; (b) dehydration; (c) hydration; (d) decarboxylation; (e) oxidation; (f) substrate-level phosphorylation.

Glycolysis + pyruvate dehydrogenase reaction + citrate cycle = **net reaction**:

Glucose + 2 H_2O + 10 NAD^+ + 2 FAD + 4 ADP + 4 Pi →

6 CO_2 + 10 NADH + 6 H^+ + 2 $FADH_2$ + 4 ATP

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 1: Condensation of oxaloacetate and acetyl-CoA by **citrate synthase** to form citrate

This reaction commits the acetate unit of acetyl-CoA to oxidative decarboxylation

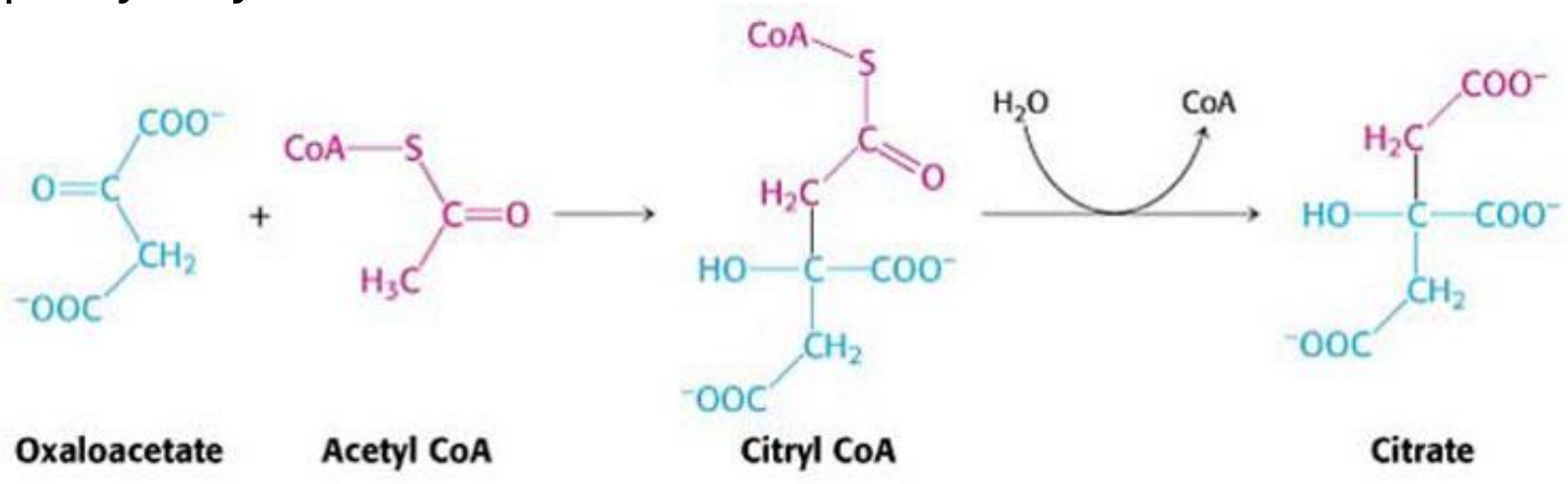
Reaction follows an ordered mechanism:

Oxaloacetate binds, inducing a conformational change in the enzyme that facilitates:

- acetyl-CoA binding

- formation of the transient intermediate, citryl-CoA

- rapid hydrolysis that releases CoA-SH and citrate



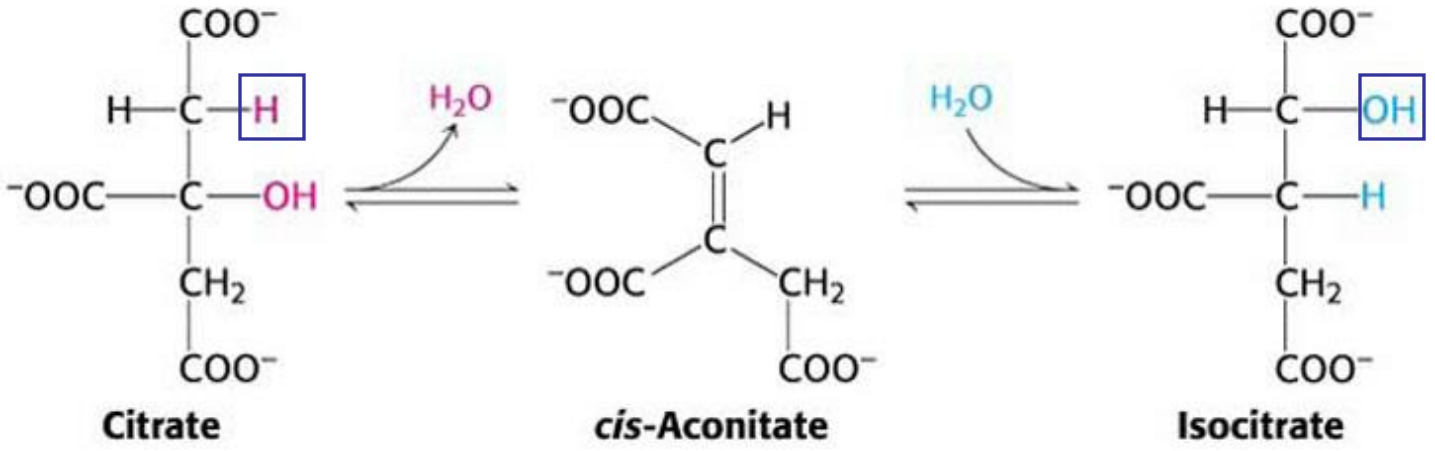
Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 2: Isomerization of citrate by **aconitase** to form isocitrate

This is a *reversible* two step isomerization reaction.

The intermediate, cis-aconitate, is formed by a dehydration reaction that requires the participation of an iron-sulfur cluster (4Fe-4S) in the enzyme active site.

H_2O is added back to convert the double bond in cis-aconitate, to a single bond with a hydroxyl group, on the terminal carbon.



Citrate *cis*-**Aconitate** **Isocitrate**

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Aconitase is one of the targets of fluorocitrate

Fluorocitrate is derived from fluoroacetate. Fluoroacetate-containing plants, such as acacia found in parts of Australia and Africa, are so deadly that Australian sheep herders have reported finding sheep with their heads still in the bush they were feeding on when they died.

Fluoracetate is the active ingredient in the poison **compound 1080** used to kill rodents and livestock predators. Sometimes, the poison is used indiscriminately, causing animal deaths.

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

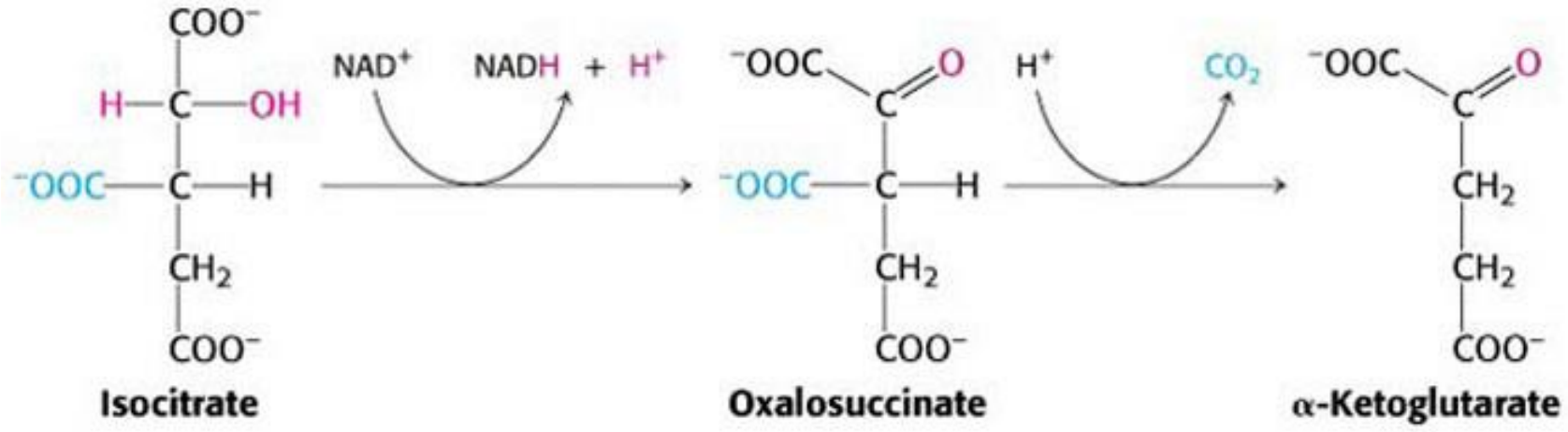
Reaction 3: Oxidative decarboxylation of isocitrate by **isocitrate dehydrogenase** to form α-ketoglutarate, CO_2 and NADH

First of two decarboxylation steps in the citrate cycle

First reaction to generate NADH used for energy conversion reactions in the electron transport system

Catalyzes an oxidation reaction that generates the transient intermediate oxalosuccinate

In the presence of the divalent cations Mg^{2+} or Mn^{2+}, oxalosuccinate is decarboxylated to form α-ketoglutarate



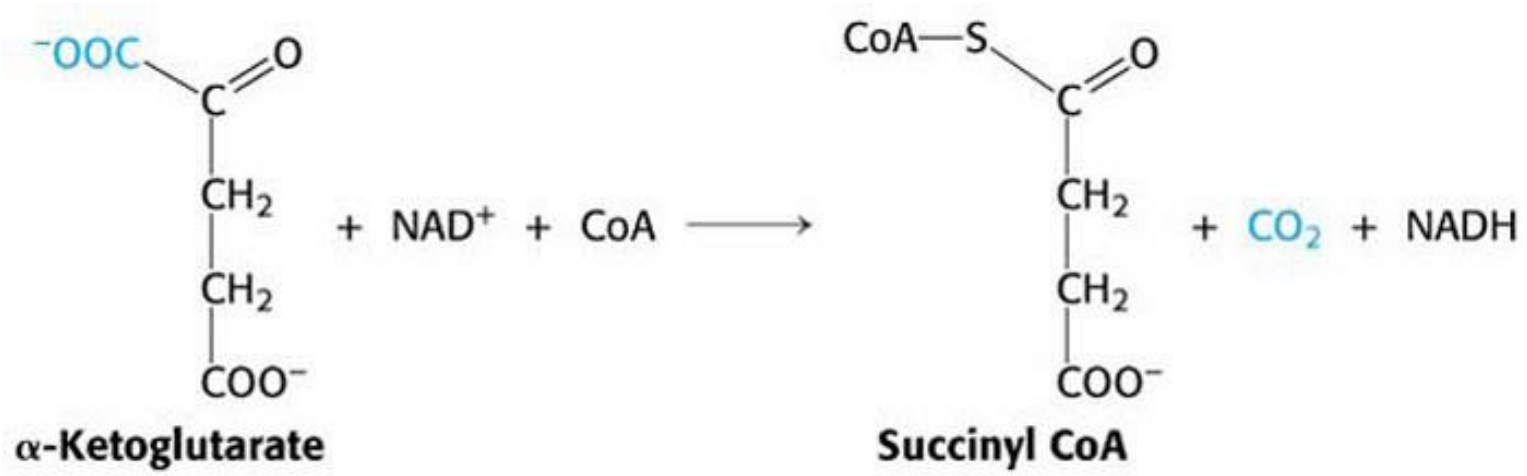
Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 4: Oxidative decarboxylation of by **α-ketoglutarate dehydrogenase** to form succinyl-CoA, CO_2 and NADH

Second oxidative decarboxylation reaction and also produces NADH.

α-Ketoglutarate dehydrogenase complex utilizes essentially the same catalytic mechanism we have already described for the pyruvate dehydrogenase reaction.

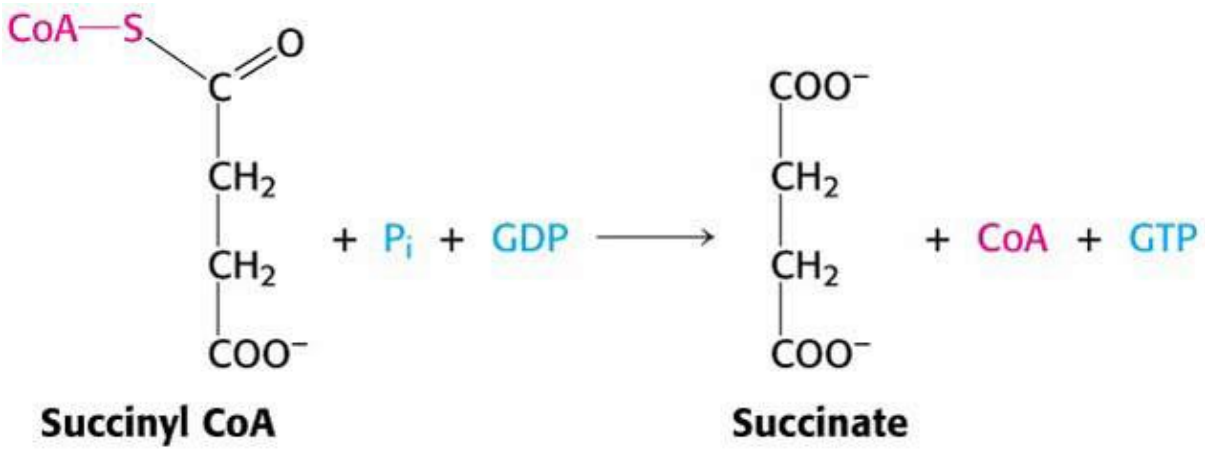
Includes the binding of substrate to an E1 subunit (α-ketoglutarate dehydrogenase), followed by decarboxylation and formation of a TPP-linked intermediate.



Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 5: Conversion of succinyl-CoA to succinate by **succinyl-CoA synthetase** in a substrate level phosphorylation reaction that generates GTP

The available free energy in the thioester bond of succinyl-CoA ($\Delta G^{o\prime}$ = -32.6 kJ/mol) is used in the succinyl-CoA synthetase reaction to carry out a phosphoryl transfer reaction ($\Delta G^{o\prime}$ = +30.5 kJ/mol), in this case, a substrate level phosphorylation reaction, that produces GTP (or ATP).



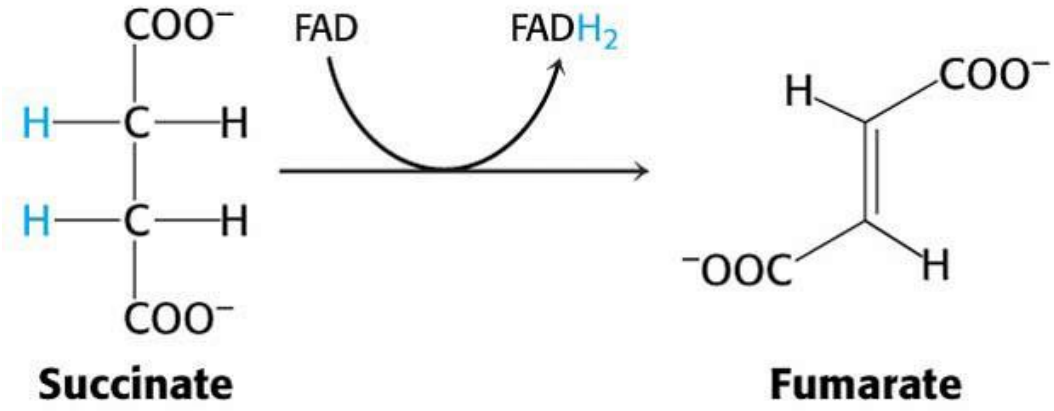
Nucleoside diphosphate kinase interconverts GTP and ATP by a readily reversible phosphoryl transfer reaction: GTP + ADP ↔ GDP + ATP ($\Delta G^{o\prime}$ = 0 kJ/mol).

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 6: Oxidation of succinate by succinate dehydrogenase to form fumarate

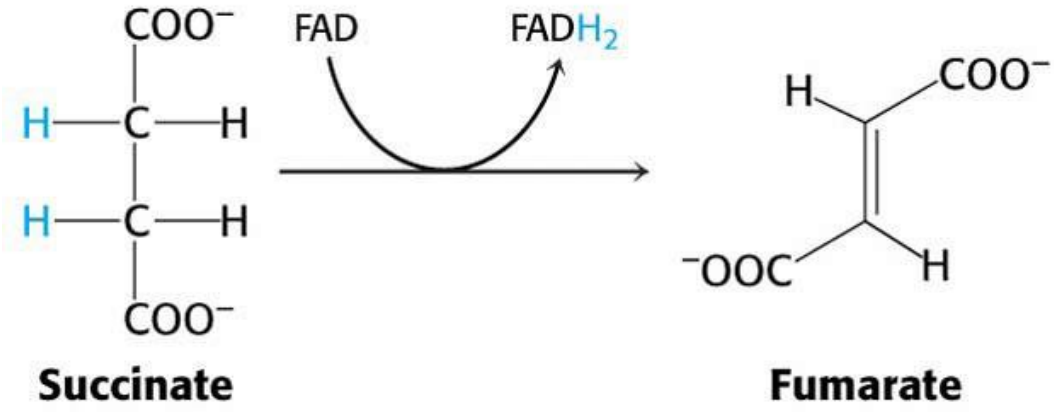
This coupled redox reaction directly links the citrate cycle to the electron transport system through the redox conjugate pair FAD/$FADH_2$ which is covalently linked to the enzyme succinate dehydrogenase, an inner mitochondrial membrane protein.

Oxidation of succinate results in the transfer of 2 *e*- to the FAD moiety, which in turn, passes the two electrons to the electron carrier coenzyme Q in complex II of the electron transport system.



Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 6: Oxidation of succinate by **succinate dehydrogenase** to form fumarate



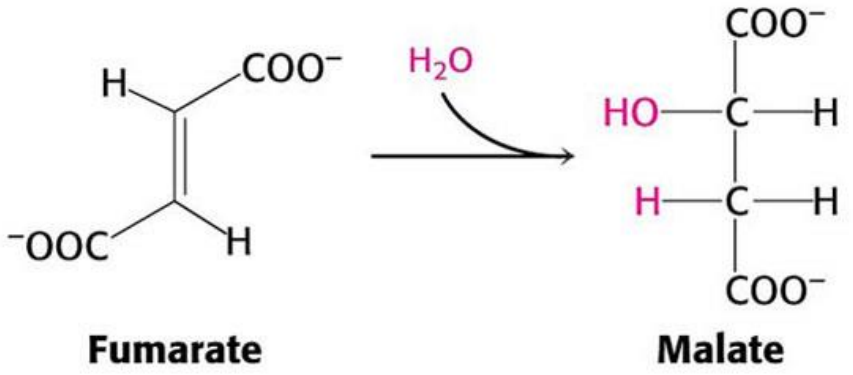
Is FAD oxidized or reduced in this redox reaction?

Is succinate the reductant or the oxidant in this reaction?

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 7: Hydration of fumarate by **fumarase** to form malate

Fumarase the reversible hydration of the C=C double bond in fumarate to generate the L-isomer of malate.



Fumarate **Malate**

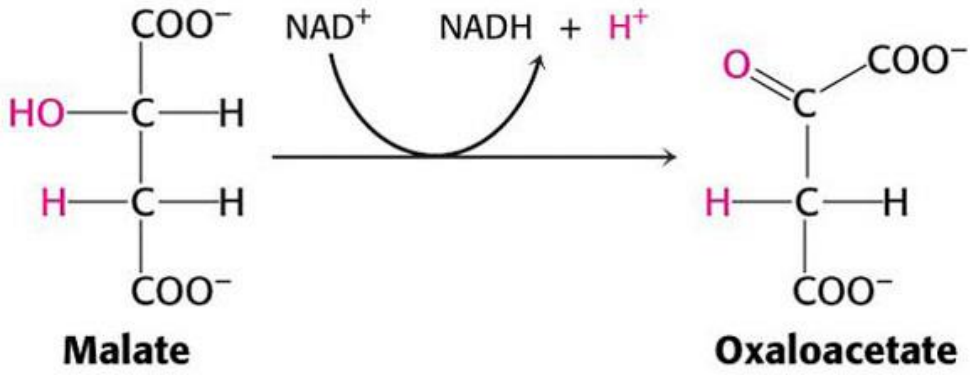
Fumarate and malate are citrate cycle intermediates that enter and exit the cycle from several different interconnected pathways.

Citric acid cycle (Tricarboxylic cycle Or Kreb's cycle)

Reaction 8: Oxidation of malate by malate dehydrogenase to form oxaloacetate

Oxidation of the hydroxyl group of malate to form oxaloacetate in a coupled redox reaction involving NAD^+/NADH. The change in standard free energy for this reaction is unfavorable

($\Delta G^{o'}$ = +29.7 kJ/mol), but the *actual* ΔG for this reaction is favorable.

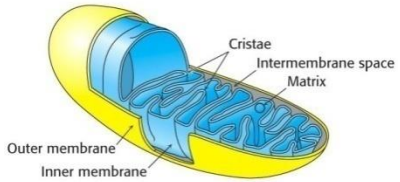


In order for this unfavorable $\Delta G^{o'}$ to allow for a favorable ΔG, the metabolite concentrations need to be far from equilibrium.

Electron Transport Chain and Oxidative Phosphorylation

Electron Transport Chain and Oxidative Phosphorylation

- This processes takes place in mitochondria in aerobic organisms
- The process involve two coupled steps



Electron Transport Chain (ETC)

- A ***series of coupled redox reactions*** where electrons in NADH and $FADH_2$ move through membrane until finally used to reduce molecular oxygen to water
  - aided by **complex cytochrome proteins**
- Proteins pump **protons (H^+)** across innermembrane **into intermembrane space**
  - This **lowers pH in innermembrane space**.

Oxidative phosphorylation

- Involve movement of **protons** via **diffusion (Proton Motive Force)** through **ATP Synthase** to make **ATP**.
- Both **NADH** and **$FADH_2$** are converted to **ATP** during this stage
  - Giving 2.5 & 1.5 **ATP respectively**.

Net reaction

$$2\ NADH + 2\ H^+ + 5\ ADP + 5\ Pi + O_2 \rightarrow 2\ NAD^+ + 5\ ATP + 2\ H_2O$$

Role:-

- Generates ATP - from oxidation of metabolic fuels accounting for 28 out of 32 ATP (88%)
- Tissue-specific expression of uncoupling protein-1 (UCP1) in brown adipose tissue of mammals short-circuits the electron transport system and thereby produces heat for thermoregulation.

Electron Transport Chain and Oxidative Phosphorylation

Protein complexes (Redox enzymes/Cytochromes)

NADH dehydrogenase (**complex I** /NADH-ubiquinone oxidoreductase)

- Catalyzes the first redox reaction in the electron transport system in which NADH oxidation is coupled to FMN reduction and pumps 4 H^+ into the inter-membrane space.

Succinate dehydrogenase (**complex II)**

Ubiquinone-cytochrome c oxidoreductase (**complex III)**

- Translocate 4 H^+ across the membrane via the **Q cycle** and has the important role of facilitating electron transfer from a two electron carrier (QH_2), to cytochrome c, a mobile protein carrier that transfers one electron at a time to complex IV.

Cytochrome c oxidase (**complex IV**)

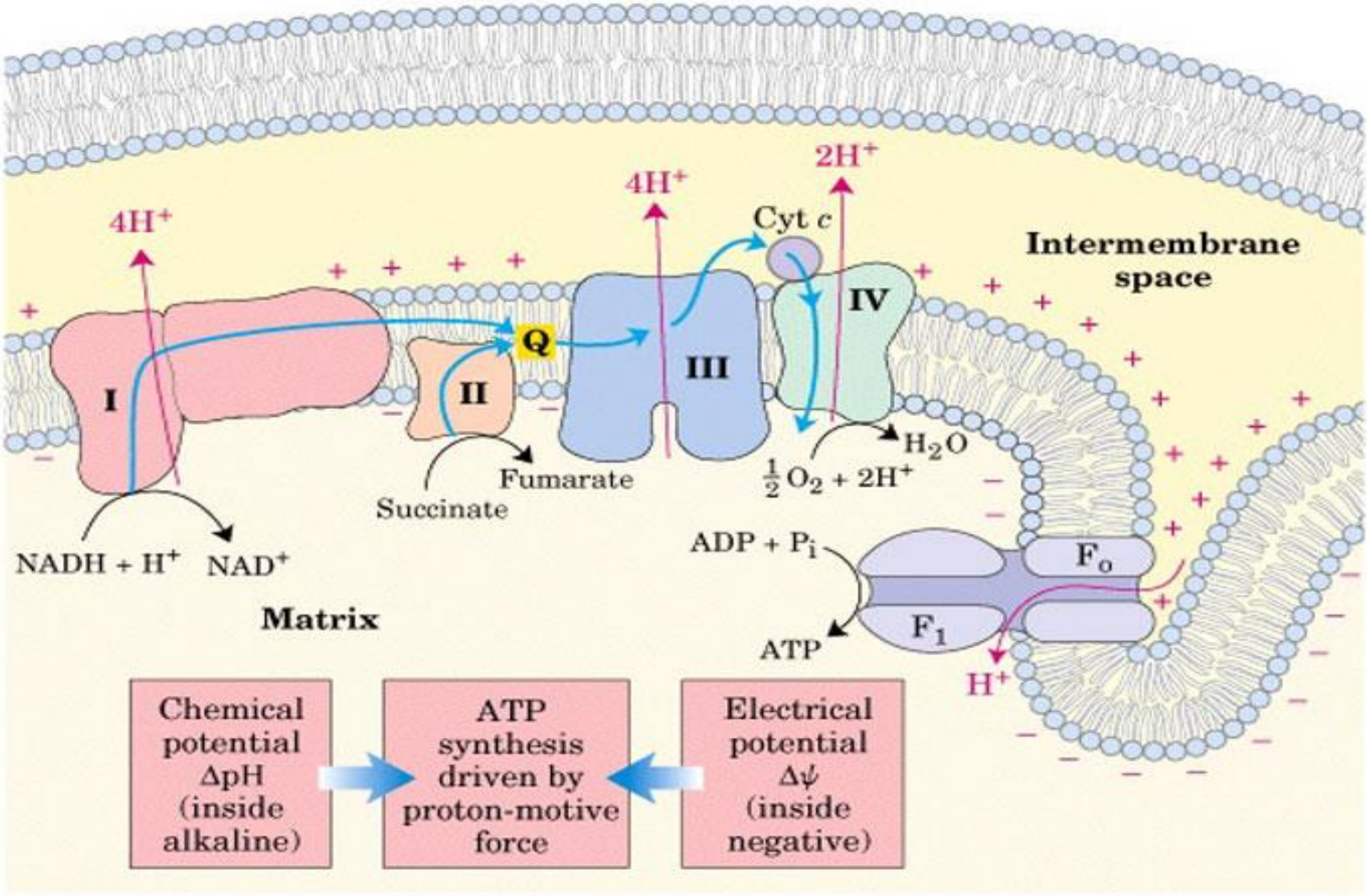
- Pumps $2H^+$ into the inter-membrane space and catalyzes the last redox reaction in the electron transport system in which cytochrome a3 oxidation is coupled to the reduction of molecular oxygen to form water ($O_2 + 2\ e^- + 2\ H^+ \rightarrow H_2O$).

ATP synthase complex (**complex V**)

- Is responsible for converting proton-motive force (energy available from the electrochemical proton gradient) into **net ATP synthesis** through a series of proton-driven conformational changes.

Note:- Specific redox reaction inhibitors (such as rotenone, antimycin A and cyanide) can have effect on the system

Electron Transport Chain and Oxidative Phosphorylation



Total ATP yields of glucose oxidation

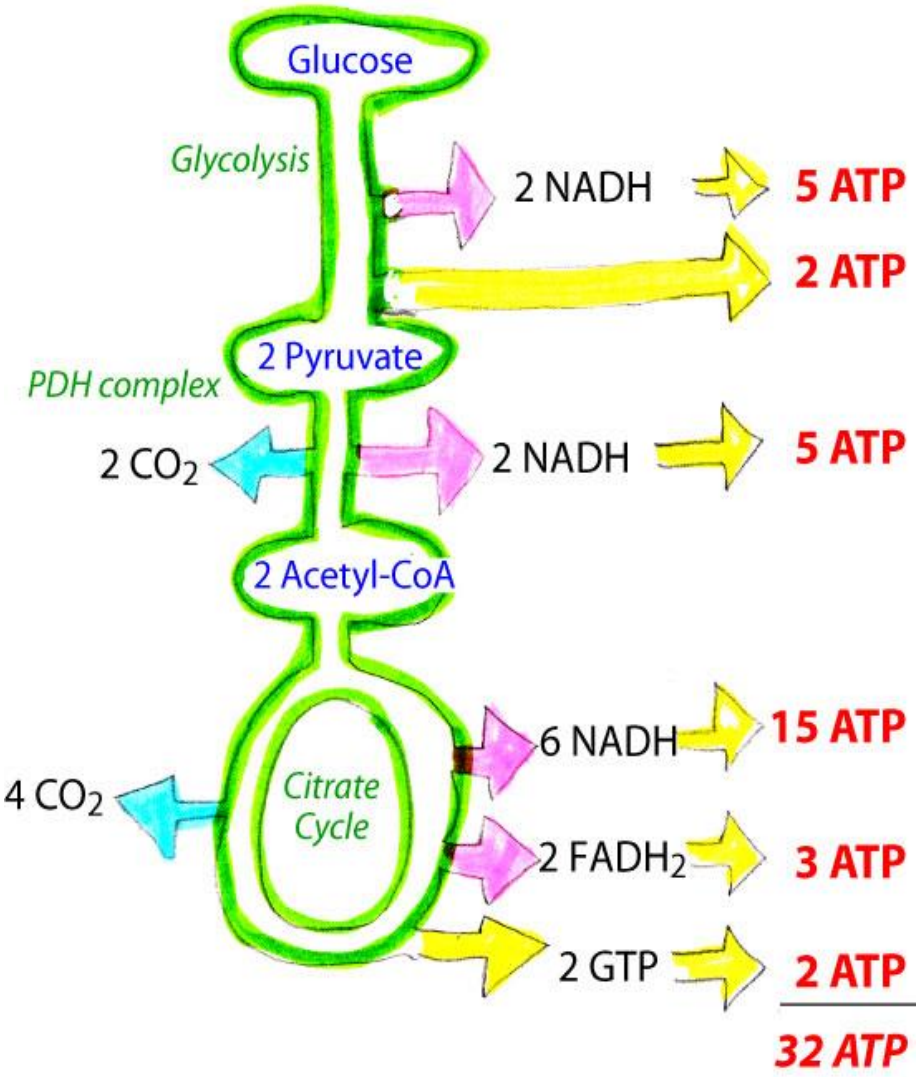
The reducing power of NADH and $FADH_2$ can be converted to ATP equivalents using the **currency exchange ratio.**

~2.5 ATP/NADH
~1.5 ATP/$FADH_2$

This yields ~28 ATP based on 3 NADH and 1 $FADH_2$

Anoter 4 ATP are synthesized by substrate phosphorylation, generates a maximum of **~32 ATP**.

The complete oxidation of glucose by the pyruvate dehydrogenase complex and the citrate cycle leads to the production of **6 CO_2 molecules as "waste"**.



Glycogen Metabolism

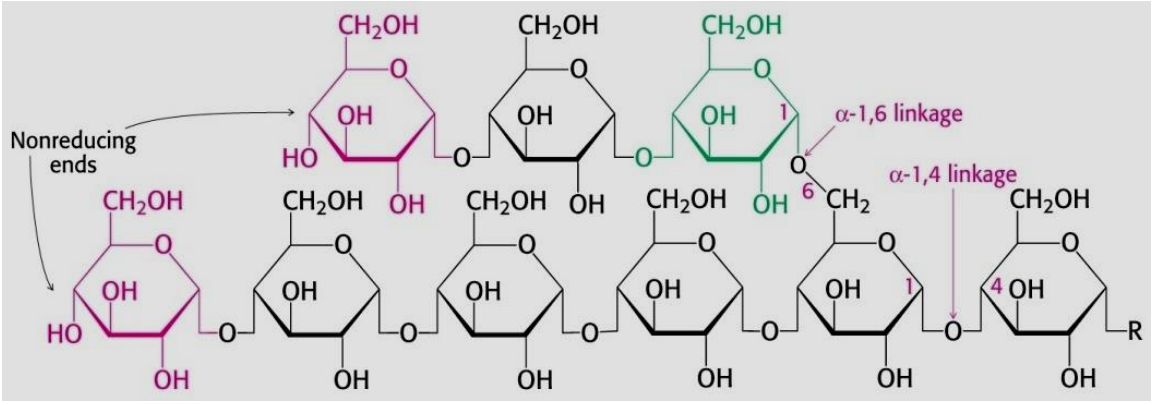
Glycogen Metabolism

- Glycogen is the major storage form of carbohydrate in animals.
  - Mainly in liver and muscles
    - ✓ to be mobilized as glucose when tissues require

- It is a branched polysaccharide composed of glucose residues

  - Glucose residues are linked by α (1-4) glycosidic bonds into chains
  - Chains branch via α (1-6) linkage-

- Branch points are frequent (after ***4-8 residues***) – allows glucose residues to be easily added or removed quicker than a linear molecule.



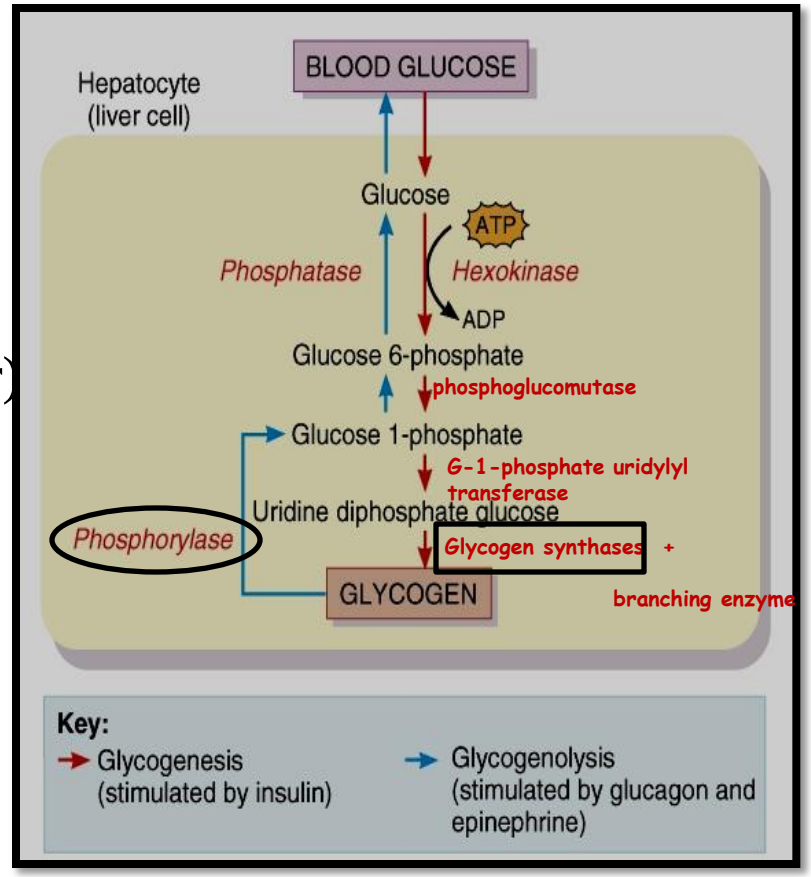
Glycogen Metabolism

Glycogenesis

- Synthesis of glycogen
- Regulated by insulin

Glycogenolysis

- Breakdown of glycogen to give glucose(liver) or glucose-6-phosphate (muscle)
- Regulated by epinephrine and glucagon
- Glucose obtained from glycogen breakdown in liver is used to maintain blood glucose levels.

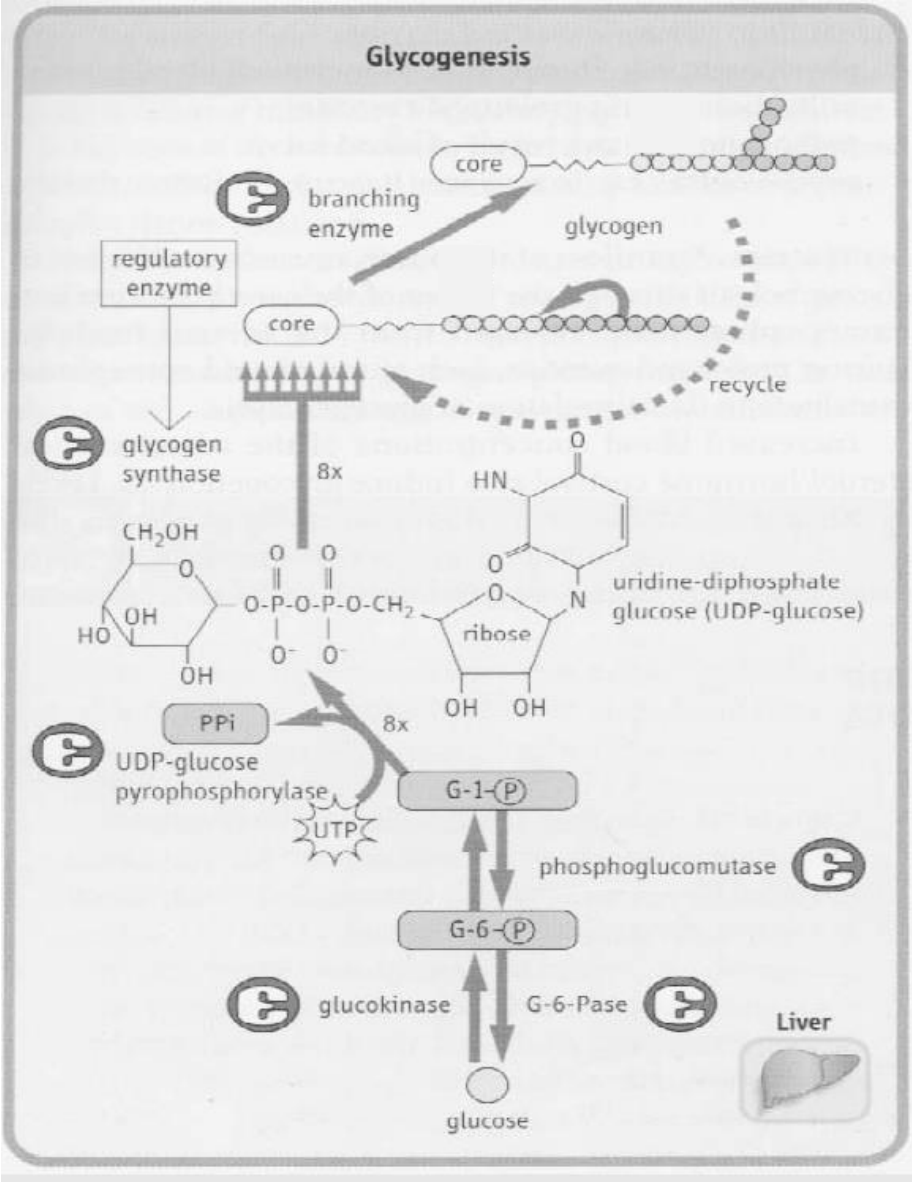


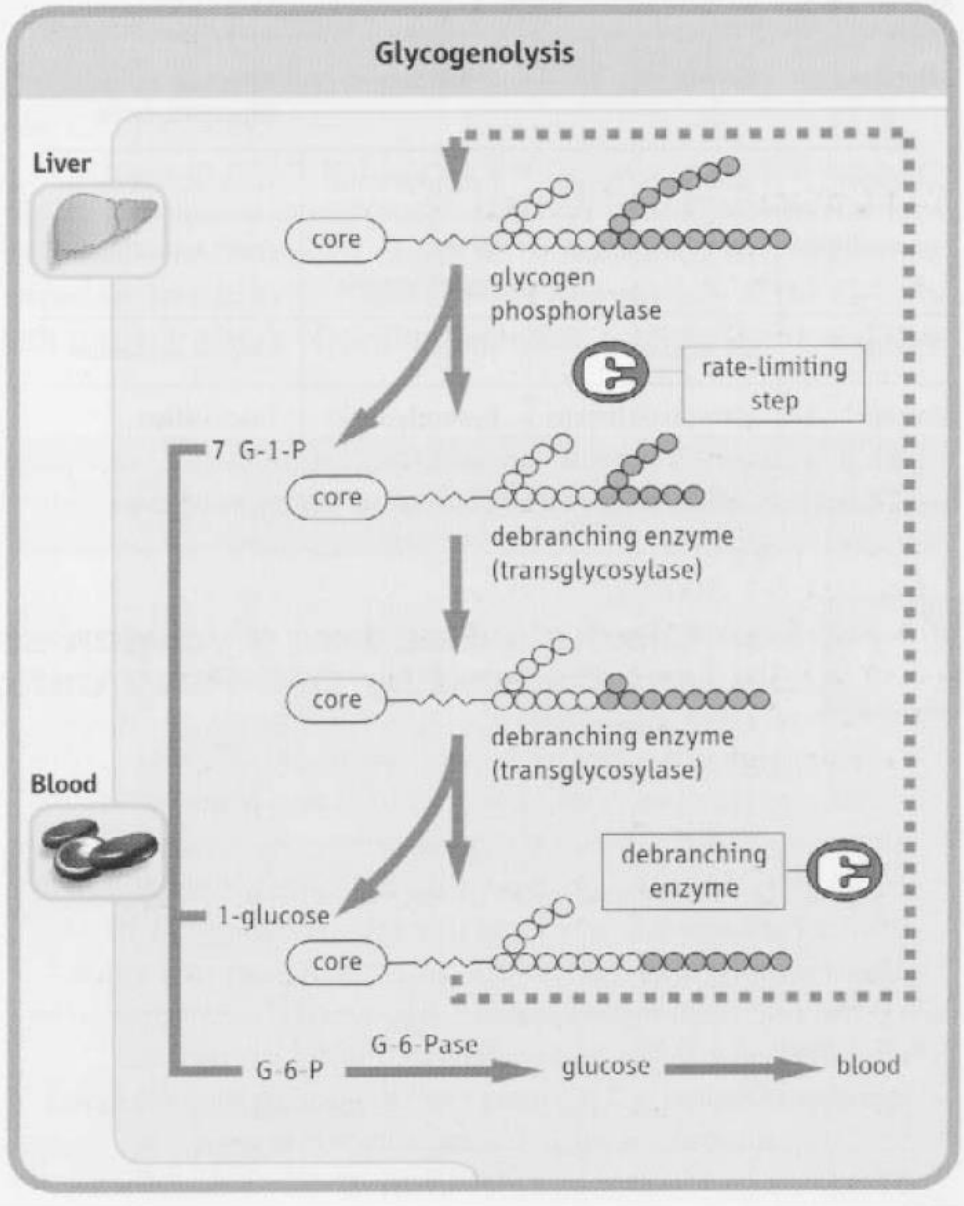
- Where as G-6-p obtained from glycogen breakdown in muscle is used for glycolysis -to meet the energy requirements of the muscle cell.

❖ Glycogen metabolism (Synthesis and breakdown) is a highly regulated process

- Involving mutual control of **glycogen phosphorylase** (GP) and **glycogen synthase** (GS)

Glycogen Metabolism





Control of Glycogen Metabolism

❖ **Glycogen phosphorylase (GP)**

- ➢ Allosterically activated by AMP and inhibited by ATP, glucose-6-P and caffeine

❖ **Glycogen synthase (GS)**

- ➢ Is stimulated by glucose-6-Phosphate

❖ **Hormonal control**

- ➢ Both enzymes are regulated by covalent modification - Phosphorylation (modulated by hormones)

Insulin (a 51 aa protein)

- ➢ Secreted by pancreas under high blood glucose level
- ➢ Stimulate glycogen synthesis in liver & increases glucose transport into muscles and adipose tissues

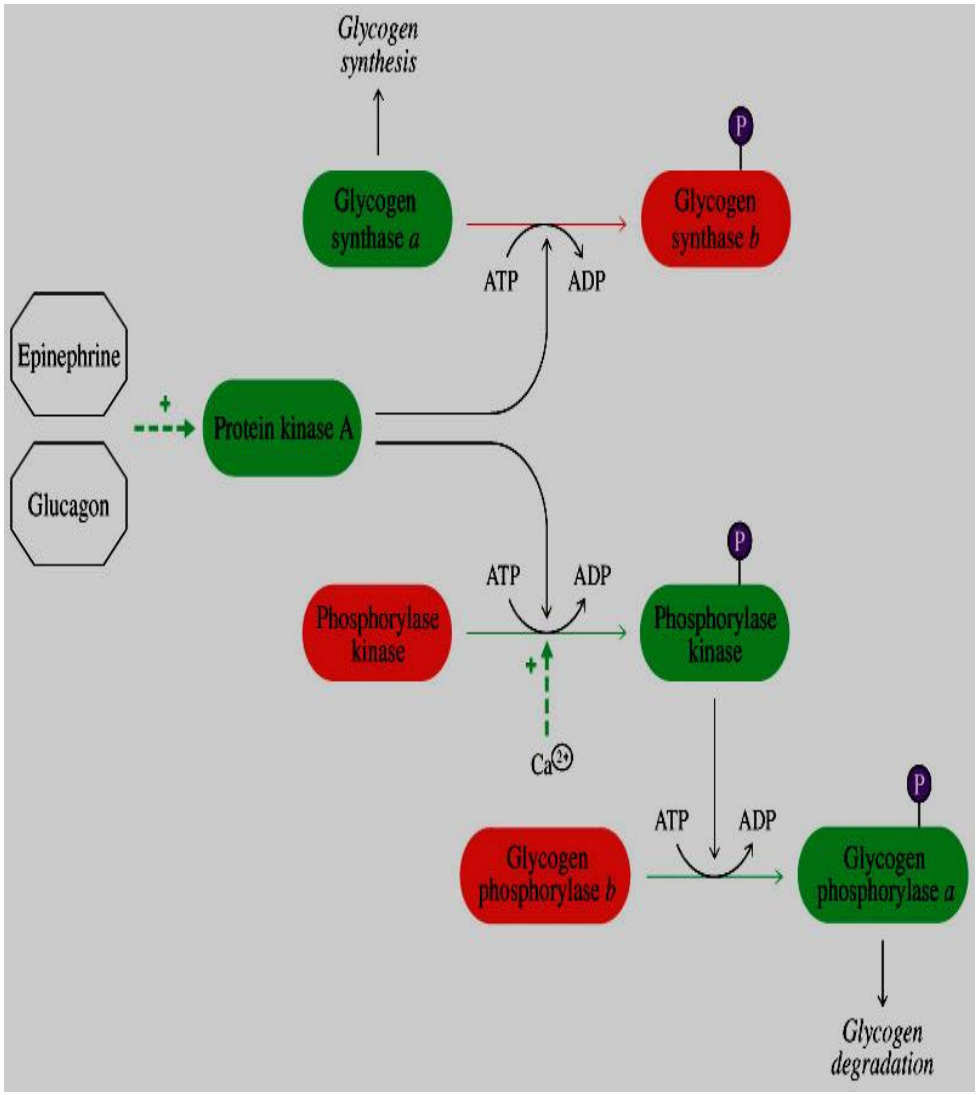
Glucagon

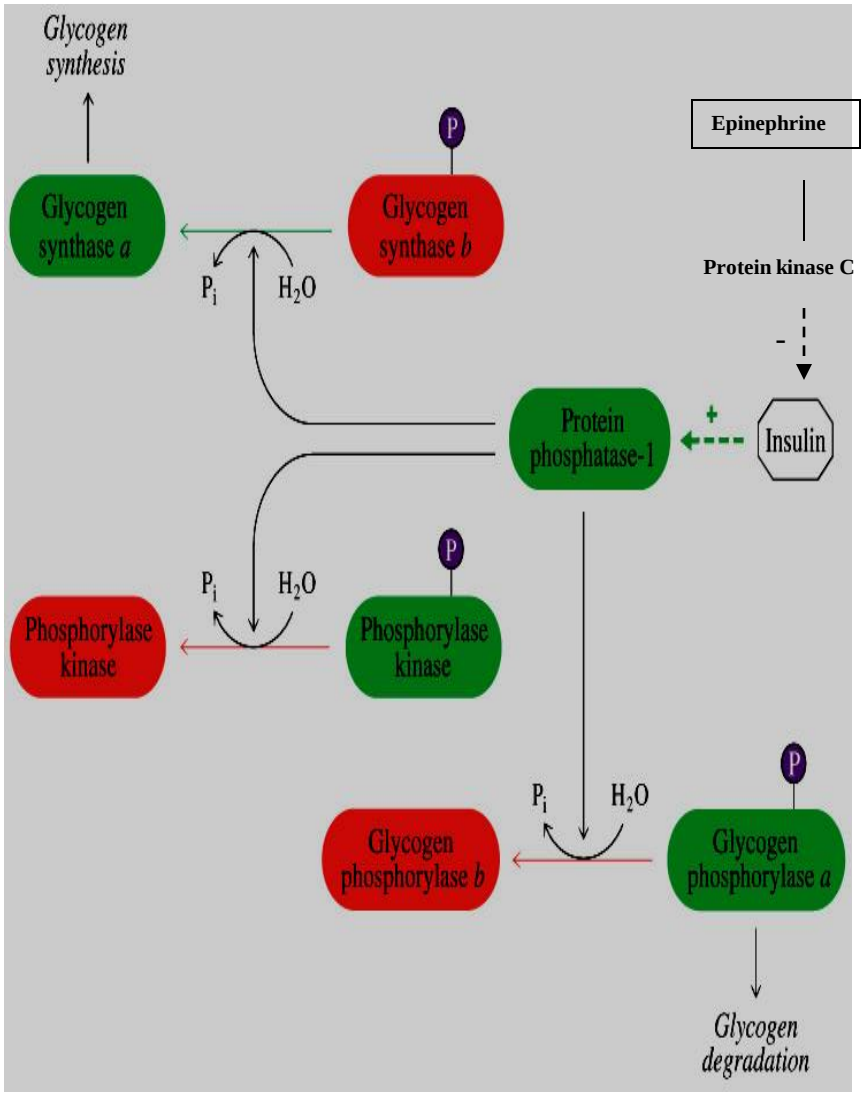
- ➢ Secreted by pancreas in response to low blood glucose level
  - ✓ Hence stimulate glycogen breakdown
- ➢ Acts primarily in liver

Ephinephrine (adrenalin)

- ➢ Secreted by adrenal gland in response to stress (“fight or flight”)
- ➢ Stimulate release of glucagon and hence glycogen breakdown
  - ✓ Increases rates of glycolysis in muscles and release of glucose from the liver

Control of Glycogen Metabolism





Gluconeogenesis

Gluconeogenesis

- Glucose is the major fuel source for the brain, nervous system, testes, erythrocytes, and kidney medulla.

- Body's daily glucose requirement is approx. 160 grams.

  - ✓≈ 20 g is present in body fluids.
  - ✓≈ 190 g is available as stored glycogen.

  This is sufficient reserves for single day requirement

- But the high glucose consumed during starvation or intense exercise need to be replenished

  - Gluconeogenesis can help to maintain the glucose level in blood

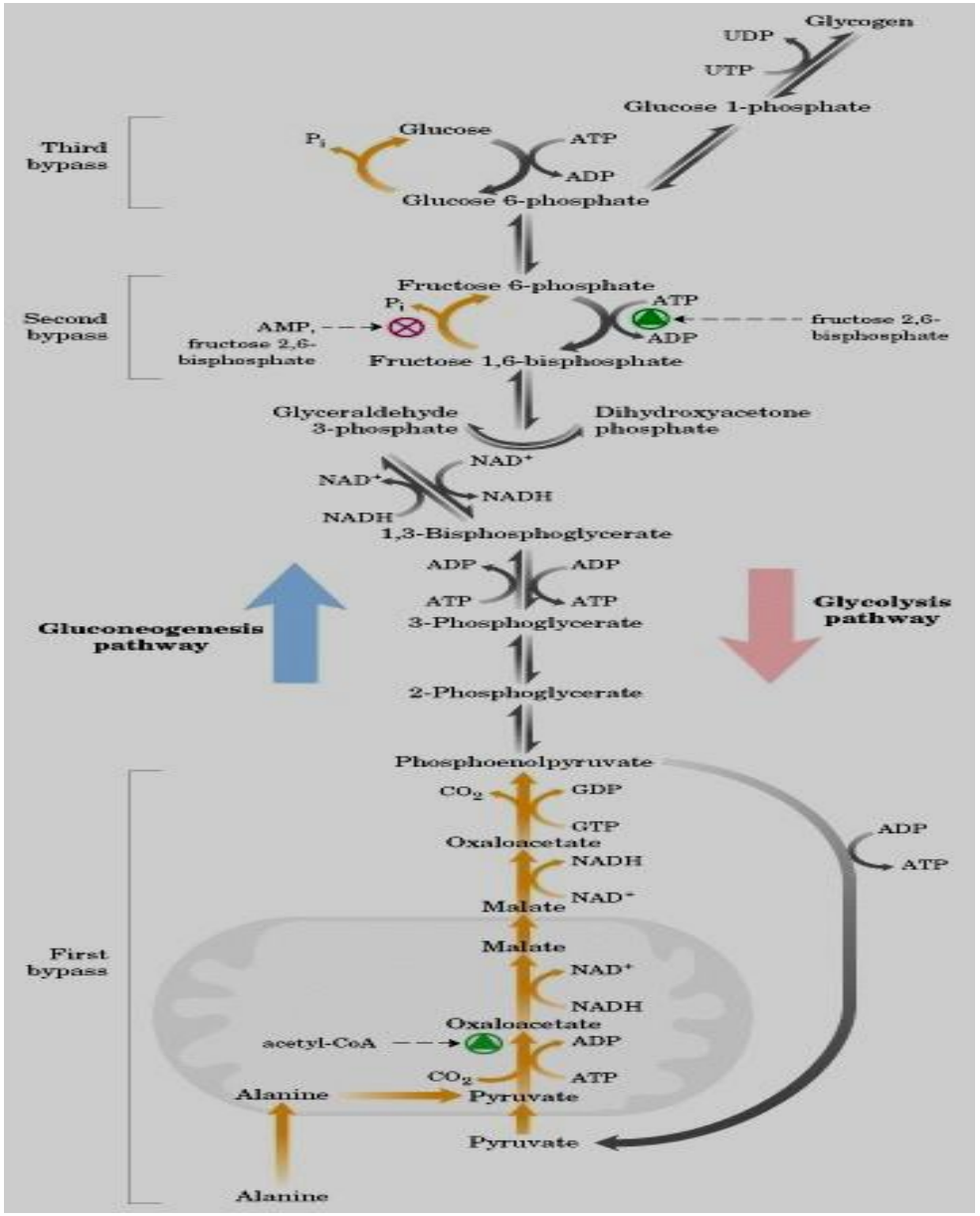
- Gluconeogenesis:- synthesis of glucose from non-carbohydrate sources

  - Such as lactate , pyruvate, glycerol, citric acid cycle intermediates, amino acids

- Gluconeogensis occur primarily in the liver (but also in kidneys).
  - In the cytosol and partly in mitochondria

Metabolic steps in Gluconeogenesis

- Involve conversion of pyruvate in to Glucose
  - Process seem inverse of glycolysis
- Seven of the steps in glycolysis which are reversible are retained in gluconeogenesis
- Three steps in glycolysis which are irreversible are replaced by four newer steps



Metabolic steps in Gluconeogenesis

I) First Bypass Reaction

- ❖ Involve convervsion of Pyruvate to Phosphoenolpyruvate (PEP)
- ❖ Requires participation of both mitochondrial and cytosolic enzymes.
- ❖ Involve six steps

Step 1: Transportation of pyruvate from the cytosol into mitochondria via the mitochondrial pyruvate transporter OR generation of pyruvate within mitochondria via deamination of alanine.

Step 2: Convertion of pyruvate into Oxaloacetate (OAA) by *pyruvate carboxylase* (require biotin)

$$\text{Pyruvate} + HCO_3^- + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{Pi} + H^+$$

Step 3: Reduction of Oxaloacetate in to malate by mitochondrial *malate dehydrogenase* .

$$\text{Oxaloacetate} + \text{NADH} + H^+ \rightarrow \text{L-malate} + NAD^+$$

Step 4: Malate exits the mitochondrion via the malate/α-ketoglutarate carrier.

Step 5: Reoxidation of malate in to oxaloacetate via cytosolic *malate dehydrogenase* .

$$\text{L-malate} + NAD^+ \rightarrow \text{oxaloacetate} + \text{NADH} + H^+$$

Step 6: Convertion of Oxaloacetate in to phosphoenolpyruvate (PEP) by *PEP carboxykinase*

$$\text{Oxaloacetate} + \text{GTP} \rightarrow \text{phosphoenolpyruvate} + CO_2 + \text{GDP}$$

Metabolic steps in Gluconeogenesis

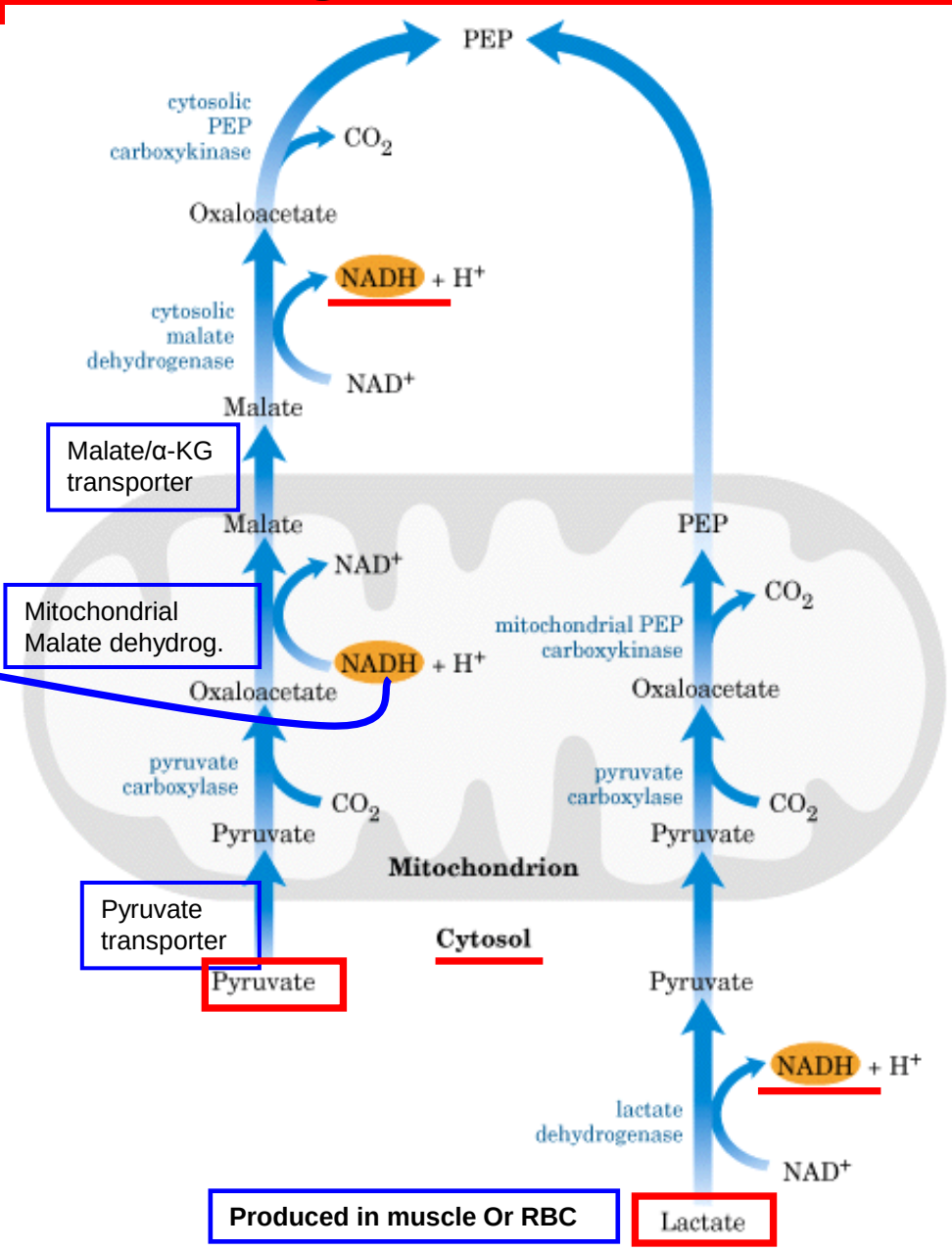
Overall reactions

$$\text{Pyruvate} + \text{ATP} + \text{GTP} + HCO_3^- \rightarrow$$

$$\text{PEP} + \text{ADP} + \text{GDP} + \text{Pi} + H^+ + CO_2$$

Mitochondria are the source of reducing equivalents that will be needed later.

<u>Note:</u> when either pyruvate or the ATP/ADP ratio is high, the reaction is pushed toward the right (i.e., in the direction of biosynthesis).



Metabolic steps in Gluconeogenesis

Second Bypass Reaction

- Involve conversion of Fructose 1,6-bisphosphate to Fructose 6-phosphate
  - by enzyme *fructose 1,6-bisphosphatase*

Fructose 1,6-bisphosphate + H_2O ⟶ fructose 6-phosphate + Pi $\Delta G^{\circ\prime} = -3.9$ kcal/mol

Third Bypass Reaction

- Involve conversion of Glucose 6-phosphate to Glucose
  - by an enzyme named *glucose 6-phosphatase.*

Glucose 6-phosphate + H_2O ⟶ glucose + Pi $\Delta G^{\circ\prime} = -3.3$ kcal/mol

Gluconeogenesis from Various Metabolites

❖ Apart from pyruvate various non-carbohydrate precursors can be used to synthesize glucose

i) **Lactate**:-Join the system after being converted into pyruvate via LD

ii) **Citric Acid Cycle Intermediates**

- Form oxaloacetate during one turn of the cycle.
- Can get net synthesis of glucose from citric acid cycle intermediates.
- 3 carbons of the resulting OAA are converted into glucose, 1 carbon is released as CO_2 by *PEP carboxykinase*.

iii) **Amino Acids**

- Except leucine and lysine all aa's are metabolized either to pyruvate or certain intermediates of the citric acid cycle.
- Hence they are glucogenic (i.e., they can undergo net conversion to glucose).

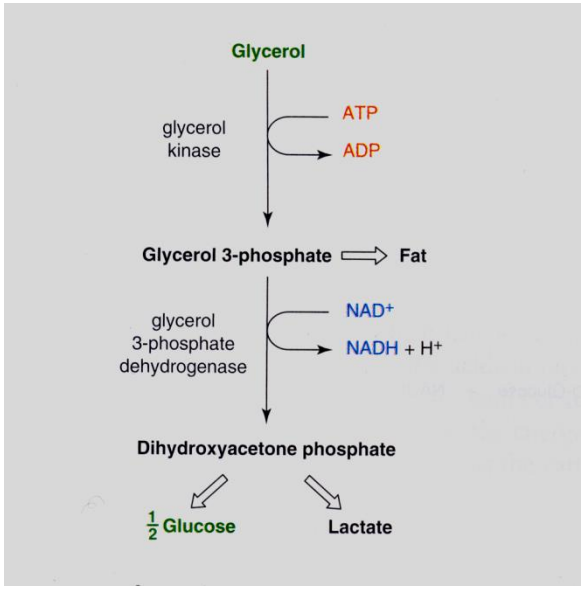
IV) **Glycerol**:-are excellent substrate for gluconeogenesis.

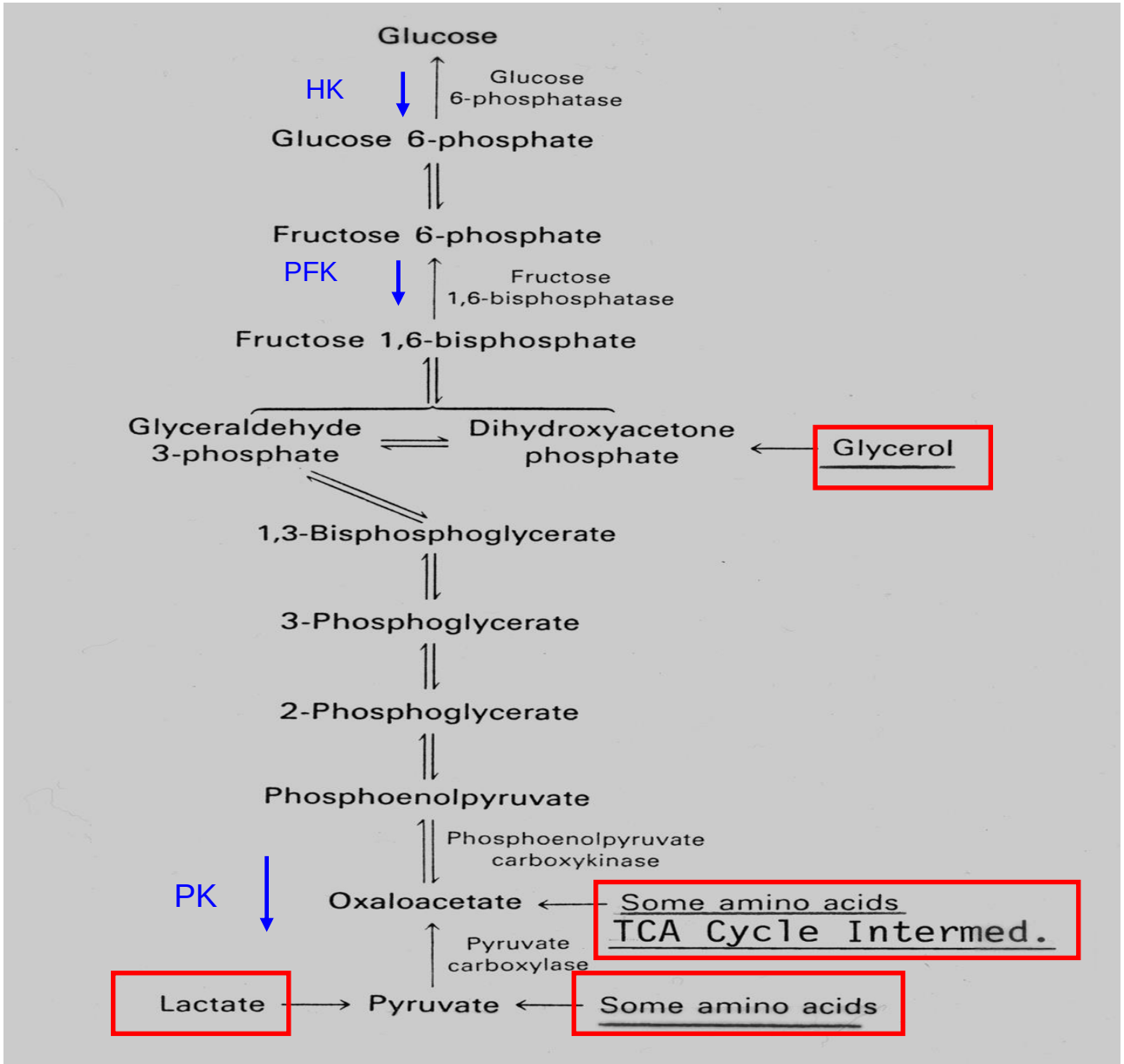
- which can be generated by hydrolysis of triacylglycerols (fat) to yield free FAs

V) **Odd numbered fatty acids**:- Are metabolized to give propionyl CoA and then succinyl CoA which enters the cycle past the decarboxylation steps.

Gluconeogenesis from Various Metabolites

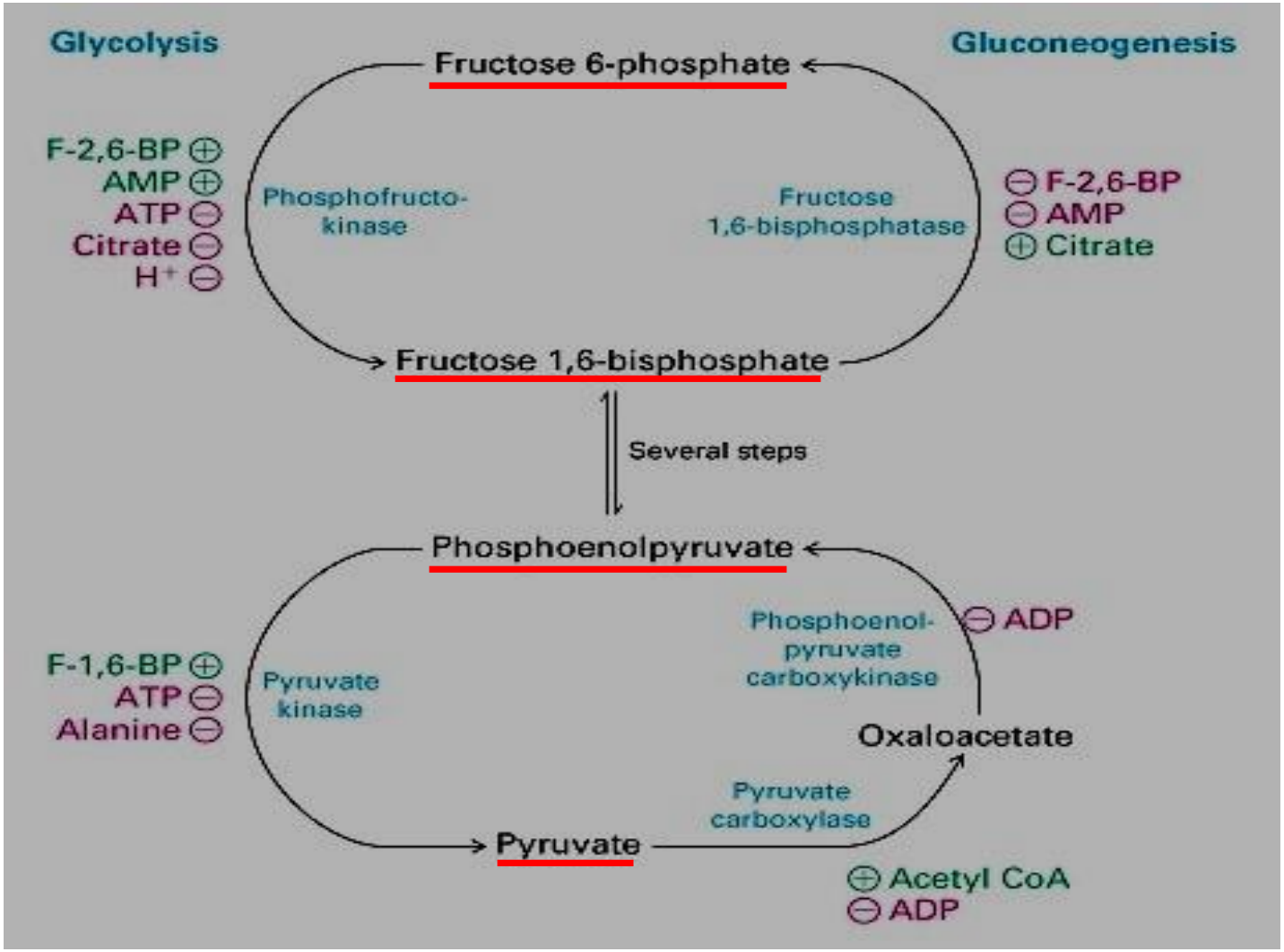
Entry points of non-carbohydrate precursors into gluconeogenesis





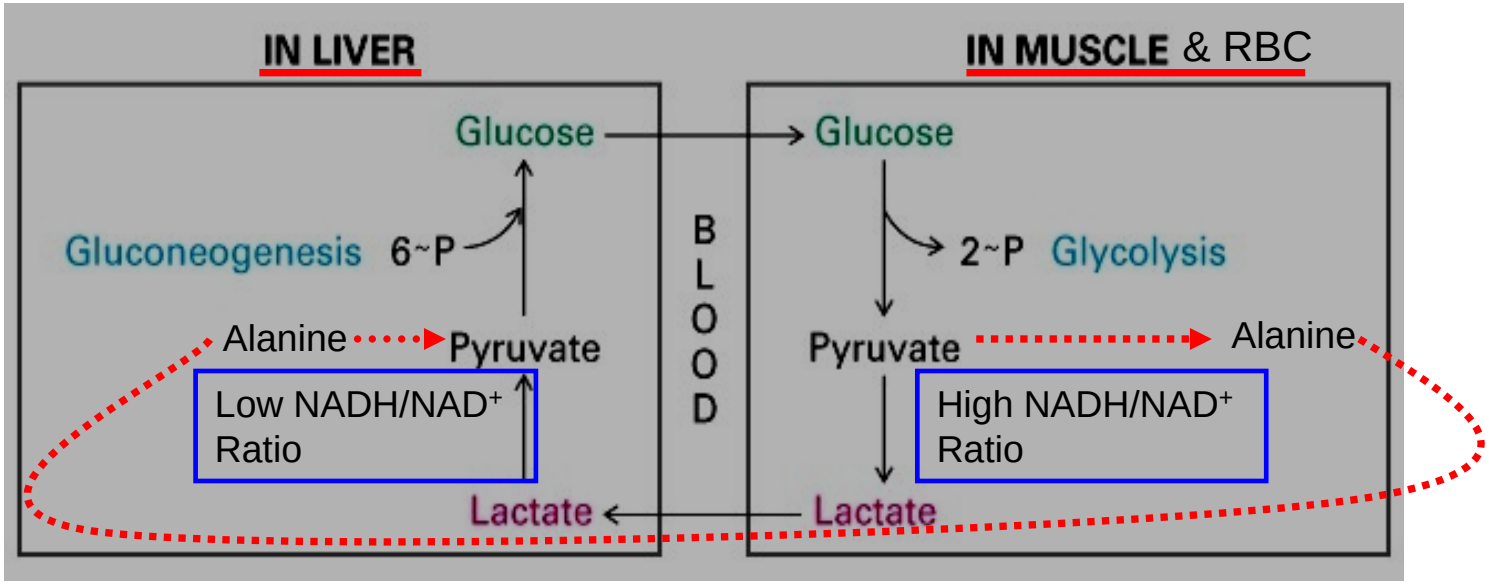
Regulation of Gluconeogenesis

- Gluconeogenesis and Glycolysis are Reciprocally Regulated



Cori's Cycle

- During heavy exercise glucose in muscle metabolized in to lactate
- Lactate produced in this way diffuses into the blood where it can reach to the liver where oxygen is surplus (unlike in muscles)
  - Where it is oxidized into glucose through pyruvate (by gluconeogenesis)
- This cycle pathway is called *Cori's cycle* or *lactic acid cycle*
- This cycle shifts part of the metabolic burden of active muscle to the liver



Pentose Phosphate Pathway

(Hexose monophosphate shunt)

Pentose Phosphate Pathway

- Is a cytosolic pathway that occur in
  - Tissues that synthesis fatty acids and sterols (***liver, mammary glands, adrenal glands, adipose tissue***) and
  - ***Red blood cells***-to maintain heme in reduced form
- Involve two basic steps : ***oxidative*** and ***non-oxidative***

- **<u>Oxidative phases</u>**
  - Involve irreversible reactions producing NADPH
    - ✓Catalysed by Glucose-6-P dehydrogenase & 6-P-gluconate dehydrogenase
  - NADPH serves as reducing power carrier
    - ✓In several synthetic pathways (***FFA's, NA's, Steroids etc***)

- **<u>Non-oxidative phases</u>**
  - Involve reversible reactions that produce
    - ✓**Ribose-5-phosphate-** important for synthesis of nucleic acids and nucleotide and
    - ✓**Several metabolites -**that feed into glycolytic pathways

Pentose Phosphate Pathway