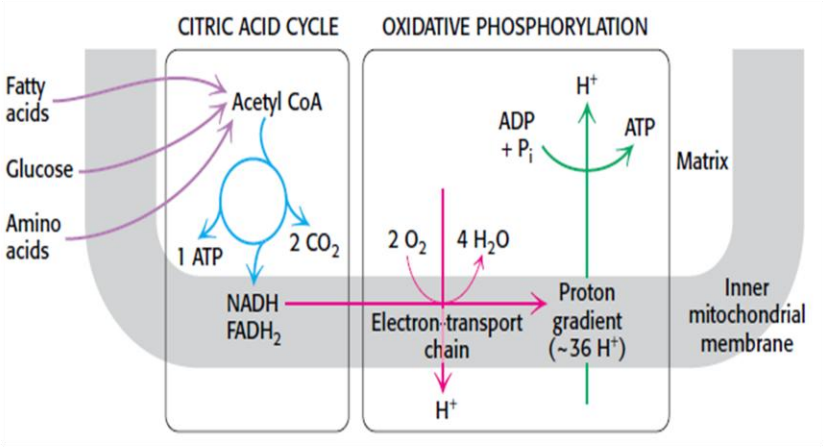
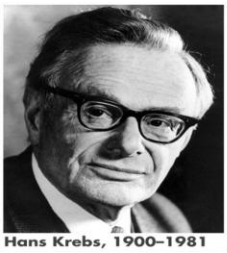
CITRIC ACID CYCLE AND OXIDATIVE PHOSPHORYLATION




Hans Krebs, 1900–1981

Yoseph Cherinet (BSc, MSc, Assistant Professor)

Learning Objectives

- Describe Krebs cycle
- Cytoplasmic Shuttles
- Common disorders of carbohydrate metabolism
- Biological oxidation
- Explain Electron Transport Chain (ETC)
- Describe the process of oxidative phosphorylation
- Energy yield in the Krebs cycle
- Inhibitors and uncouplers
- Recognize the key regulatory steps in pathways

Where does exhaled CO_2 come from?

Exhaled CO_2 ,a waste product of cellular metabolism, is generated mostly by operation of the TCA cycle.

Overview

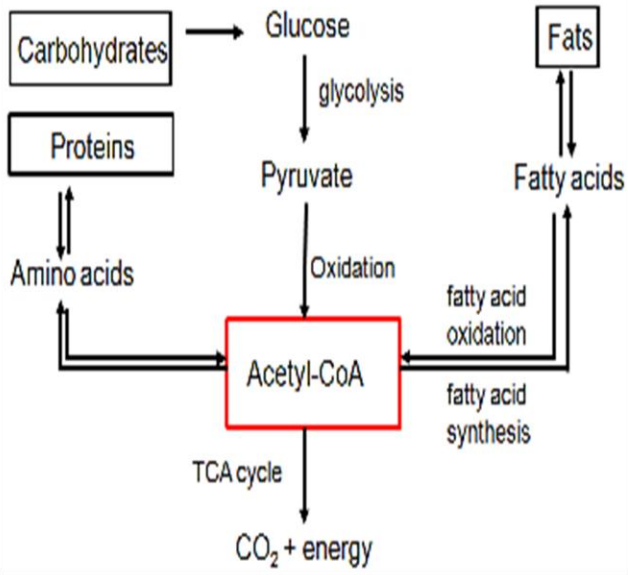
- In 1937 Hans Krebs discovered the cycle
- The tricarboxylic acid (TCA) or citric acid cycle is a series of reactions in mitochonodrial matrix that brings about the catabolism of acetyl CoA, librating hydrogen equivalents in the form of NADH and $FADH_2$ which up on oxidation in ETC lead to the production of ATP.
- One acetyl-CoA molecule is oxidized at a time.

- TCA cycle is a hub in metabolism; Amphibolic: with degradative pathways leading in and anabolic pathways leading out.
- Acetyl CoA joins with oxaloacetate to form citric acid (Tri-carboxylic acid, TCA) and completes with regeneration of oxaloacetate.
- Throughout the entire cycle, acetyl-CoA changes into Citrate, Isocitrate,α-Ketoglutarate, Succinyl-CoA, Succinate, Fumarate, Malate and finally Oxaloacetate.

- The TCA cycle is an 8 step process involving 8 different enzymes.
    1. Citrate synthase
    2. Aconitase
    3. Isocitrate dehydrogenase
    4. α-Ketoglutarate dehydrogenase
    5. Succinyl-CoA synthase
    6. Succinate dehydrogenase
    7. Fumarase
    8. Malate dehydrogenase

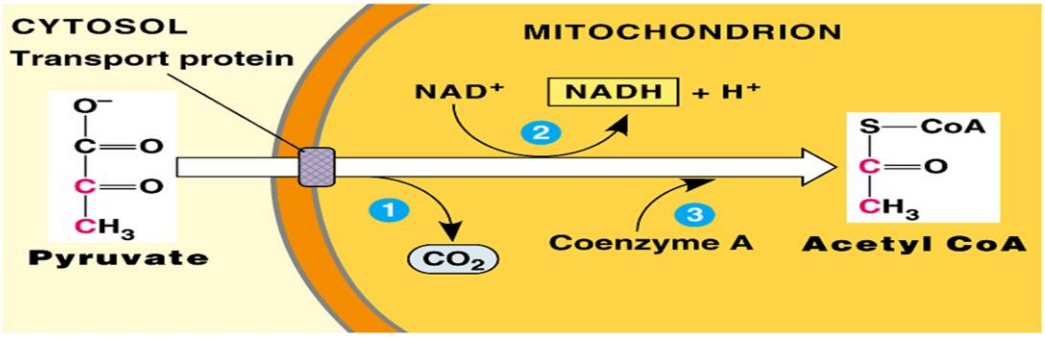
SOURCES OF ACETYL CoA

- Metabolic reactions linked to production of acetyl-CoA
  - Glycolysis
  - Oxidation of fatty acids
  - Amino acid deamination
  - Ketone bodies utilization
  - Ethanol metabolism



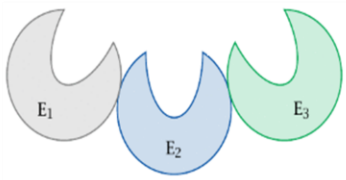
Transport of Pyruvate & Production of Acetyl-CoA

- Pyruvate, the end product of aerobic glycolysis, must be transported into the mitochondria.
- This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane.
- Pyruvate is then oxidized to acetyl-CoA by the pyruvate dehydrogenase (PDH) complex

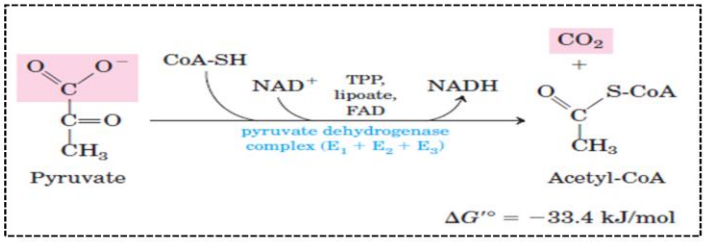


Pyruvate dehydrogenase complex (PDH)

- PDC is a multifunctional enzyme complex, individual enzymes are linked by non-covalent bonds,which act in sequence to catalyze the transformation of pyruvate to acetyl-CoA
- **Three enzymes of PDH**
  - E1; Pyruvate dehydrogenase
    - Decarboxylation of pyruvate
  - E2; Dihydrolipoyl transacetylase
    - Transfer of acetyl group to CoASH
  - E3; Dihydrolipoyl dehydrogenase
    - Regeneration of oxidized form of lipoamide and transfer of electrons from FADH2 to NAD+
- **Five coenzymes of PDH**
  - Thiamine pyrophosphate
  - Lipoamide
  - FAD
  - NAD+
  - Coenzyme A (CoASH)



THE FIVE REACTIONS OF THE PYRUVATE DEHYDROGENASE COMPLEX



- The five reactions of the pyruvate dehydrogenase multienzyme complex. E1 (pyruvate dehydrogenase) contains TPP & catalyzes Reactions 1 & 2. E2 (dihydrolipoyl transacetylase) contains lipoamide and catalyzes reaction 3. E3 (dihydrolipoyl dehydrogenase) contains FAD catalyzes Reactions 4 and 5.

E1

1. Decarboxylates pyruvate and acetyl group is bound to TPP (acetyl-TPP).
2. Next is acetyl group from acetyl-TPP transferred to lipoamide and forms acyl-lipoamide

E2

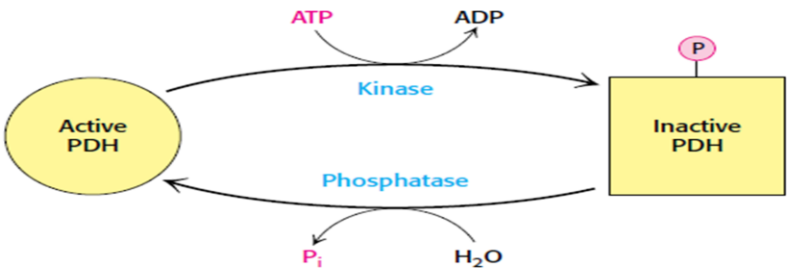
3. Transfers the acetyl group from acyl-lipoamide to CoA results in the formation of acetyl CoA and the resulting lipoamide with two–SH groups (reduced)

E3

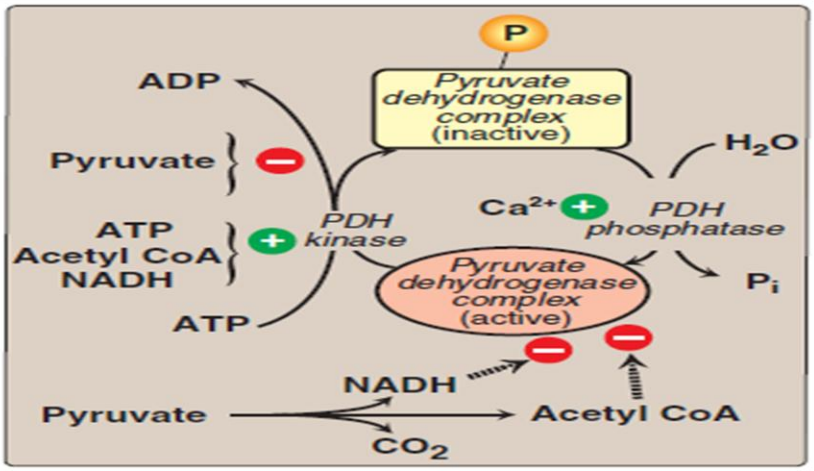
4. Lipoamide with two–SH groups are reoxidized by E3 –FAD forming E3 –FADH2 and oxidized Lipoamide with two–S .
5. Finally E3 –FADH2 reduces NAD+ to NADH

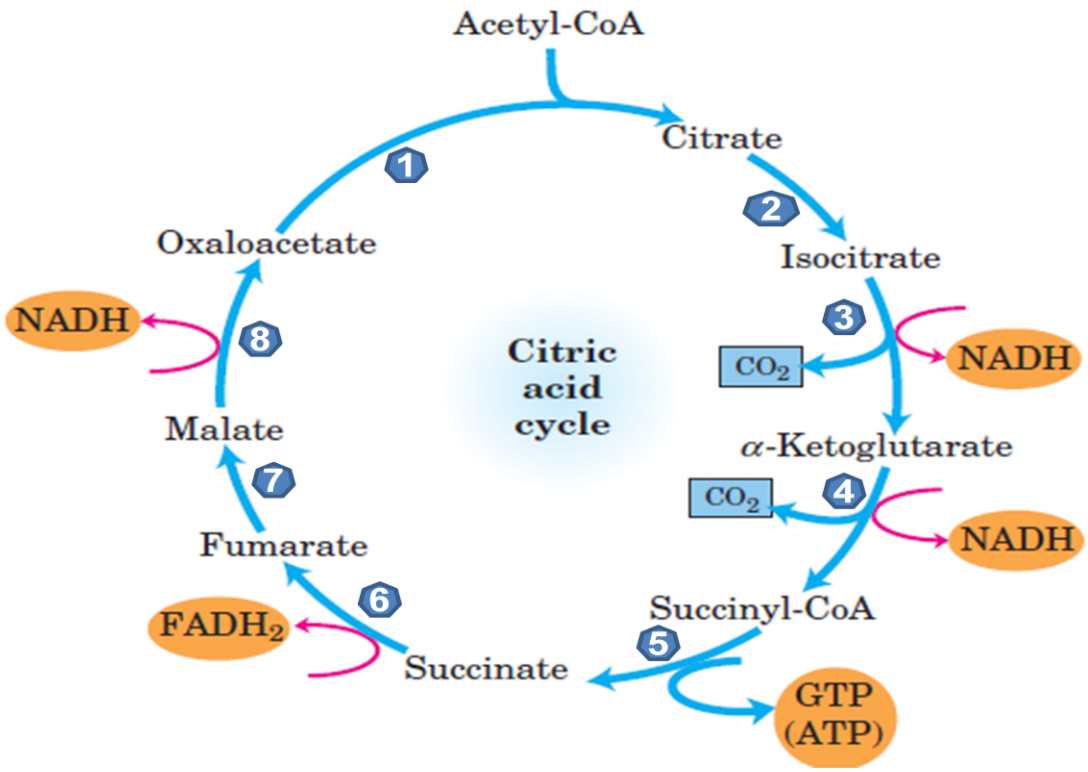
Regulation of the PDH

- Covalent modification by the two regulatory enzymes that are part of the complex alternately activate and inactivate E1 (PDH).
- A specific kinase phosphorylates and inactivates PDH, and a phosphatase activates the PDH by removing the phosphoryl group.



- PDH kinase itself is allosterically activated by ATP, acetyl CoA, & NADH.
- Pyruvate is inhibitor of PDH kinase.
- Calcium is activator of PDH phosphatase
- Although covalent regulation by the kinase and phosphatase is key, the complex is also subject to product (NADH, acetyl CoA) inhibition.



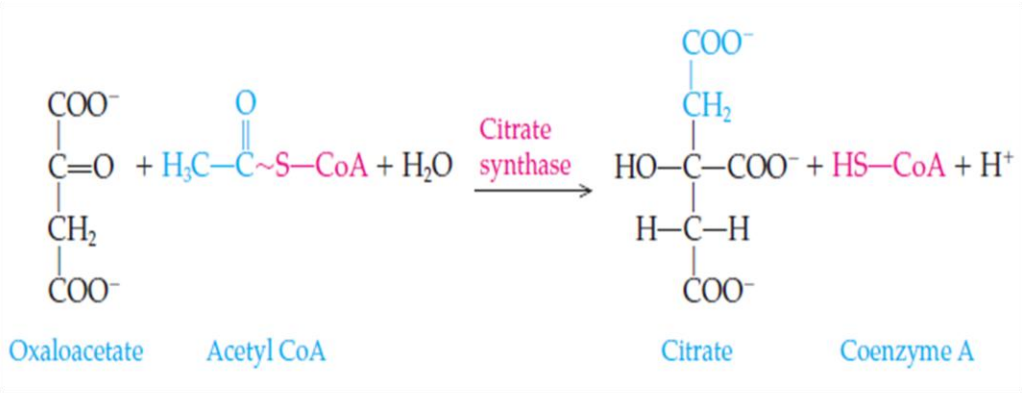


Reactions of the TCA Cycle

Individual reactions of the TCA Cycle

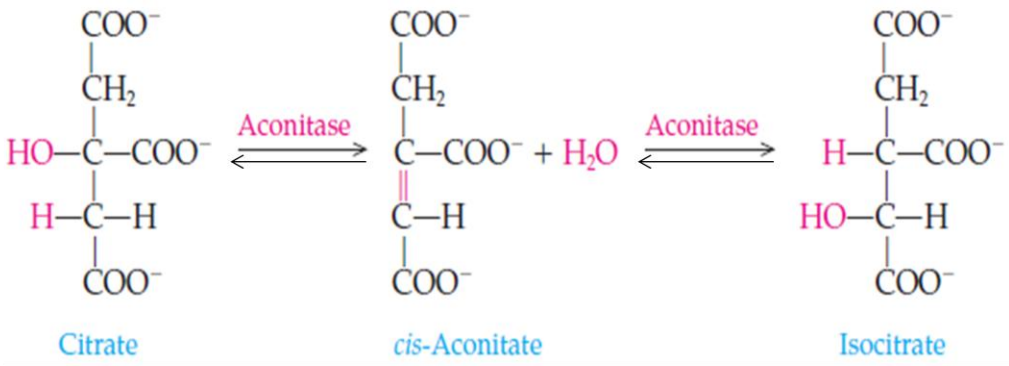
1. Formation of Citrate

- The first reaction of the cycle is the condensation of acetyl-CoA with oxaloacetate to form citrate, catalyzed by citrate synthase.



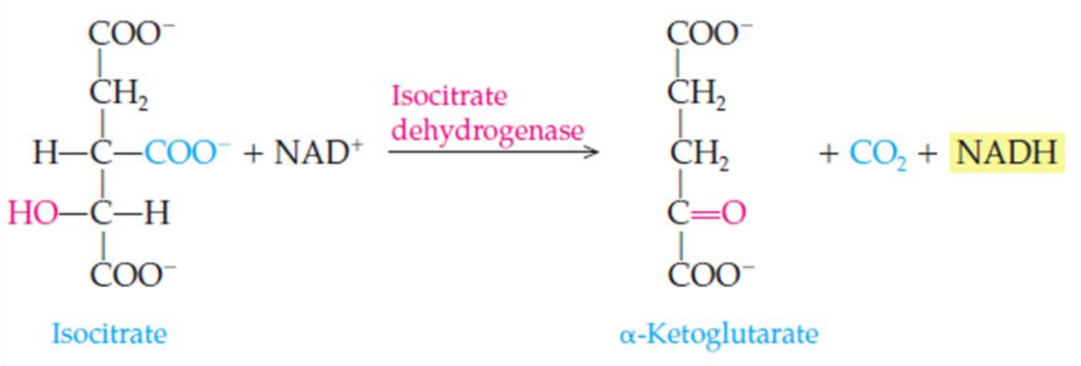
2. Formation of Isocitrate

- The isomerization of citrate to isocitrate is catalyzed by the enzyme aconitase.
- The OH group of citrate is moved to the adjacent carbon.



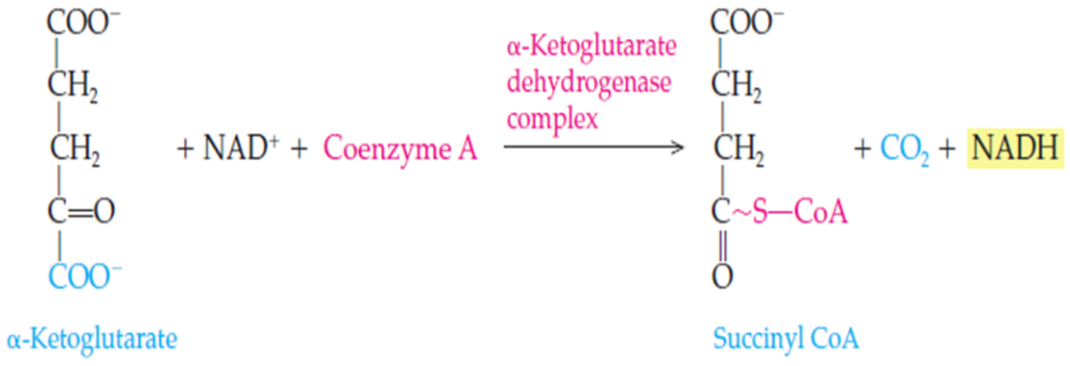
3. Oxidation of Isocitrate to α-Ketoglutarate

- Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate to α-ketoglutarate and yields the first of three NADH molecules produced by the cycle.



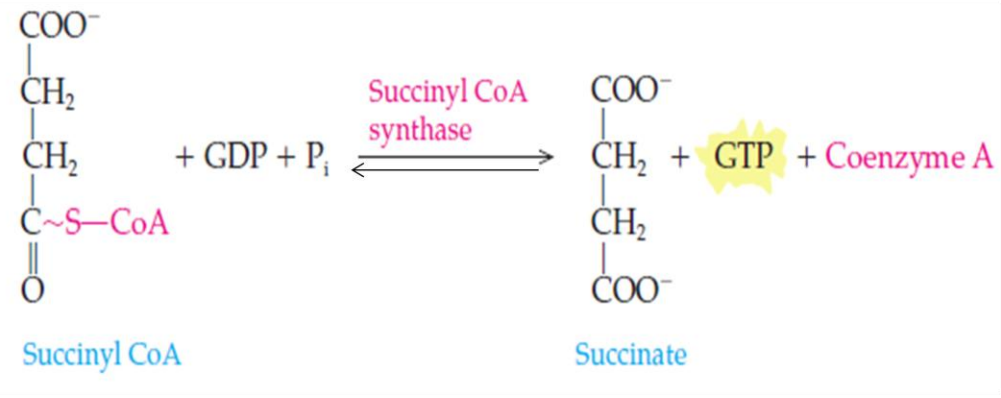
4. Oxidation of α-Ketoglutarate to Succinyl-CoA

- α-ketoglutarate undergoes irreversible oxidative decarboxylation catalyzed by α-ketoglutarate dehydrogenase to succinyl-CoA.
- NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.



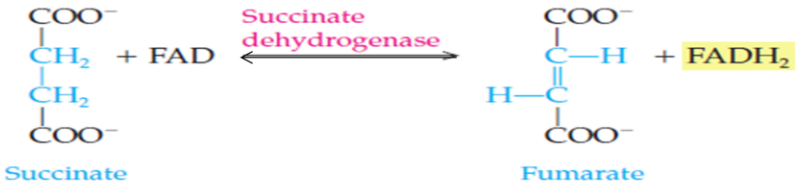
5. Conversion of Succinyl-CoA to Succinate

- Succinyl-CoA synthase cleaves the thioester bond of succinyl CoA. The reaction is coupled to phosphorylation of GDP to GTP (Substrate-level phosphorylation).
- The only reaction in the cycle where high energy phospahte (GTP) compound is formed.
  - **ATP is then formed from GTP by <u>nucleoside diphosphokinase.</u>**



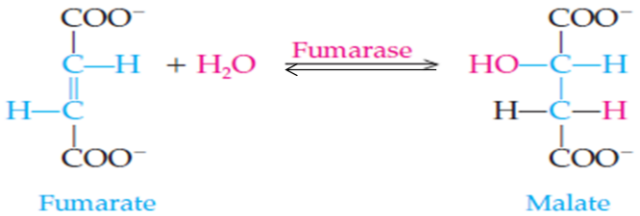
6. Oxidation of Succinate to Fumarate

- The succinate formed from succinyl-CoA is oxidized to fumarate by the succinate dehydrogenase.



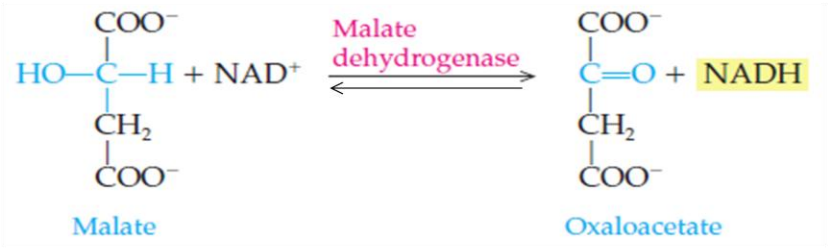
7. Hydration of Fumarate to Malate

- Fumarate is then hydrated by fumarase to malate



8. Oxidation of Malate to Oxaloacetate

- Malate is then oxidized to oxaloacetate by malate dehydrogenase, completing the TCA cycle and bringing it full circle.



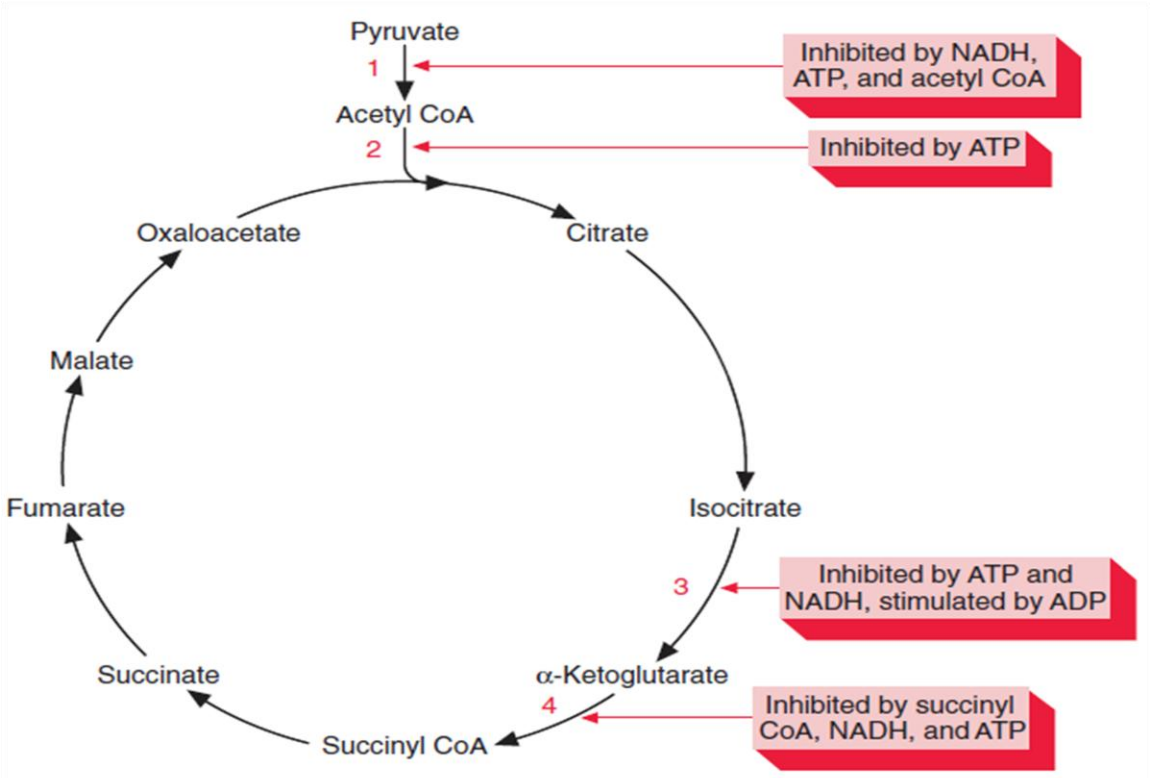
TCA Cycle as a source of biosynthetic intermediates

- TCA cycle plays a key role in anabolism.
- Cataplerotic reactions steals intermediates for biosynthesis.
  - Citrate - Malate
  - Oxaloacetae
  - Ketoglutarate
  - Succinyl CoA
- Anaplerotic reactions replenish TCA cycle intermediates.
  - Aspartate - Fumarate
  - Pyruvate
  - Glutamate
  - Odd chain fatty acids

REGULATION OF THE TCA CYCLE

- The pathway speeds up when there is a greater demand for ATP, and it slows down when ATP energy is in excess.
- Citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase are involved in the regulation of TCA cycle
- The flow of pyruvate into TCA cycle is also under regulation (PDH complex)
- The activities of enzymes controlled by substrate availability and inhibition by cycle intermediates.

Regulation of TCA cycle



Energetics of the TCA cycle

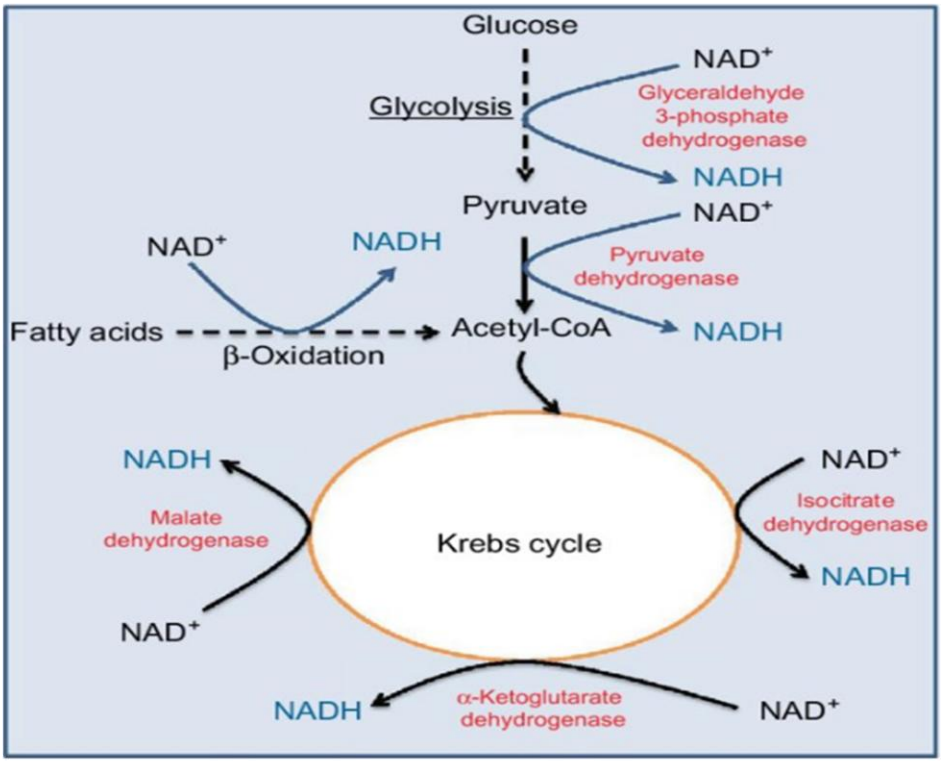
- Number of ATP generated by oxidation of 3 NADH = 9 ATP
- Number of ATP generated by oxidation of 1 $FADH_2$ = 2 ATP
- Number of ATP generated from GTP 1 = 1 ATP
- Total of 12 ATP are generated in TCA for each acetyl-CoA.
- Since glucose gives rise to two acetyl-CoA molecules aerobic oxidation of glucose yields 24 ATPs.

ATP Generation during oxidation of glucose

Process	No of ATP/mol of glucose
Glycolysis	6/8
Pyruvate dehydrogenase C	6
TCA cycle	24
Total	36/38

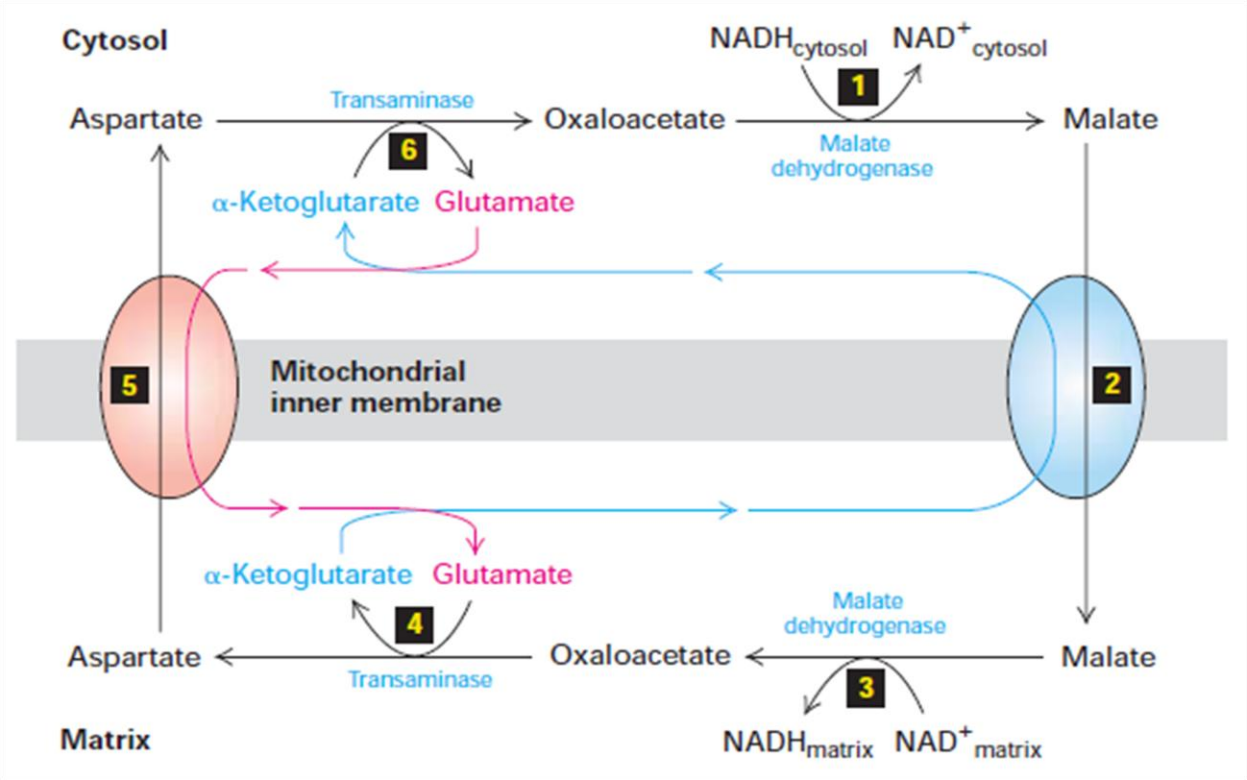
ATP yield variation depends on shuttle used for the transfer of NADH from cytosol to mitochondria.

Metabolic pathways & enzymes involved in NADH production using NAD + as their cofactor.

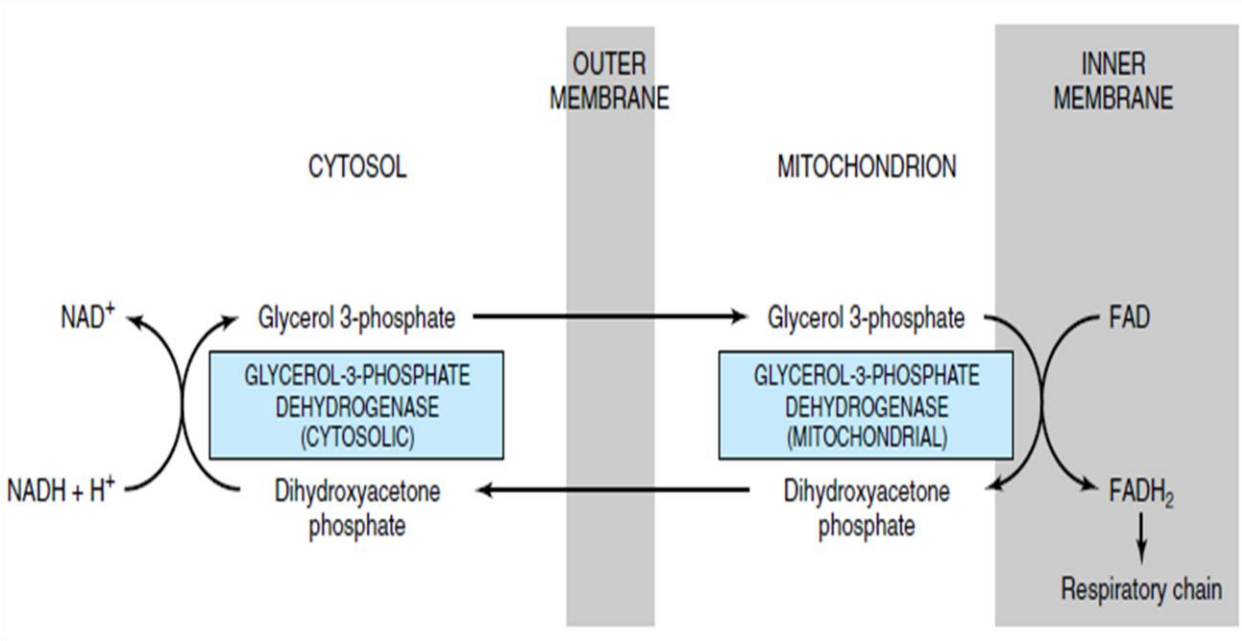


Cytoplasmic Shuttle Systems "Transport" NADH

- NADH is generated in the mitochondrial matrix via the TCA cycle, β-oxidation & pyruvate dehydrogenase complex reactions. NADH also generated by glycolysis in the cytosol.
- However inner mitochondrial membrane lacks an NADH transport protein.
- Two shuttle systems for the transport of electrons across the inner mitochondrial membrane exists.
  - Malate–Aspartate Shuttle
    - **Most active in liver, kidney and heart**
  - Glycerol-Phosphate Shuttle
    - **Most active in skeletal muscle and brain**



Malate–Aspartate Shuttle



Glycerol-Phosphate Shuttle

ATP Yield in Malate–aspartate & Glycerol phosphate shuttle

Process	Malate–aspartate shuttle *	Glycerol phosphate shuttle*	Malate–aspartate shuttle **	Glycerol phosphate shuttle **
Glycolysis	8	6	7	5
Pyruvate DHC	6	6	5	5
TCA cycle	24	24	20	20
ATP/Glucose	38	36	32	30

* = ATP per NADH & $FADH_2$ is 3 & 2 respectively (old concept)

** = ATP per NADH & $FADH_2$ is 2.5 &1.5 respectively (new concept)

Common disorders of Carbohydrate Metabolism

- **Diabetes Mellitus**
  - Impaired ability of the body to produce or respond to insulin .
- **Glycogen storage diseases**
  - Deficiency of enzymes affecting glycogenolysis and glycogenesis.
- **Galactosemia**
  - Impaired ability to utilize galactose.
- **Fructose intolerance**
  - Defect in absorption of fructose/ deficiency of enzymes aldolase.

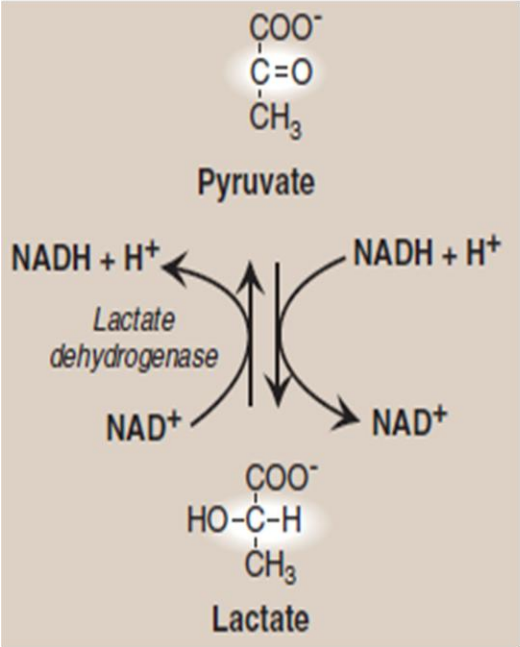
- **Lactose intolerance**
  - It is a consequence of lactase deficiency
- **Pompe disease**
  - Lysosomal storage disorder due to acid maltase deficiency
- **Pyruvate dehydrogenase deficiency**
  - It is characterized by buildup of lactic acid

BIOLOGICAL OXIDATION

- In many reaction one substance transfers electrons to another. This type of reaction is called **REDOX** reactions (reduction and oxidation reaction occur at the same time)
- In any **REDOX** reaction, one reactant (called oxidizing agent or oxidant) is reduced as it gains electrons.
- The other reactant (called reducing agent or reductant) is oxidized as it gives up electrons.

$$A_{oxidized} + B_{reduced} \rightleftharpoons A_{reduced} + B_{oxidized}$$

Example: Reduction of Pyruvate to Lactate



Pyruvate is oxidant

NADH is reductant

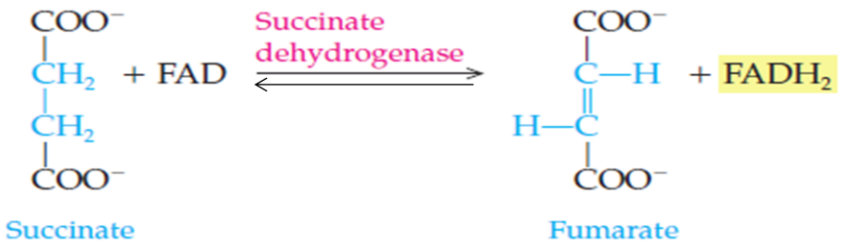
⇕

Lactate reductant

NAD+ is oxidant

- Pyruvate & NAD+ are oxidants (oxidizing agents)
- Lactate & NADH are reductants (reducing agents).

Practice: Consider the following reaction and identify the species as oxidant, reductant, reducing agent, oxidizing agent

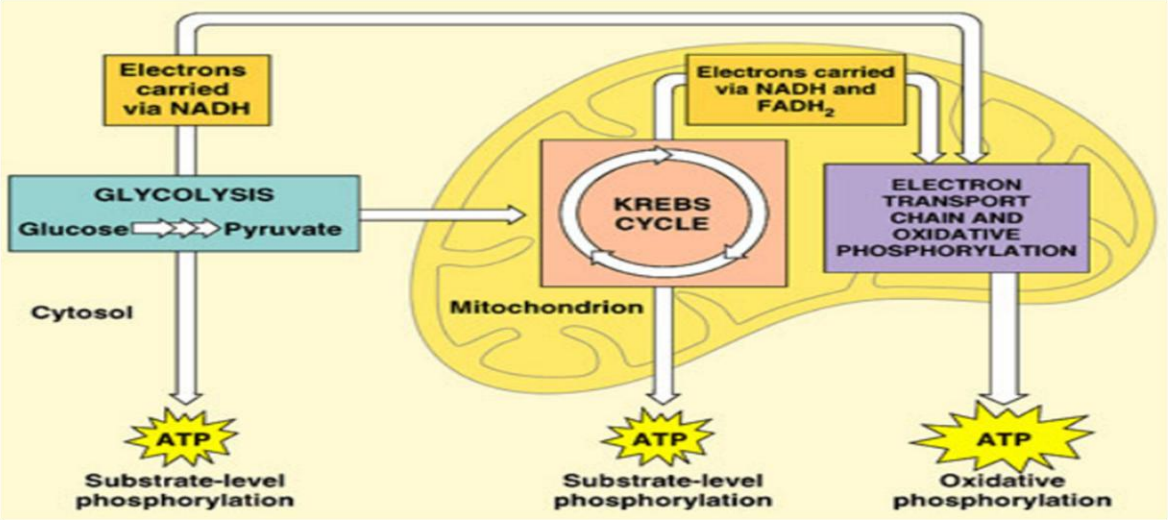


- **Succinate is reductant.**
- **FAD is oxidant.**
- **Fumarate is oxidant.**
- **$FADH_2$ is reductant.**
- **Succinate & FADH2 are reductant (reducing agents).**
- **Fumarate & FAD are oxidants (oxidizing agents)**

ELECTRON TRANSPORT CHAIN

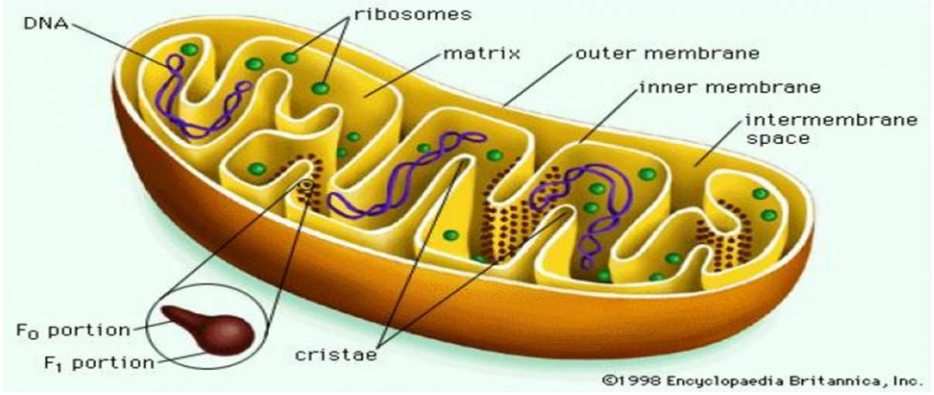
&

OXIDATIVE PHOSPHORYLATION



Structure of Mitochondria

- Enclosed by an outer and inner membrane; in between intermembrane space
- Inner membrane is folded into numerous cristae
- Internal space enclosed by inner mitochondrial membrane is called the matrix.



Electron Transport Chain

- Electron transport chain (ETC) is a series of compounds that transfer electrons from electron donors to electron acceptors via redox reactions and couples this electron transfer with the transfer of protons (H^+ ions) across a membrane.
- This creates an electrochemical proton gradient that drives ATP synthesis.
- The process of making ATP by the capture of energy produced during ETC is called oxidative phosphorylation.

- ETC represents the final stage in the oxidation of carbohydrates, fats, and amino acids.
- This pathway transfers the reducing equivalents in NADH and $FADH_2$ to molecular oxygen.
- ETC and oxidative phosphorylation occur within all cells except RBCs,lack mitochondria.
- ETC and oxidative phosphorylation are most active when there is an increased need for ATP.

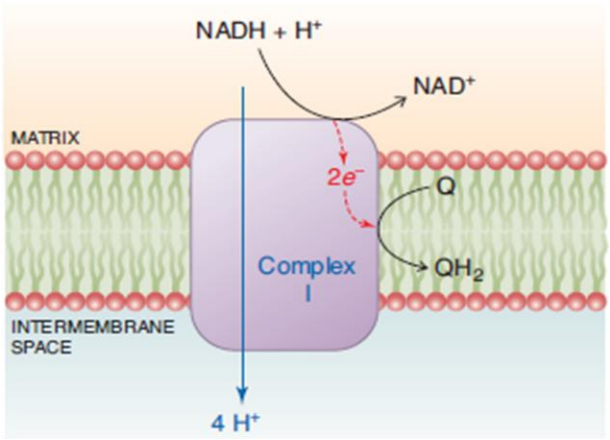
Components of Electron Transport Chain

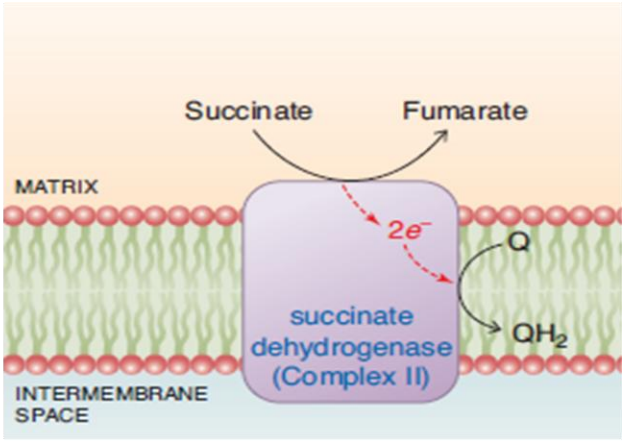
- Complexes I, III and IV span the inner mitochondrial membrane
- Complex II embedded in but does not span the inner mitochondrial membrane.
- Electrons are transferred between complexes by two smaller electron carriers: Coenzyme Q & cytochrome c

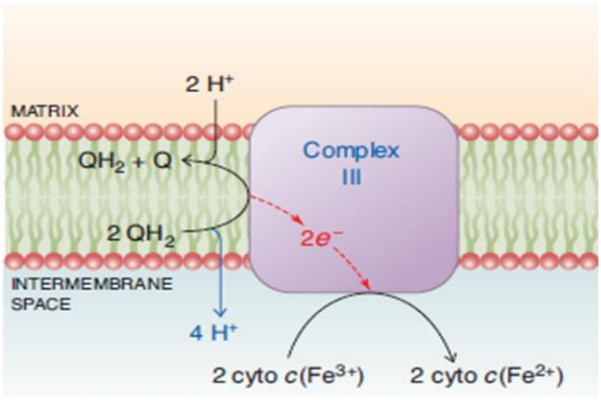
Reaction Sequences of ETC

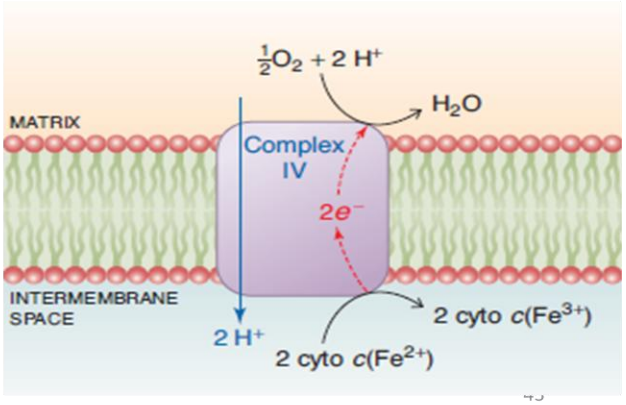
- In ETC, electrons from NADH & $FADH_2$ are passed through complex I,III,IV and II, III, IV respectively.

1. Electrons from NADH are passed to Complex I then to CoQ.
   - Complex I is also called NADH dehydrogenase
2. Electrons from $FADH_2$ are passed to In Complex II then to CoQ.
   - Complex II is also called succinate dehydrogenase
3. CoQ passes electrons to Complex III.
4. Complex III passes electrons to cytochrome c.
5. Electrons from cytochrome c are passed to complex IV.
6. Complex IV utilizes the electrons to reduce O_2 to H_2O.

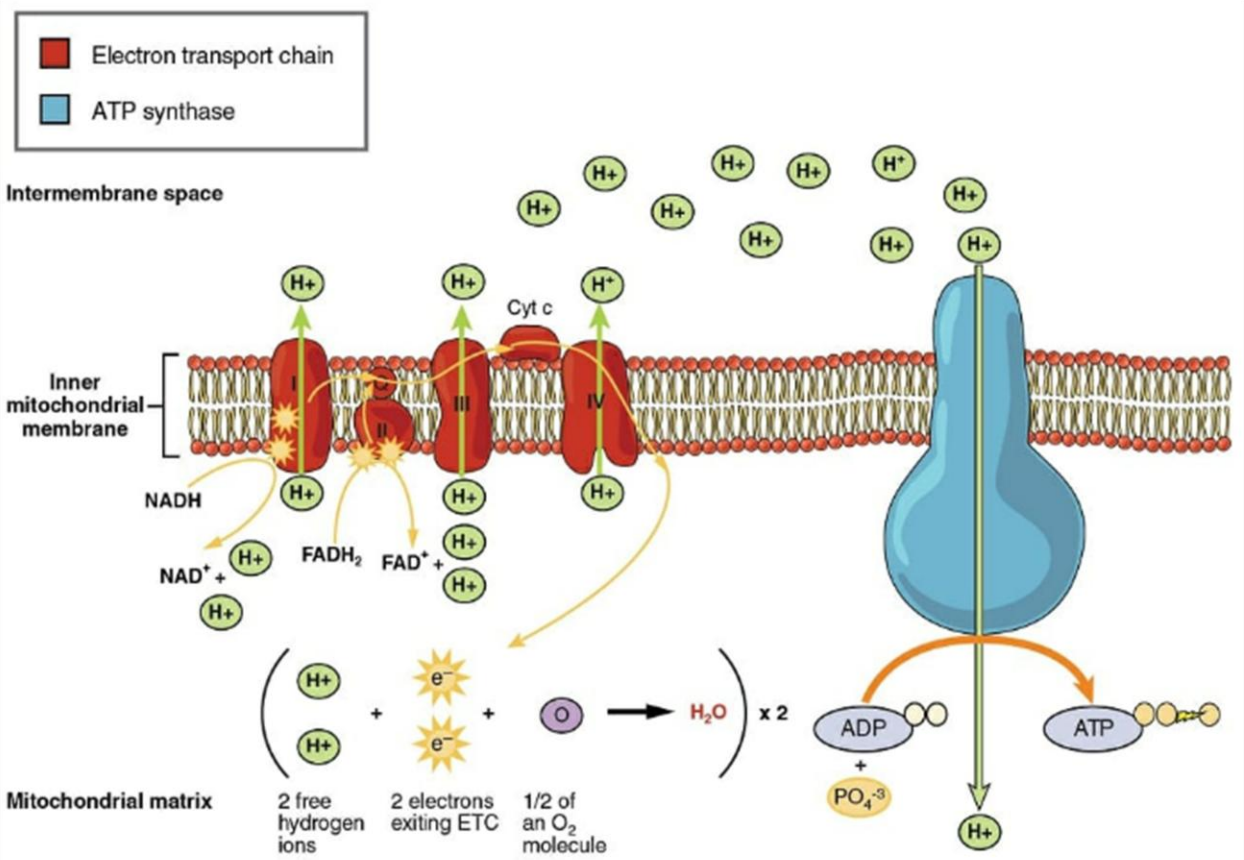








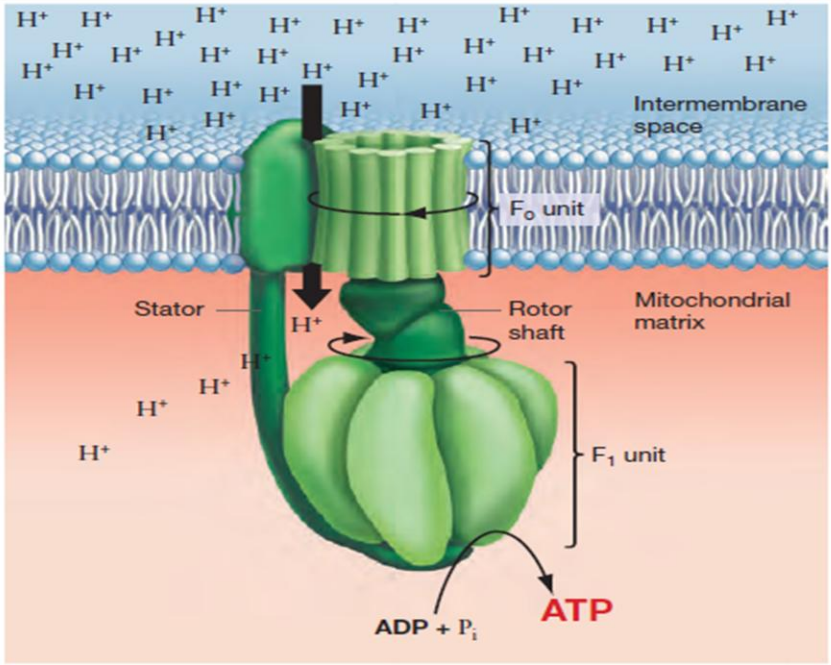
- Electron transport through complexes I, III, and IV is coupled to the transport of protons from the matrix to the intermembrane space, which <u>establishes a proton gradient</u> across the inner membrane.
  - Complex I ($4H^+$), complex III ($4H^+$) and complex IV ($2H^+$)
- Complex II does not span the inner mitochondrial membrane; so not coupled to the transport of protons.
- Then movement of protons back into the cell, down their concentration gradient, is coupled to the synthesis of ATP



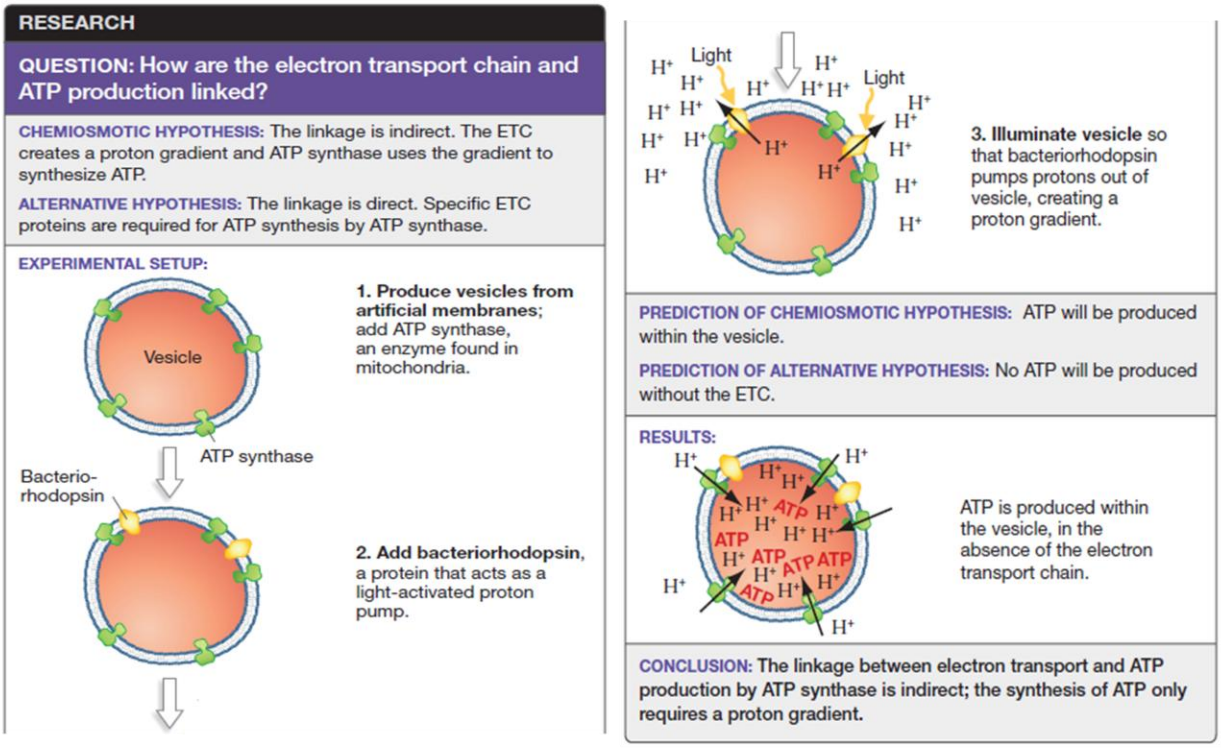
OXIDATIVE PHOSPHORYLATION

- Oxidative phosphorylation is the name given to the synthesis of ATP that occurs when NADH and $FADH_2$ are oxidized by ETC.
- Oxidative phosphorylation is the combination of oxidation and phosphorylation.
- Formation of ATP from ADP and phosphate using energy released when electrons flow in the ETC is catalyzed by membrane bound enzyme known as **ATP synthase**.
  - It is often referred as another **complex V** of ETC

- ATP synthase is organized into two structurally distinct components, F_0 and F_1,
- The **F_0 subunit** spans the inner membrane and provides a channel through which protons are able to flow back from the intermembrane space to the matrix.
  - Activated by electrochemical potential difference
- The energetically favorable return of protons to the matrix is coupled to ATP synthesis by the **F_1 subunit**, which catalyzes the synthesis of ATP from ADP and phosphate ions (P_i).
  - Because protons are electrically charged particles, the potential energy stored in the proton gradient is electric as well as chemical in nature.



Oxidative Phosphorylation involves the ATP Synthase Motor and a Proton Gradient. ATP synthase has two major components, designated Fo and F1, connected by a shaft. The Fo unit spins as protons pass through. The shaft transmits the rotation to the F1 unit, causing it to make ATP from ADP and Pi.
Its action seems just like hummingbird shown here.



Evidence for the Chemiosmotic Hypothesis. SOURCE: Racker, E., and W. Stoeckenius. 1974. Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation. ***Journal of Biological Chemistry 249: 662–663.***

Why do NADH and $FADH_2$ produces 3 and 2 ATPs respectively ?????

- The difference in ATP production by $FADH_2$ is due to bypassing the complex I of the ETC.
- The chance to pump protons is missed, which establishes less proton gradient across the inner membrane leads to less ATP formation than NADH.

Regulation of Oxidative Phosphorylation

- Oxidative phosphorylation is subjected to regulation like any metabolic pathway.
- The rate of oxidative phosphorylation depends on availability of substrates like ADP, P_i, NADH, $FADH_2$, & O_2.
- When cell has enough ATP the oxidative phosphorylation occurs at lower rate.
- When the cell is deficient in ATP rate of oxidative phosphorylation is more active.

Learning Check

1. Which of the following is a true statement?

   A. Oxidative phosphorylation & ETC are unrelated

   B. Oxidative phosphorylation drives ETC

   C. Oxidative phosphorylation relies on ETC

2. What would happen to a cell if there is no ETC?

   A. The cell would have no energy

   B. The cell would have more energy

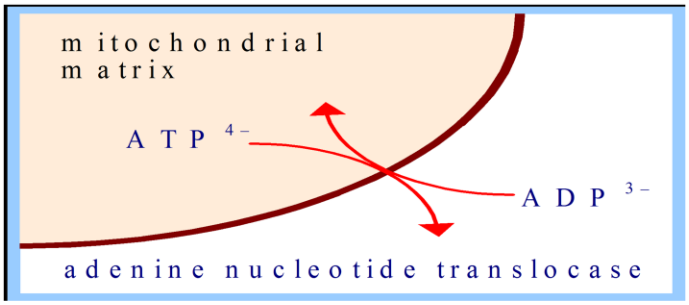
   C. The cell would have less energy

Answers

1. **C**
2. **C**

Membrane transport of ATP and ADP

- ATP and ADP do not diffuse freely across the inner mitochondrial membrane.
- A specific transport protein ADP-ATP translocase (also called adenine nucleotide translocase, ANT) is a antiporter that exchanges each ATP from the matrix for ADP from the cytosol. This transporter export ATP and import ADP
- The flow of ATP and ADP are coupled in that ADP can only enter the mitochondrial matrix if ATP exits.



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Inhibitors & Uncouples

A. Uncouplers

- Uncouplers are substances which uncouple phosphorylation of ADP from ETC
- These means that ETC continues to function, leading to oxygen consumption but phosphorylation of ADP is inhibited.
- Protons pumped out are carried by uncouplers back into the mitochondrial matrix preventing development of electrochemical gradient.
- 2,4-dinitrophenol, Pentachorophenol & Aspirin are examples of uncouplers.

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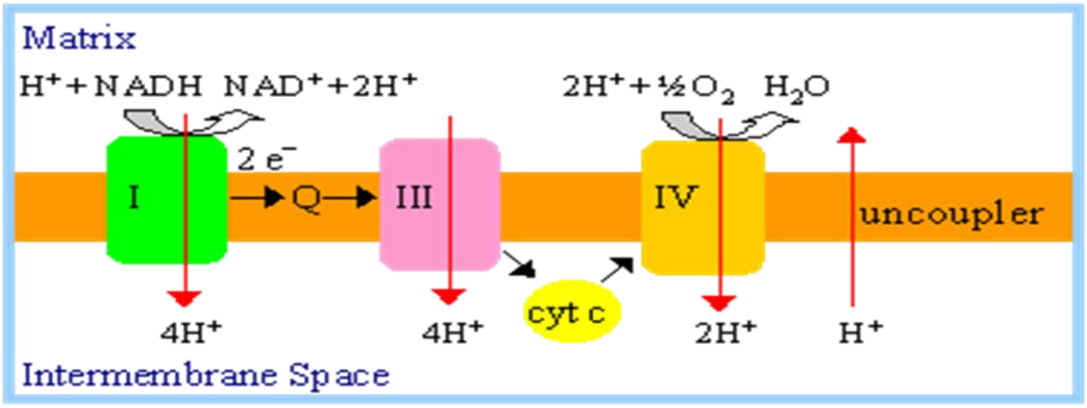
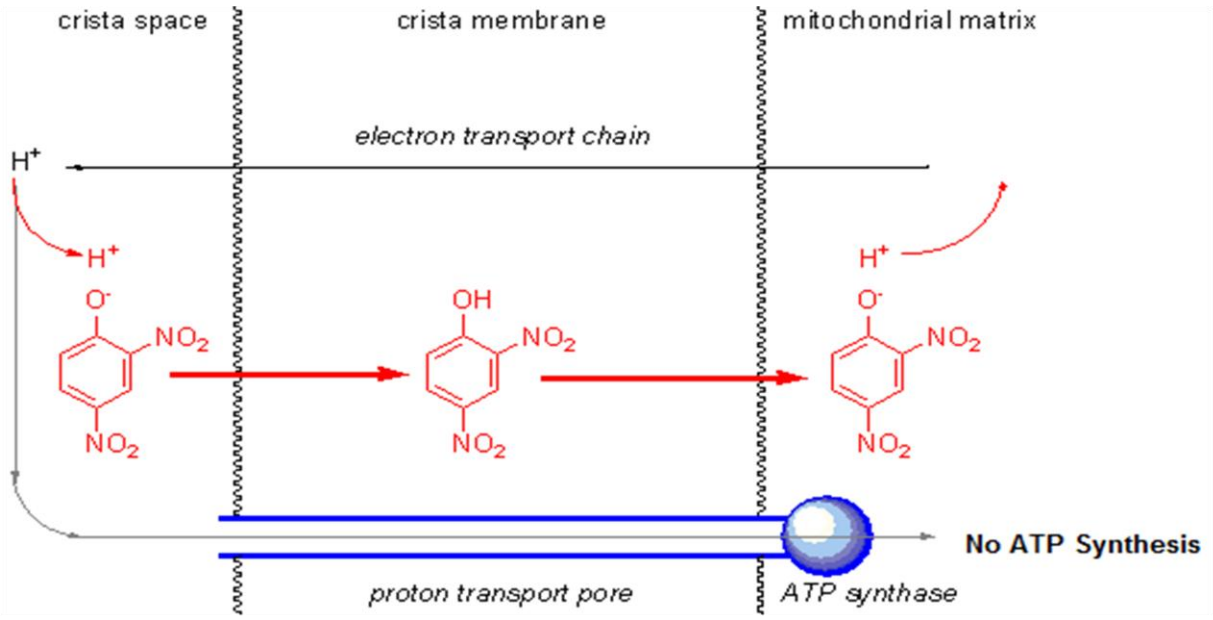
- Uncouplers are weak acids with lipophilic properties that permit them to diffuse readily across mitochondrial membranes.
- After entering the matrix in the protonated form, they can release a proton, thus dissipating the proton gradient.



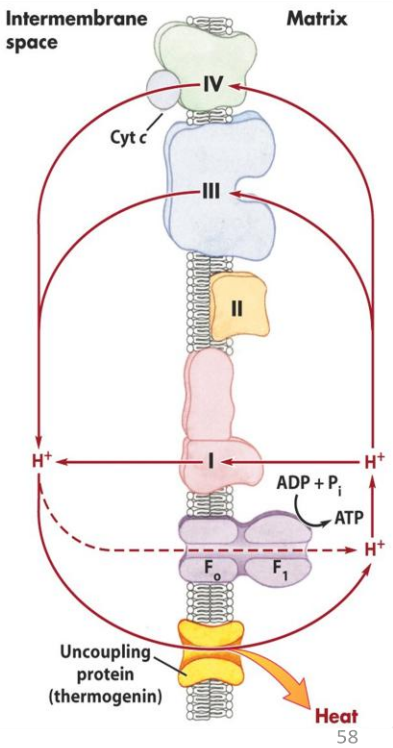
Role of uncouplers: 2,4-dinitrophenol



- In the presence of uncouplers:
  - Electron transport proceeds
  - Proton translocation by proton pumps proceeds
  - Oxygen consumption proceeds
  - Aerobic oxidations proceed without control
  - No ATP synthesis (ATP synthase not activated)

Natural Uncoupler - Thermogenin

- In brown adipose tissue, endogenous protein called thermogenin uncouples ATP synthesis from ETC.
- It is a path for protons in to matrix with out passing ATP synthase; so energy is not conserved as ATP but lost as heat.
- Its function is to generate heat especially in small mammals and hibernating animals. Thermogenin also high in neonates, lost as we grow up



B. Inhibitors

- Chemicals that inhibit the ETC or oxidative phosphorylation at specific points are called inhibitors.
- Several drugs, poisons and toxins work act as inhibitors.

Inhibitor	Complex inhibited
Rotenone	Complex I
Amytal	Complex II
Antimycin A	Complex III
Cynide	Complex IV
Carbon monoxide	Complex IV
Oligomycin	Complex V

P/O Ratio

- P/O ratio is the no of ATP formed in oxidative phosphorylation per two electrons flowing through a defined segment of ETC, terminated by reduction of an O_2.
- Ratio depends on the ratio of H transported out of the matrix per two electrons passed from NADH/FADH2 to 1/2 O2 in ETC and on the number of H pass through ATP synthase to synthesize ATP.
- Most experiments have yielded P/O ratios of between 2 and 3 when NADH used and between 1 and 2 when $FADH_2$ used.
- There is a strong evidence now to suggest a P/O of 2.5 for NADH, and 1.5 for FADH2.

- 4 protons are translocated by complex I, 4 by complex III, and 2 by complex IV. Thus, for each pair of electrons that pass through these complexes from NADH to O2 a total of 10 protons are moved across the membrane while 6 protons for FADH2
- Since four protons are moved back across the membrane for each molecule of cytoplasmic ATP,
- The P/O ratio for NADH is 10 ÷ 4 = 2.5. The P/O ratio for FADH2 is 6 ÷ 4 = 1.5

$$\left(\frac{1\ \text{ATP}}{4\ \text{H}^+}\right)\left(\frac{10\ \text{H}^+}{2\ e^-\ [\text{NADH}\rightarrow\frac{1}{2}\text{O}_2]}\right) = \frac{10}{4} = \frac{\text{P}}{\text{O}}$$

The role of vitamins in carbohydrate metabolism

- Vitamins are needed for generating energy from macromolecules
- Do not directly produce energy; often function as coenzymes
- B-vitamins are particularly important in assisting energy metabolism

Vitamin	Role
Thiamine	Carrier of activated aldehyde group
Riboflavin	Part of FADH2
Niacin	Part of NADH
Pantothenic acid	Part of CoA
Biotin	Coenzyme in gluconeogenesis
Pyridoxal	Coenzyme in glycogenolysis

READING ASSIGNMENT

- RAPAPORT – LEUBERING CYCLE & ITS SIGNIFICANCE
- EFFECT OF ETHANOL ON GLUCONEOGENESIS SUBSTRATES