Carbohydrate Metabolism

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Syllabus

Carbohydrate metabolism discussed with respect to the structures of intermediates, enzymes and cofactors, energy yield/requirements and regulation. Examples of drugs modulating carbohydrate metabolism.

1.1 Glycolysis (Embden Meyerhoff Pathway), TCA cycle (Kreb's Cycle, Citric acid Cycle) and glyoxalate shunt. Entry of sugars other than glucose into glycolytic pathway. Discussion of shuttle systems to transfer NADH to the mitochondria.

1.2 Electron Transport Chain discussed with respect to the components of the ETC, explanation of oxidative phosphorylation vs substrate level phosphorylation. Discussion of proton motive force and generation of ATP using proton gradients. Discussion of uncouplers of oxidative phosphorylation.

GLOSSARY

Anabolic Pathways:-Which are those involved in the synthesis of larger and more complex compounds from smaller precursors- eg., Synthesis of protein from amino acids

Catabolic pathways:-Which are involved in the breakdown of larger molecules, commonly involving oxidative reactions

- ***Amphibolic pathways:-***which occur at the crossroads of metabolism, acting as link between the anabolic and catabolic pathways, eg.,citric acid cycle. ***Glycolysis:-***is the cytosolic pathway of all mammalian cells for the metabolism of glucose(or glycogen) to pyruvate and lactate
- ***Glycogenesis:-***Stores glucose by converting glucose to glycogen, when glucose level is high
- ***Glyconeogenesis:-***is the process of synthesizing glucose or glycogen from noncarbohydrates precursors. significant substrates are amino acids, lactase, glycerol and propionate.
- ***Glycogenolysis:-***breakdown of glycogen when glucose level is low
- **ATP:**- Adenosine Tri Phosphate -**ADP:**- Adenosine Di Phosphate

Adenosine Tri-Phosphate (ATP)

- Link between energy releasing and energy requiring mechanisms
  - "rechargeable battery"

    ADP + P + Energy ⟷ ATP
- Substrate-level phosphorylation
  - Substrate transfers a phosphate group directly
  - Requires enzymes

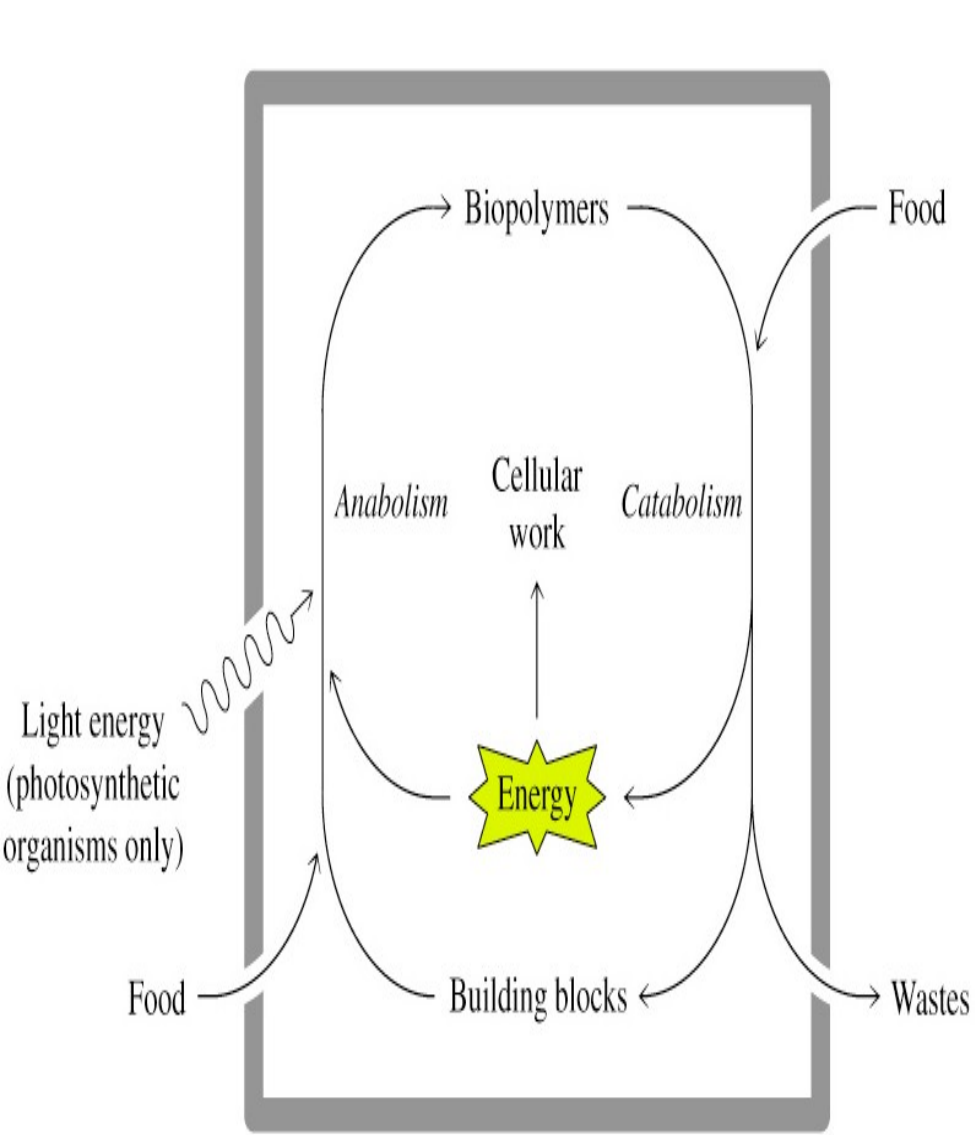
    Phosphocreatine + ADP → Creatine + ATP
- Oxidative phosphorylation
  - Method by which most ATP formed
  - Small carbon chains transfer hydrogens to transporter (NAD or FADH) which enters the electron transport chain

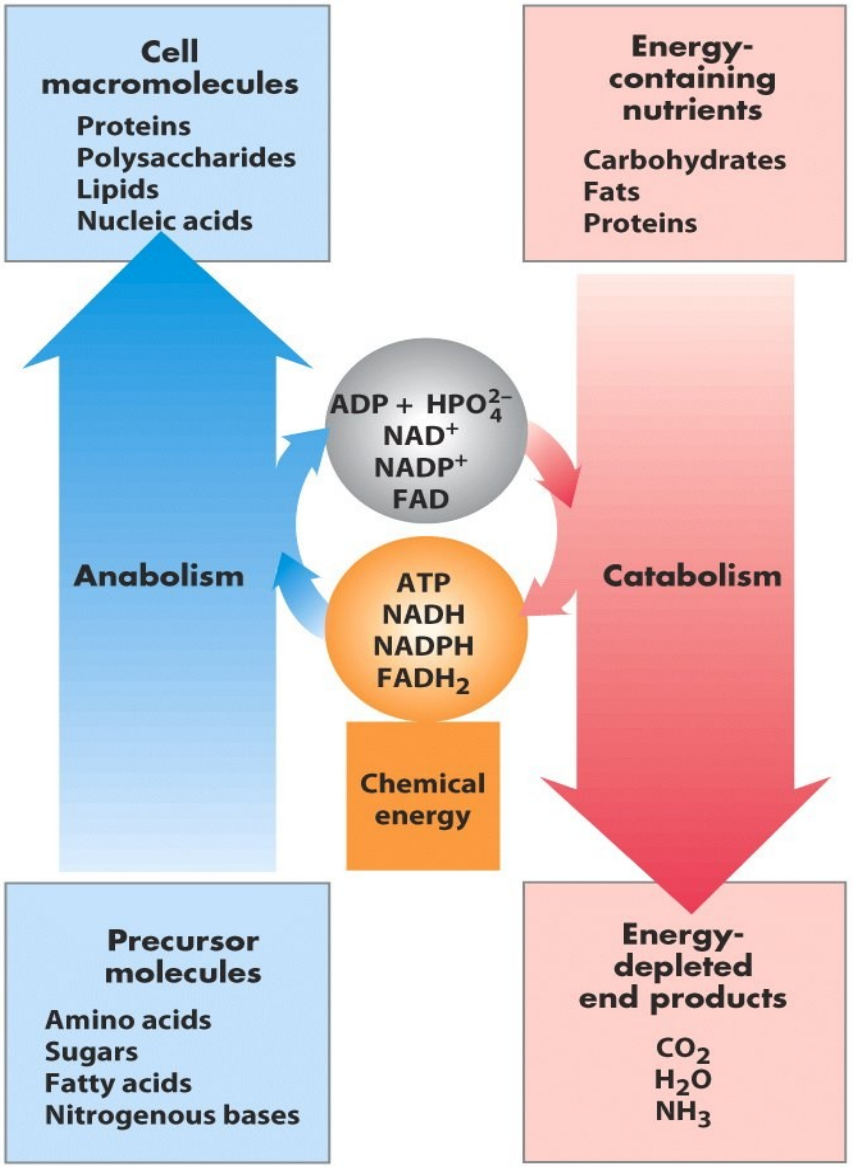
Metabolism

- Metabolism is all the chemical reactions that occur in an organism
- Cellular metabolism
  - Cells *break down* excess carbohydrates first, then lipids, finally amino acids if energy needs are not met by carbohydrates and fat
  - Nutrients not used for energy are used to *build up* structure, are stored, or they are excreted
  - 40% of the energy released in catabolism is captured in ATP, the rest is released as heat

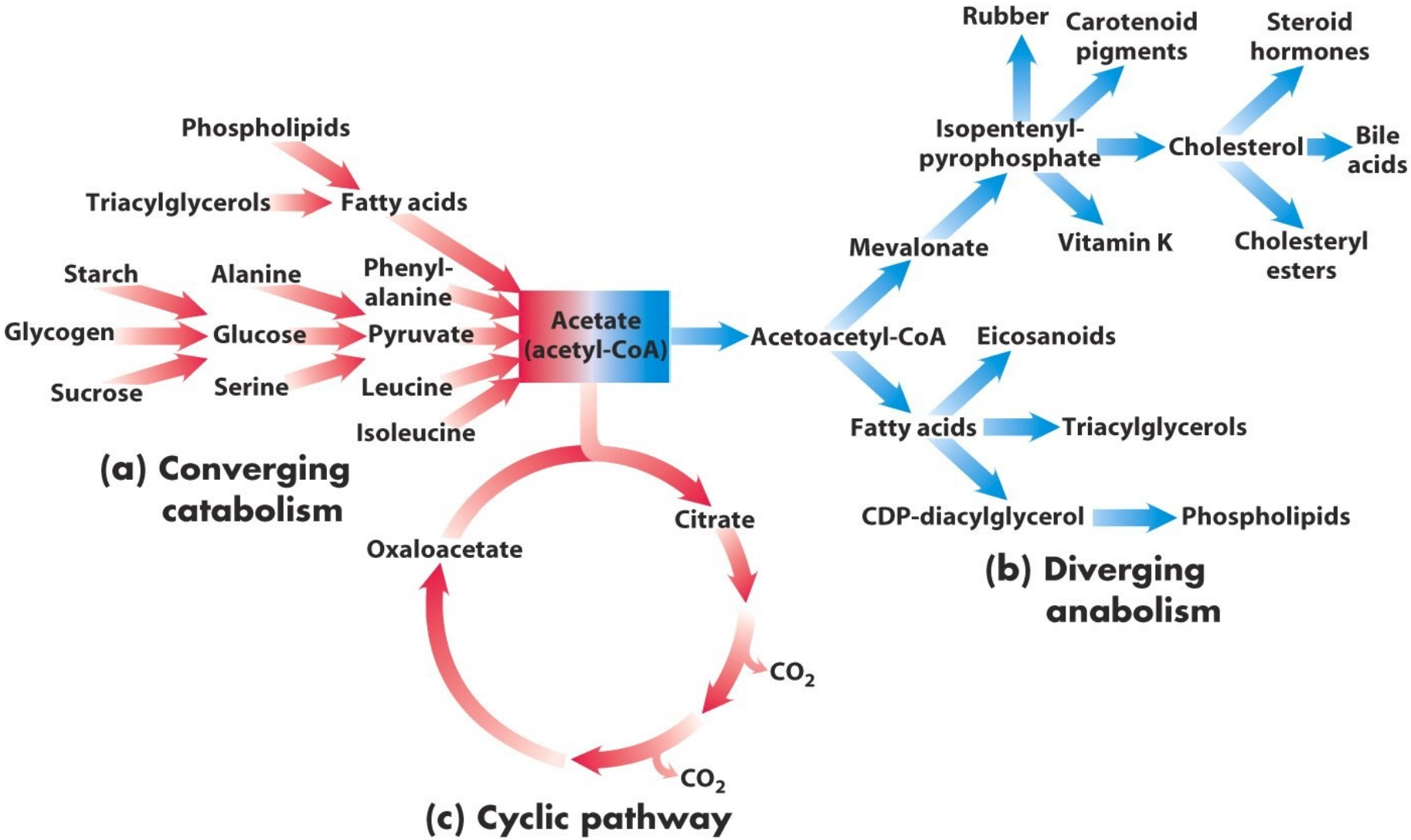
ANABOLISM	CATABOLISM
•Performance of structural maintenance and repairs. •Support of growth •Production of secretions •Building of nutrient reserves	•Breakdown of nutrients to provide energy (in the form of ATP) for body processes • Nutrients directly absorbed • Stored nutrients

Anabolism and catabolism

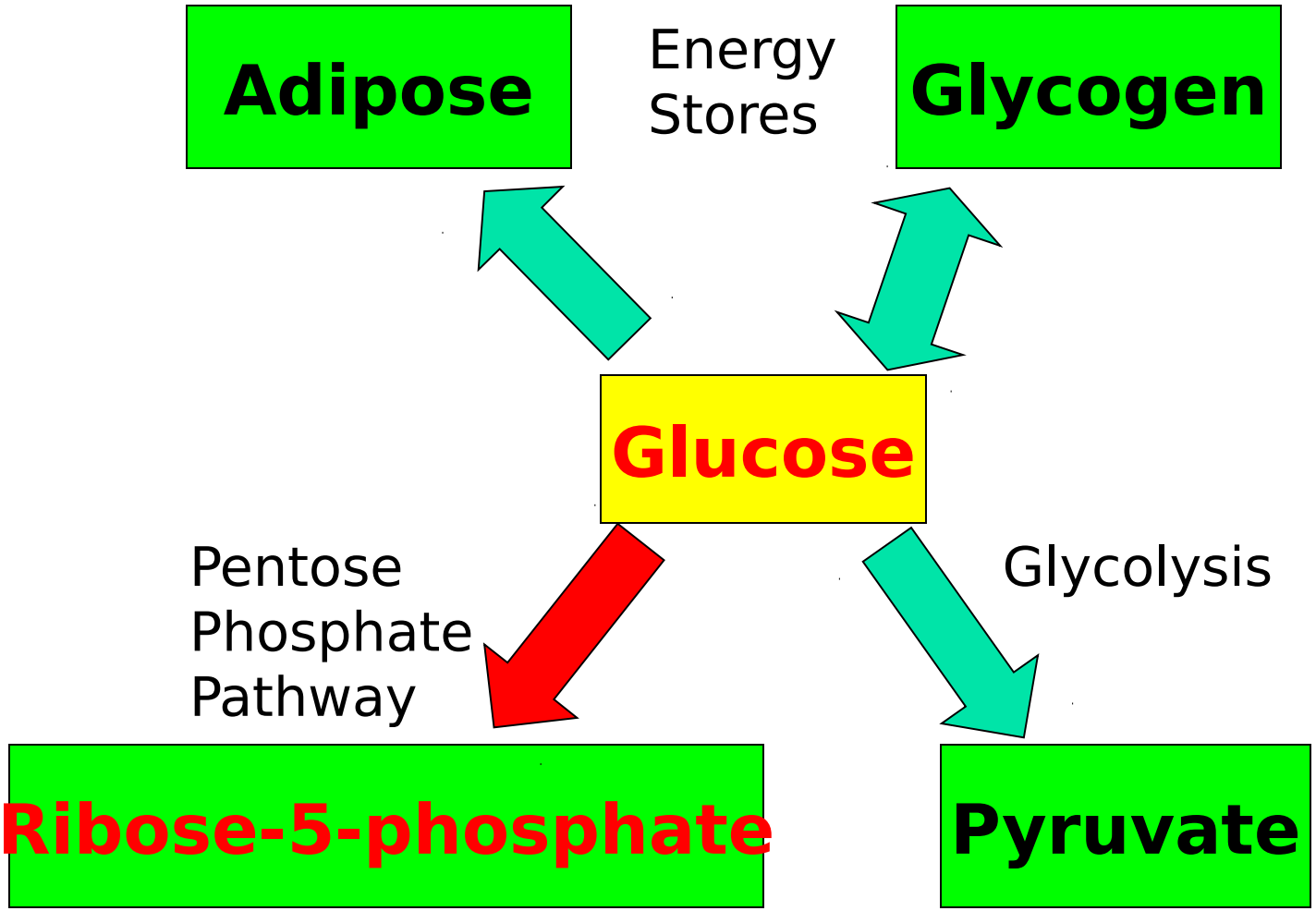




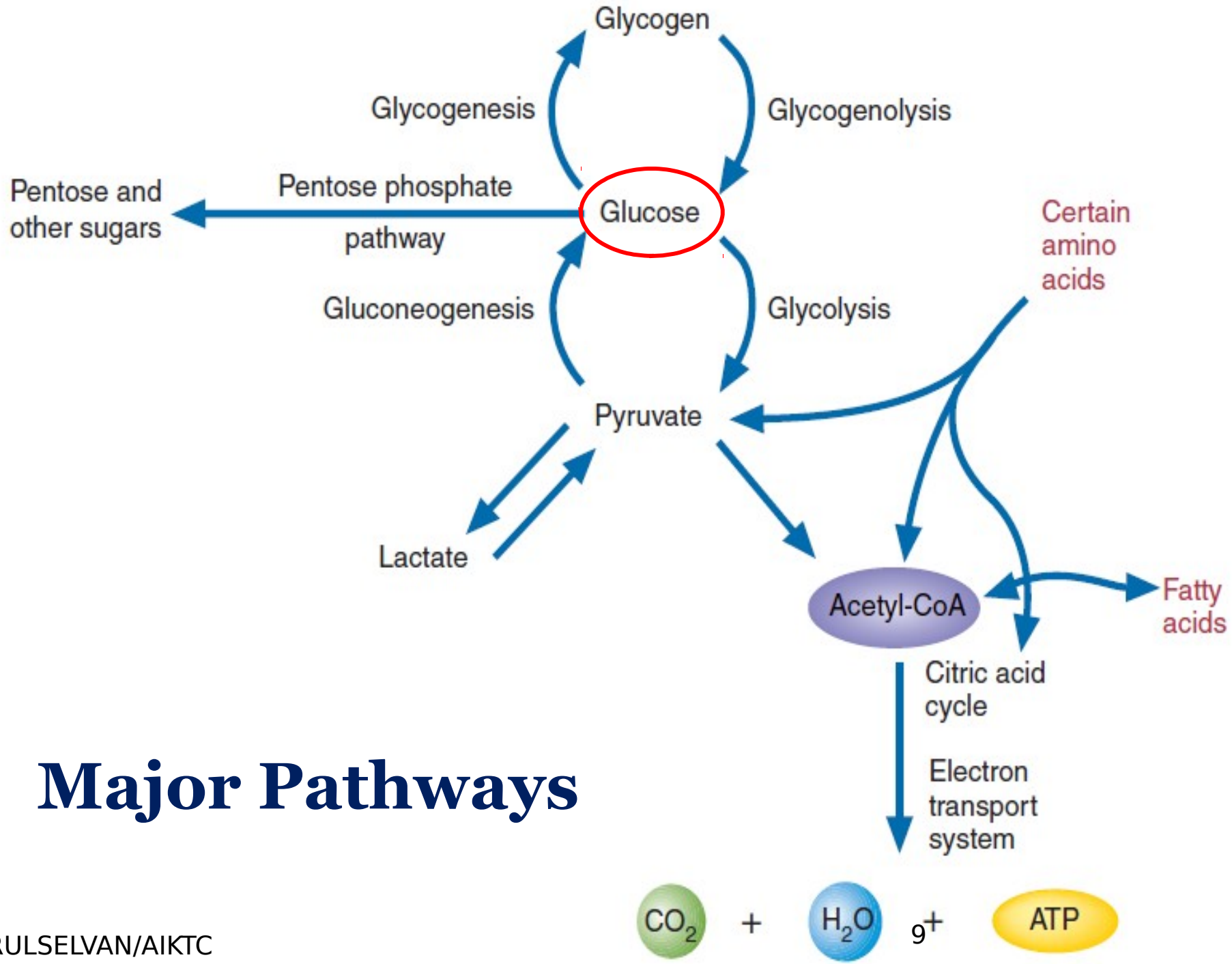
Anabolism and catabolism



Glucose Utilization



Major Pathways



Glucose Metabolism

- Four major metabolic pathways:
  - Immediate source of energy
  - Pentophosphate pathway
  - Glycogen synthesis in liver/muscle
  - Precursor for triacylglycerol synthesis
- Energy status (ATP) of body regulates which pathway gets energy
- Same in ruminants and non-ruminants
- 1st Priority: glycogen storage
  - Stored in muscle and liver
- 2nd Priority: provide energy
  - Oxidized to ATP
- 3rd Priority: stored as fat
  - Only excess glucose
  - Stored as triglycerides in adipose

Glycolysis

- Glycolysis is an anaerobic process

- Two stages (stage 1 and 2): energy investment and energy producing
  - Glycolytic Pathway: D-Glucose + 2 ADP + 2 P_i + 2 NAD^+ □ 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O

    - In eukaryotes, the enzymes for this pathway are in the cytosol. They are all homodimers or homotetramers.

Glucose → 2 Pyruvate → Lactate (anaerobic)

Glucose → 2 Pyruvate → Acetyl-CoA (TCA cycle)

I. Glycolysis (Embden Meyerhof Pathway)

A. Definition:

1. Glycolysis means oxidation of glucose to give pyruvate (in the presence of oxygen) or lactate (in the absence of oxygen).

B. Site:

Cytoplasm of all tissue cells, but it is of physiological importance in:

1. Tissues with no mitochondria: mature RBCs, cornea and lens.
2. Tissues with few mitochondria: Testis, leucocytes, medulla of the kidney, retina, skin and gastrointestinal tract.
3. Tissues undergo frequent oxygen lack: skeletal muscles especially during exercise.

I. Glycolysis (Embden Meyerhof Pathway)

C. Steps:

Stages of glycolysis

1. Stage one (the energy requiring stage):

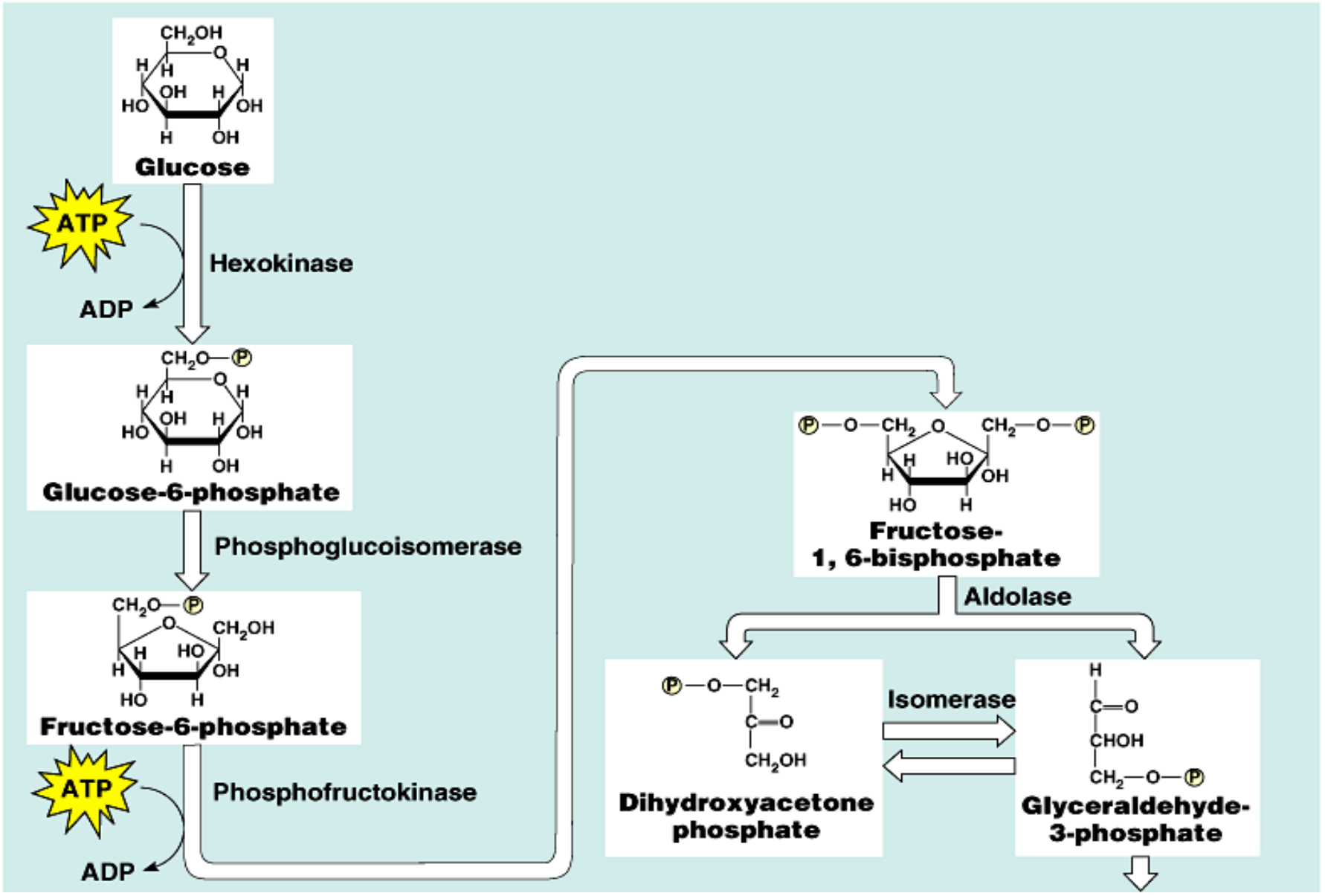
   a) One molecule of glucose is converted into two molecules of glycerosldhyde-3-phosphate.

   b) These steps requires 2 molecules of ATP (energy loss)

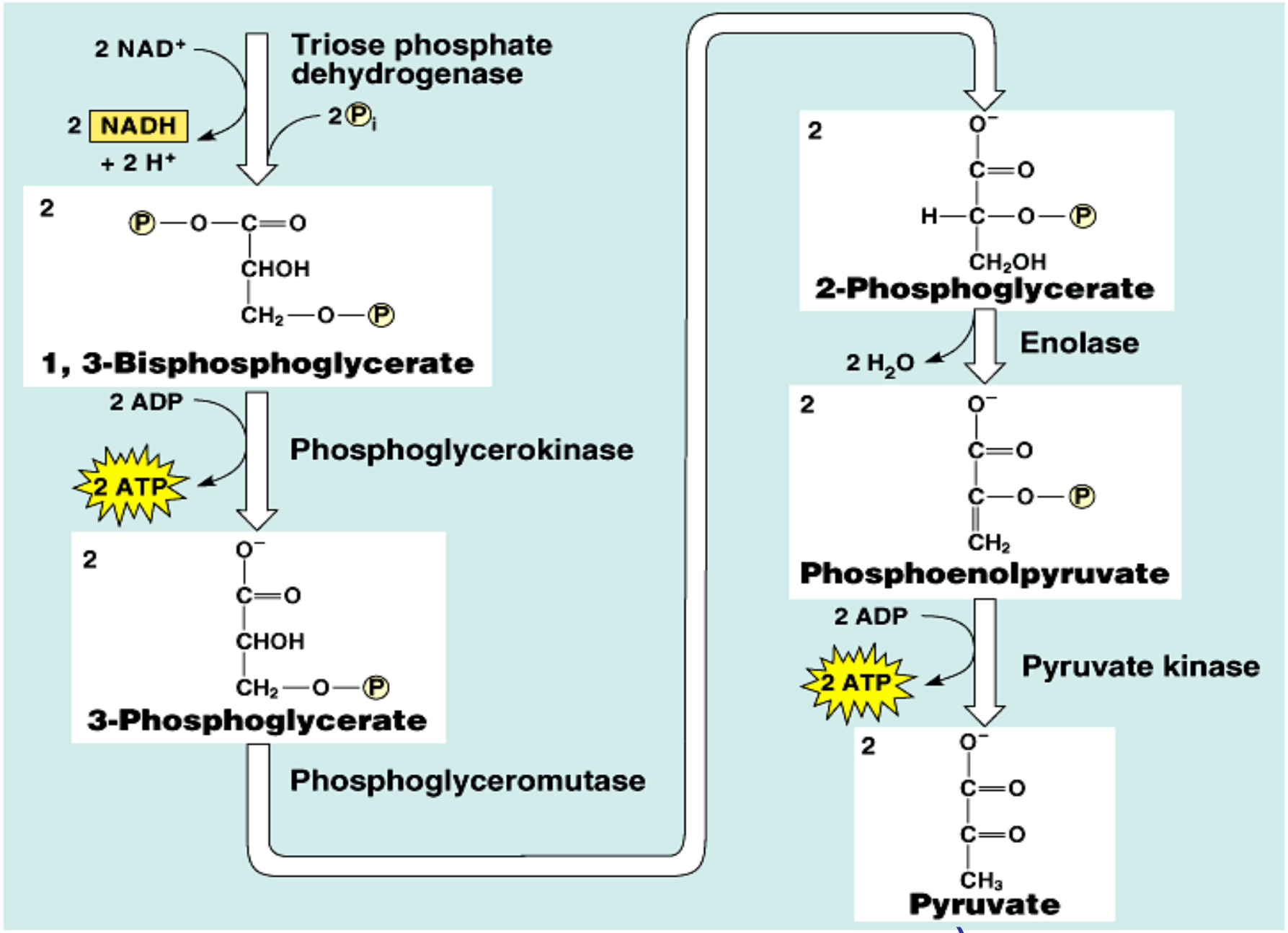
2. Stage two (the energy producing stage(:

   a) The 2 molecules of glyceroaldehyde-3-phosphate are converted into pyruvate (aerobic glycolysis) or lactate (anaerobic glycolysis).

   b) These steps produce ATP molecules (energy production).



Energy Investment Phase (steps 1-5)



Energy-Payoff Phase (Steps 6-10)

D. Energy production of Glycolysis:

Net energy	ATP utilized	ATP produced	
2 ATP	2ATP From glucose to glucose -6-p. From fructose -6-p to fructose 1,6 p.	4 ATP (Substrate level phosphorylation) 2ATP from 1,3 DPG. 2ATP from phosphoenol pyruvate	In absence of oxygen (anaerobic glycolysis)
6 ATP Or 8 ATP	2ATP -From glucose to glucose -6-p. From fructose -6-p to fructose 1,6 p.	4 ATP (substrate level phosphorylation) 2ATP from 1,3 BPG. 2ATP from phosphoenol pyruvate.	In presence of oxygen (aerobic glycolysis)
		+ 4ATP or 6ATP (from oxidation of 2 NADH + H in mitochondria).	

E. Oxidation of extra-mitochondrial $NADH^+H^+$:

1. Cytoplasmic NADH+H+ cannot penetrate mitochondrial membrane, however it can be used to produce energy (4 or 6 ATP) by respiratory chain phosphorylation in the mitochondria.
2. This can be done by using special carriers for hydrogen of $NADH^+H^+$ .These carriers are either dihydroxyacetone phosphate (Glycerophosphate shuttle) or oxaloacetate (aspartate malate shuttle).

a) Glycerophosphate shuttle:

1) It is important in certain muscle and nerve cells.

2) The final energy produced is 4 ATP.

3) Mechanism:

- The coenzyme of cytoplasmic glycerol-3- phosphate dehydrogenase is NAD^+.
- The coenzyme of mitochodrial glycerol-3-phosphate dehydogenase is FAD.
- Oxidation of FADH, in respiratory chain gives 2 ATP. As glycolysis gives 2 cytoplasmic NADH + H+ $\rightarrow$ 2 mitochondrial FADH,

2 x 2 ATP $\rightarrow$ = 4 ATP.

b) Malate – aspartate shuttle:

1) It is important in other tissues patriculary liver and heart.

2) The final energy produced is 6 ATP.

Differences between aerobic and anaerobic glycolysis

Anaerobic	Aerobic	
Lactate	Pyruvate	1. End product
2 ATP	6 or 8 ATP	2 .Energy
Through Lactate formation	Through respiration chain in mitochondria	3. Regeneration of NAD^+
Not available as lactate is cytoplasmic substrate	Available and 2 Pyruvate can oxidize to give 30 ATP	4. Availability to TCA in mitochondria

Importance of lactate production in anaerobic glycolysis:

1. In absence of oxygen, lactate is the end product of glycolysis:

Glucose $\rightarrow$ Pyruvate $\rightarrow$ Lactate

2. In absence of oxygen, NADH $^{+}$ H^{+} is not oxidized by the respiratory chain.
3. The conversion of pyruvate to lactate is the mechanism for regeneration of NAD^{+}.
4. This helps continuity of glycolysis, as the generated NAD+ will be used once more for oxidation of another glucose molecule.

Substrate level phosphorylation

- This means phosphorylation of ADP to ATP at the reaction itself in glycolysis there are 2 examples:
  - 1.3 Bisphosphoglycerate + ADP 3 Phosphoglycerate + ATP
  - Phospho-enol pyruvate + ADP Enolpyruvate + ATP

I. Special features of glycolysis in RBCs:

1. Mature RBCs contain no mitochondria, thus:
   a) They depend only upon glycolysis for energy production (=2 ATP).
   b) Lactate is always the end product.
2. Glucose uptake by RBCs is independent on insulin hormone.
3. Reduction of met-hemoglobin: Glycolysis produces $NADH^{+}H^{+}$, which used for reduction of met-hemoglobin in red cells.

Biological importance (functions) of glycolysis

1. Energy production:
   a) anaerobic glycolysis gives 2 ATP.
   b) aerobic glycolysis gives 8 ATP.
2. Oxygenation of tissues:
   Through formation of 2,3 bisphosphoglycerate, which decreases the affinity of Hemoglobin to O_2.
3. Provides important intermediates:
   a) Dihydroxyacetone phosphate: can give glycerol-3phosphate, which is used for synthesis of triacylglycerols and phospholipids (lipogenesis).
   b) 3 Phosphoglycerate: can be used for synthesis of amino acid serine.
   c) Pyruvate: which can be used in synthesis of amino acid alanine.
4. Aerobic glycolysis provides the mitochondria with pyruvate, which gives acetyl CoA (Krebs' cycle).

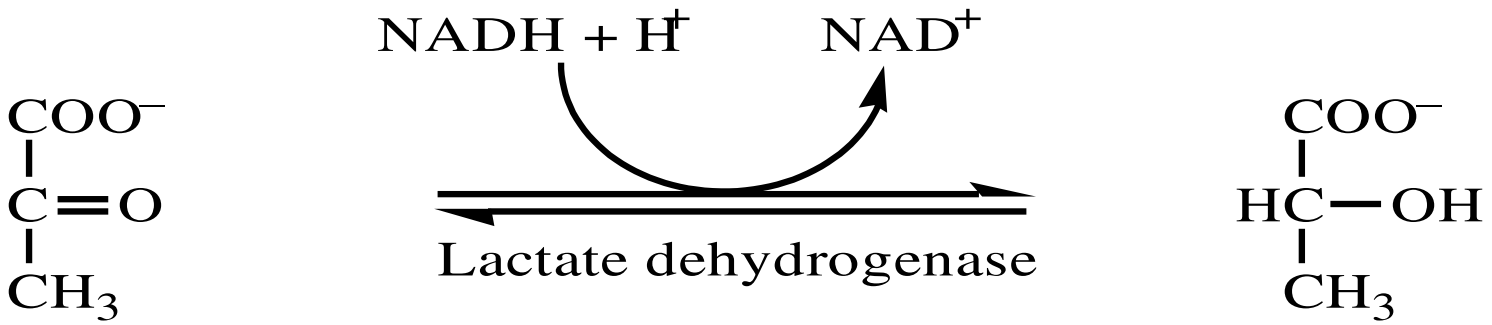
Pyruvate Metabolism

- FATES OF PYRUVATE
  - Conversion to lactate (anaerobic) -Conversion to alanine (amino acid)
  - Entry into the TCA cycle via pyruvate dehydrogenase pathway

1.Anaerobic Metabolism of Pyruvate to Lactate

Problem:

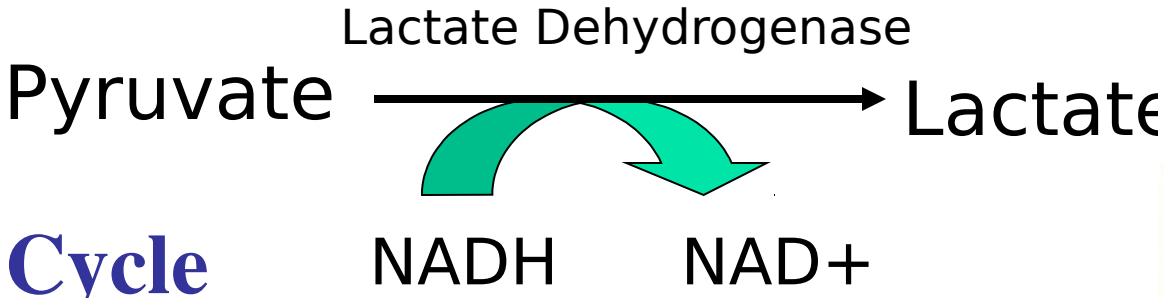
- During glycolysis, NADH is formed from NAD^+
- Without O_2, NADH cannot be oxidized to NAD^+
- No more NAD^+ All converted to NADH
- Without NAD^+, glycolysis stops…



Pyruvate *(oxidized)* Lactate *(reduced)*

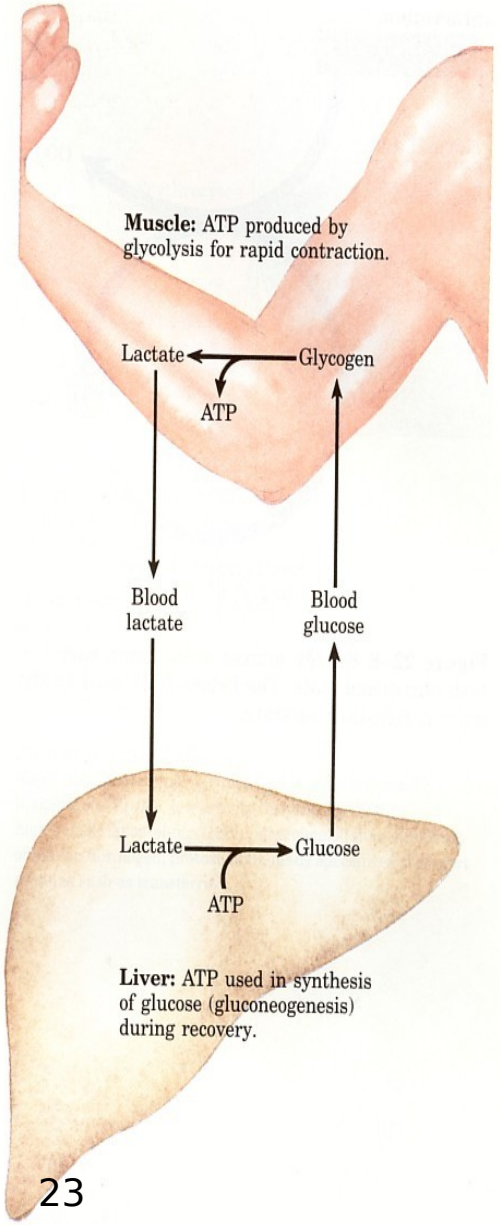
Anaerobic Metabolism of Pyruvate

- ATP yield
  - Two ATPs (net) are produced during the anaerobic breakdown of one glucose
    - The 2 NADHs are used to reduce 2 pyruvate to 2 lactate
  - Reaction is fast and doesn't require oxygen
- Lactate can be transported by blood to liver and used in gluconeogenesis



Cori Cycle

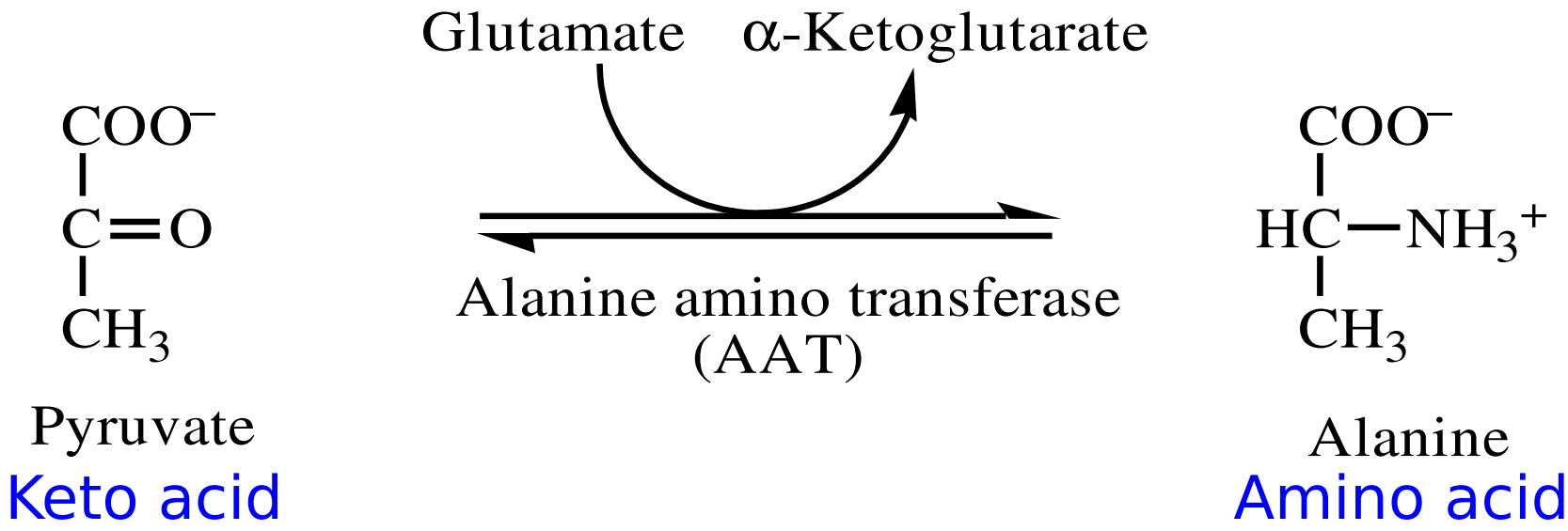
- Lactate is converted to pyruvate in the liver



Pyruvate metabolism

2.Conversion to Alanine (Amino acid)

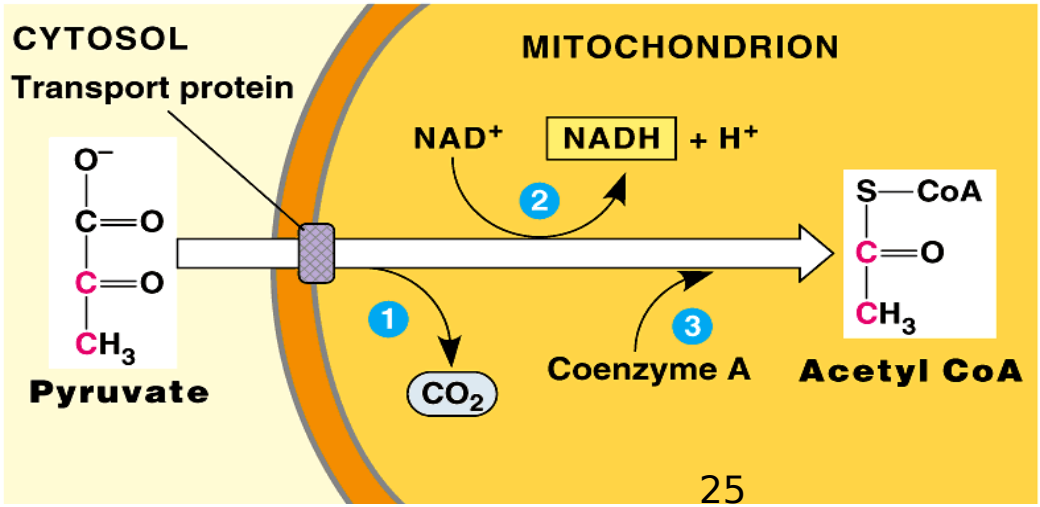
-pyruvate is Converted to alanine by alanine amino transferase (AAT) enzyme and export to blood



Pyruvate metabolism

3.Entry into the TCA cycle via pyruvate dehydrogenase pathway

- As pyruvate enters the mitochondrion, a multienzyme complex modifies pyruvate to acetyl CoA which enters the Krebs cycle in the matrix.
  - A carboxyl group is removed as CO_2.
  - A pair of electrons is transferred from the remaining two-carbon fragment to NAD^+ to form NADH.
  - The oxidized fragment, acetate, combines with coenzyme A to form acetyl CoA.



Glycolysis Regulation

- **Regulation of Glycolysis**
  - The rate of the glycolytic pathway in a cell is controlled by the allosteric enzymes:
    - **Hexokinases I, II, and III**
    - **PFK-1**
    - **Pyruvate kinase**
  - Allosteric enzymes are sensitive indicators of a cell's metabolic state regulated locally by effector molecules
  - The peptide hormones **glucagon** and **insulin** also regulate glycolysis

Glycolysis Regulation

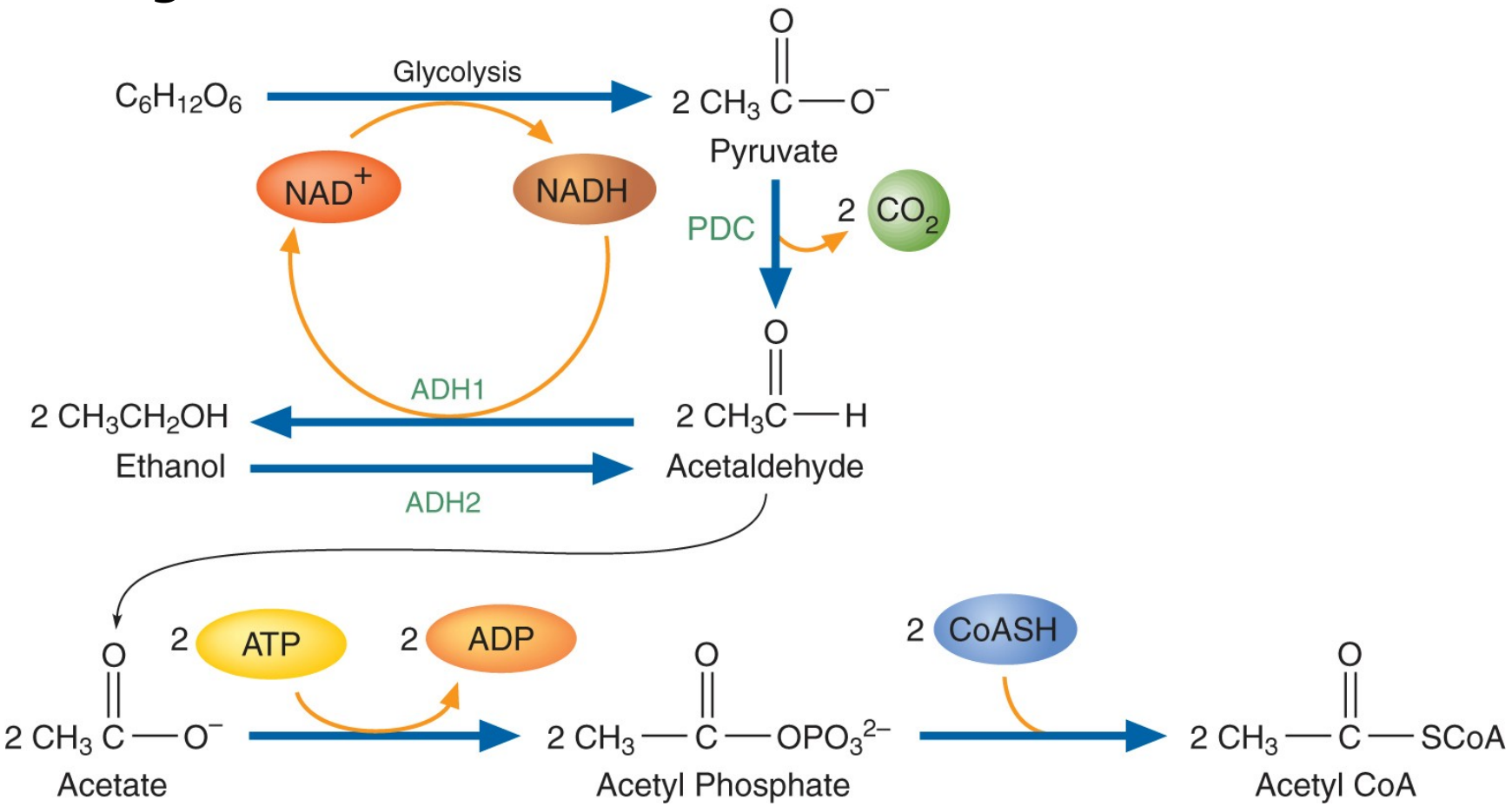
TABLE 8.1 Allosteric Regulation of Glycolysis

Enzyme	Activator	Inhibitor
Hexokinase		Glucose-6-phosphate, ATP
PFK-1	Fructose-2,6-bisphosphate, AMP	Citrate, ATP
Pyruvate kinase	Fructose-1,6-bisphosphate, AMP	Acetyl-CoA, ATP

- **Regulation of Glycolysis Continued**
  - High AMP concentrations activate pyruvate kinase
  - Fructose-2,6-bisphosphate, produced via hormone-induced covalent modification of PFK-2, activates PFK-1
  - Accumulation of fructose-1,6-bisphosphate activates PFK-1 providing a feed-forward mechanism

Glycolysis Regulation

Figure 8.9 Fructose-2,6-Bisphosphate Level Regulation

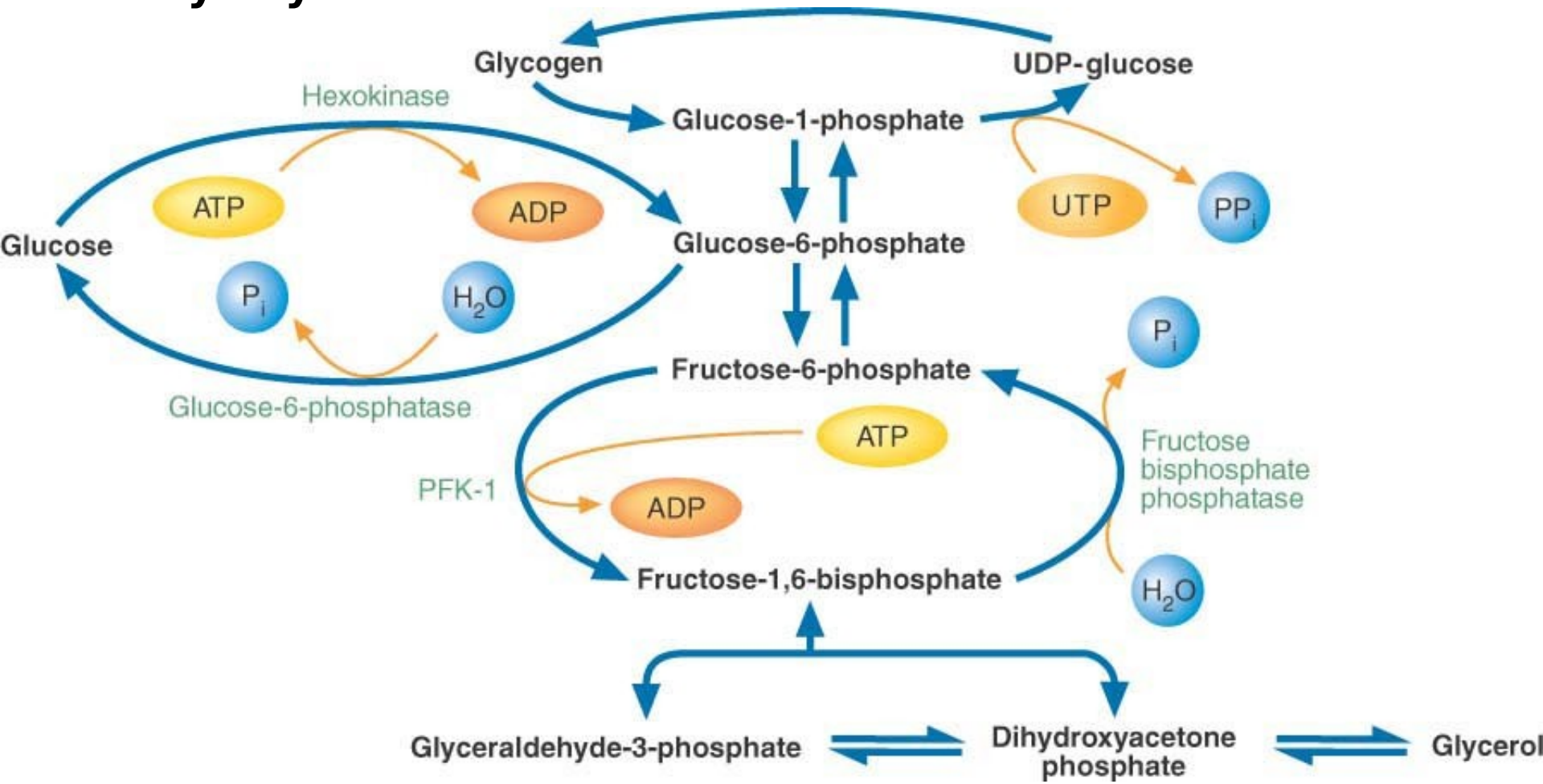


Gluconeogenesis

- Gluconeogenesis is the formation of new glucose molecules from precursors in the liver
  - Precursor molecules include lactate, pyruvate, and α-keto acids
- **Gluconeogenesis Reactions**
  - Reverse of glycolysis except the three irreversible reactions

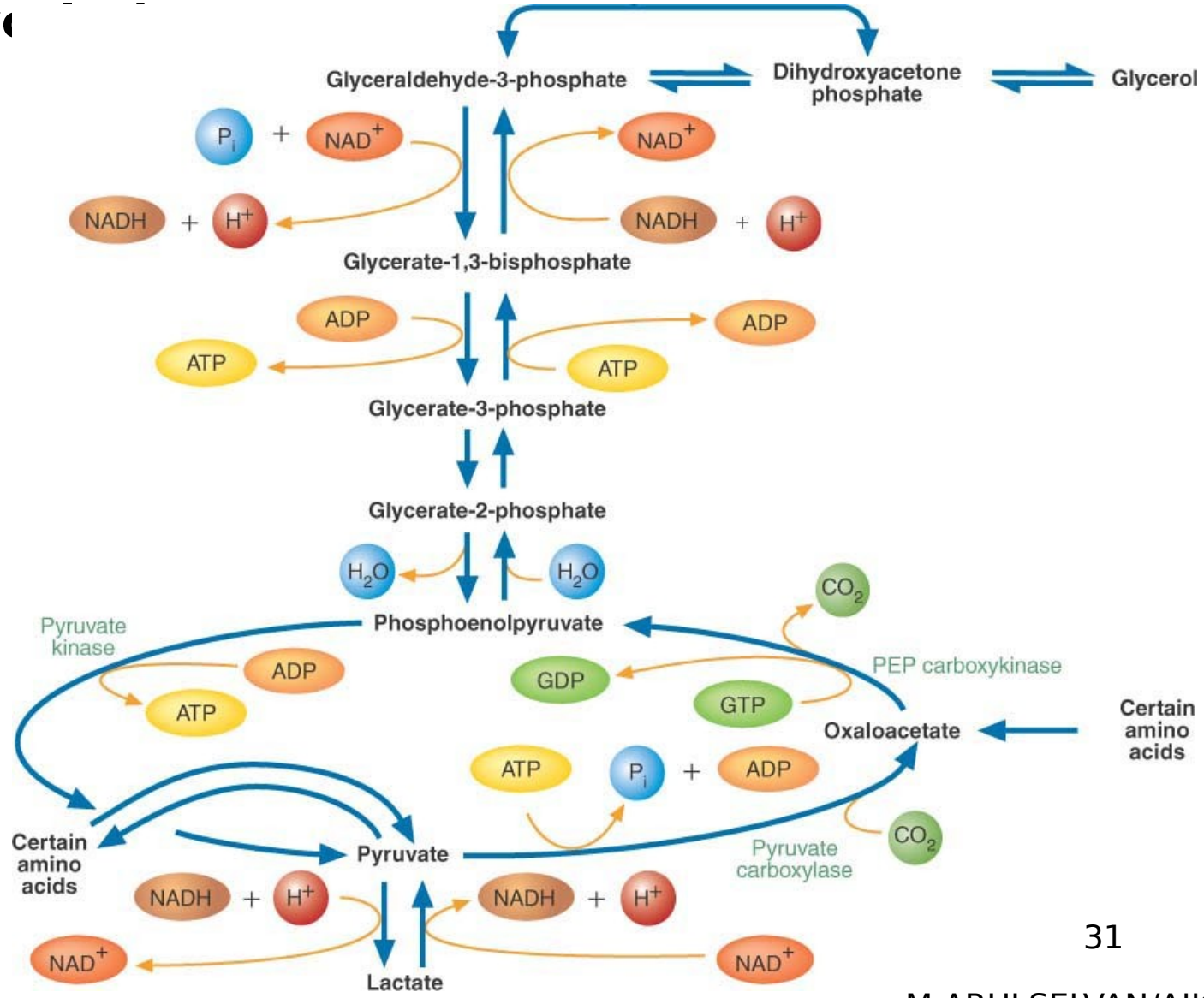
Gluconeogenesis

Carbohydrate Metabolism: Gluconeogenesis and Glycolysis



Gluconeogenesis

Carbohydrate Metabolism: Gluconeogenesis and Gly



Gluconeogenesis

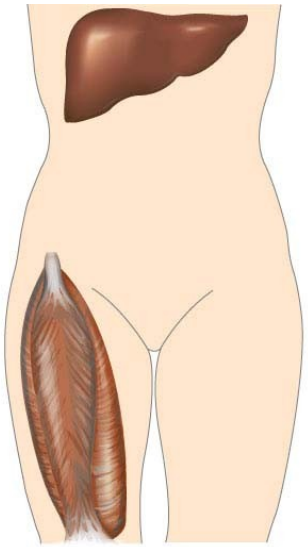
- **Gluconeogenesis Reactions Continued**
  - Three bypass reactions:

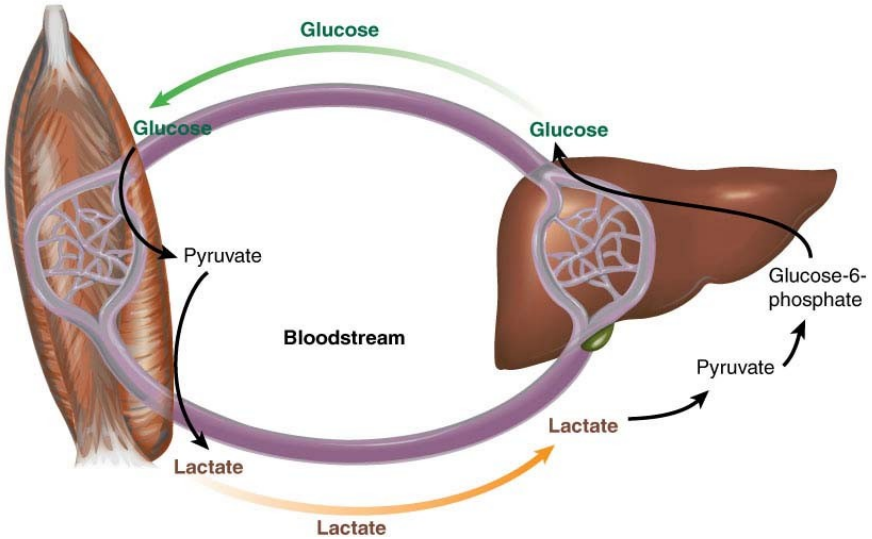
    1. Synthesis of phosphoenolpyruvate (PEP) via the enzymes pyruvate carboxylase and pyruvate carboxykinase

    2. Conversion of fructose-1,6-bisphosphate to fructose-6-phosphate via the enzyme fructose-1,6-bisphosphatase

    3. Formation of glucose from glucose-6-phosphate via the liver and kidney-specific enzyme glucose-6-phosphatase

Gluconeogenesis

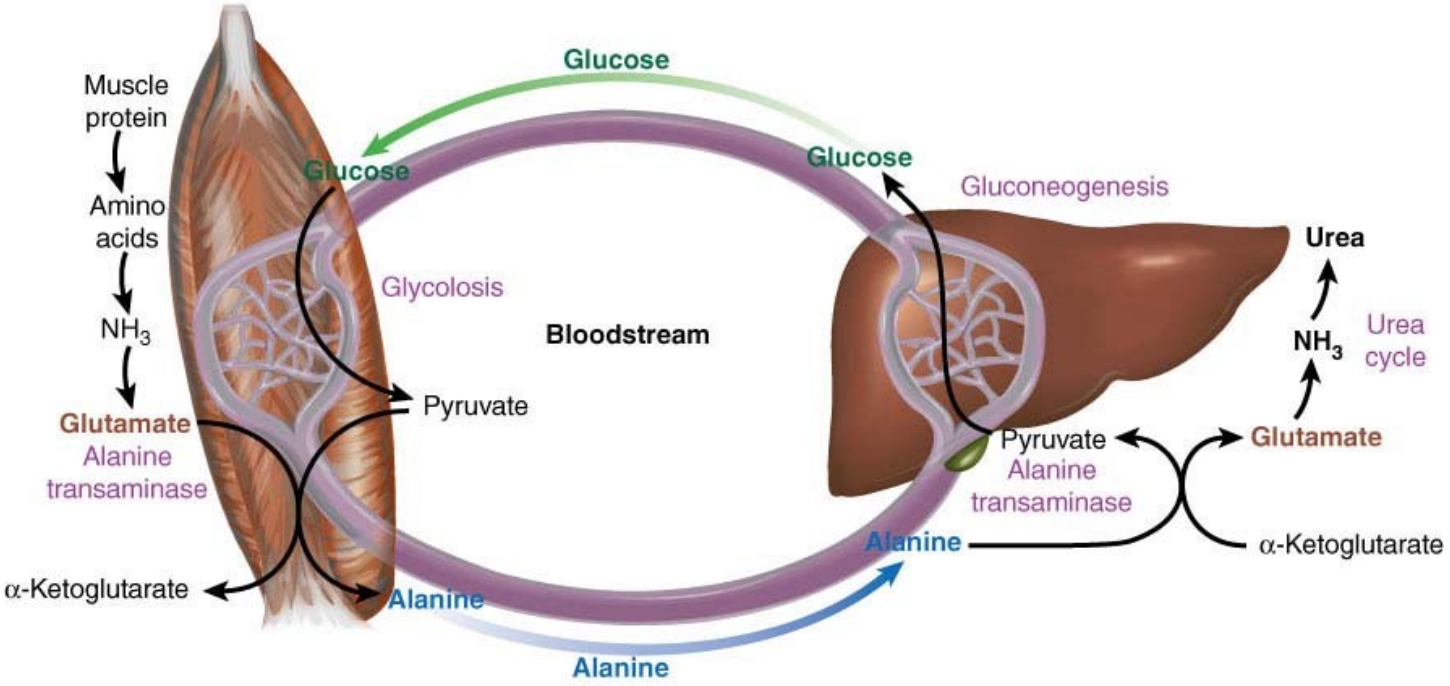




Cori Cycle

- **Gluconeogenesis Substrates**
  - Three of the most important substrates for gluconeogenesis are:
    - **1. Lactate—**released by skeletal muscle from the **Cori cycle**
      - After transfer to the liver lactate is converted to pyruvate, then to glucose
    - **2. Glycerol—**a product of fat metabolism

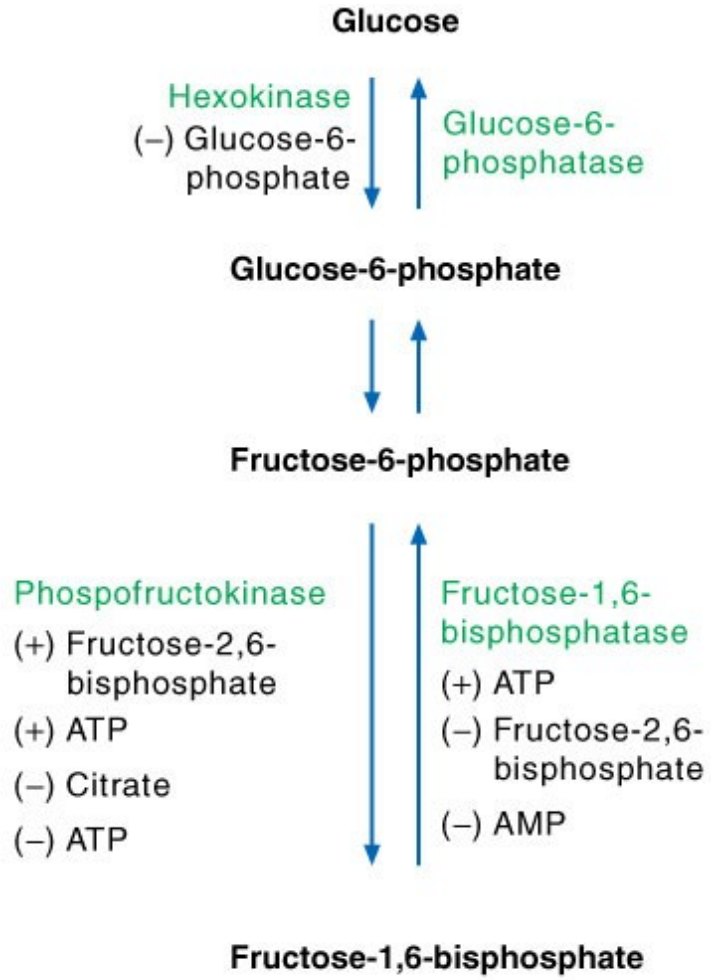
Gluconeogenesis



The Glucose Alanine Cycle

- **Gluconeogenesis Substrates Continued**
  - **3. Alanine—**generated from pyruvate in exercising muscle
    - Alanine is converted to pyruvate and then glucose in the liver

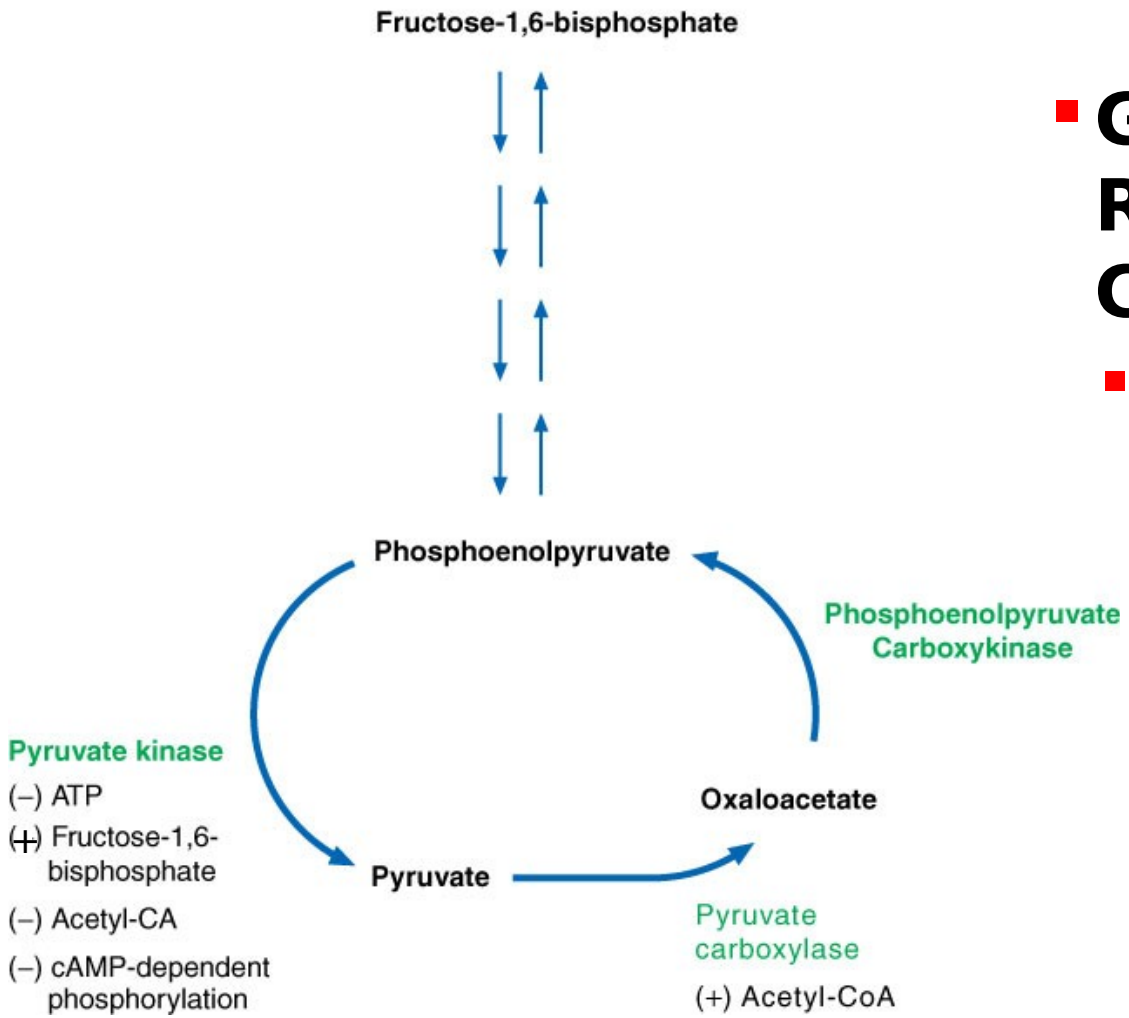
Gluconeogenesis Regulation



Allosteric Regulation of Glycolysis and Gluconeogenesis

- **Gluconeogenesis Regulation**
  - Substrate availability
  - Hormones (e.g., cortisol and insulin)

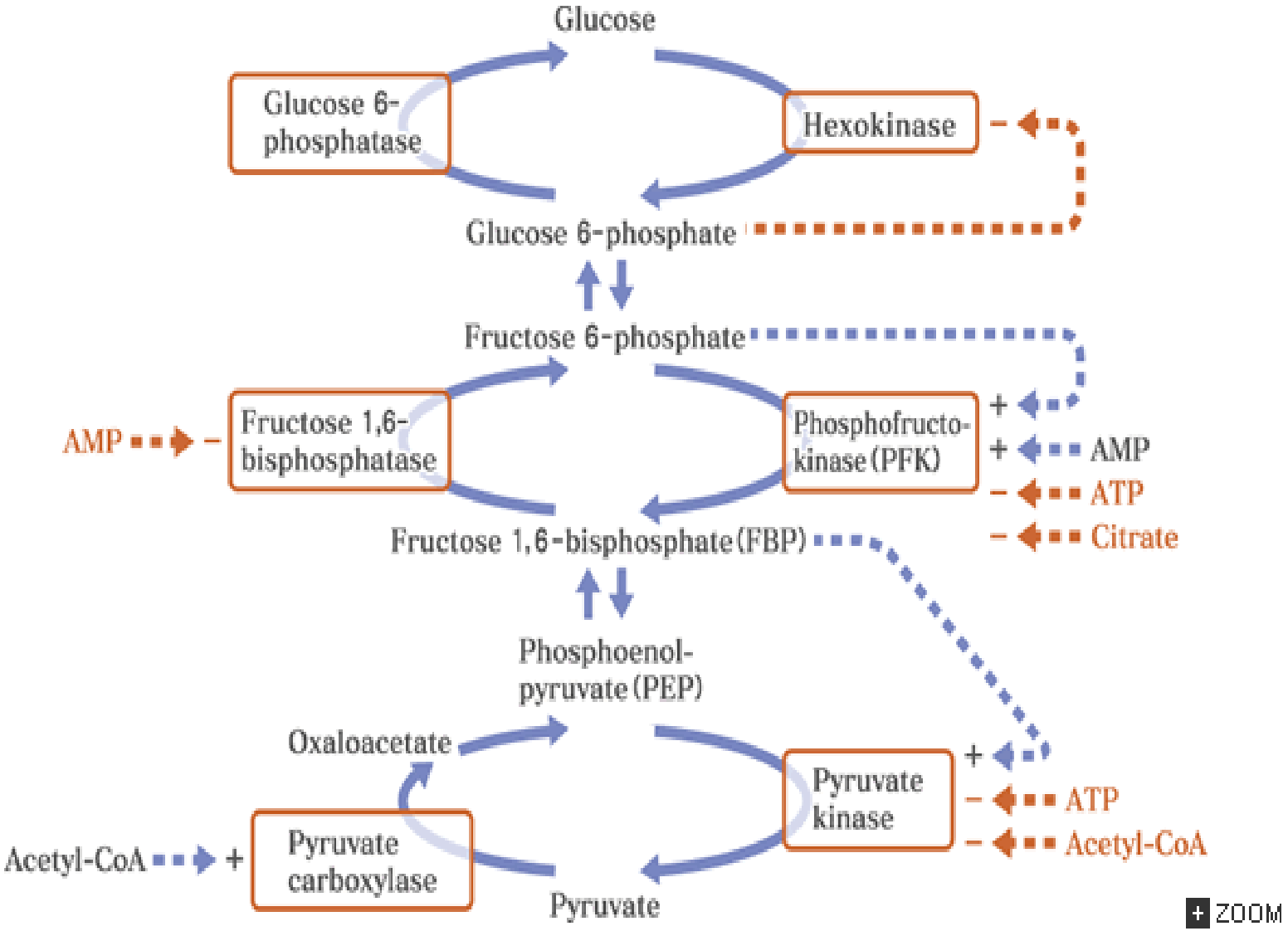
Gluconeogenesis Regulation

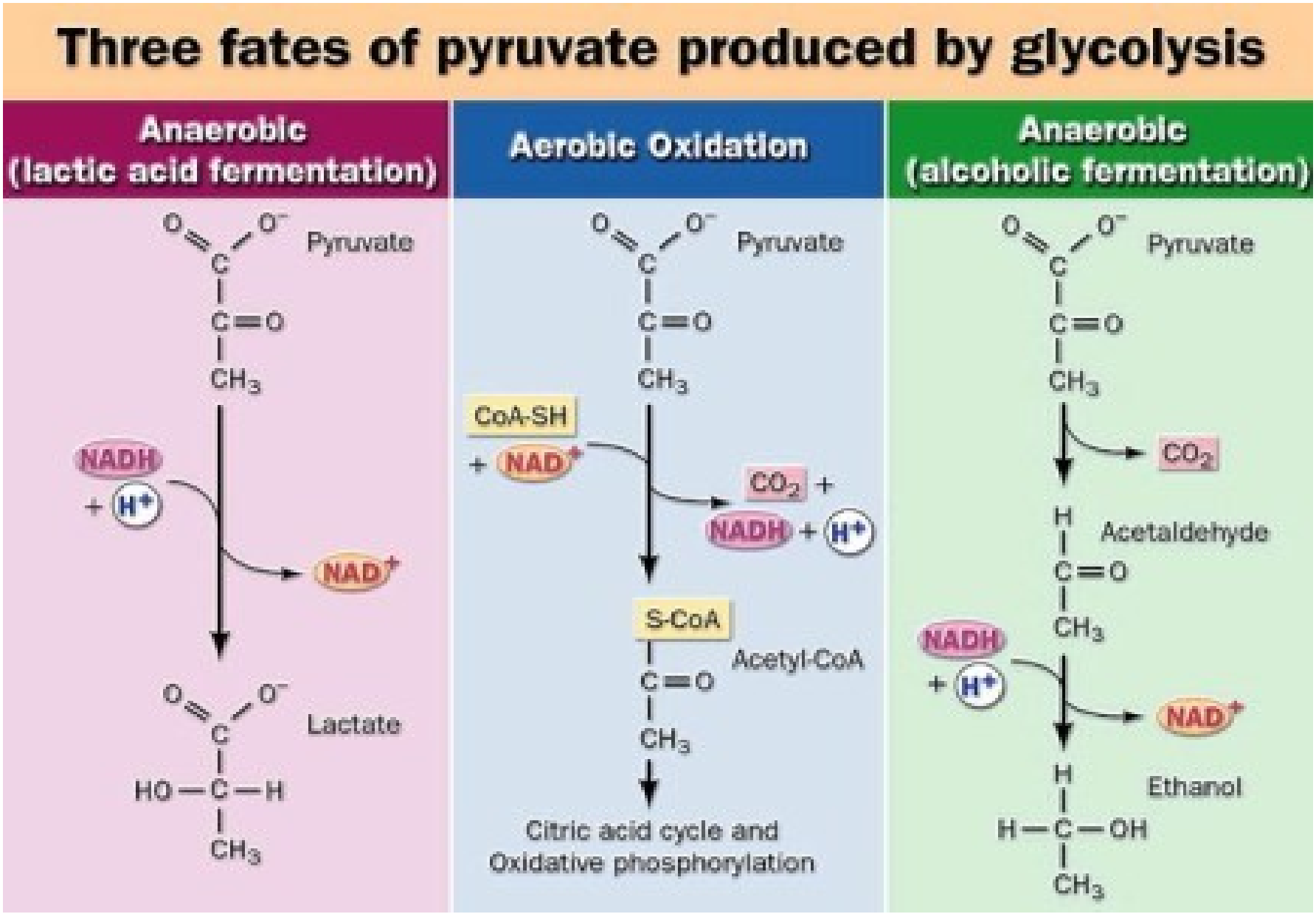


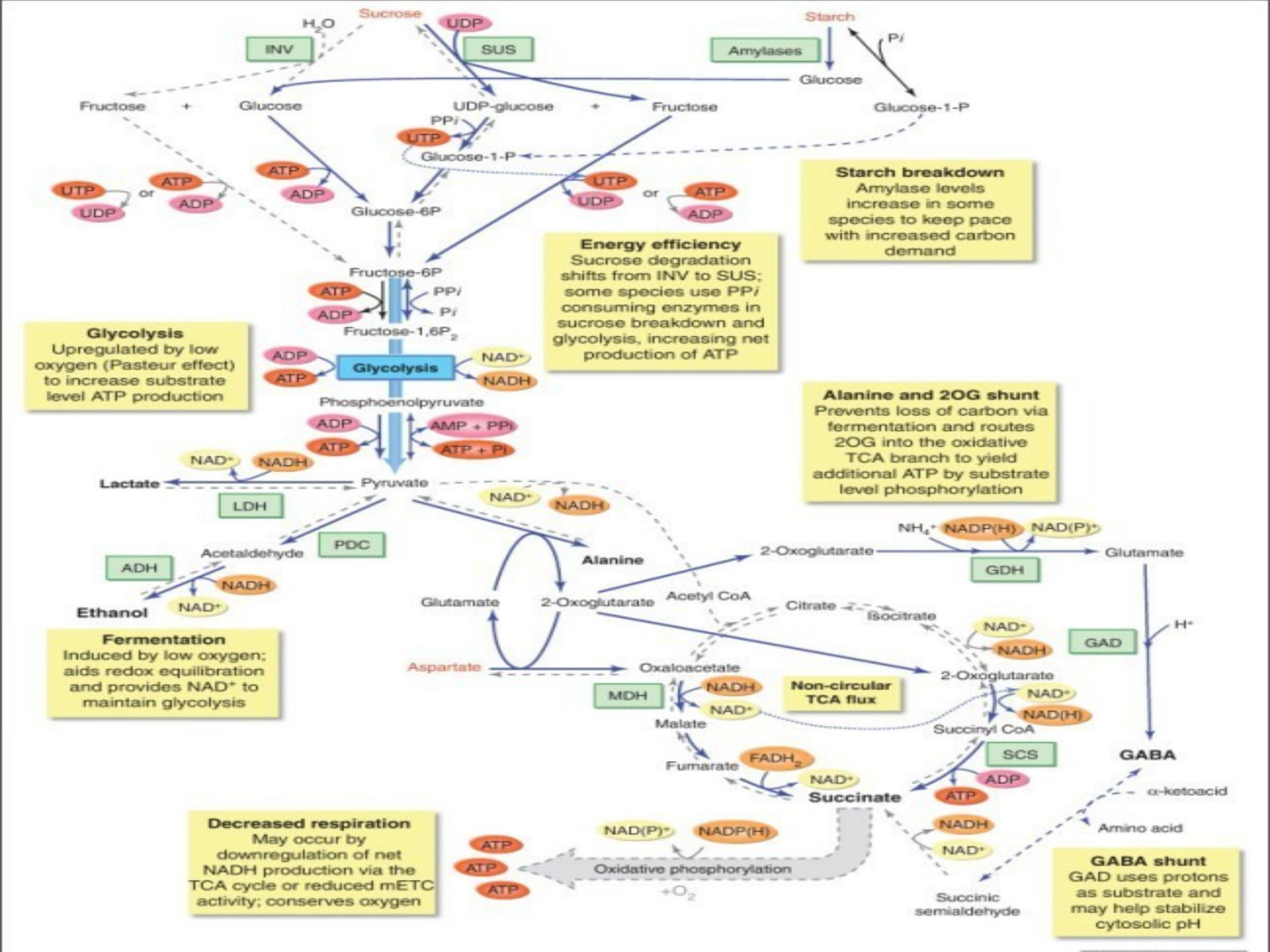
Allosteric Regulation of Glycolysis and Gluconeogenesis

- **Gluconeogenesis Regulation Continued**
  - Allosteric enzymes (pyruvate carboxylase, pyruvate carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase)

Regulation of Glycolysis and Gluconeogenesis




Three fates of pyruvate produced by glycolysis
Anaerobic (lactic acid fermentation)
Aerobic Oxidation
Anaerobic (alcoholic fermentation)
Pyruvate
NADH
H+
NAD+
Lactate
CoA-SH
CO2
S-CoA
Acetyl-CoA
Citric acid cycle and Oxidative phosphorylation
Acetaldehyde
Ethanol


Sucrose
H_2O
UDP
INV
SUS
Starch
Amylases
Pi
Glucose
Fructose
+
Glucose
UDP-glucose
+
Fructose
Glucose-1-P
PPi
UTP
Glucose-1-P
ATP
ADP
UTP
UDP
or
ATP
ADP
UTP
UDP
or
ATP
ADP
Glucose-6P
Starch breakdown
Amylase levels increase in some species to keep pace with increased carbon demand
Energy efficiency
Sucrose degradation shifts from INV to SUS; some species use PPi consuming enzymes in sucrose breakdown and glycolysis, increasing net production of ATP
Fructose-6P
ATP
ADP
PPi
Pi
Fructose-1,6P2
Glycolysis
Upregulated by low oxygen (Pasteur effect) to increase substrate level ATP production
ADP
ATP
Glycolysis
NAD+
NADH
Phosphoenolpyruvate
ADP
ATP
AMP + PPi
ATP + Pi
NAD+
NADH
Lactate
Pyruvate
LDH
NAD+
NADH
Alanine and 2OG shunt
Prevents loss of carbon via fermentation and routes 2OG into the oxidative TCA branch to yield additional ATP by substrate level phosphorylation
Acetaldehyde
PDC
ADH
NADH
NAD+
Ethanol
Fermentation
Induced by low oxygen; aids redox equilibration and provides NAD+ to maintain glycolysis
Alanine
Glutamate
2-Oxoglutarate
Acetyl CoA
Citrate
Isocitrate
NH4+
NADP(H)
NAD(P)+
2-Oxoglutarate
GDH
Glutamate
NAD+
NADH
GAD
H+
Aspartate
Oxaloacetate
MDH
NADH
NAD+
Non-circular TCA flux
2-Oxoglutarate
NAD+
NAD(H)
Malate
Succinyl CoA
SCS
GABA
Fumarate
FADH2
NAD+
Succinate
ADP
ATP
α-ketoacid
Amino acid
NADH
NAD+
Decreased respiration
May occur by downregulation of net NADH production via the TCA cycle or reduced mETC activity; conserves oxygen
ATP
ATP
ATP
NAD(P)+
NADP(H)
Oxidative phosphorylation
+O2
Succinic semialdehyde
GABA shunt
GAD uses protons as substrate and may help stabilize cytosolic pH

The Entry of Fructose and Galactose into Glycolysis

- Fructose and galactose—can be funneled into the glycolytic pathway .Much of the ingested fructose is metabolized by the liver, using the *fructose 1-phosphate pathway*.
- The first step is the phosphorylation of *fructose* to *fructose 1-phosphate* by *fructokinase*. Fructose 1-phosphate is then split into *glyceraldehyde* and *dihydroxyacetone phosphate*, an intermediate in glycolysis. This aldol cleavage is catalyzed by a specific *fructose 1-phosphate aldolase*.
- Glyceraldehyde is then phosphorylated to *glyceralde-hyde 3-phosphate*, a glycolytic intermediate, by *triose kinase*. Alternatively, *fructose can be phosphorylated to fructose 6-phosphate by hexokinase*.
- Little fructose 6-phosphate is formed in the liver because glucose is so much more abundant in this organ. Moreover, glucose, as the preferred fuel, is also trapped in the muscle by the hexokinase reaction. Because liver and muscle phosphorylate glucose rather than fructose, adipose tissue is exposed to more fructose than glucose most of the fructose in adipose tissue is metabolized through fructose 6-phosphate.

Fructose Metabolism & Entry Points in Glycolysis for Galactose and Fructose

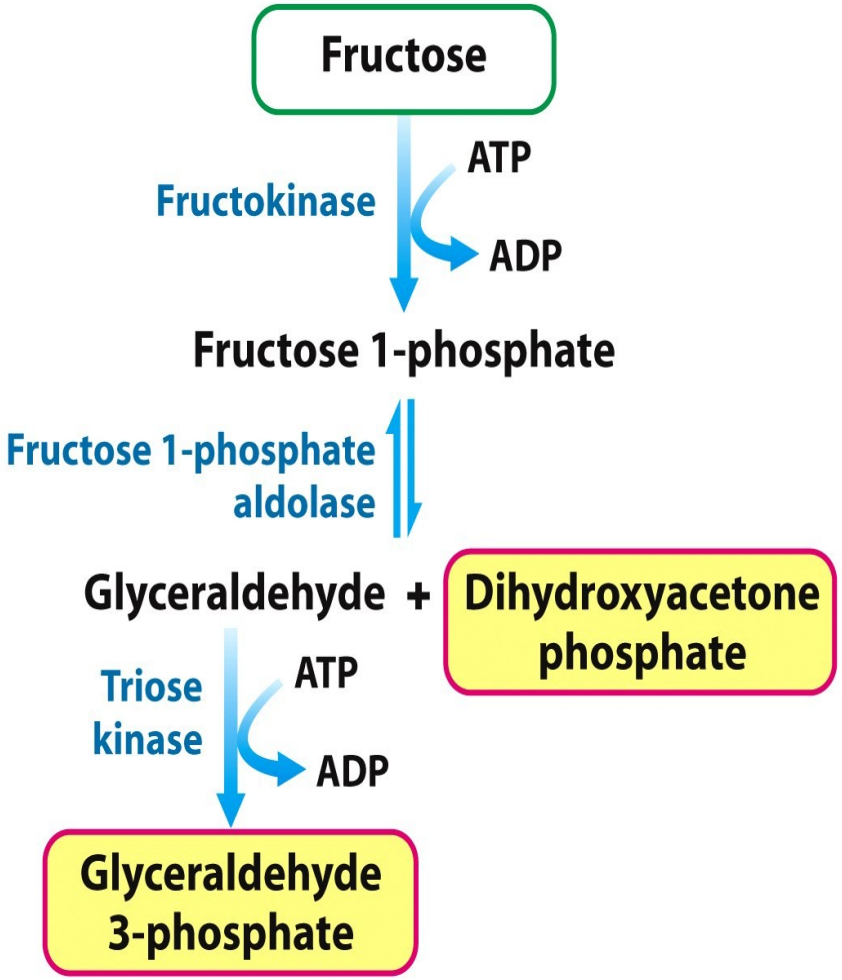


Figure 16.14
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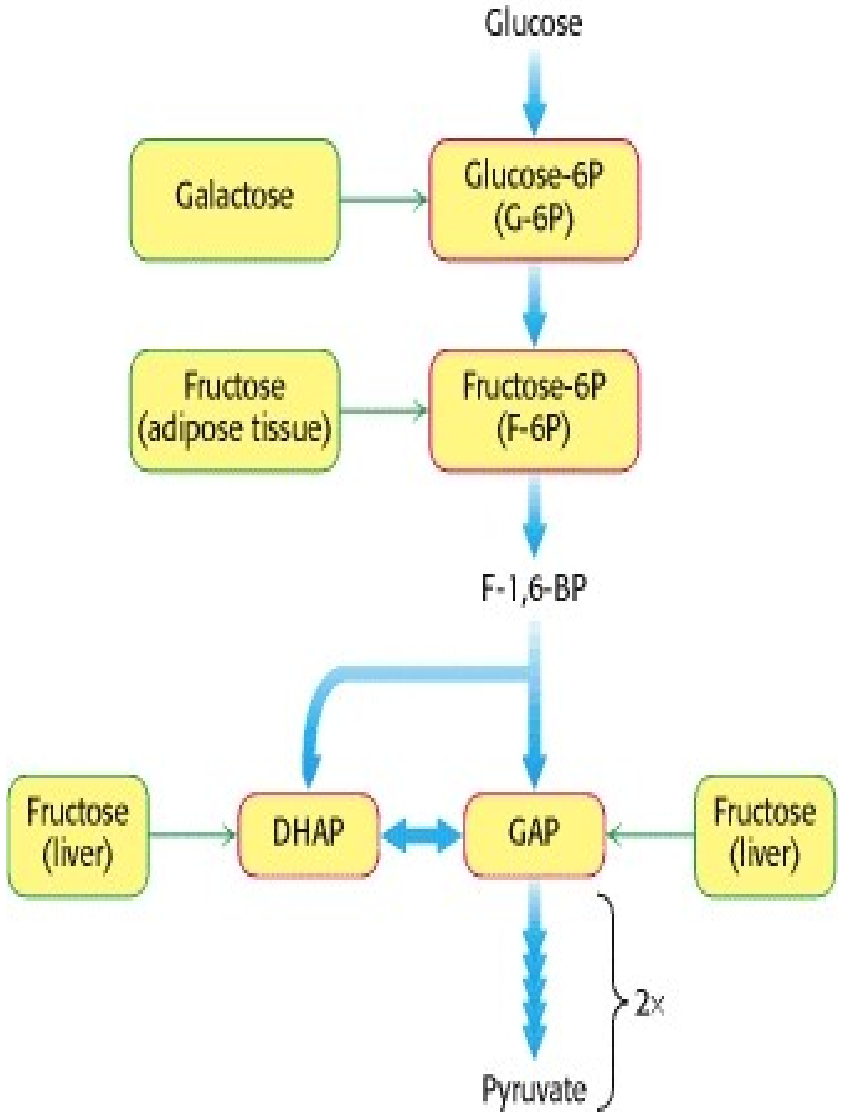
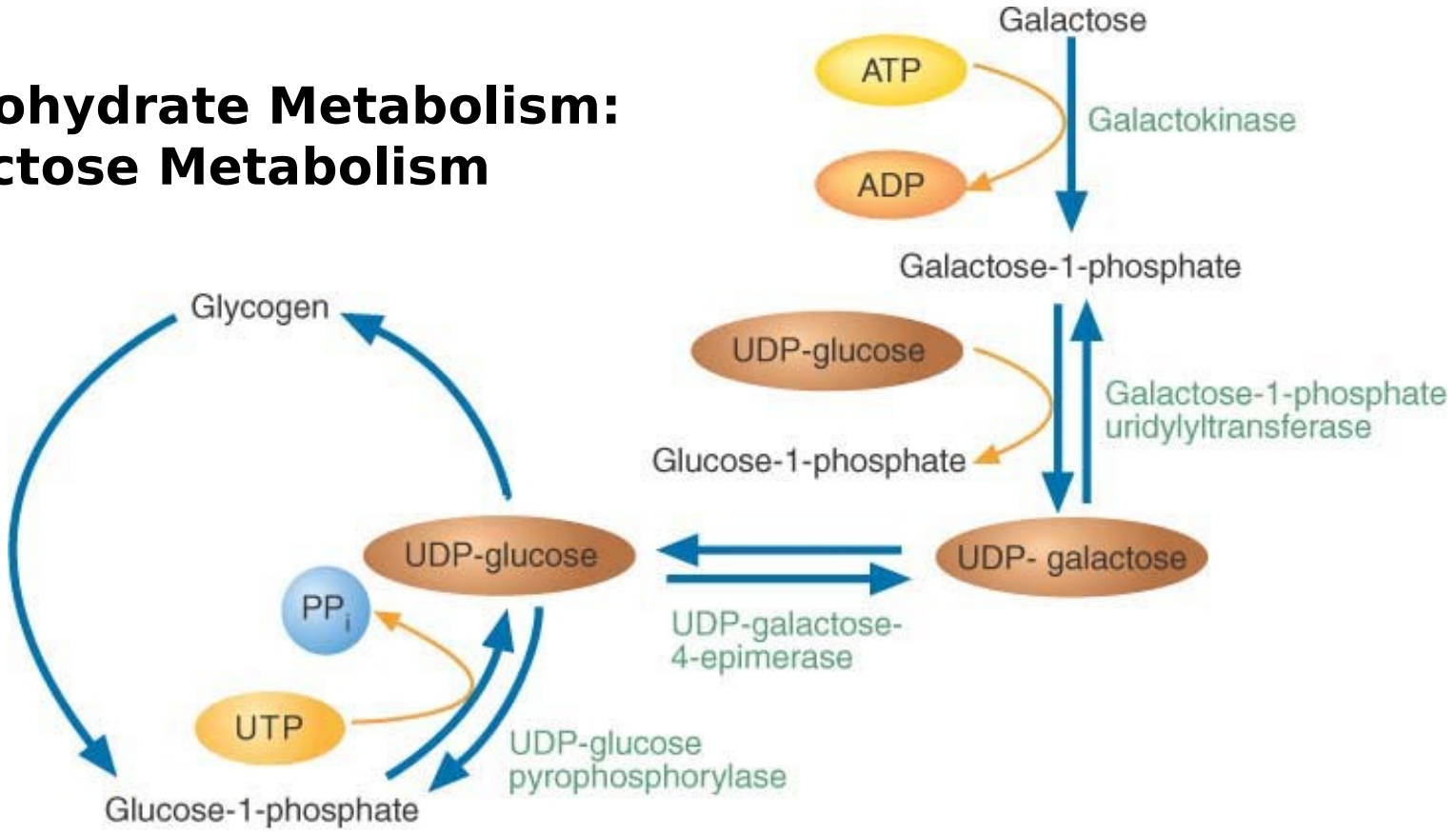


Metabolism of Other Important Sugars

Carbohydrate Metabolism: Galactose Metabolism

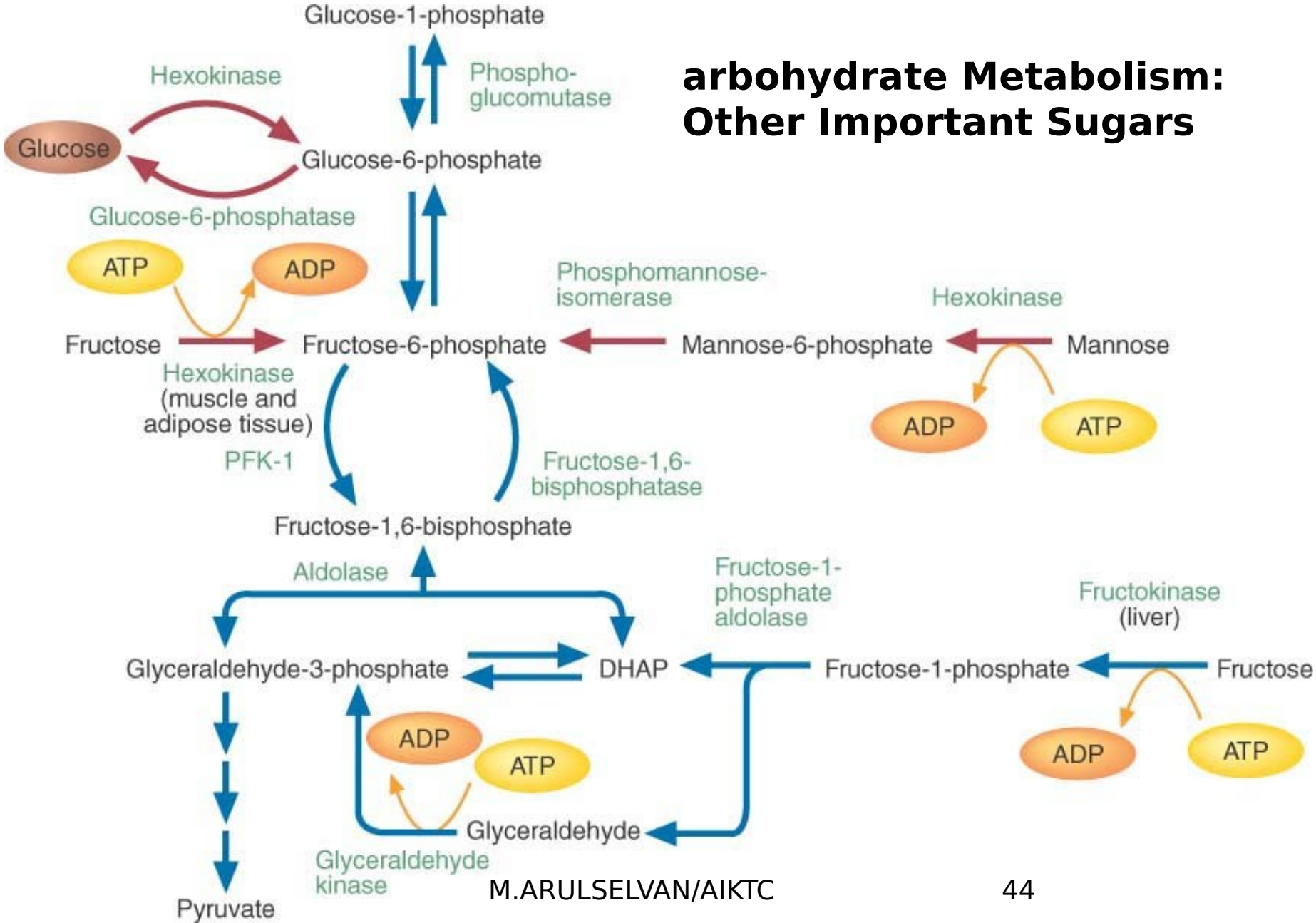


- Fructose, mannose, and galactose are also important sugars for vertebrates
  - Most common sugars found in oligosaccharides besides glucose

Metabolism of Other Important Sugars

- **Fructose Metabolism**
  - Second to glucose in the human diet
  - Can enter the glycolytic pathway in two ways:
    - Through the liver (multi-enzymatic process)
    - Muscle and adipose tissue (hexokinase)

Metabolism of Other Important Sugars



arbohydrate Metabolism: Other Important Sugars

Mitochondrial Shuttle

- The **mitochondrial shuttles** are systems used to transport reducing agents across the inner mitochondrial membrane.
- NADH cannot cross the membrane, but it can reduce another molecule that can cross the membrane, so that its electrons can reach the electron transport chain.
- The two main systems in humans are

Name	In, to mitochondrion	To ETC	Out, to cytosol
Glycerol phosphate shuttle	glycerol 3-phosphate	QH_2 (~1,5 ATP)	dihydroxyacetone phosphate
Malate-aspartate shuttle	malate	NADH (~3 ATP)	oxaloacetate/ aspartate

The Glycerophosphate Shuttle

- This shuttle system uses two distinct glycerol 3- phosphate dehydrogenases. The first is found in the cytoplasm, the other is found on the intermembrane side of the inner mitochondrial membrane.

- In the first step, NADH produced in the cytosol transfers its electrons to dihydroxyacetone phosphate to form glycerol-3-phosphate. Blycerol-3-phosphate enters the intermitochondrial space through a porin.

- Glycerol-3-phosphate is then reoxidized into dihydroxyacetone phosphate by an FAD-dependent mitochondrial membrane glycerol 3-phosphate dehydrogenase. In this shuttle the electrons of NADH are transferred to FAD to form $FADH_2$.

- The two electrons bound by the $FADH_2$ are transferred directly to coenzyme Q forming QH_2 . QH_2 carries the electrons to complex III. The result of this shuttle is 1.5 ATP/NADH.

The Glycerophosphate Shuttle

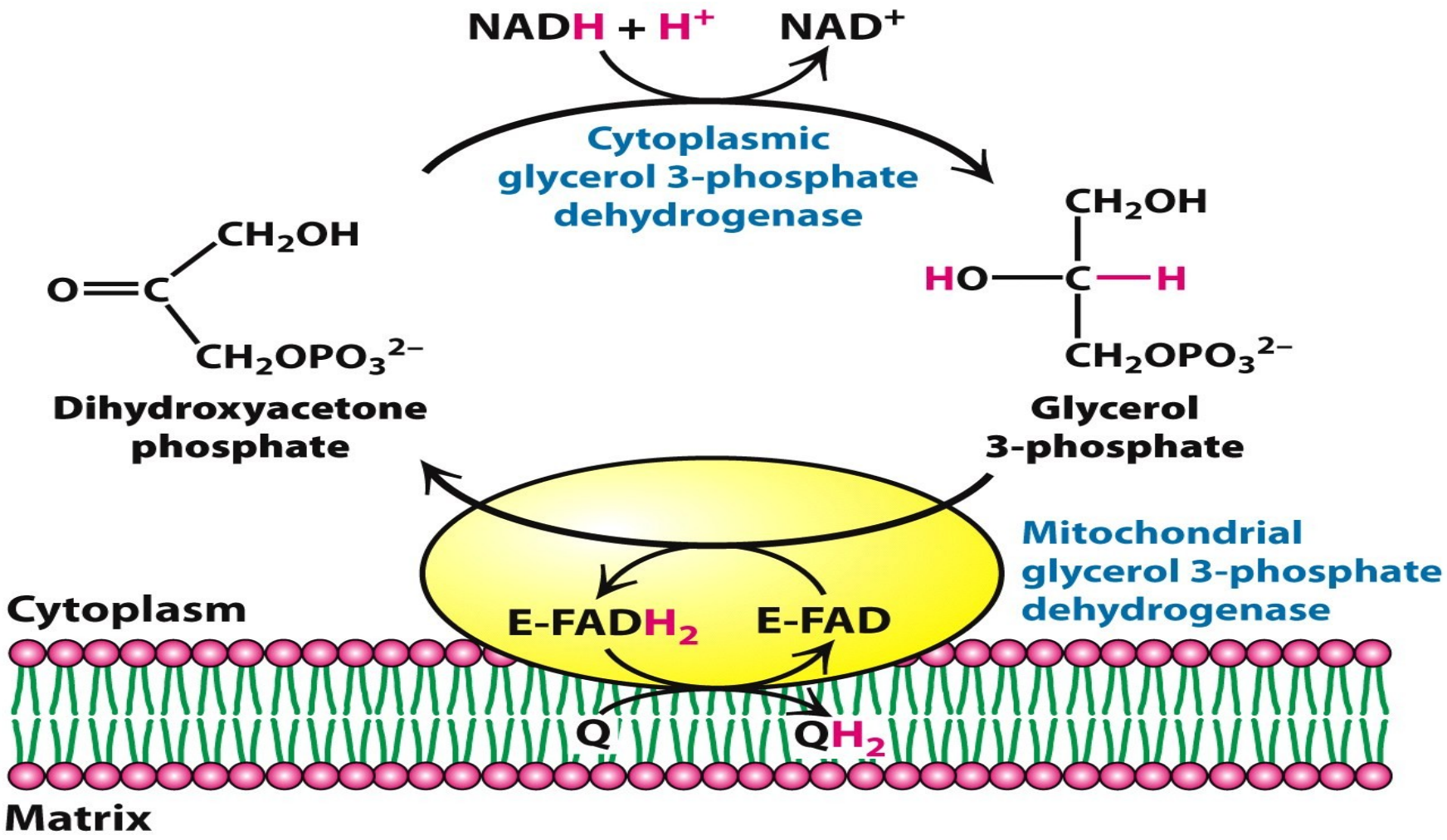


Figure 18.34
***Biochemistry*, Seventh Edition**

Malate-aspartate shuttle

- A biochemical system for translocating electrons produced during glycolysis across the semipermeable inner membrane of the mitochondrion for oxidative phosphorylation in eukaryotes.
- These electrons enter the electron transport chain of the mitochondria via reduction equivalents to generate ATP.
- The shuttle system is required because the mitochondrial inner membrane is impermeable to NADH, the primary reducing equivalent of the electron transport chain.
- To circumvent this, malate carries the reducing equivalents across the membrane.
- The shuttle consists of four protein parts:

-malate dehydrogenase in the mitochondrial matrix and intermembrane space.

-aspartate aminotransferase in the mitochondrial matrix and intermembrane space.

-malate-alpha-ketoglutarate antiporter in the cytosol.

-glutamate-aspartate antiporter in the inner membrane.

Malate-aspartate shuttle

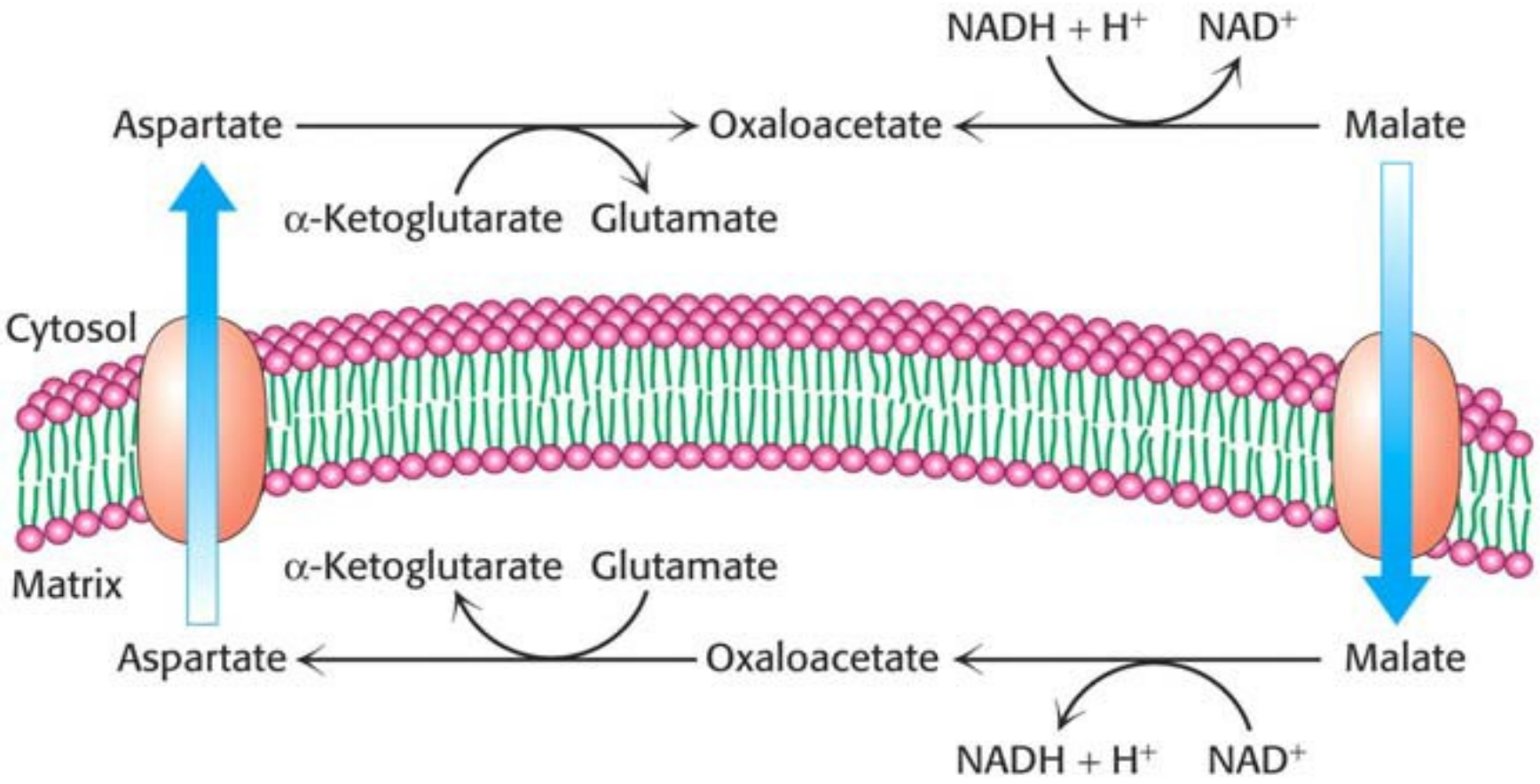
- In the cytosol, oxaloacetate is reduced to malate by malate dehydrogenase which uses NADH as the reductant. Malate is transported across the inner mitochondrial membrane by the dicarboxylic acid or tricarboxylic acid carrier.

- Now in the matrix, the malate is reoxidized by malate dehydrogenase to generate oxaloacetate and NADH which can now transfer its electrons to Complex I.

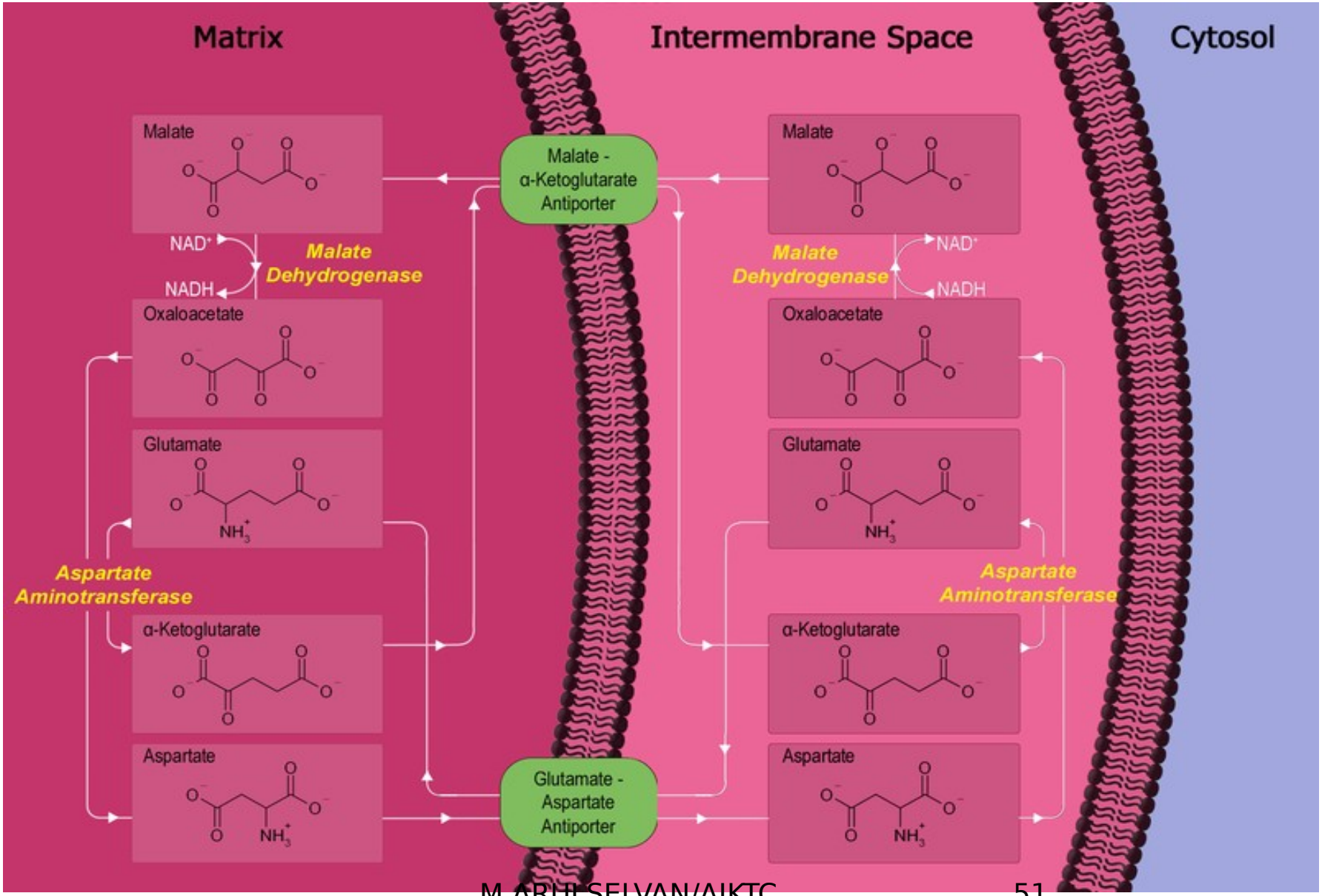
- The oxaloacetate is transaminated by glutamine to form aspartate and α-ketoglutarate. Aspartate can be transported across the inner mitochondrial membrane by the dicarboxylic acid carrier.

- In the cytosol aspartate transaminates α-ketoglutarate to reform oxaloacetate completing the cycle.This shuttle system generates 2.5 ATP/NADH and is completely reversible.

Malate-aspartate shuttle



Malate-aspartate shuttle



The net yield of ATP by glucose oxidation depends on the shuttle used.

Glycolysis Glucose → 2 pyruvate		2ATP
	2NADH	
Glycerophosphate shuttle		
$2NADH \rightarrow 2FADH_2$		
1.5 ATP/$FADH_2$		3 ATP
Conversion of 2pyruvate into 2acetyl CoA in the mitochondria	2NADH	
2.5 ATP/NADH		5 ATP
Citric Acid cycle oxidation of 2 acetyl CoA	2GTP = $2FADH_2$ +6NADH	2 ATP
1.5 ATP/$FADH_2$		3 ATP
2.5 ATP/NADH		15 ATP
		30 ATP

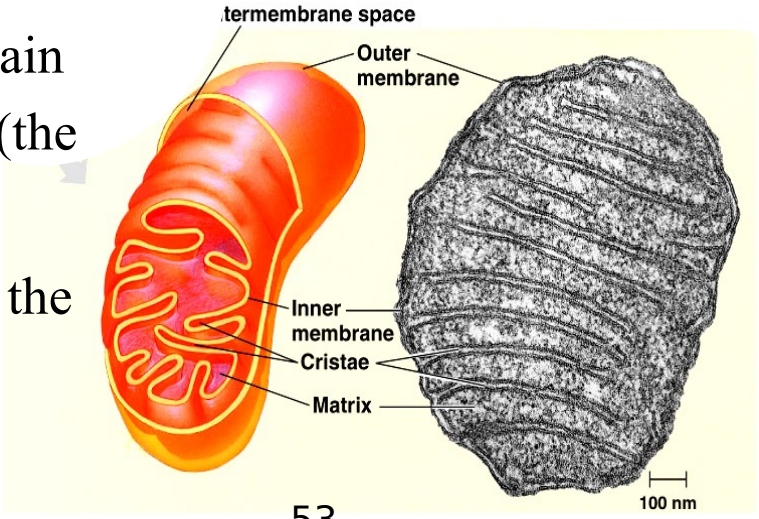
Glycolysis Glucose → 2 pyruvate		2ATP
	2NADH	
Malate-Aspartate shuttle		
2.5 ATP/NADH		5 ATP
Conversion of 2pyruvate into 2acetyl CoA in the mitochondria	2NADH	
2.5 ATP/NADH		5 ATP
Citric Acid cycle oxidation of 2 acetyl CoA	2GTP = $2FADH_2$ +6NADH	2 ATP
1.5 ATP/$FADH_2$		3 ATP
2.5 ATP/NADH		15 ATP
		32 ATP

phosphorylation

The inner mitochondrial membrane couples electron transport to ATP synthesis (90% of ATP)

- Only 4 of 38 ATP ultimately produced by respiration of glucose are derived from substrate-level phosphorylation (2 from glycolysis and 2 from Krebs Cycle).
- The vast majority of the ATP (90%) comes from the energy in the electrons carried by NADH and $FADH_2$.
- The energy in these electrons is used in the electron transport chain to power ATP synthesis.

- Thousands of copies of the electron transport chain are found in the extensive surface of the cristae (the inner membrane of the mitochondrion).
- Electrons drop in free energy as they pass down the electron transport chain.



- Electrons carried by NADH are transferred to the first molecule in the electron transport chain (the flavoprotein; FMN).
- The electrons continue along the chain which includes several Cytochrome proteins and one lipid carrier.
- The electrons carried by $FADH_2$ have lower free energy and are added to a later point in the chain.

- Electrons from NADH or $FADH_2$ ultimately pass to oxygen.
- The electron transport chain generates no ATP directly. Rather, its function is to break the large free energy drop from food to oxygen into a series of smaller steps that release energy in manageable amounts.

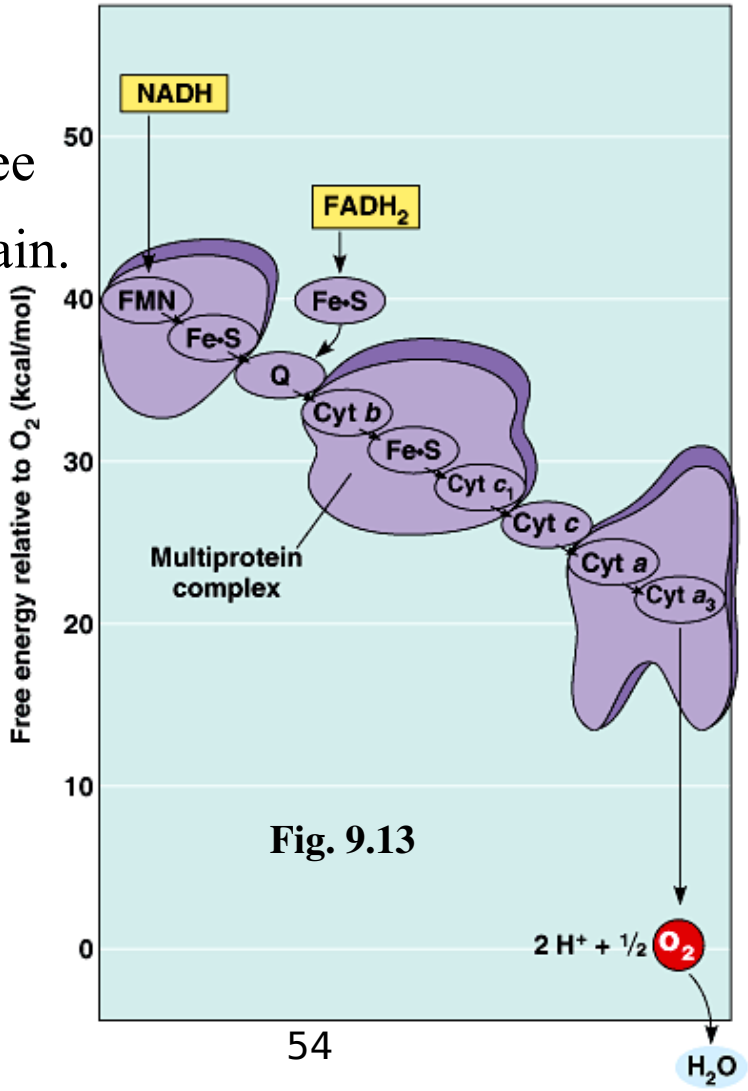
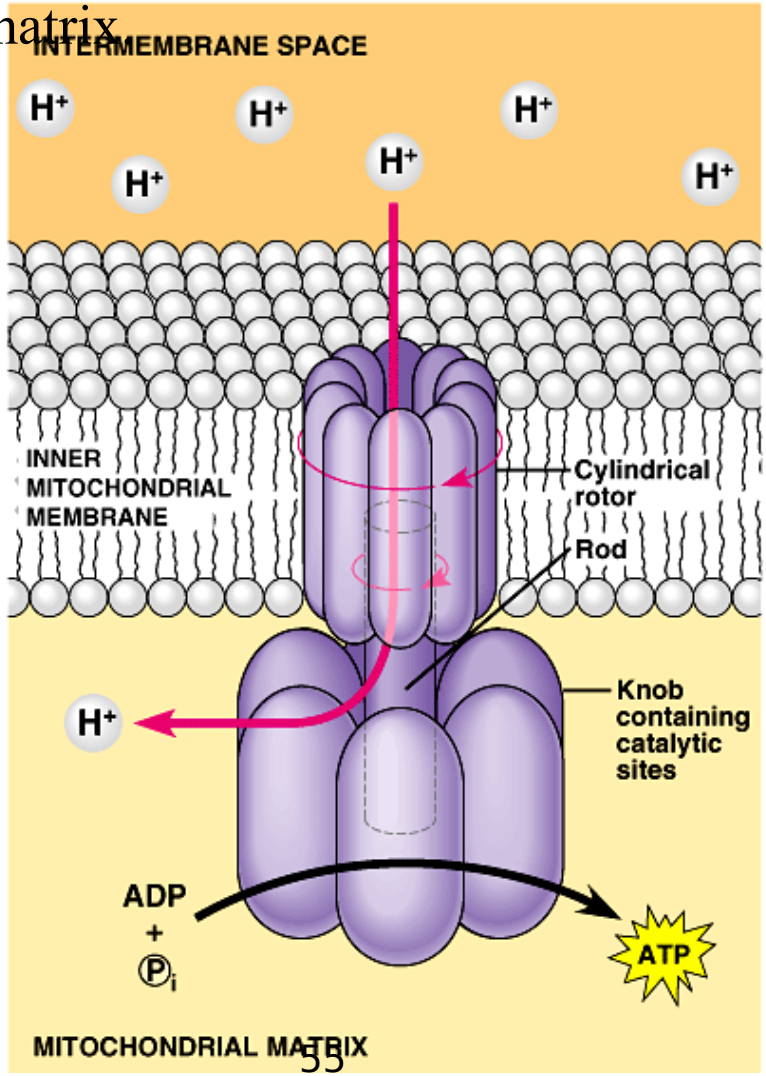


Fig. 9.13

- ATP-synthase, in the cristae actually makes ATP from ADP and P_i.
- ATP used the energy of an existing proton gradient to power ATP synthesis.
  - This proton gradient develops between the intermembrane space and the matrix.
  - This concentration of H^+ is the proton-motive force.



- The ATP synthase molecules are the only place that will allow H^+ to diffuse back to the matrix (exergonic flow of H^+).
- This flow of H^+ is used by the enzyme to generate ATP a process called chemiosmosis.
- Chemiosmosis: (osmos = puch)

  It is the oxidative phosphorylation that results in ATP production in the inner membrane of mitochondria.

Oxidative Phosphorylation

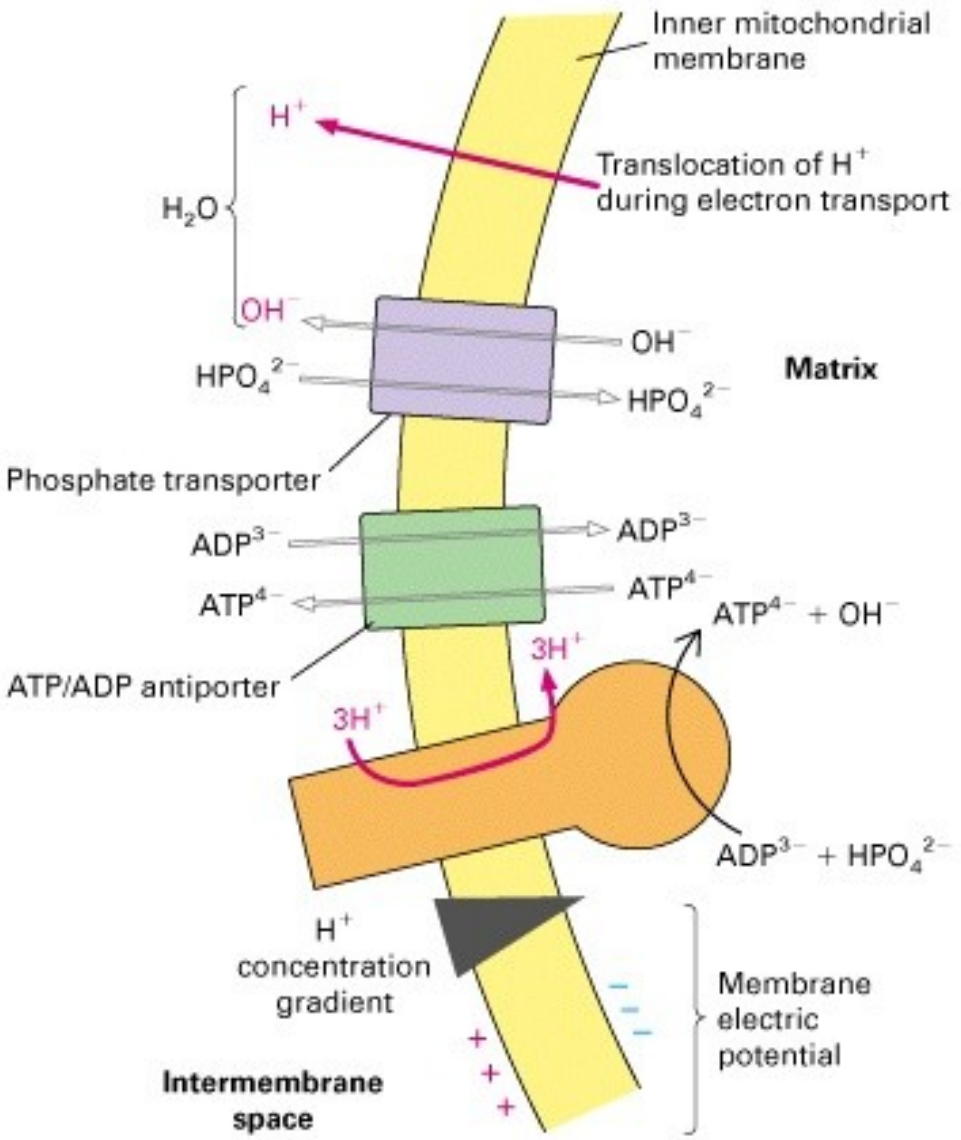
- oxidative phosphorylation to describe how 2 molecules of $FADH_2$ and NADH (produced in the Citric Acid cycle) are used to make ATP. We use the term "oxidative" because oxygen accepts an electron while the gradient made by the movement of electrons powers the creation ATP.

- ***Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electrons from NADH or $FADH_2$ to O_2 by a series of electron carriers.***

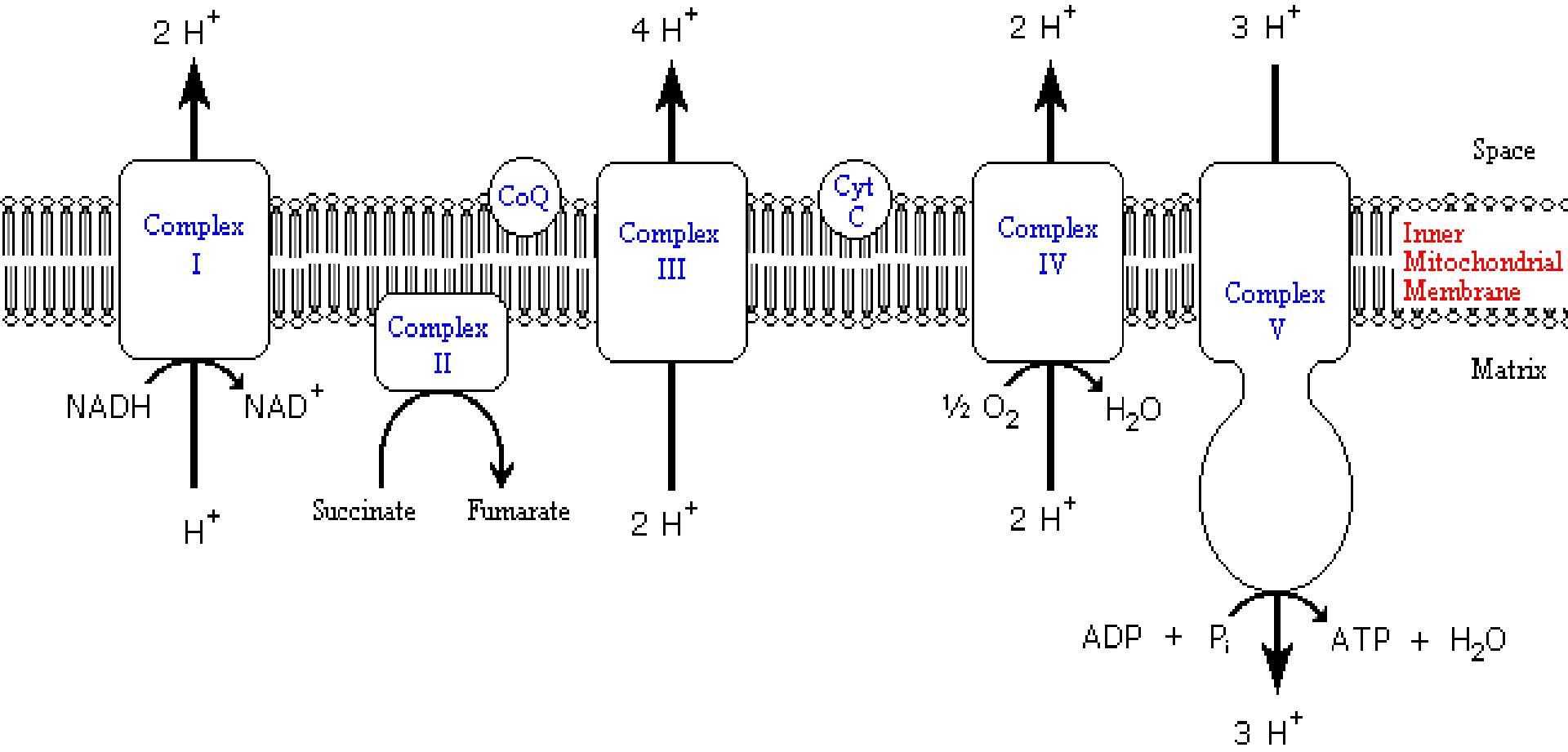
- Series of enzyme complexes (electron carriers) embedded in the inner mitochondrial membrane, which oxidize $NADH_2$ and $FADH_2$ and transport electrons to oxygen is called respiratory electron-transport chain (ETC).

Oxidative Phosphorylation

- H^+ transport results in an electrochemical gradient
- **Proton motive force**: energy released by flow of H^+ down its gradient is used for ATP synthesis
- **ATP synthase**: H^+ channel that couples energy from H^+ flow with ATP synthesis

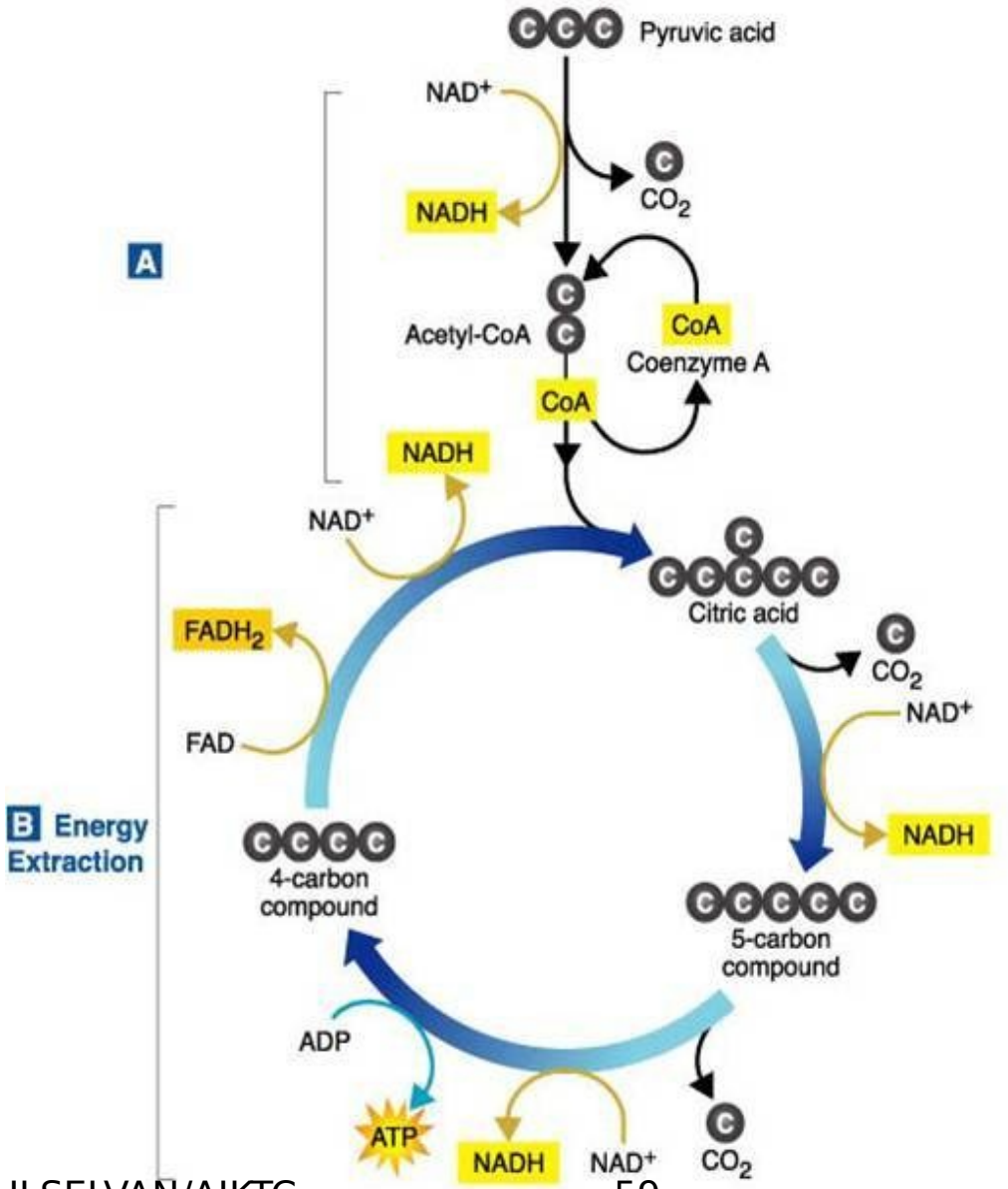


Overview of oxidative phosphorylation



TCA Cycle

- In aerobic conditions TCA cycle links pyruvate to oxidative phosphorylation
- Occurs in mitochondria
- Generates 90% of energy obtained from feed
  - ➢ Includes metabolism of carbohydrate, protein, and fat
- Oxidize acetyl-CoA to CO_2 and capture potential energy as NADH (or $FADH_2$) and some ATP



CITRIC ACID CYCLE

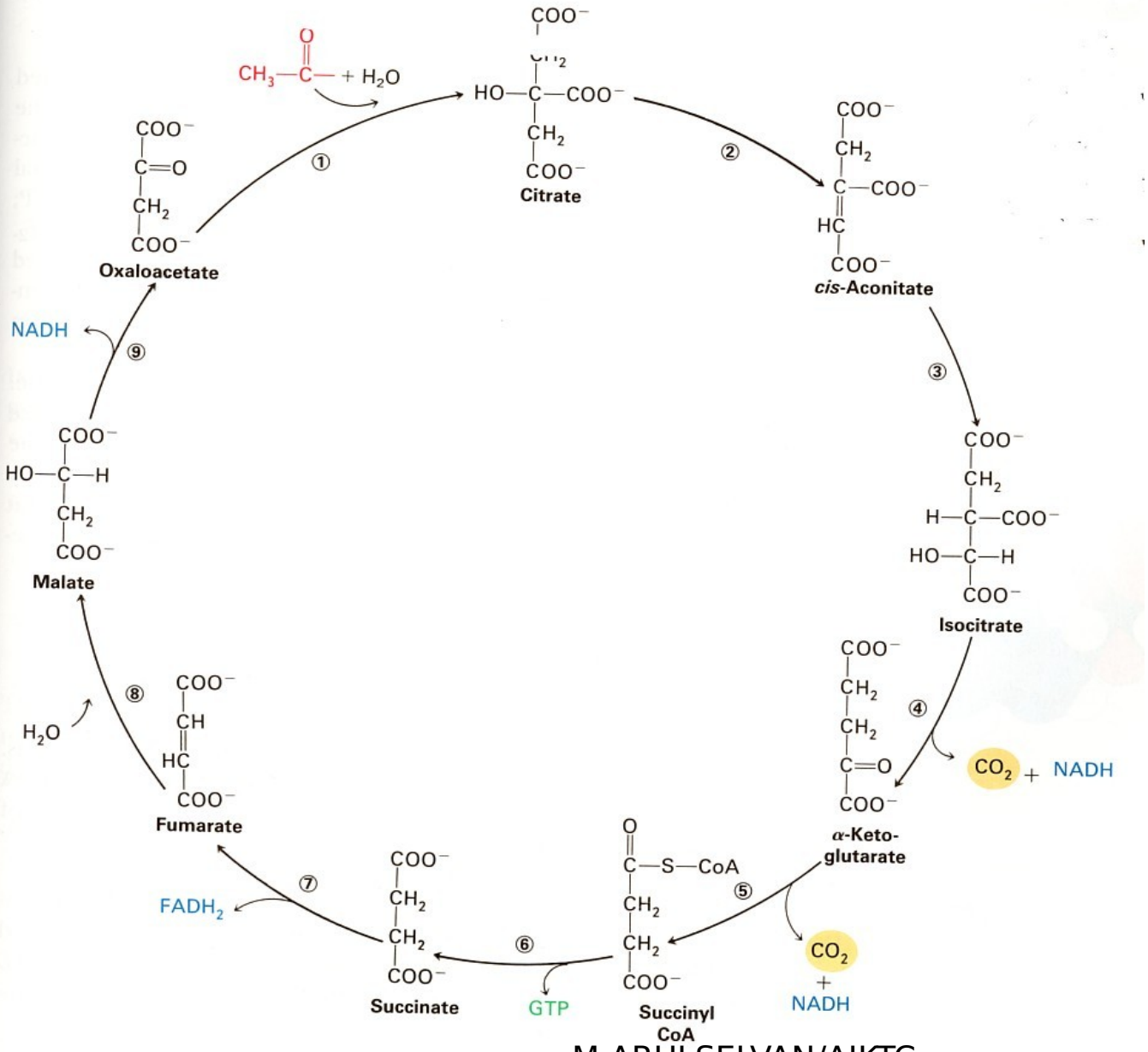
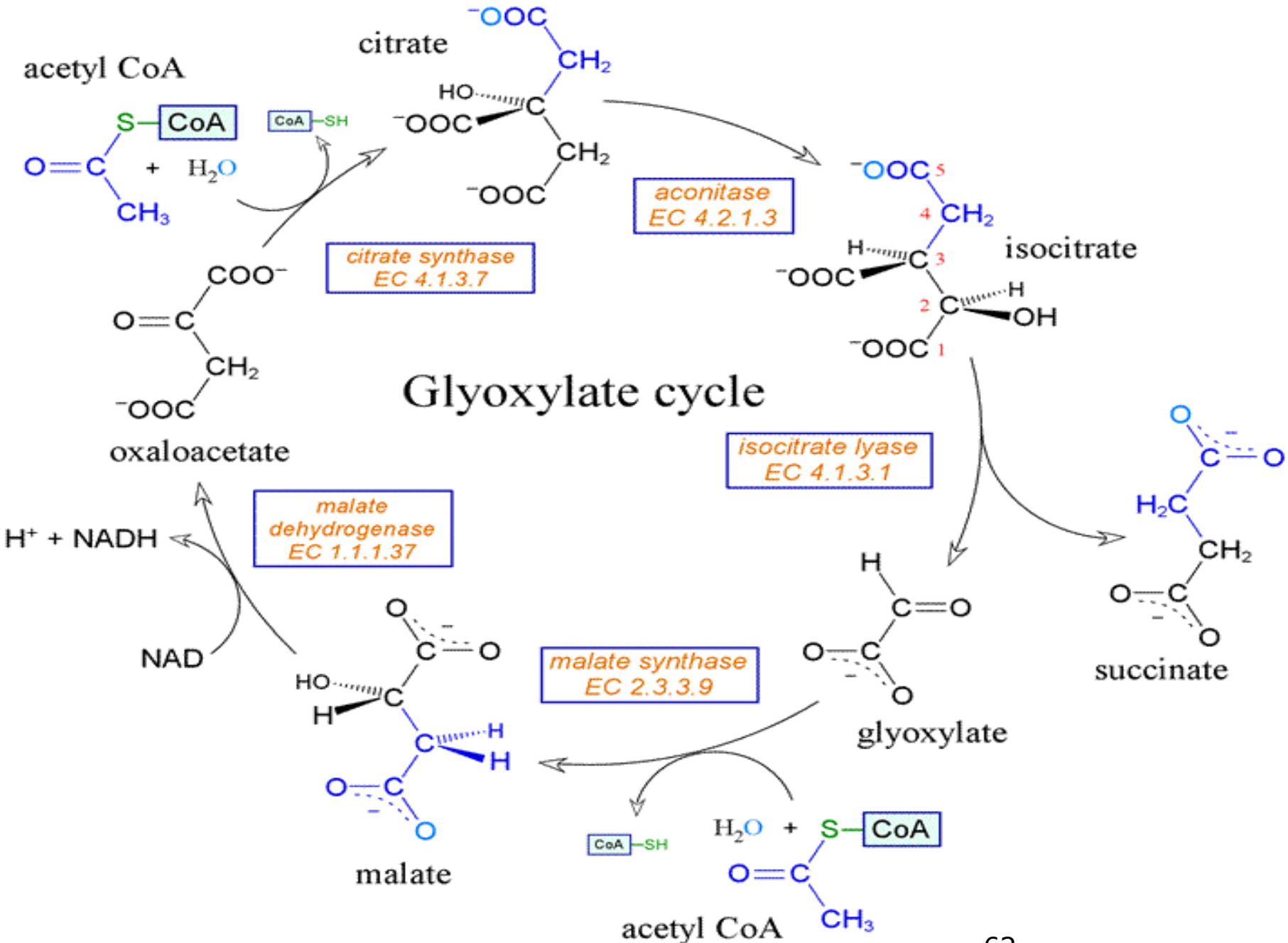


Figure 20-5
Citric acid cycle. This series of reactions is catalyzed by the following enzymes, as numbered in the diagram:

1 Citrate synthase
2 Aconitase
3 Aconitase
4 Isocitrate dehydrogenase
5 α-Ketoglutarate dehydrogenase complex
6 Succinyl CoA synthetase
7 Succinate dehydrogenase
8 Fumarase
9 Malate dehydrogenase

Glyoxalate cycle

- Glyoxylate is a simple two-carbon species consisting of a carboxylate (ionized carboxyl) group attached to an aldehyde functional group.
- In plants and certain microorganisms, acetyl CoA can be reformed into succinate. The net synthesis of succinate from two molecules of acetyl CoA takes place in what is called the **glyoxylate cycle**.
- The cycle formally uses three enzymes from the citric acid cycle, citrate synthase, aconitase, and malate dehydrogenase. The enzymes **isocitrate lyase** [EC 4.1.3.1] and **malate synthase** [EC 2.3.3.9] are unique to the cycle.
- Bacteria and some species of higher plants are able to obtain a net increase in malate or oxaloacetate through expression of enzymes of the glyoxylate cycle or glyoxylate shunt.
- The two additional enzymes that permit the glyoxylate shunt are *isocitrate lyase* and *malate synthase,* which convert isocitrate to succinate or to malate via glyoxylate .
- The glyoxylate cycle is also called the ***glyoxylate bypass or glyoxylate shunt.***


Glyoxylate cycle
acetyl CoA
citrate
aconitase
EC 4.2.1.3
isocitrate
citrate synthase
EC 4.1.3.7
oxaloacetate
isocitrate lyase
EC 4.1.3.1
succinate
malate
dehydrogenase
EC 1.1.1.37
H+ + NADH
NAD
malate synthase
EC 2.3.3.9
glyoxylate
malate
acetyl CoA

Glyoxalate cycle

-The anaplerotic glyoxylate pathway is active when growth on 2 carbon compounds requires conservation of 4 carbon TCA intermediates. Two molecules of acetylCoA are taken up per turn of the glyoxylate cycle, and acetylCoA is generated by acetate thiokinase in the reaction:

acetate + CoA + ATP = acetyl-CoA + AMP + Pi

-Alternatively, acetylCoA is generated by β oxidation of fatty acids. The glyoxylate cycle is repressed during growth on glucose, and induced by growth on acetate.

The net reaction is

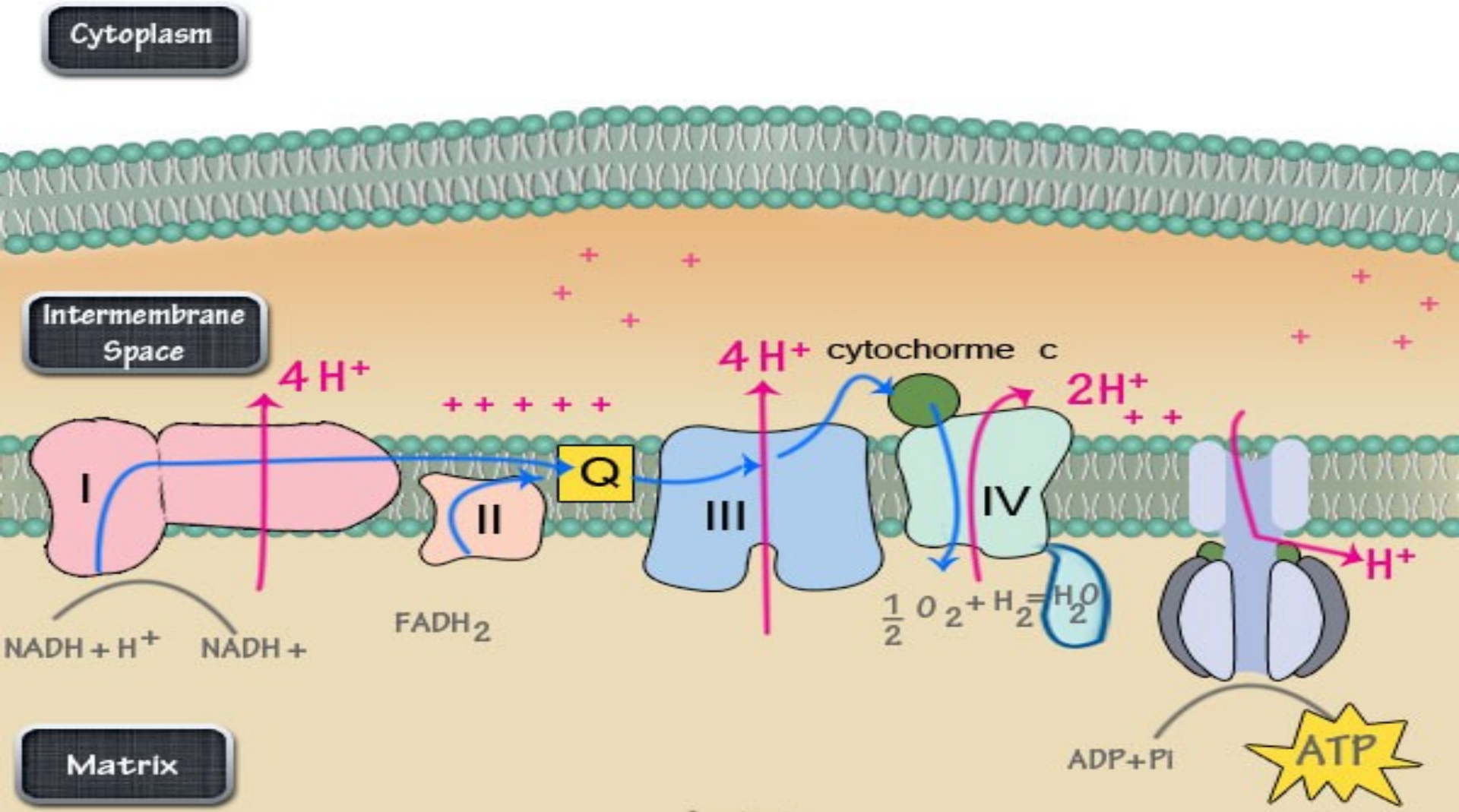
2 acetyl-CoA + NAD^+ → succinate + 2 CoA + NADH + H^+

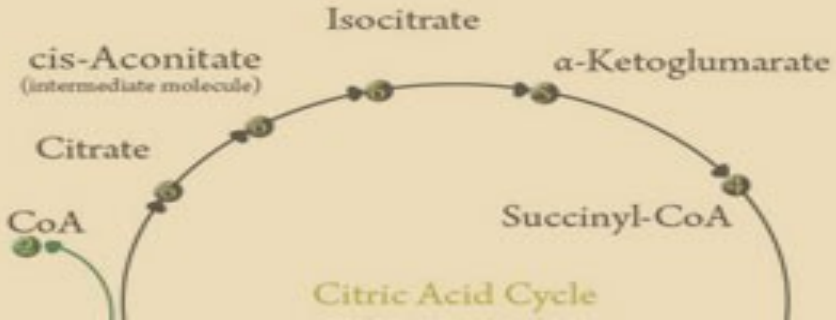
Substrate-level Phosphorylation

- **Substrate-level phosphorylation** is a type of chemical reaction that results in the formation and creation of adenosine triphosphate (ATP) by the direct transfer and donation of a phosphoryl (PO_3) group to adenosine diphosphate (ADP) from a reactive intermediate.
- While technically the transfer is PO_3, or a phosphoryl group, convention in biological sciences is to refer to this as the transfer of a phosphate group. In cells, it occurs primarily and firstly in the cytoplasm (in glycolysis) under both aerobic and anaerobic conditions.
- Unlike oxidative phosphorylation, here the oxidation and phosphorylation are not coupled or joined, although both types of phosphorylation result in ATP.
- It should be noted that there is an oxidation reaction coupled to phosphorylation, however this occurs in the generation of 1,3-bisphosphoglycerate from 3-phosphoglyceraldehyde via a dehydrogenase. ATP is generated in a separate step (key difference from oxidative phosphorylation) by transfer of the high energy phosphate on 1,3-bisphosphoglycerate to ADP via a kinase.

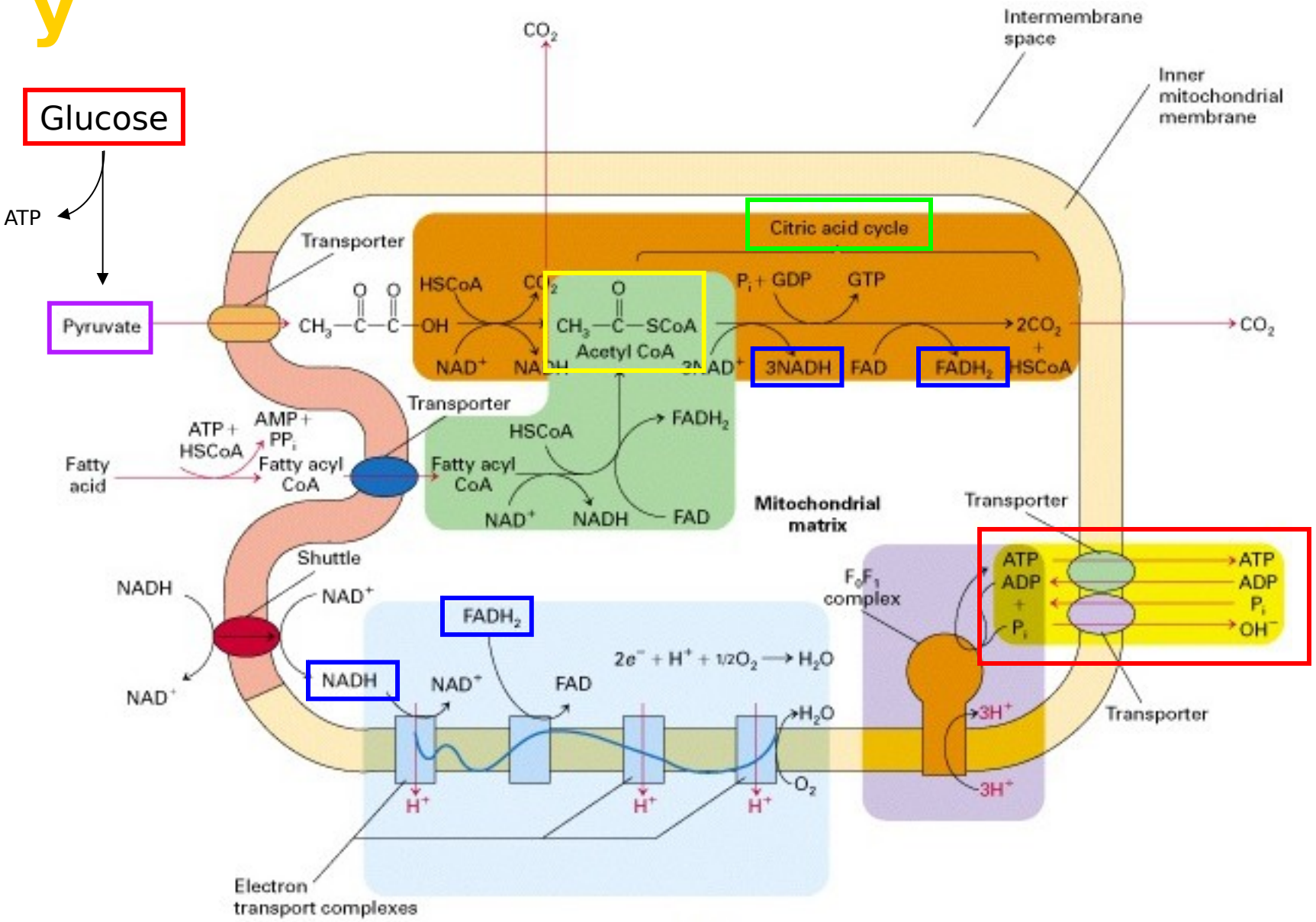
Cellular respiration generates many ATP molecules for each sugar molecule it oxidizes

- During respiration, most energy flows from glucose ➡ NADH electron transport chain ➡ proton-motive force ➡ ATP.
- Some ATP is produced by substrate-level phosphorylation during glycolysis and the Krebs cycle, but most ATP comes from oxidative phosphorylation (through electron transport chain).
- Energy produced in Glycolysis and Krebs cycle gives a maximum yield of 4 ATP by substrate-level phosphorylation.
- Energy produced in electron transport chain gives a maximum yield of 34 ATP by oxidative phosphorylation via ATP-synthase.
- Substrate-level phosphorylation and oxidative phosphorylation give a bottom line of 38 ATP.


Cytoplasm
Intermembrane Space
Matrix
4 H+
4 H+
cytochorme c
2H+
I
II
Q
III
IV
NADH + H+
NADH +
FADH2
½ O2 + H2 = H2O
H+
ADP+Pi
ATP


Isocitrate
cis-Aconitate
(intermediate molecule)
α-Ketoglumarate
Citrate
CoA
Succinyl-CoA
Citric Acid Cycle

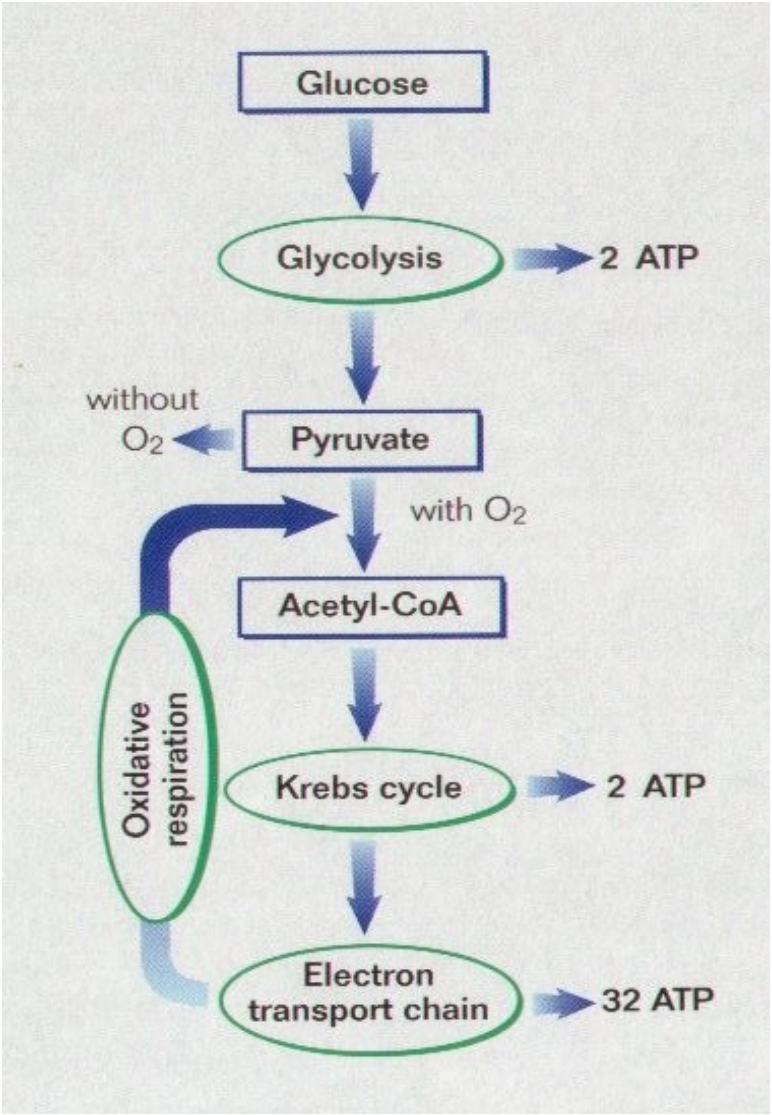
Summary


Glucose
ATP
Pyruvate
CO_2
Intermembrane space
Inner mitochondrial membrane
Transporter
Citric acid cycle
HSCoA
CO_2
P_i + GDP
GTP
CH_3—C—C—OH
CH_3—C—SCoA
Acetyl CoA
NAD^+
NADH
3NAD^+
3NADH
FAD
$FADH_2$
$2CO_2$ + HSCoA
CO_2
Transporter
ATP + HSCoA
AMP + PP_i
Fatty acid
Fatty acyl CoA
HSCoA
$FADH_2$
Fatty acyl CoA
NAD^+
NADH
FAD
Mitochondrial matrix
Transporter
Shuttle
NADH
NAD^+
NAD^+
$FADH_2$
NADH
NAD^+
FAD
$2e^- + H^+ + 1/2O_2 \rightarrow H_2O$
H_2O
O_2
H^+
H^+
H^+
F_0F_1 complex
ATP
ADP
+
P_i
ATP
ADP
P_i
OH^-
$3H^+$
$3H^+$
Transporter
Electron transport complexes

Total energy yield

- Glycolysis 2 ATP
- Krebs Cycle 2 ATP
- ETC 32 ATP

- Total 36 ATP



Rate of ATP Production(Fastest to Slowest)

- Substrate-level phosphorylation
  - Phosphocreatine + ADP ⇨ Creatine + ATP
- Anaerobic glycolysis
  - Glucose ⇨ Pyruvate ⇨ Lactate
- Aerobic carbohydrate metabolism
  - Glucose ⇨ Pyruvate ⇨ CO_2 and H_2O
- Aerobic lipid metabolism
  - Fatty Acid ⇨ Acetate ⇨ CO_2 and H_2O

Potential Amount of Energy Produced (Capacity for ATP Production)

- Aerobic lipid metabolism
  - Fatty Acid ⇨ Acetate ⇨ CO_2 and H_2O
- Aerobic carbohydrate metabolism
  - Glucose ⇨ Pyruvate ⇨ CO_2 and H_2O
- Anaerobic glycolysis
  - Glucose ⇨ Pyruvate ⇨ Lactate
- Substrate-level phosphorylation
  - Phosphocreatine + ADP ⇨ Creatine + ATP

Summary of Electron Transport Chain

1. General electron pathway food→NADH→ETC→oxygen.
2. ETC is a series of electron carriers located in the inner membrane of the mitochondria
3. NADH supplies two electrons to the ETC $NAD^+ + 2H \rightarrow NADH + H^+$.
4. In the ETC electrons move through the chain reducing and oxidizing the molecules as they pass.
5. The ETC is made mostly of proteins.
6. The NADH molecules transport the electrons to the ETC -$FADH_2$ is added at a lower energy level.
7. The electrons move down the mitochondrial membrane through the electron carriers
8. A concentration gradient is generated -positive in the intermembrane space.
9. At the end of the ETC oxygen accepts hydrogen and one electron to form water.
10. The H^+ ions that passed through the proteins into the cytoplasm flow through ATP synthase into the mitochondrial matrix.
11. The energy generated by the proton movement creates ATP by joining ADP and P_i.
12. NADH produces 3 ATP per molecule.
13. $FADH_2$ produces 2 ATP per molecule

Summary of Cellular Respiration

- Glycolysis occurs in the cytosol and breaks glucose into two pyruvates

- Krebs Cycle takes place within the mitochondrial matrix, and breaks a pyruvate into CO_2 and produce some ATP and NADH.

- Some steps of Glycolysis and Krebs Cycle are Redox in which dehydrogenase enzyme reduces NAD^+ into NADH.

- Some of ATP is produced at these tow steps via (substrate-level-phosphorylation).

- Electron Transport Chain accepts e^- from NADH and passes these e^- from one protein molecule to another.

- At the end of the chain, e^- combine with both H^+ and O_2 to form H_2O and release energy.

- These energy are used by mitochondria to synthesis 90% of the cellular ATP *via* ATP-synthase, a process called Oxidative Phosphorylation, in the inner membrane of mitochondria.

Uncouplers of oxidative phosphorylation

- The coupling between electron transport and oxidative phosphorylation depends on the impermeability of the inner mitochondrial membrane to H^+ translocation.
- The only way for protons to go from the intermembrane space to the matrix is through ATP synthase.
- Uncouplers uncouple electron transport from oxidative phosphorylation. They collapse the chemiosmotic gradient by dissipating protons across the inner mitochondrial membrane.
- All of the uncouplers shown to the left, collapse the pH gradient by binding a proton on the acidic side of the membrane, diffusing through the inner

mitochondrial membrane and releasing the proton on the membranes alkaline side.

- Uncouplers of oxidative phophorylation
    - 2,4 – dinitrophenol - 2,4 – dinitrocresol
    - cccp (most active)chloro carbonyl cyanide phenyl hydrazone
    - dicoumarol(vit. k analogue) - valino mycin
    - Calcium

Uncouplers of oxidative phosphorylation

PHYSIOLOGICAL UNCOUPLERS

- An endogenous protein called thermogenin uncouples ATP synthesis from electron transport by opening up a passive proton channel (UCP-1) through the inner mitochondrial membrane. The collapse of the pH gradient generates heat.

- UCP1(thermogenin) activation of fatty acid oxidation
  heat production in brown adipose tissue
- excessive thyroid hormone
- efa deficiency
- long chain fatty acids in adpose tissue
- conjugated hyperbilirubinemia

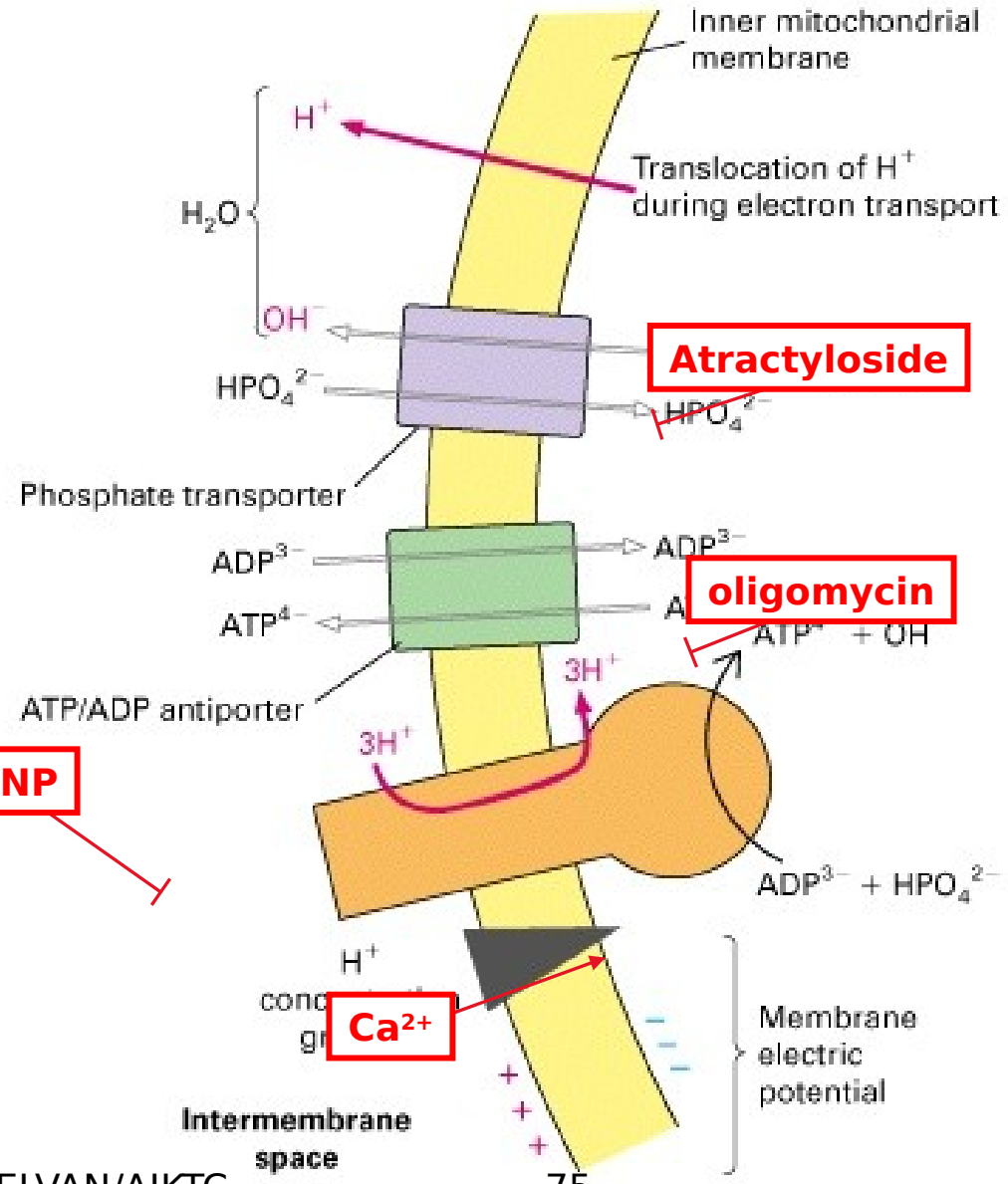
Inhibitors and uncouplers of oxidative phosphorylation

Inhibitors

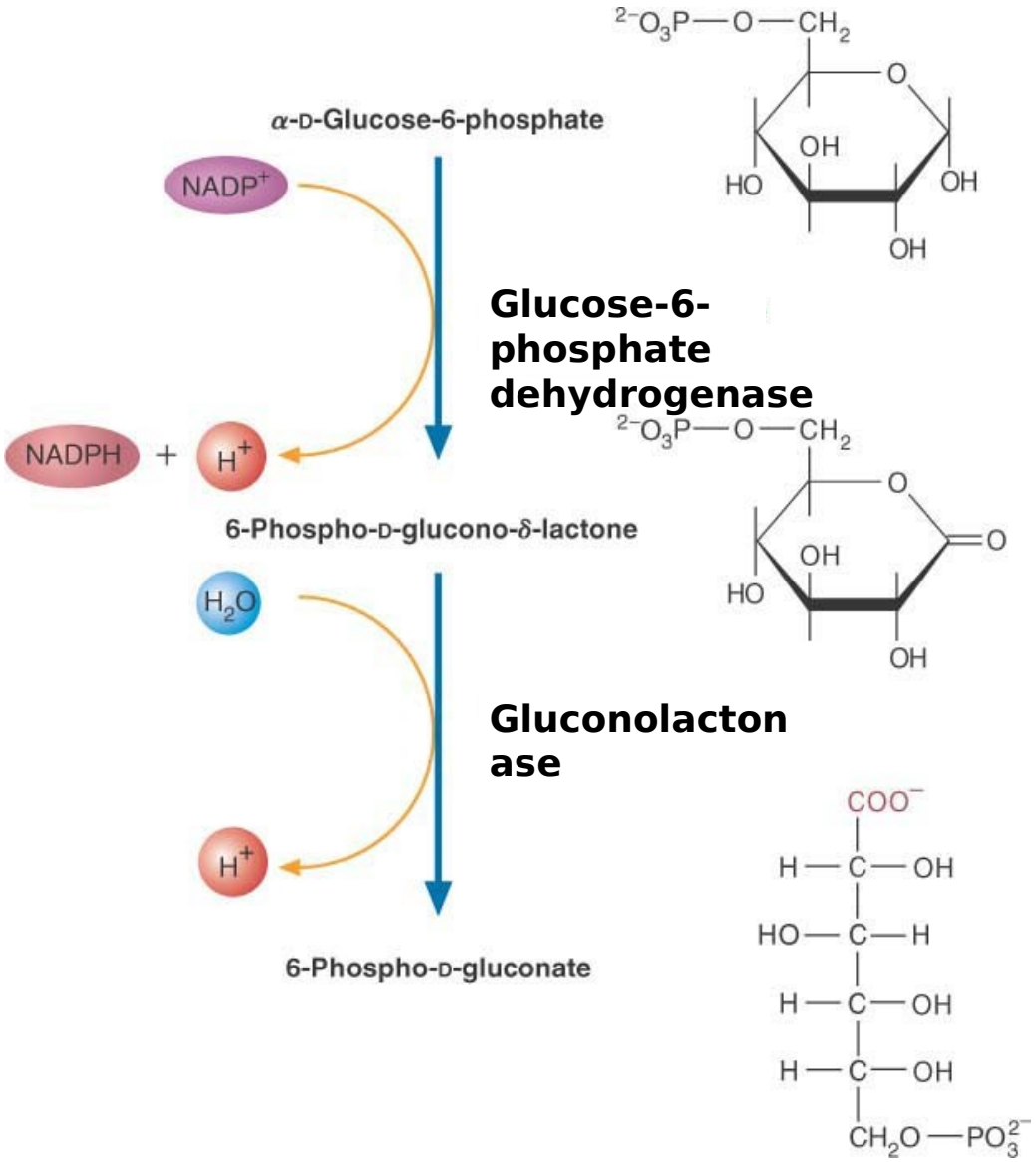
- **Atractyloside:** ADP/ATP antiporter
- **Oligomycin:**ATP synthase

Uncouplers

- **DNP** shuttles H^+ across inner membrane, dissipates gradient
- **$CaCl_2$** stimulates oxidative phosphorylation and ATP production



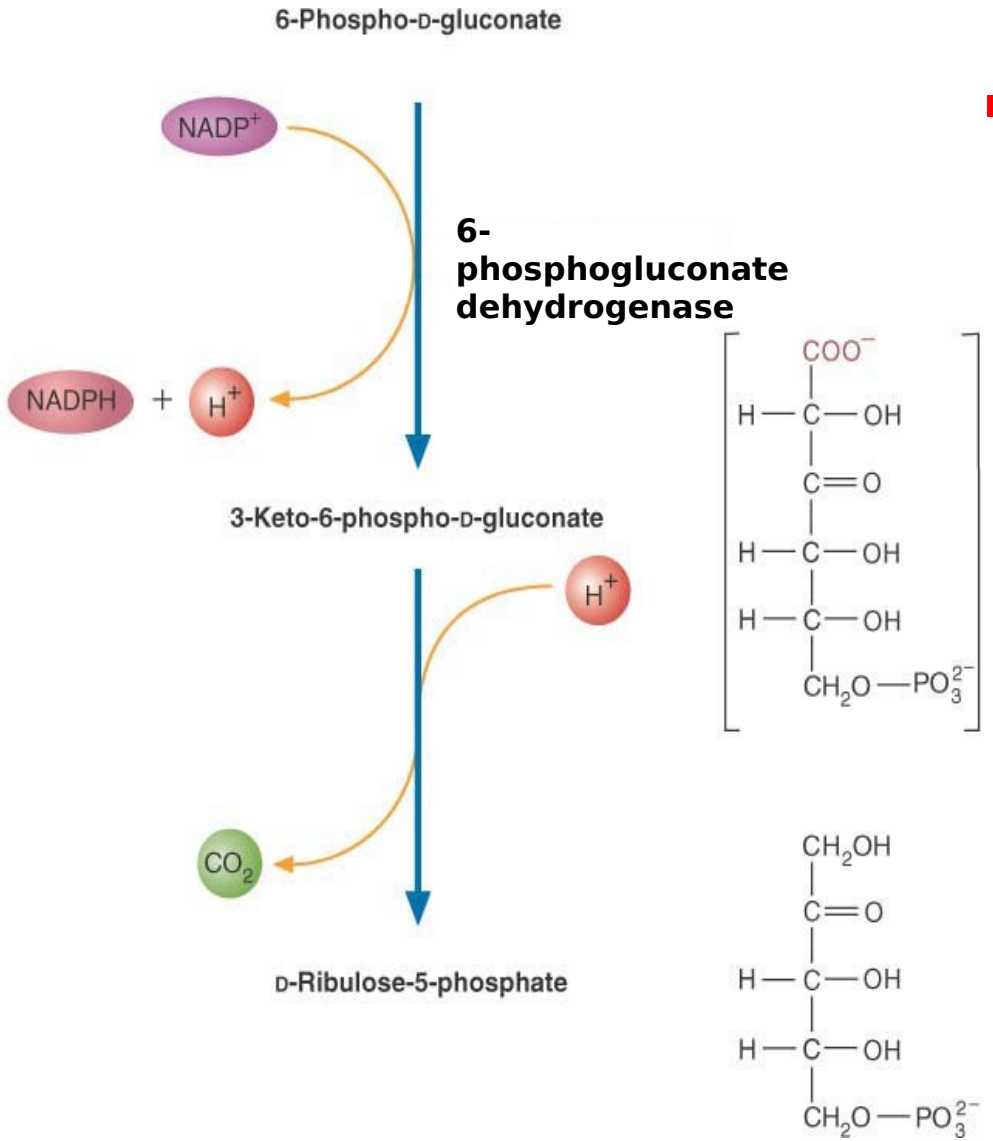
Pentose Phosphate Pathway



- **Pentose Phosphate Pathway**
  - Alternate glucose metabolic pathway
  - Products are NADPH and ribose-5-phosphate
  - Two phases: oxidative and nonoxidative

The Pentose Phosphate Pathway (oxidative)

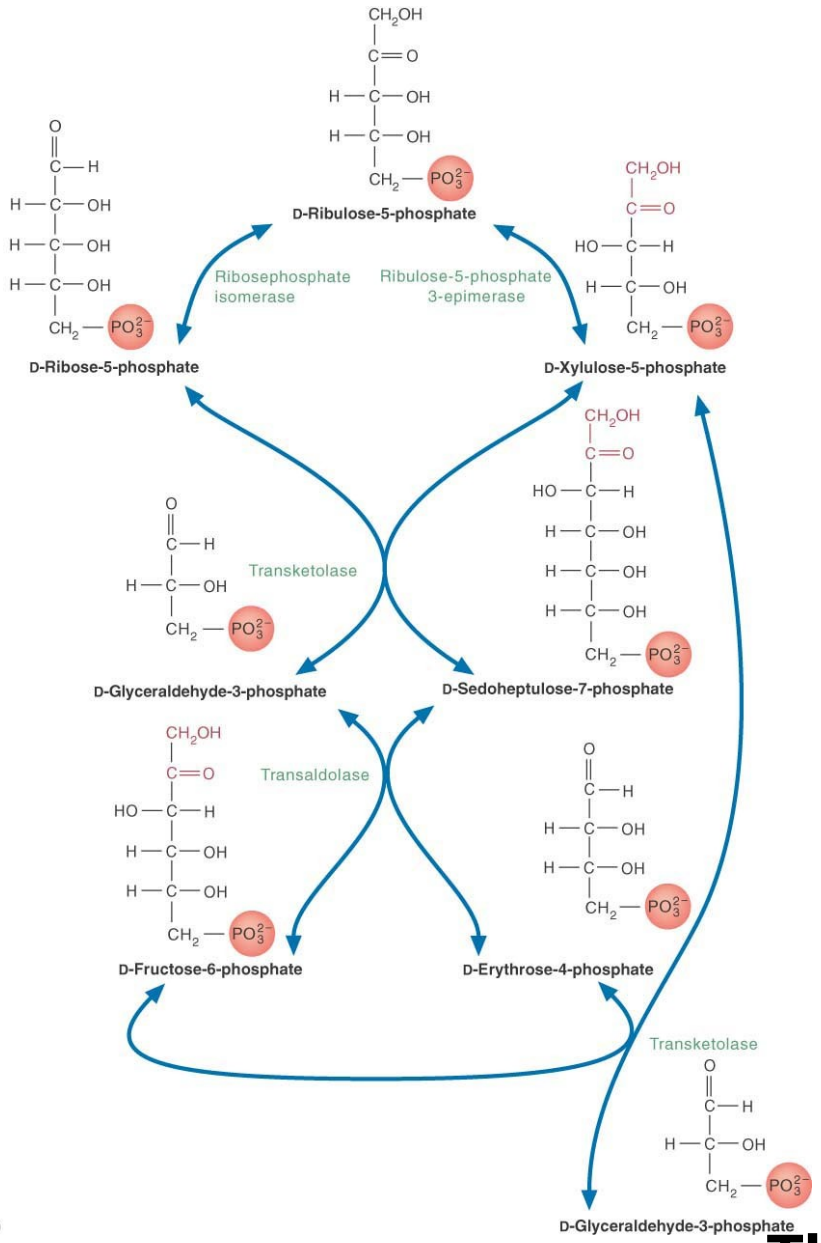
Pentose Phosphate Pathway



- **Pentose Phosphate Pathway: Oxidative**
  - Three reactions
  - Results in ribulose-5-phosphate and two NADPH
  - NADPH is a reducing agent used in anabolic processes

The Pentose Phosphate Pathway (oxidative)

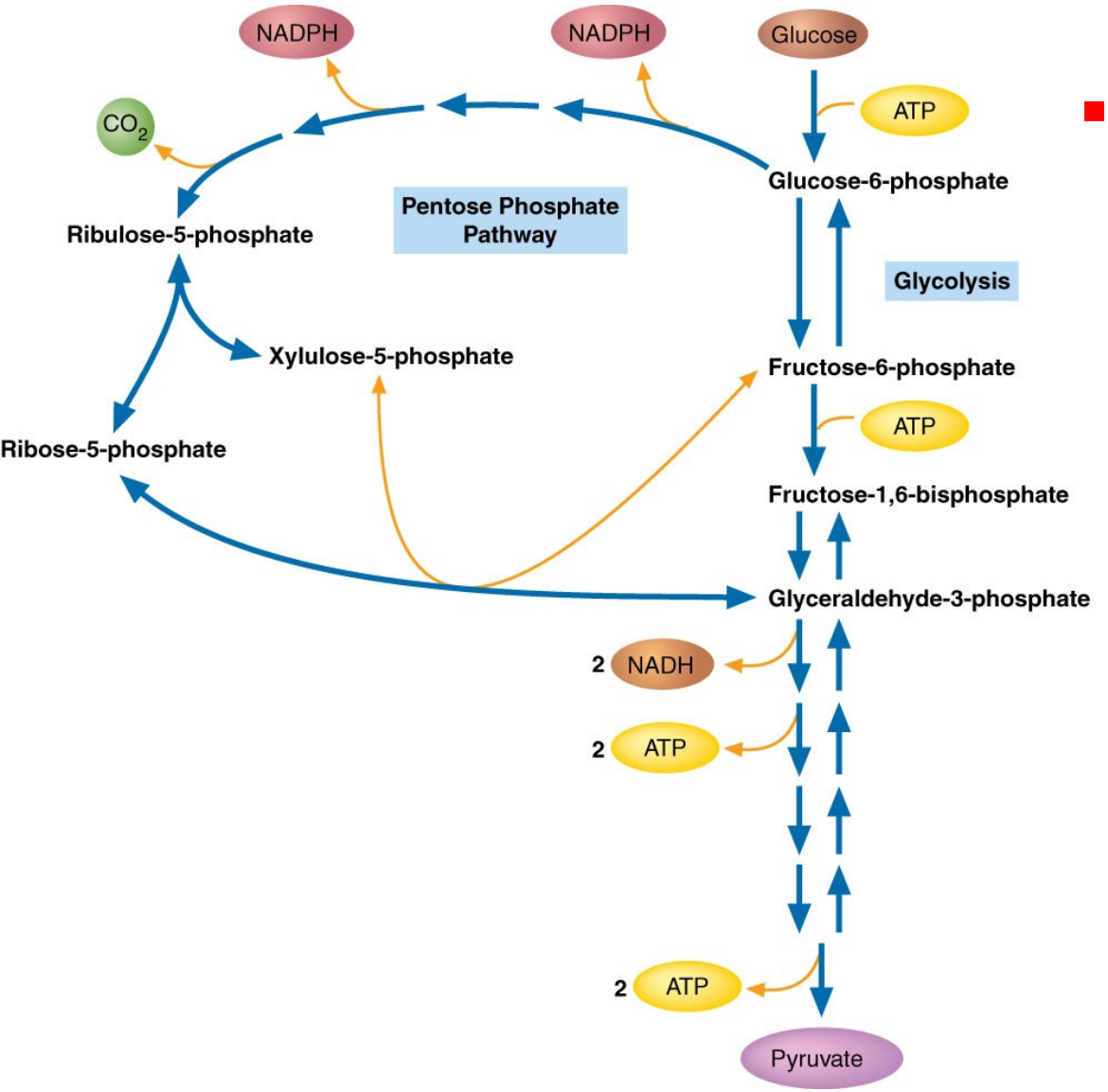
Pentose Phosphate Pathway



- **Pentose Phosphate Pathway: Nonoxidative**
  - Produces important intermediates for nucleotide biosynthesis and glycolysis
    - Ribose-5-phosphate
    - Glyceraldehyde-3-phosphate
    - Fructose-6-phosphate

The Pentose Phosphate Pathway

Pentose Phosphate Pathway



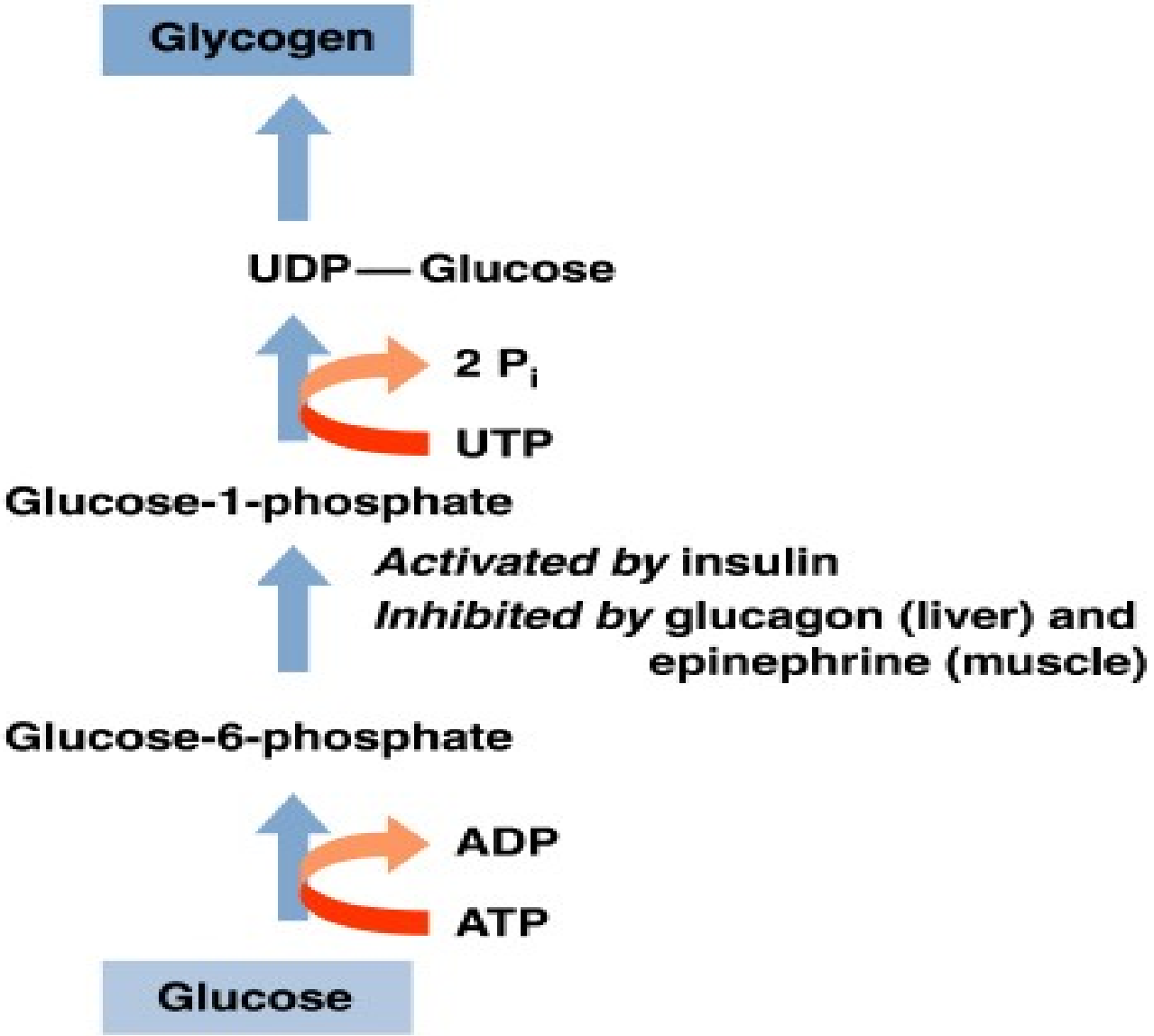
- **Pentose Phosphate Pathway**
  - If the cell requires more NADPH than ribose molecules, products of the nonoxidative phase can be shuttled into glycolysis

Glycogenesis:

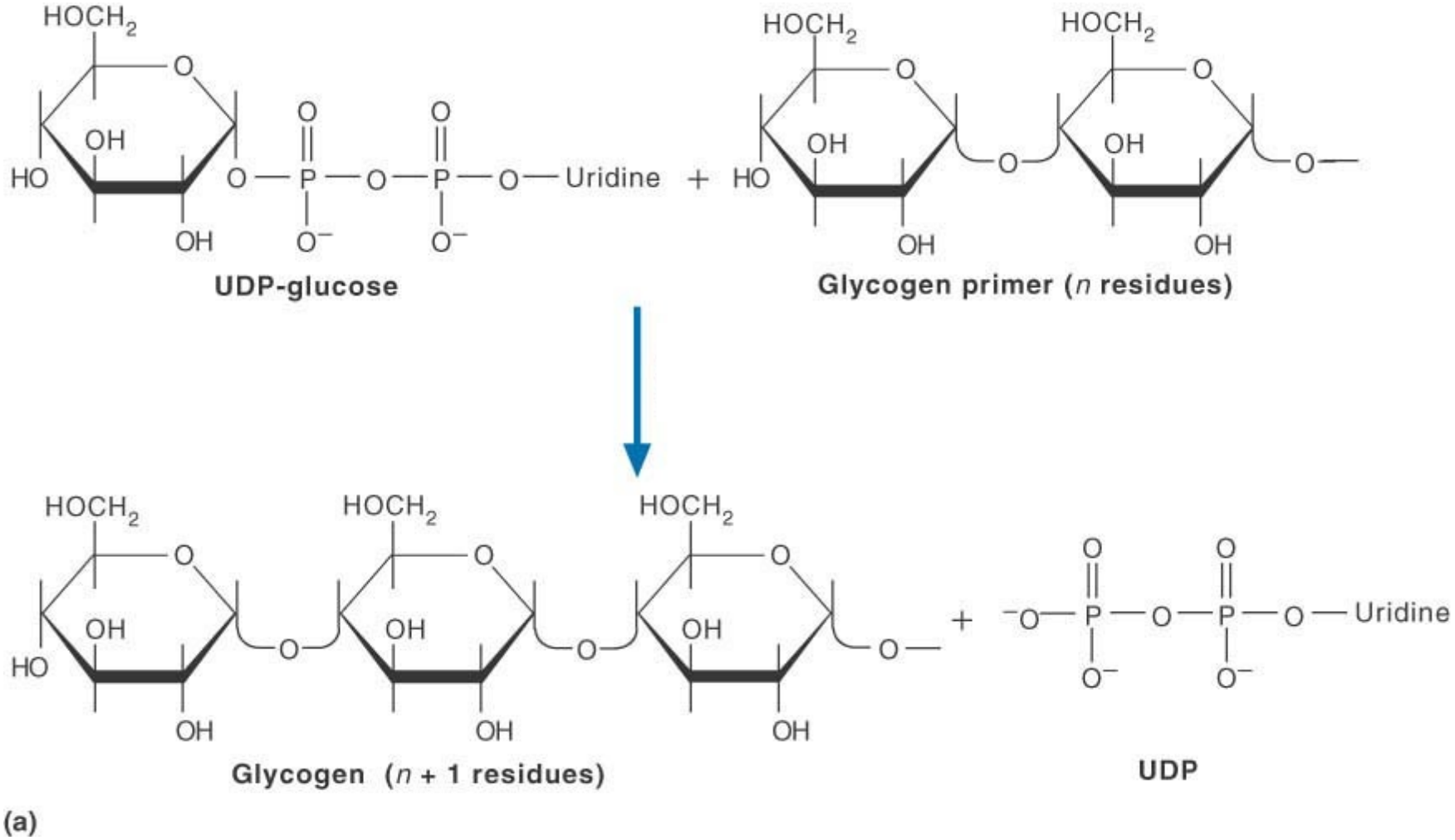
- Synthesis of glycogen, the storage form of glucose, occurs after a meal

- Requires a set of three reactions (1 and 2 are preparatory and 3 is for chain elongation):
  - **1. Synthesis of glucose-1-phosphate** (**G1P**) from glucose-6-phosphate by phosphoglucomutase
  - **2. Synthesis of UDP-glucose** from G1P by UDP-glucose phosphorylase
  - **3. Synthesis of Glycogen from UDP-glucose** requires two enzymes:
  - Glycogen synthase to grow the chain

GLYCOGENESIS



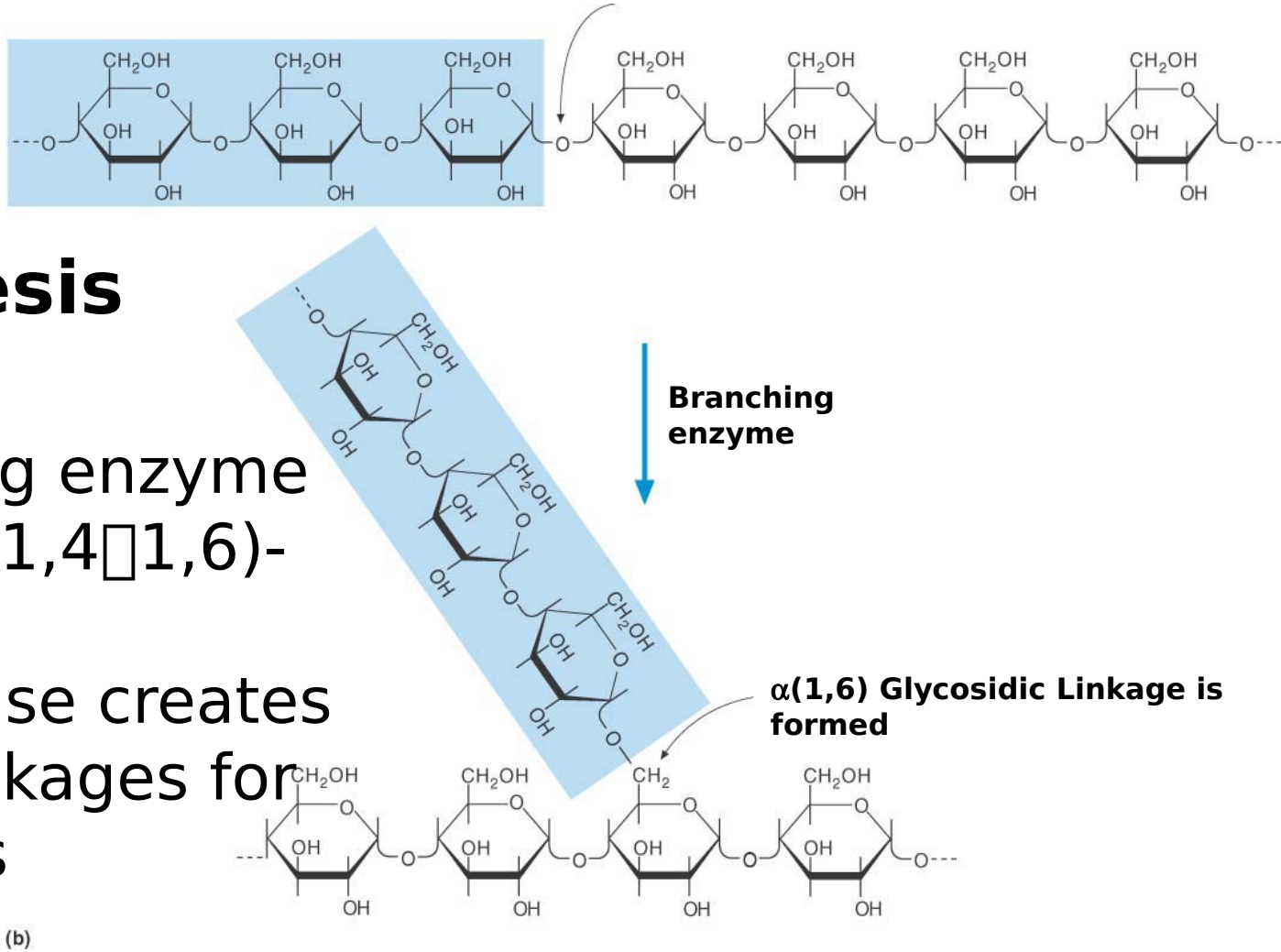
Glycogen Metabolism



a. Glycogen Synthesis

Glycogen Metabolism

- **Glycogenesis Continued**
  - Branching enzyme amylo-α(1,4→1,6)-glucosyl transferase creates α(1,6) linkages for branches



b. Glycogen Synthesis

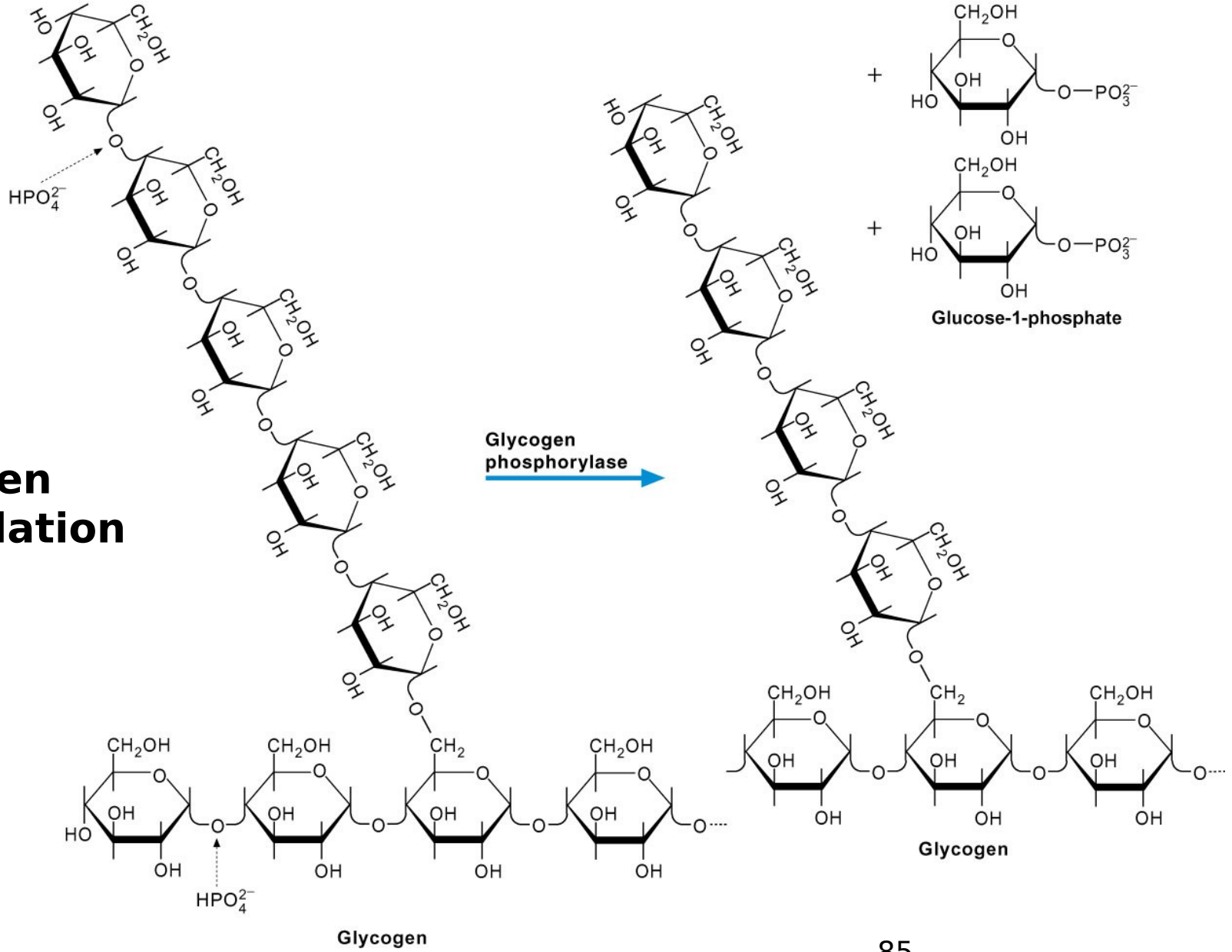
Glycogen Metabolism

- **Glycogenolysis**
  - Glycogen degradation requires two reactions:

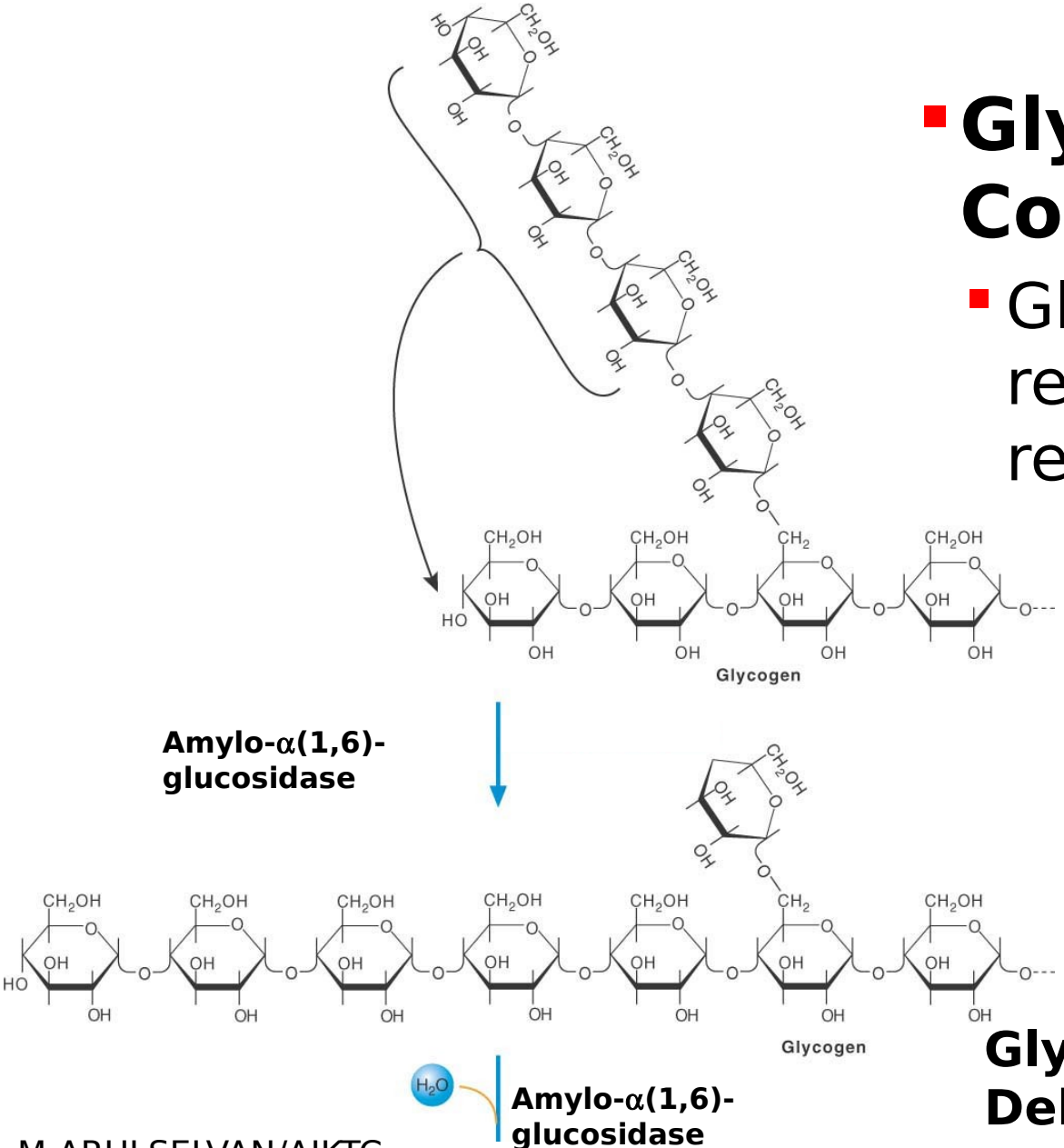
    1. Removal of glucose from nonreducing ends (glycogen phosphorylase) within four glucose of a branch point

Glycogen Metabolism

Glycogen Degradation



Glycogen Metabolism

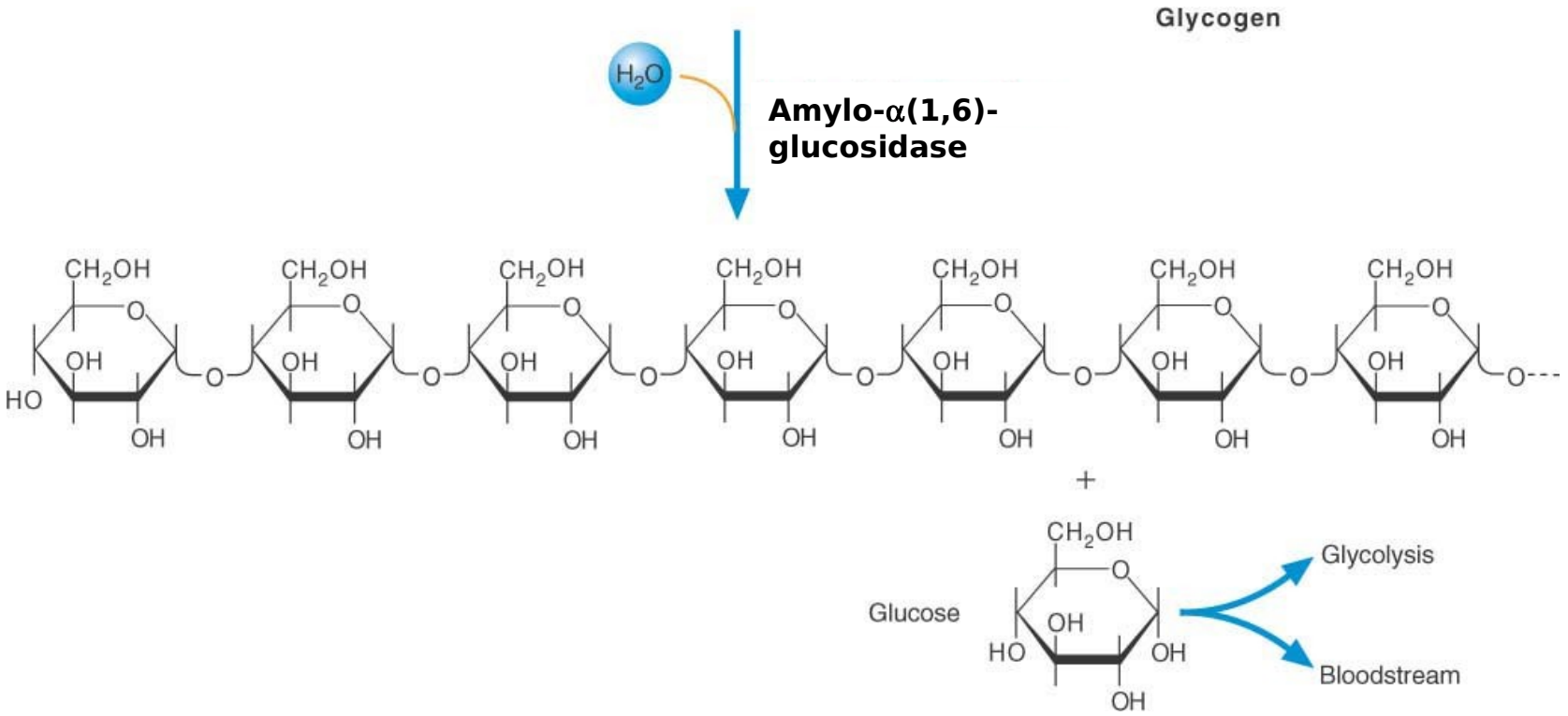


- **Glycogenolysis Cont.**
  - Glycogen degradation requires two reactions:

2. Hydrolysis of the α(1,6) glycosidic bonds at branch points by amylo-α(1,6)-glucosidase (debranching enzyme)

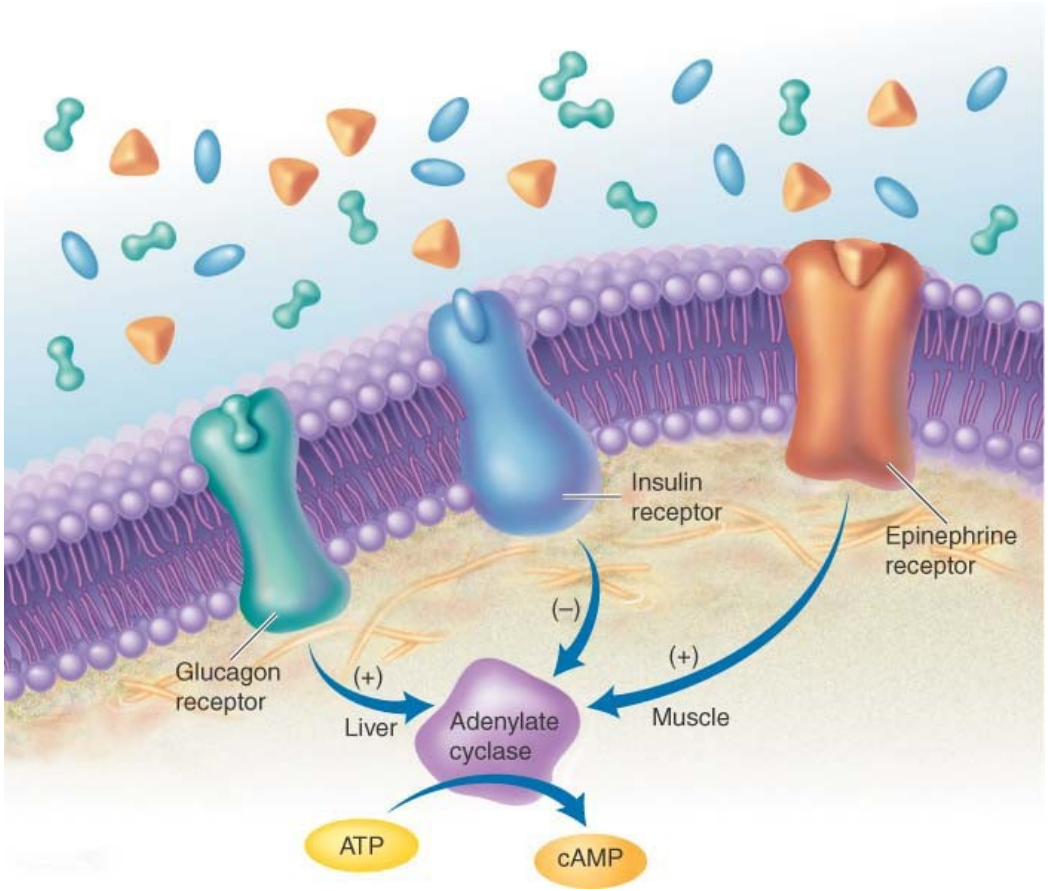
Glycogen Degradation via Debranching Enzyme

Glycogen Metabolism



Glycogen Degradation via Debranching Enzyme

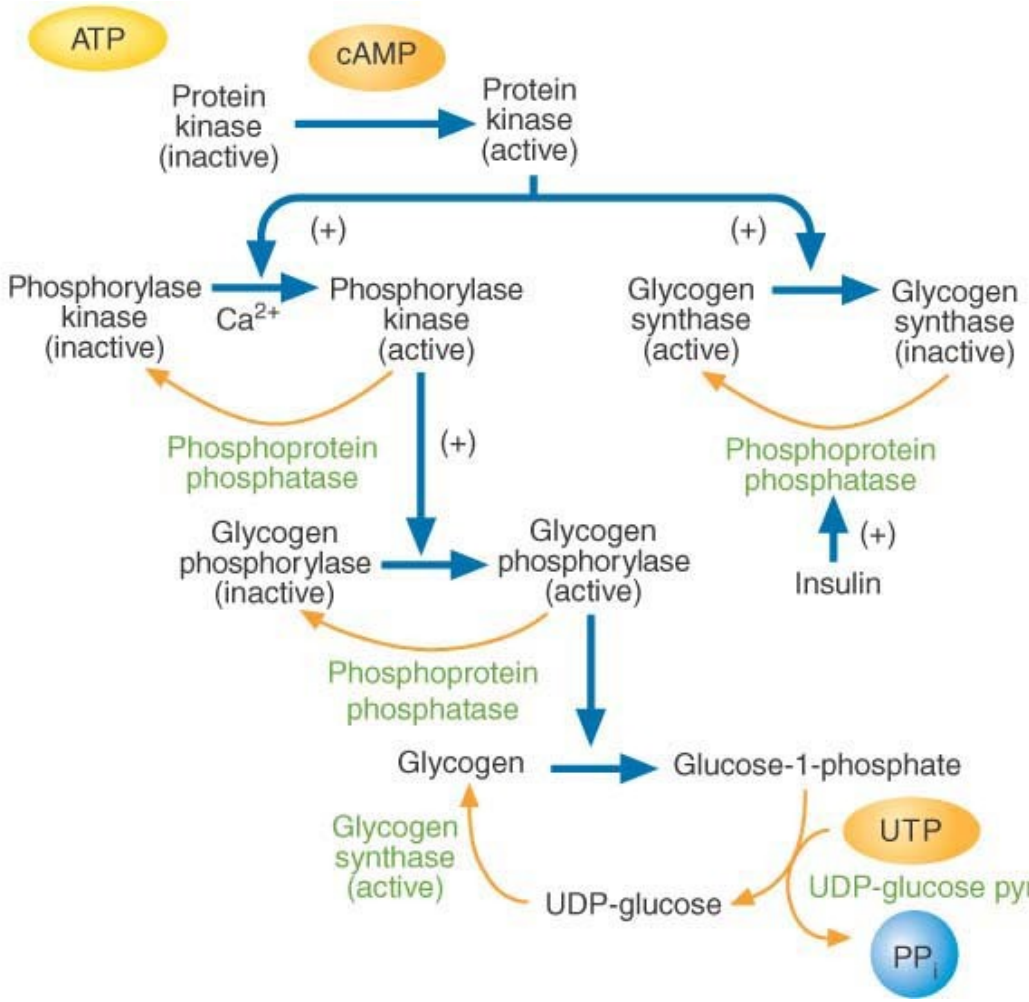
Glycogen Metabolism



Major Factors Affecting Glycogen Metabolism

- **Regulation of Glycogen Metabolism**
  - Carefully regulated to maintain consistent energy levels
  - Regulation involves **insulin**, **glucagon**, **epinephrine**, and **allosteric effectors**

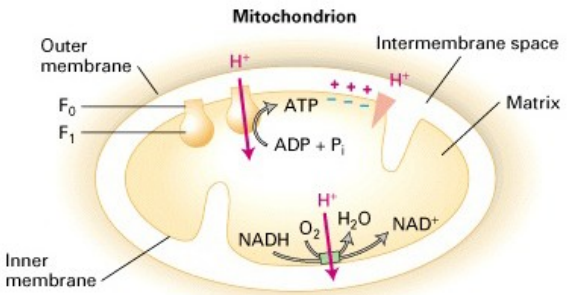
Glycogen Metabolism



- Glucagon activates glycogenolysis
- Insulin inhibits glycogenolysis and activates glycogenesis
- Epinephrine release activates glycogenolysis and inhibits glycogenesis

Major Factors Affecting Glycogen Metabolism

Summary of Cellular Energetics



Glucose

High [ATP] (Pasteur effect)

N-ethylmaleimide

Glycolysis

Pyruvate

Acetyl CoA ← ← EtOH

NADH

$FADH_2$

Malate

Succinate

Citric Acid Cycle

Uncouplers
Ca^{+2}, DNP

$NADH + FADH_2$

Rotenone

Antimycin A

Electron transport chain

Ascorbate + TMPD

Sodium Azide

Energy released used to pump H^+ creating an elecrochemical gradient

O_2 → H_2O

Flow of protons down the gradient fuels ATP synthase

Oligomycin

Atractyloside

ADP + P_i → **ATP**

Oxidative Phosphorylation

Q.P DISCUSSION

1 Mark

1.Define Glycolysis

2.Write in detail about of phosphofructokinase in glycolysis

3.Draw the structure of adenine

4.Define Glycogenesis

5.Draw the structure of AMP

6.Draw the structure of ADP

3 Marks

1.Differentiate between oxidative phosphorylation and substrate phosphorylation

2.Draw Embden Meyerhoff Pathway

4 Marks

1.Define Glycogenesis & discuss in brief reaction involved in it?

2.Give the name and structure of the substrate and products of the following enzyme

a) citrate synthase b) Succinate dehydrogenase

b) phosphogluconate dehydrogenase

3.Discuss in brief about Kreb's cycle?