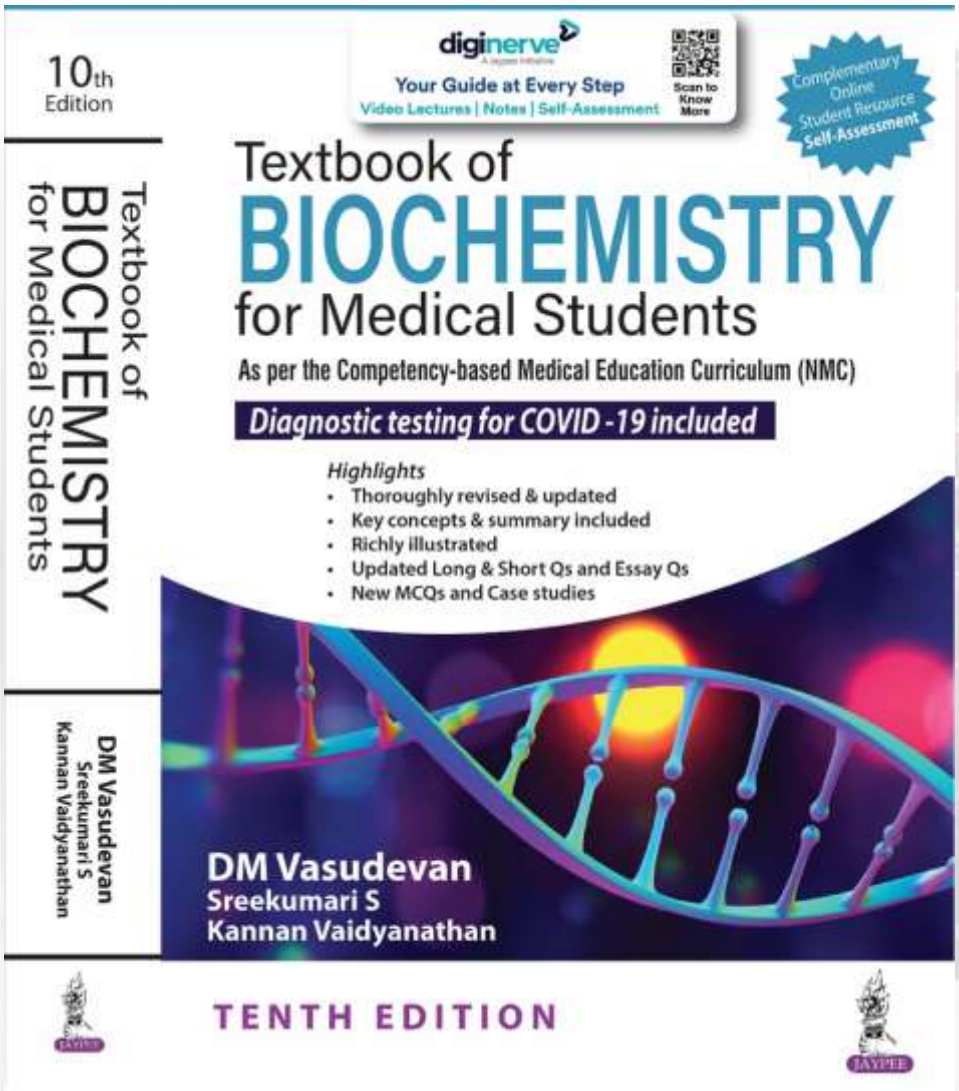


Chapter 07:

Citric acid cycle, Electron transport chain

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

Stages of Oxidation of Foodstuffs

First Stage

Digestion in the gastrointestinal tract converts the macromolecules into small units. This is called **primary metabolism.**

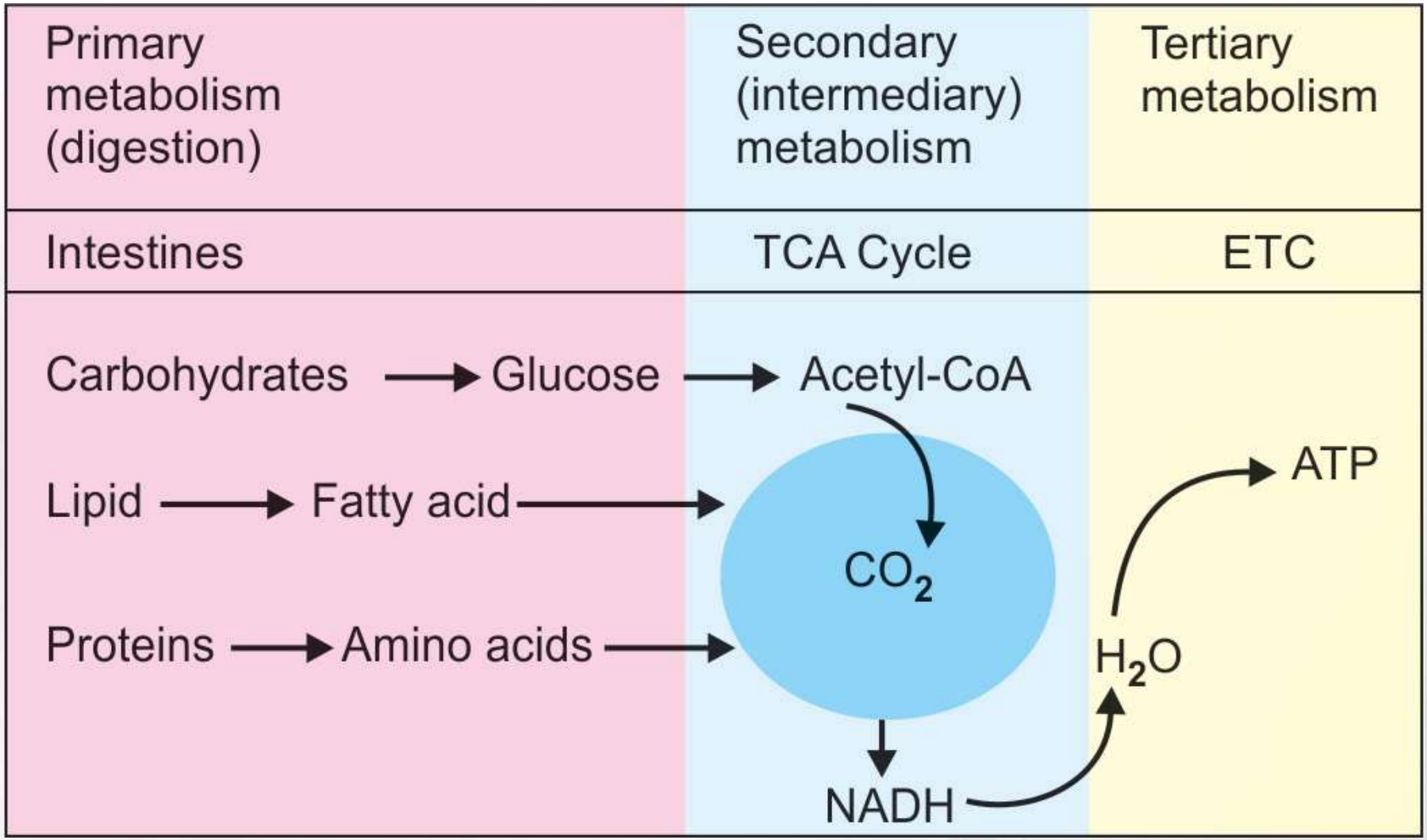
Second Stage

The products of digestion are absorbed, catabolized to smaller components, and ultimately oxidized to CO_2 in the citric acid cycle. In this process, NADH and $FADH_2$ are generated. This is called **secondary or intermediary metabolism**.

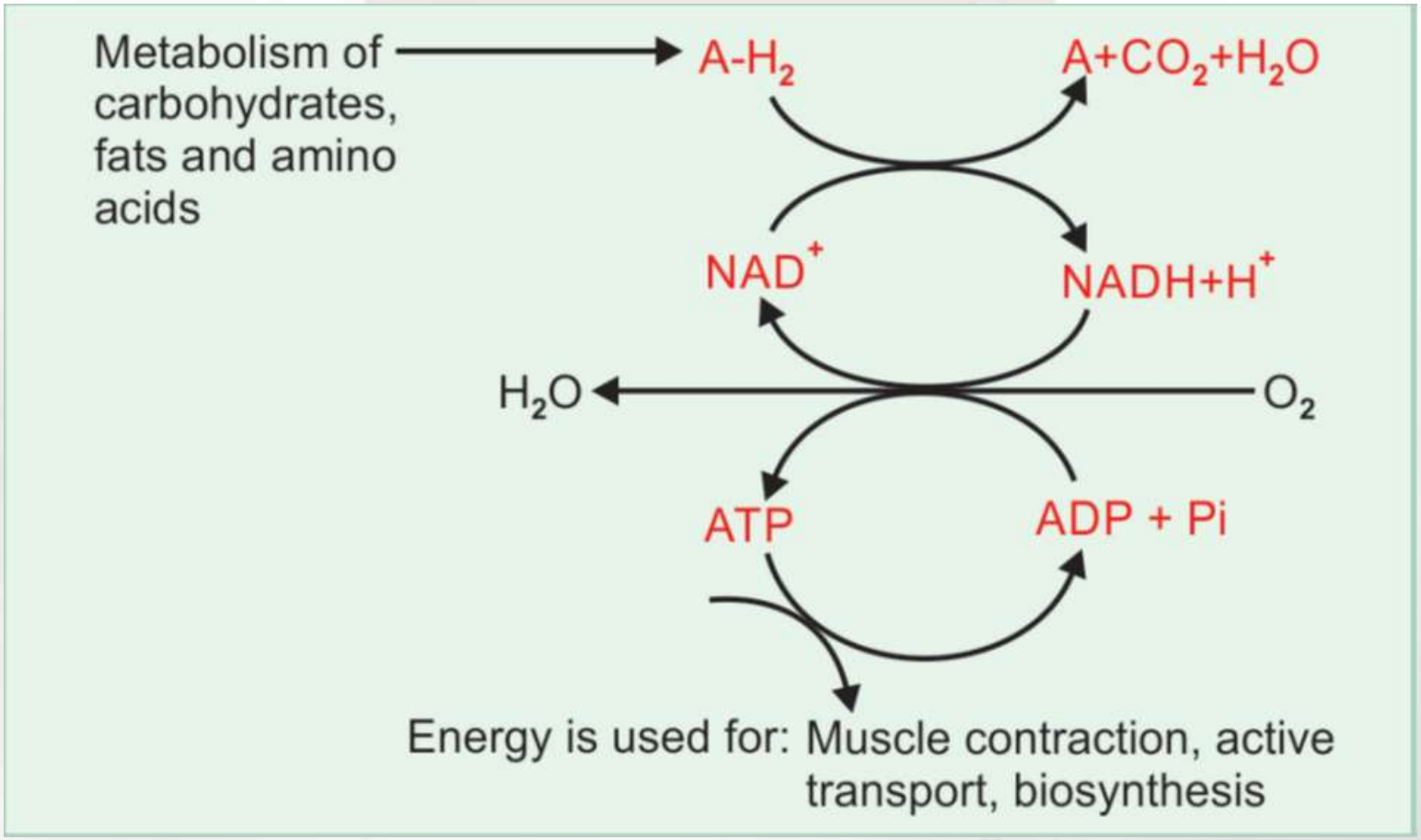
Third Stage

These reduced equivalents (NADH and $FADH_2$) enter into the **electron transport chain** (ETC), or **Respiratory** chain, where energy is released. This is the **tertiary metabolism** or **internal respiration** or cellular respiration

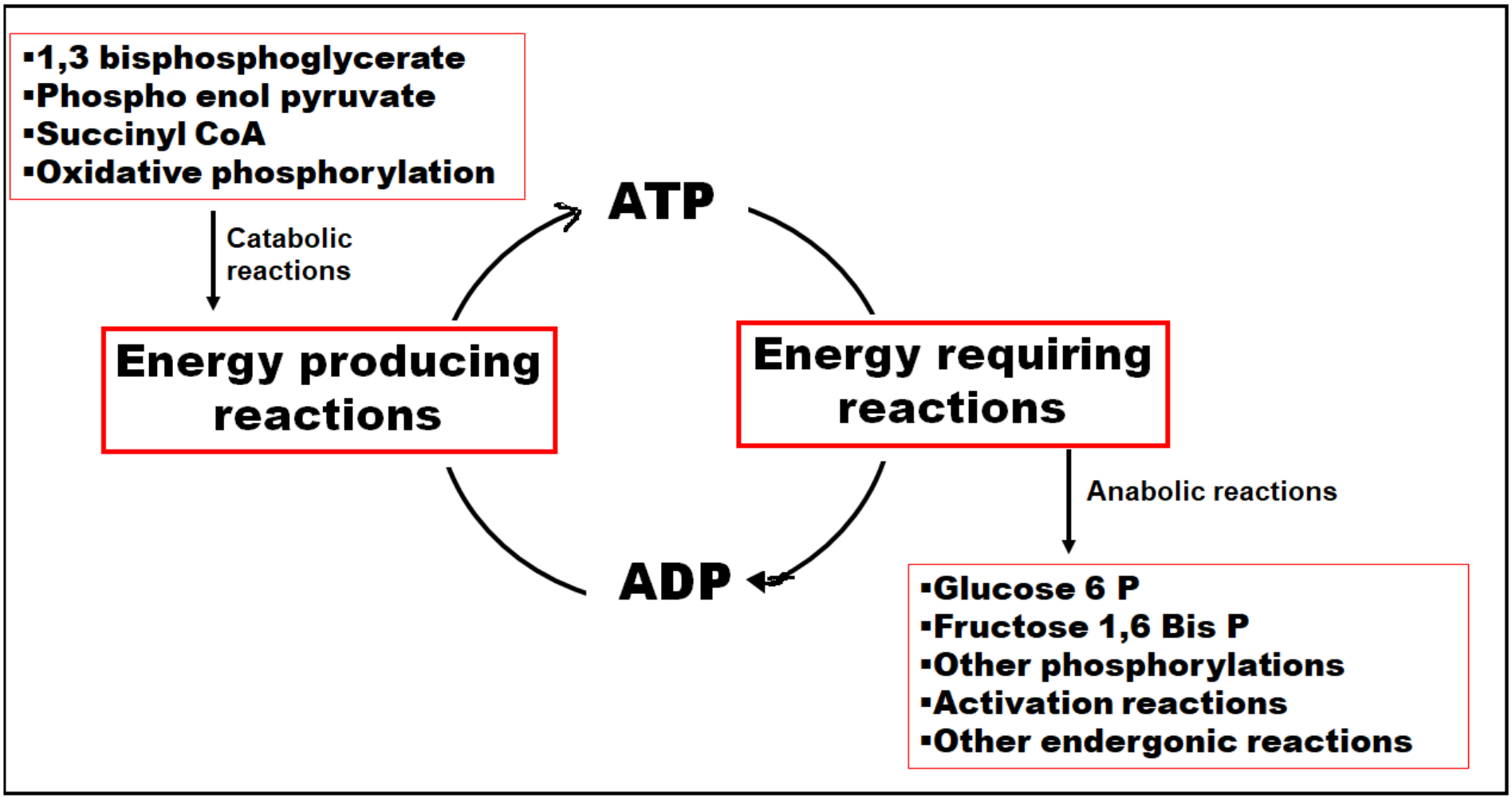
Oxidation of Foodstuffs in Three Stages



Energy from food is trapped as ATP

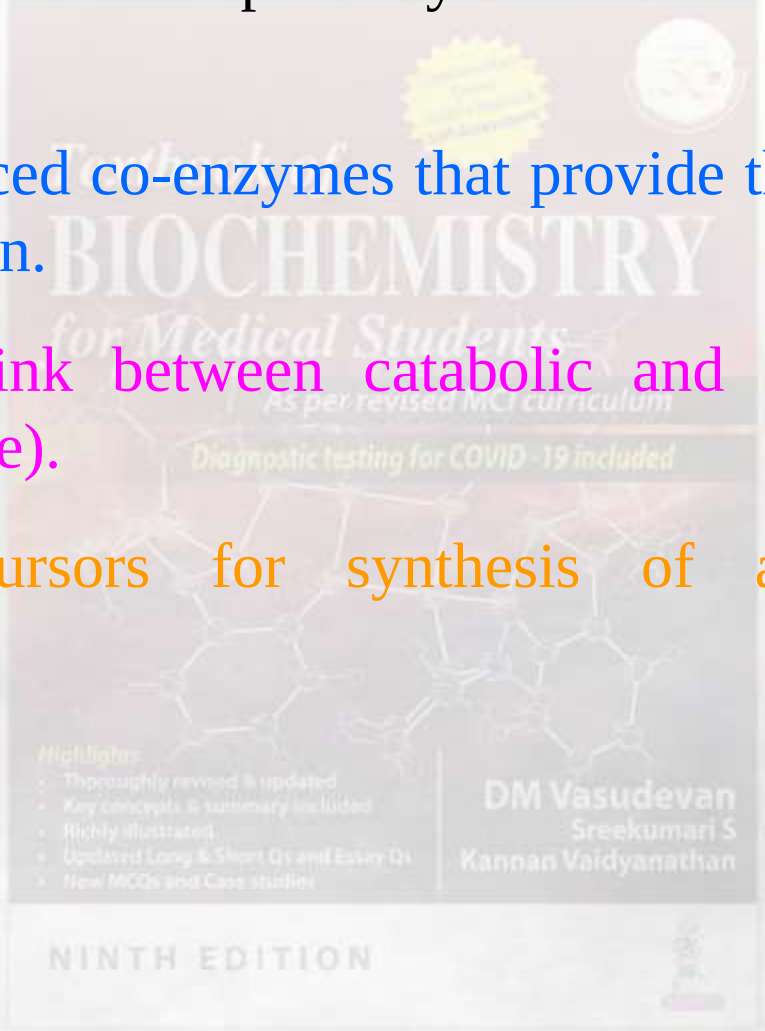


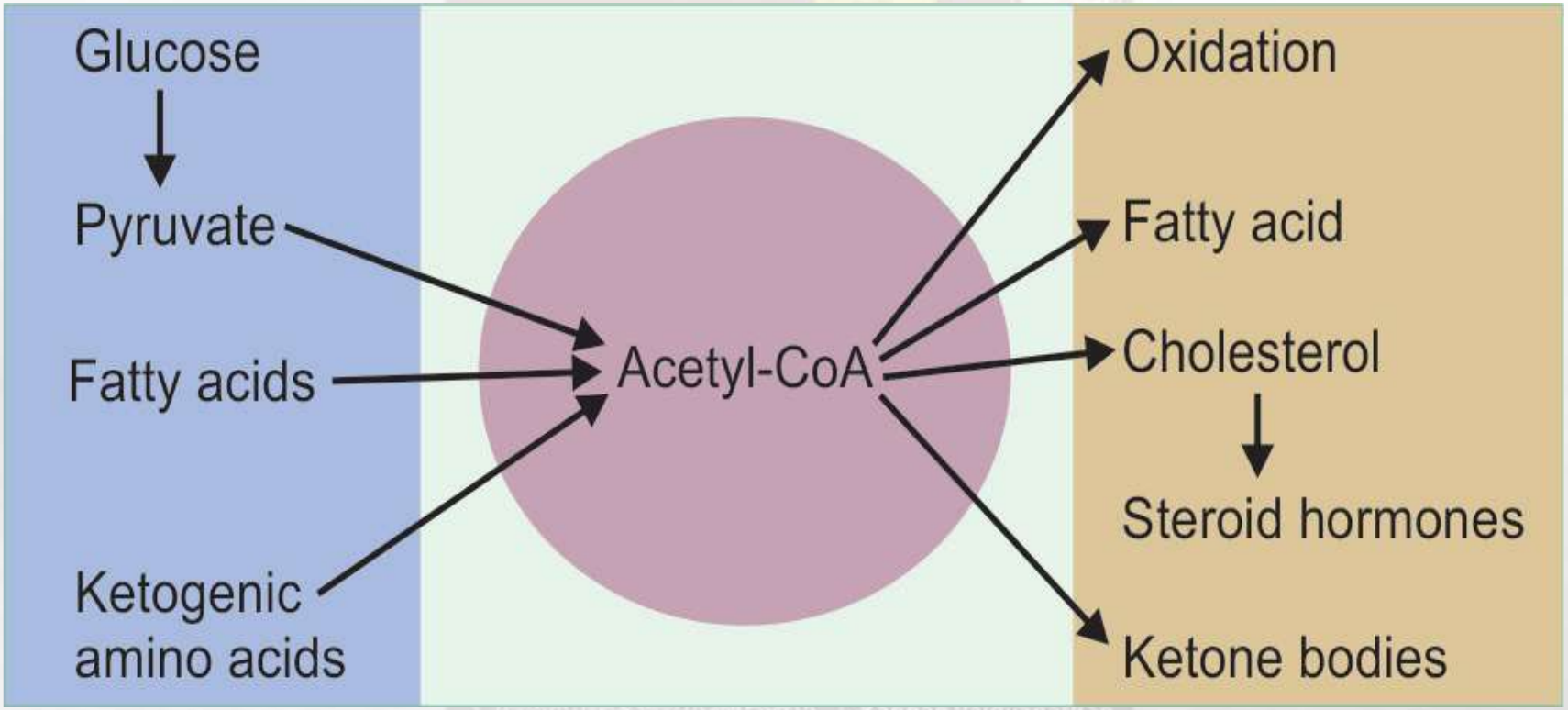
ATP hydrolysis is used to provide energy



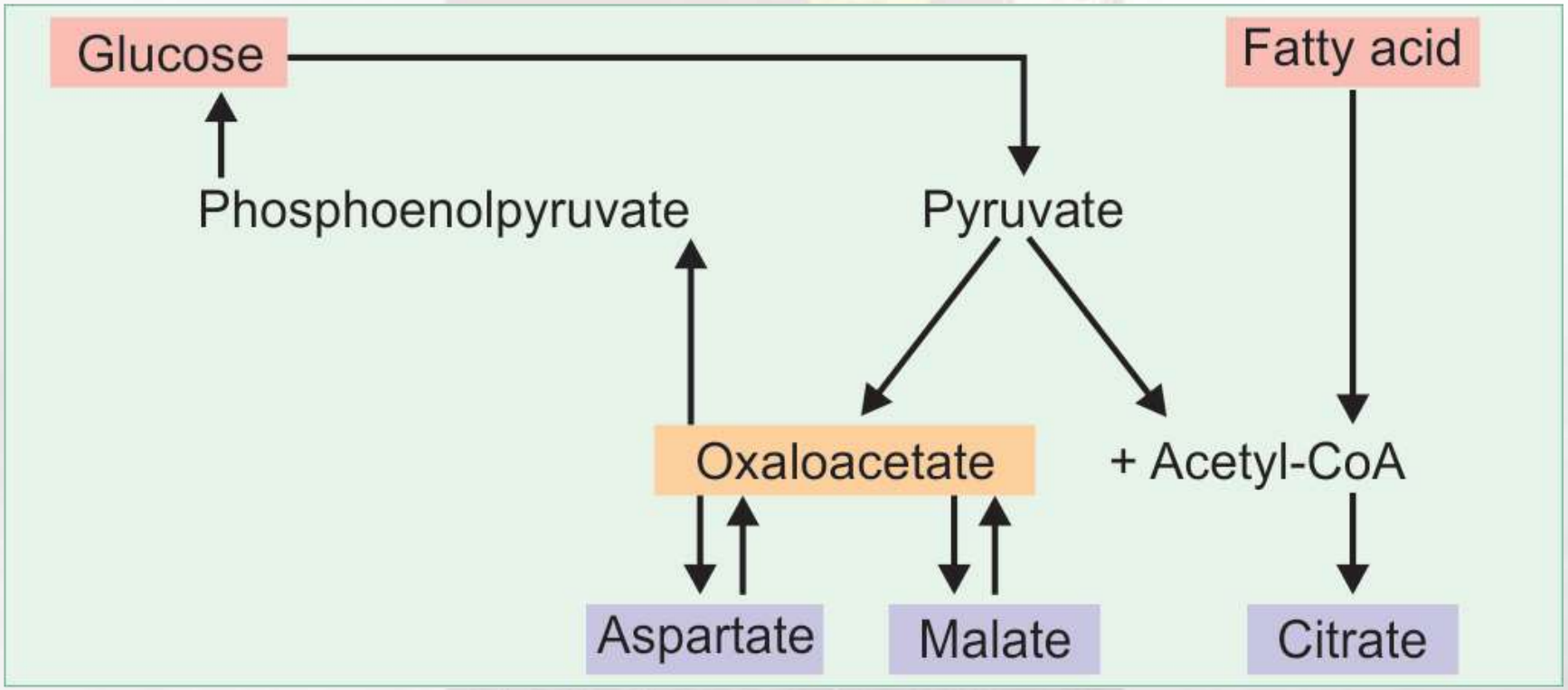
Functions of the Citric Acid Cycle

1. Final common oxidative pathway that oxidises acetyl CoA to CO2.
2. **S**ource of reduced co-enzymes that provide the substrate for the respiratory chain.
3. It acts as a link between catabolic and anabolic pathways (amphibolic role).
4. Provides precursors for synthesis of amino acids and nucleotides.

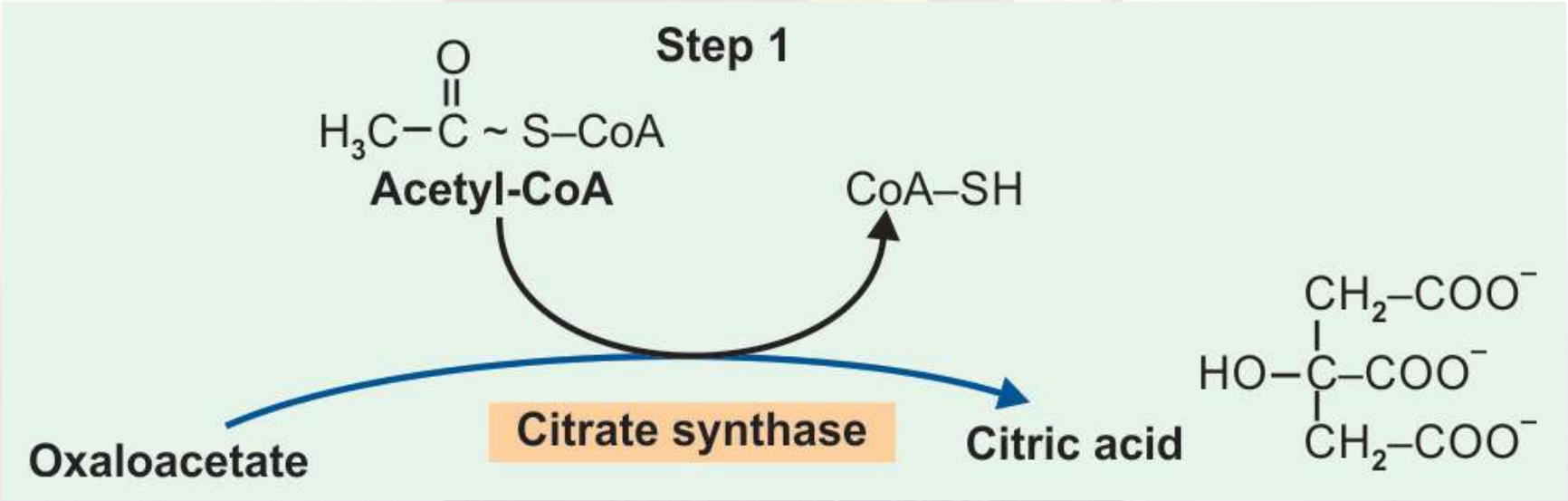




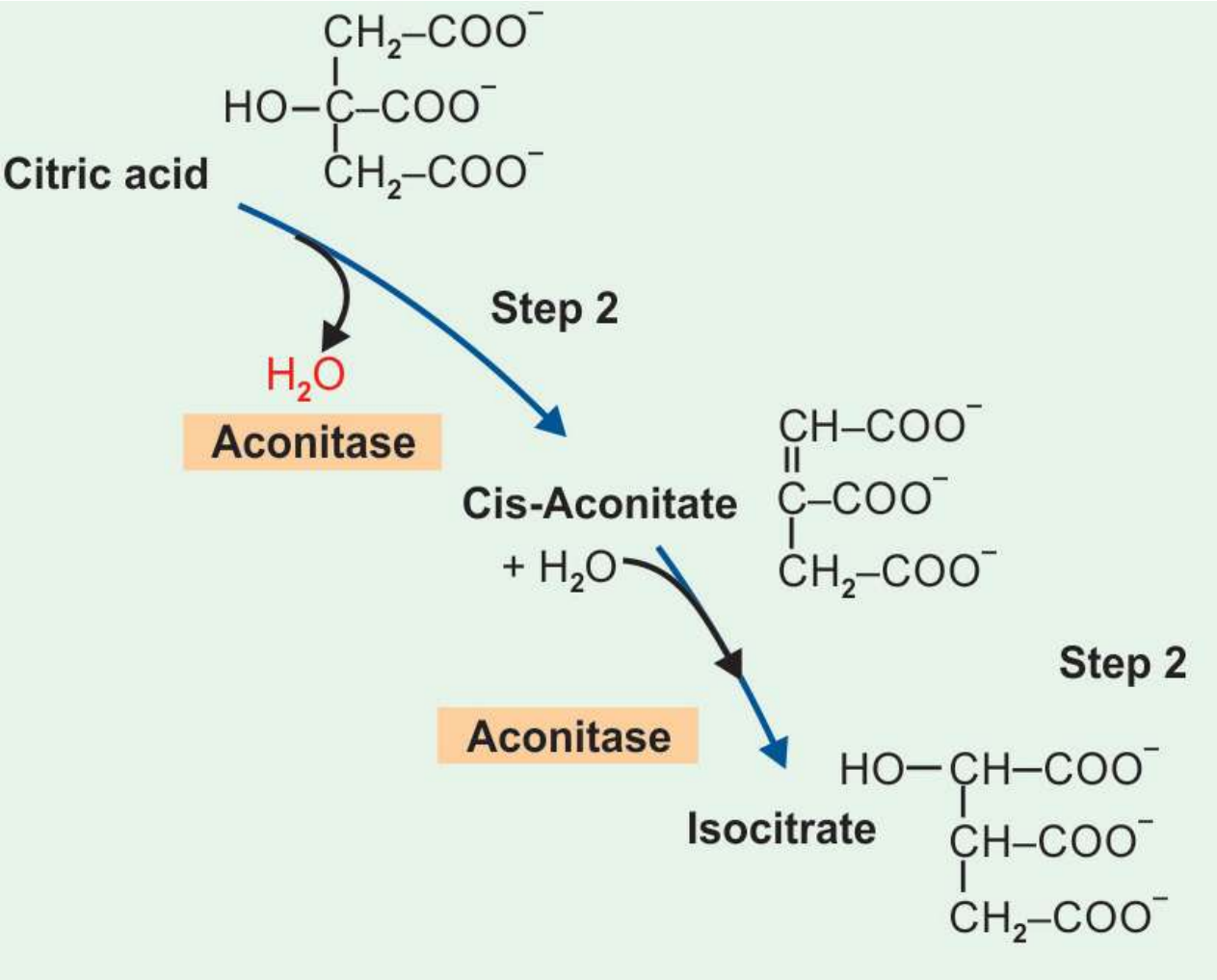
Sources and utilization of acetyl-CoA.



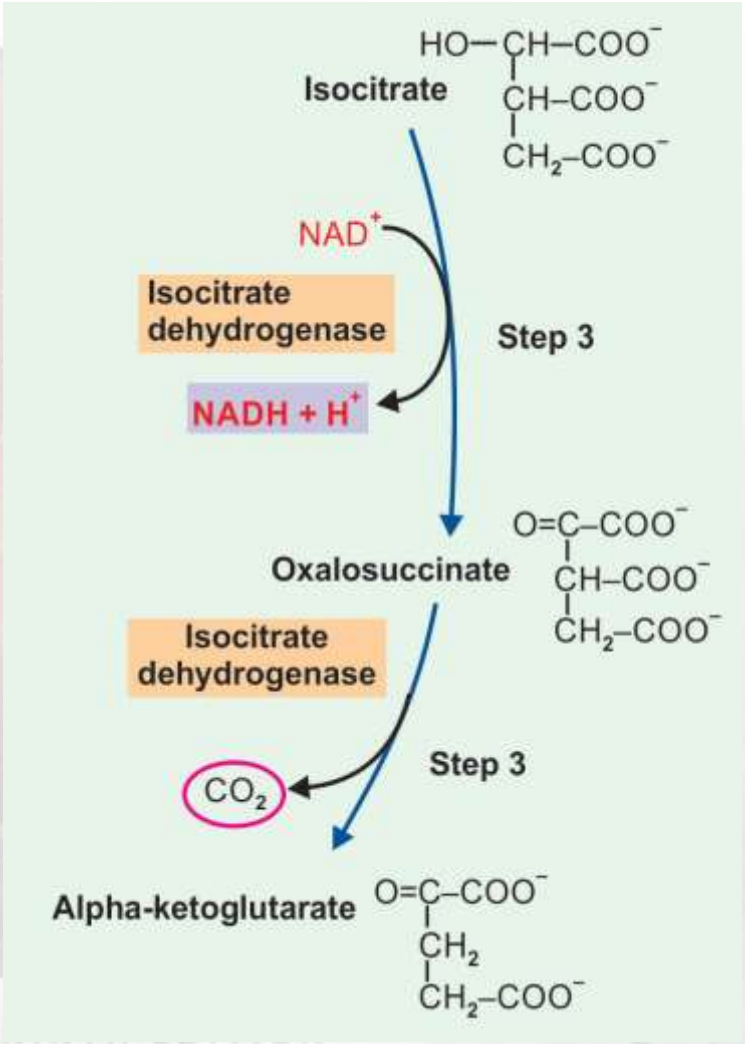
Reactions of oxaloacetate



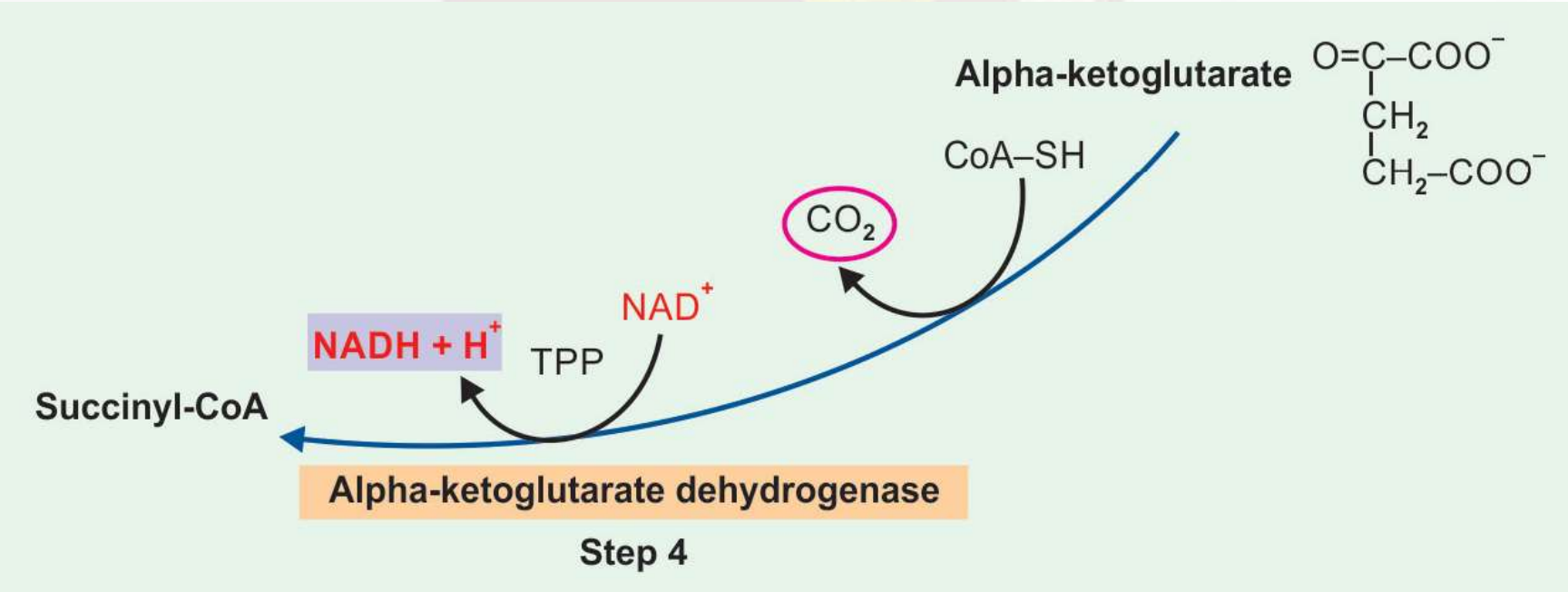
Step 1 of citric acid cycle.



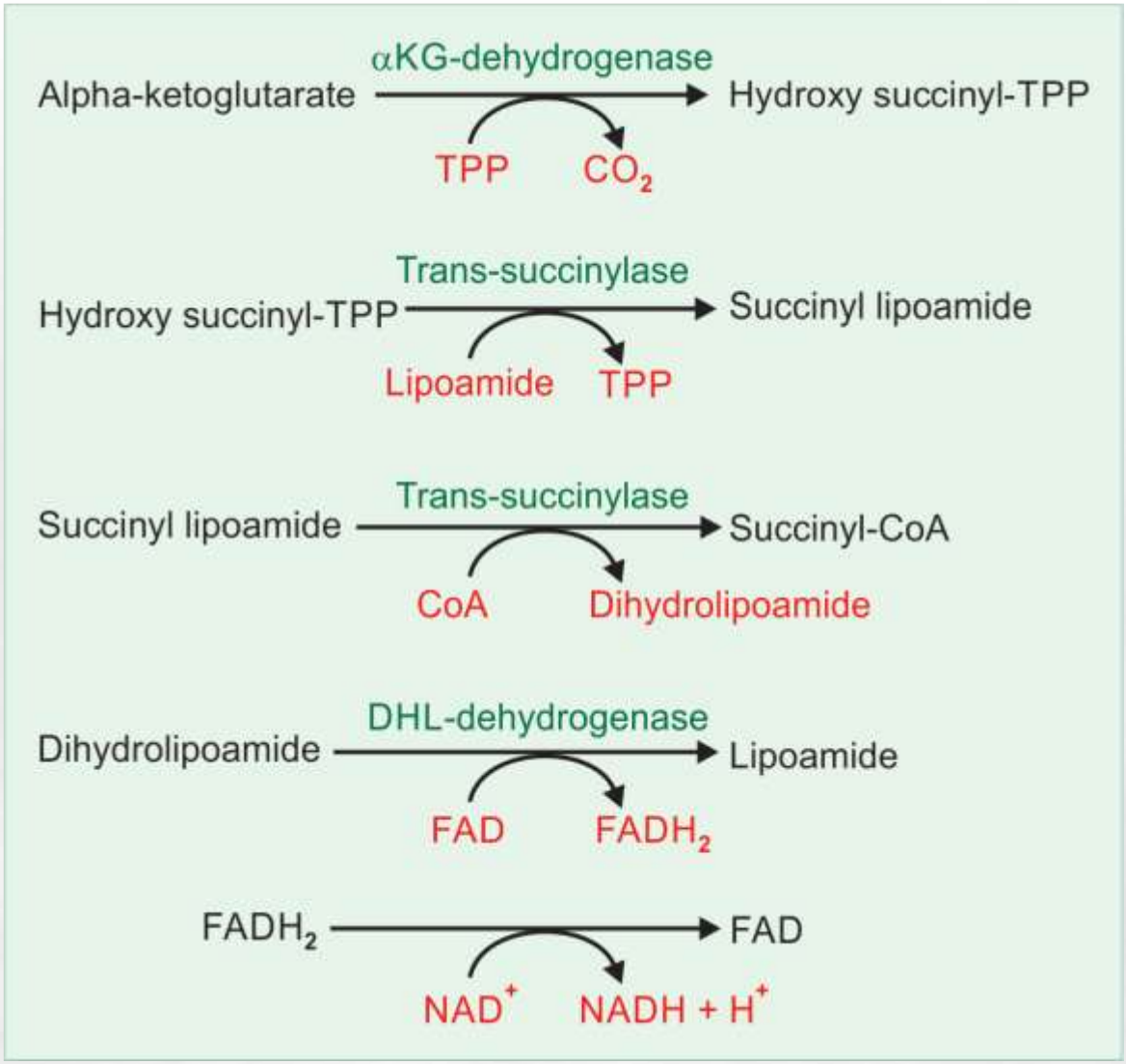
Step 2 of citric acid cycle.

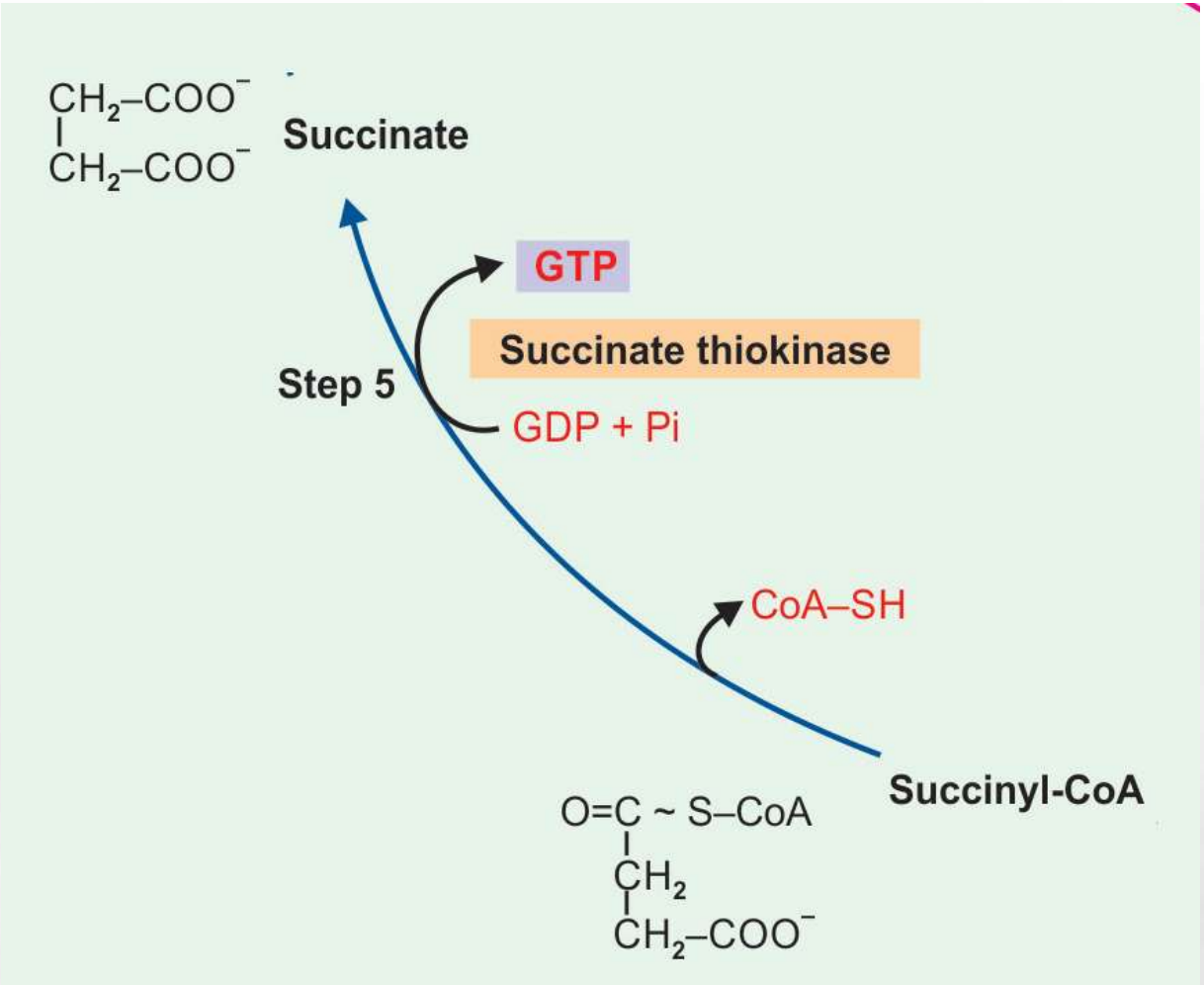


Step 3 of citric acid cycle.

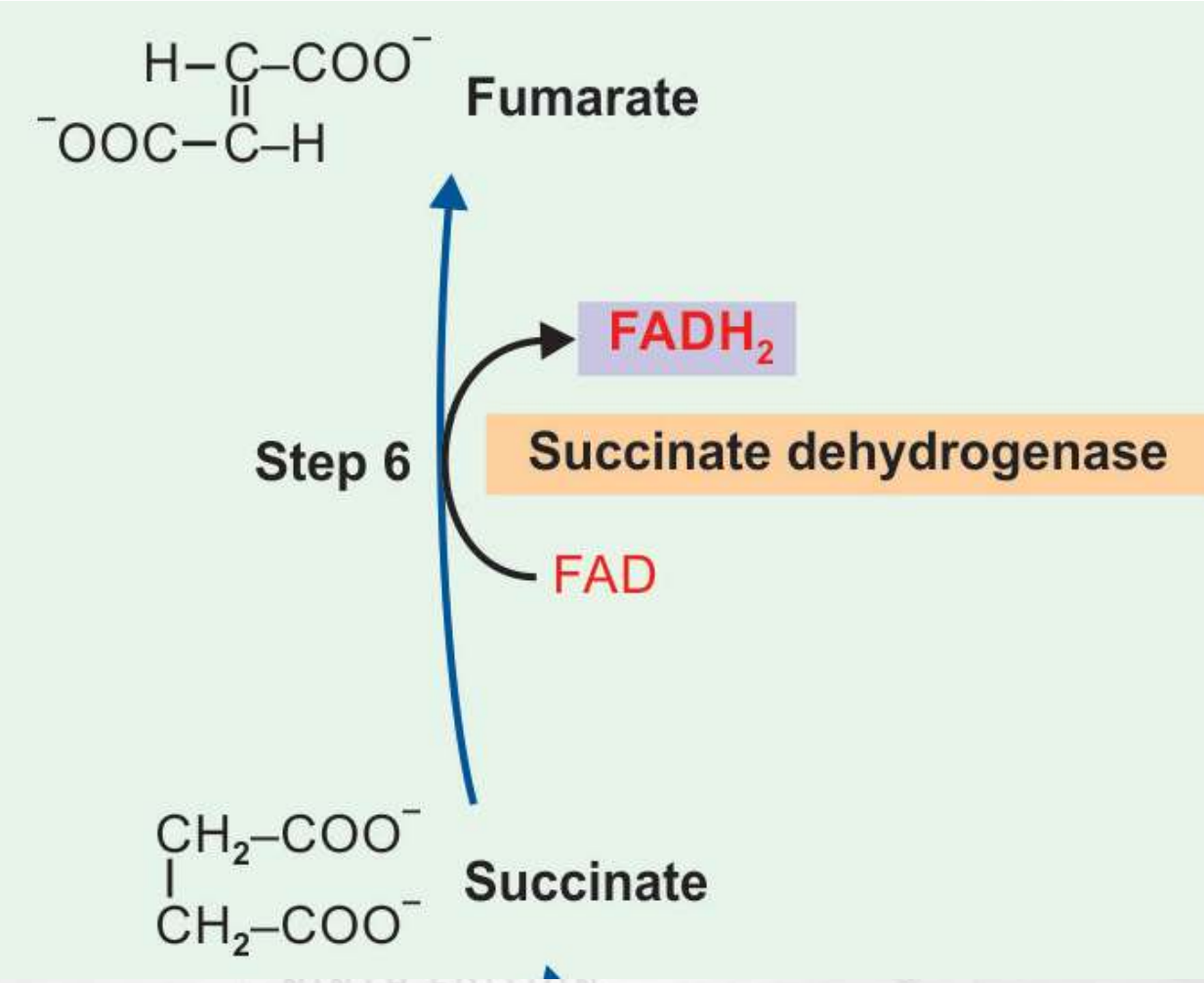


Step 4 of citric acid cycle.

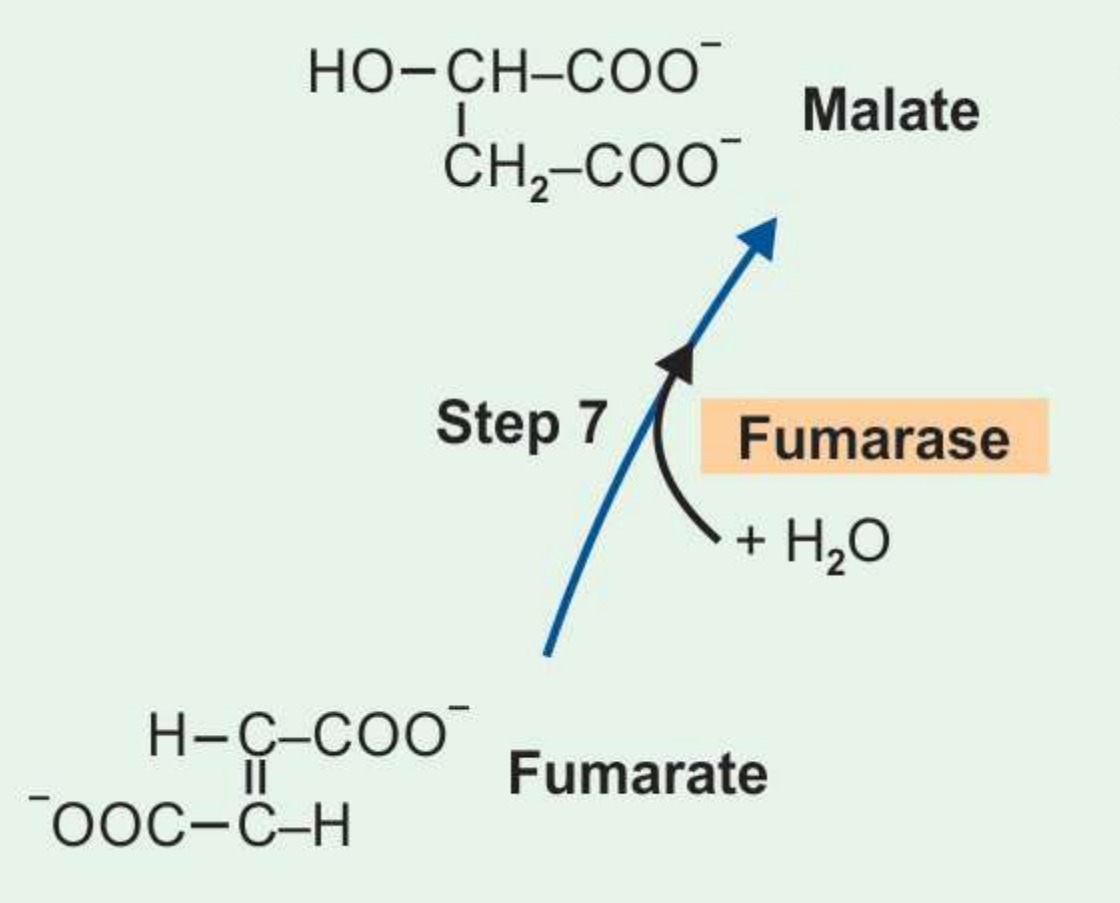

αKG-dehydrogenase
Alpha-ketoglutarate
Hydroxy succinyl-TPP
TPP
CO_2
Trans-succinylase
Hydroxy succinyl-TPP
Succinyl lipoamide
Lipoamide
TPP
Trans-succinylase
Succinyl lipoamide
Succinyl-CoA
CoA
Dihydrolipoamide
DHL-dehydrogenase
Dihydrolipoamide
Lipoamide
FAD
$FADH_2$
$FADH_2$
FAD
NAD^+
$NADH + H^+$



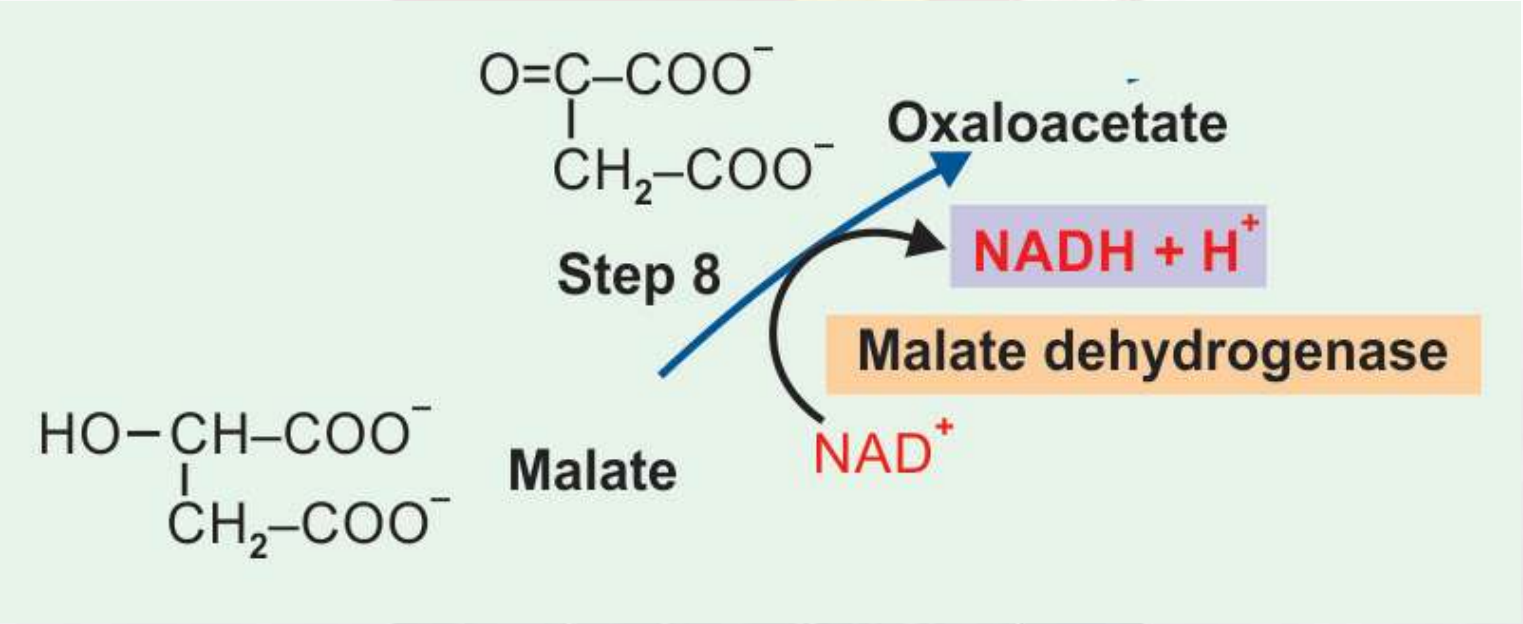
Step 5 of citric acid cycle.



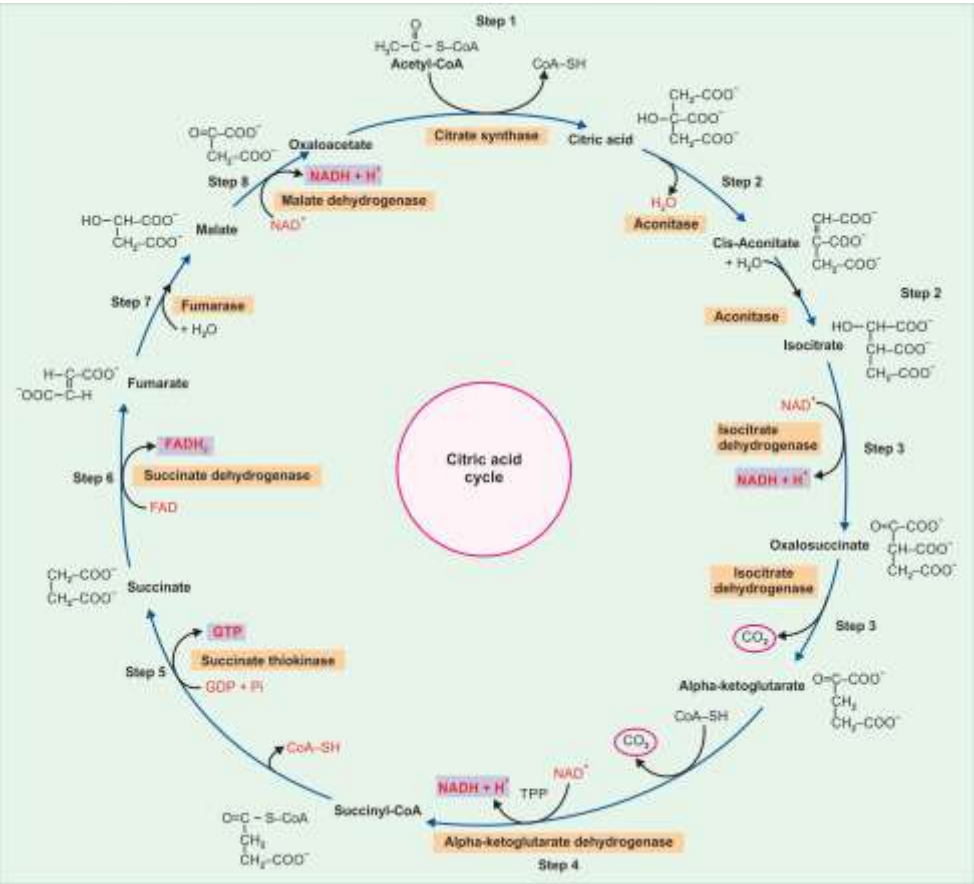
Step 6 of citric acid cycle.



Step 7 of citric acid cycle.



Step 8 of citric acid cycle.



Acetyl-CoA (2 carbon), enters the cycle. These carbon atoms are released as CO2 in the steps 3 and 4. So acetyl-CoA is completely oxidized by the time the cycle reaches alpha-ketoglutarate.

PK

Glucose → Pyruvate → Acetyl Co A

↓

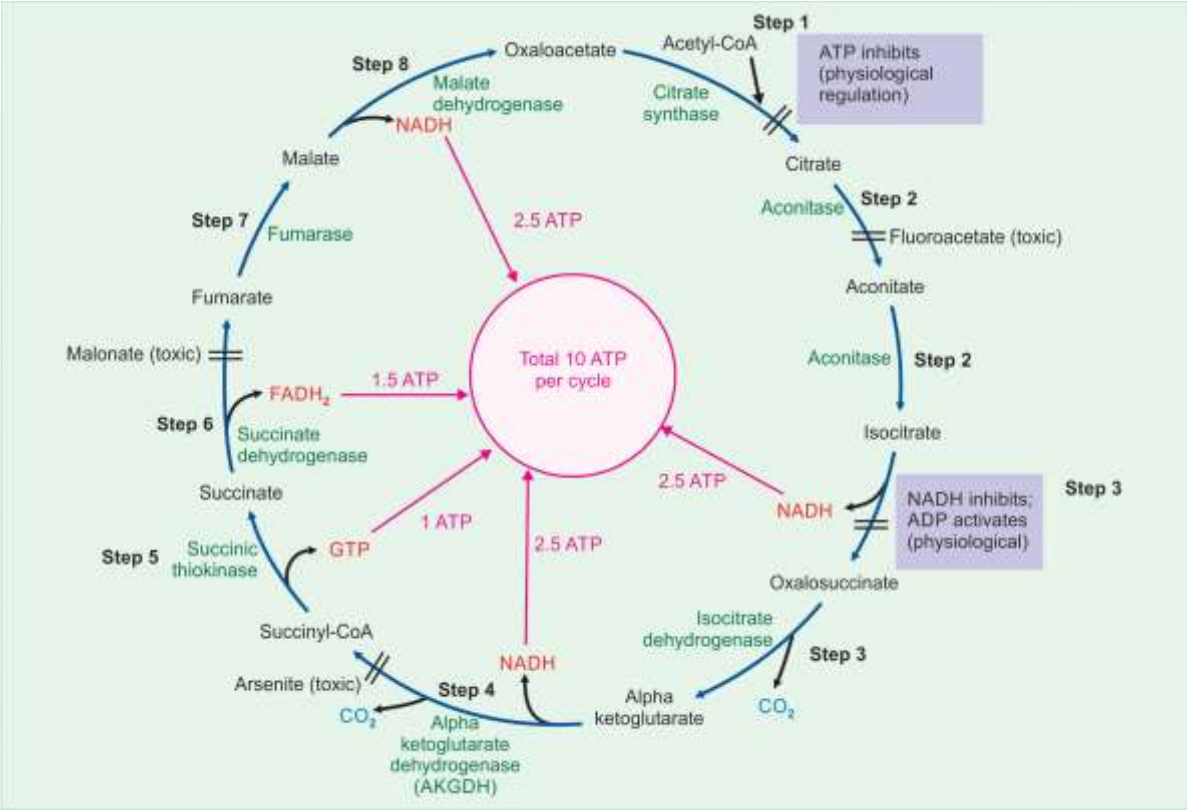
Fatty acid

Fatty acid → Acetyl Co A → CO_2

~~↓~~

Glucose

No net synthesis of carbohydrates from fat



Physiological regulatory steps are: step no.1 (citrate synthase) is physiologically inhibited by ATP. Step 3 (ICDH) is inhibited by NADH and activated by ADP. Steps where energy is trapped are marked with the coenzyme and the number of ATP generated during that reaction. A total of 10 ATPs are generated during one cycle.

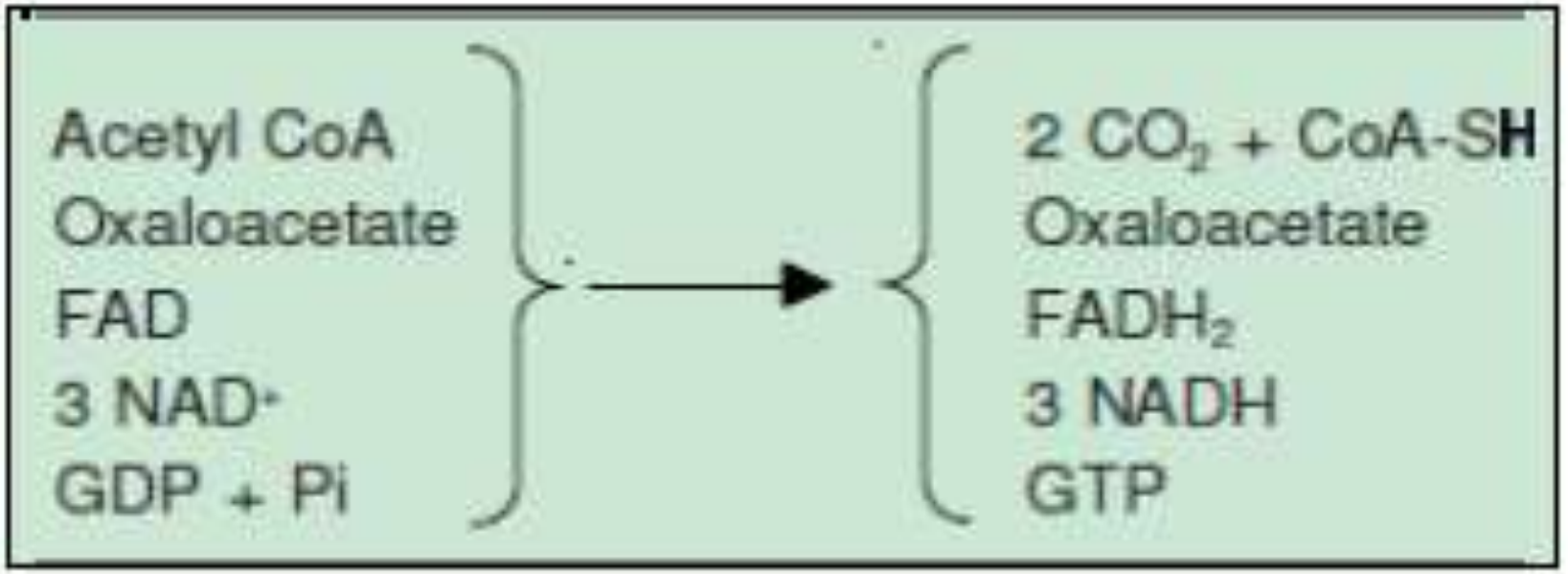
ATP Generation Steps of Citric Acid Cycle

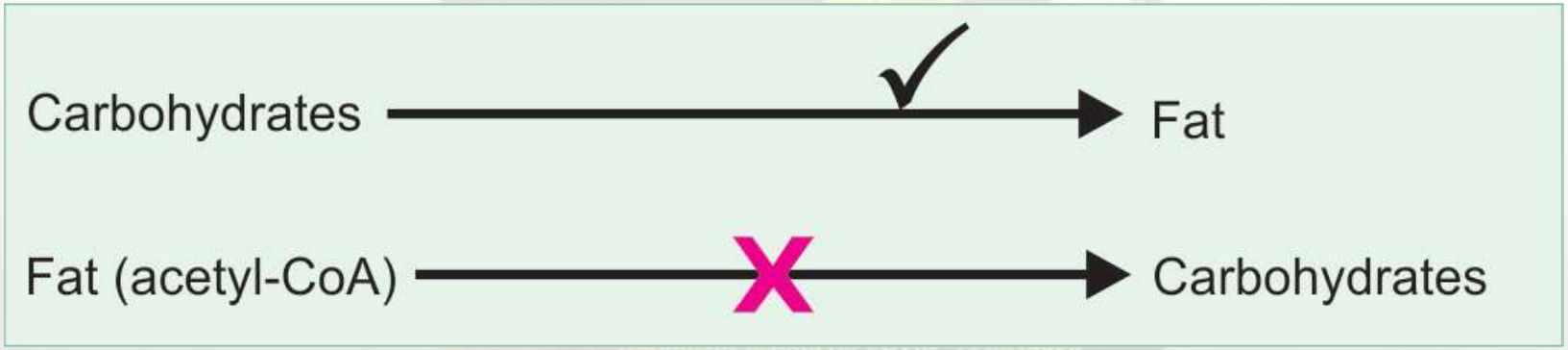
Step No.	Reactions no.	Co-enzyme	ATPs generated
3	Isocitrate → alpha ketoglutarate	NADH	2.5
4	Alpha ketoglutarate → succinyl CoA	NADH	2.5
5	Succinyl CoA → Succinate	GTP	1
6	Succinate → Fumarate	FADH2	1.5
8	Malate → Oxaloacetate	NADH	2.5
		Total	10

Significance of Citric Acid Cycle

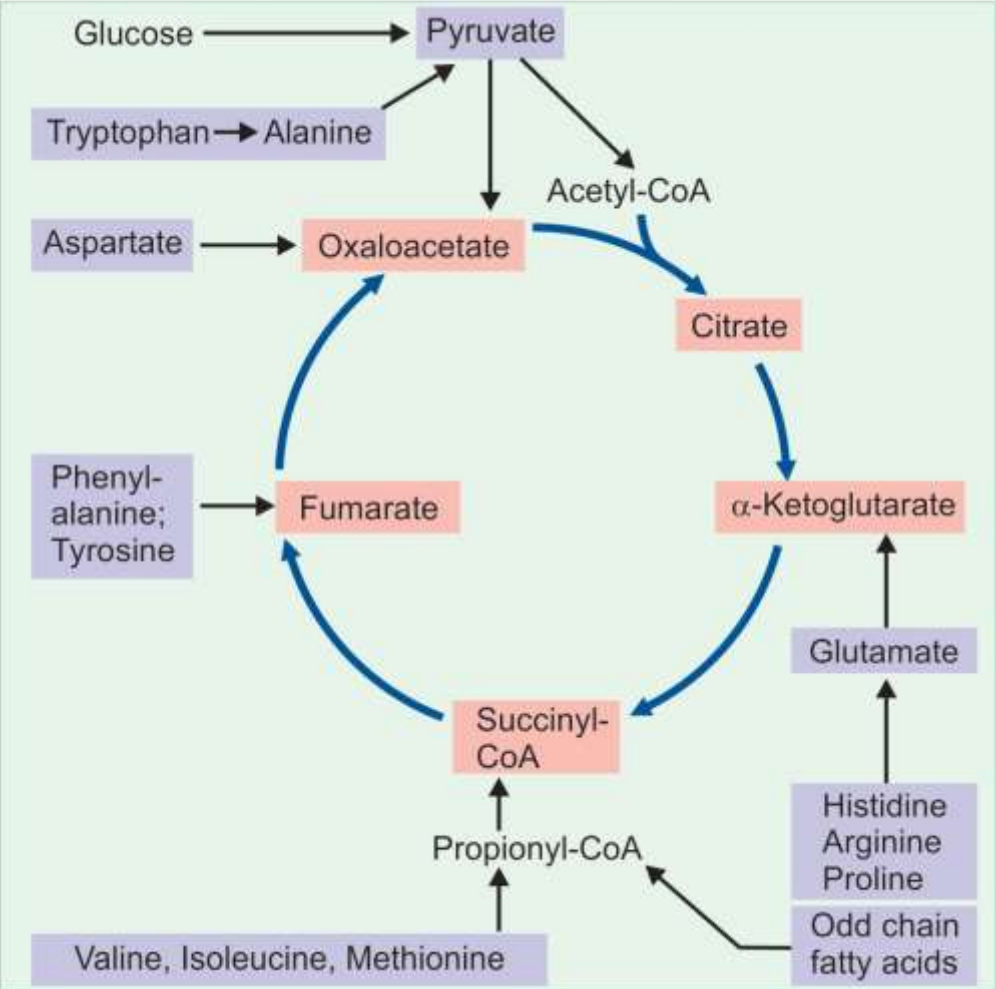
1. Complete oxidation of acetyl-CoA
2. ATP generation
3. Final common oxidative pathway
4. Integration of major metabolic pathways
5. Fat is burned on the wick of carbohydrates
6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle
9. Amphibolic pathway
10. Anaplerotic role

Stoichiometry of the Citric Acid Cycle

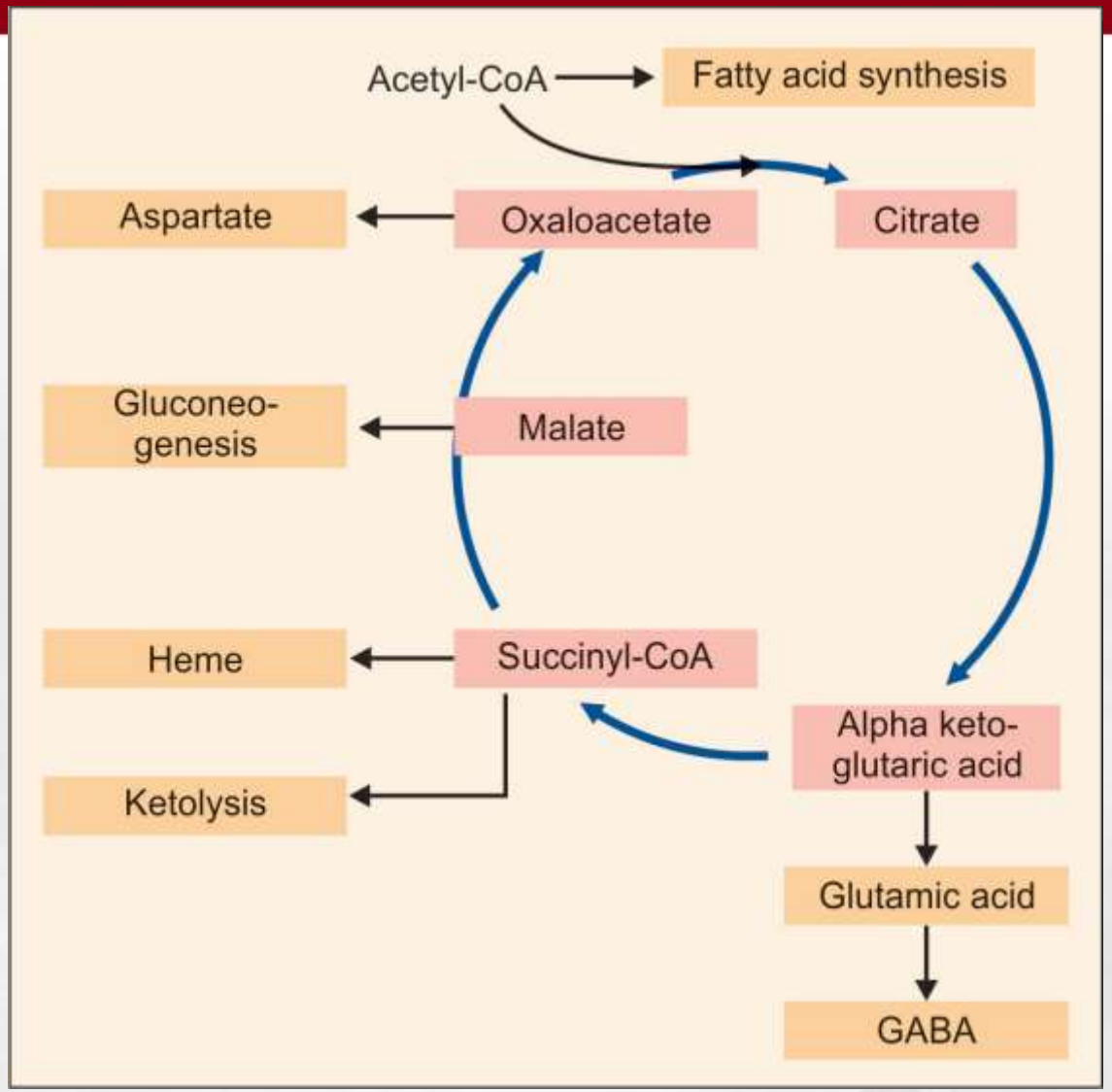




Fat cannot be converted to glucose



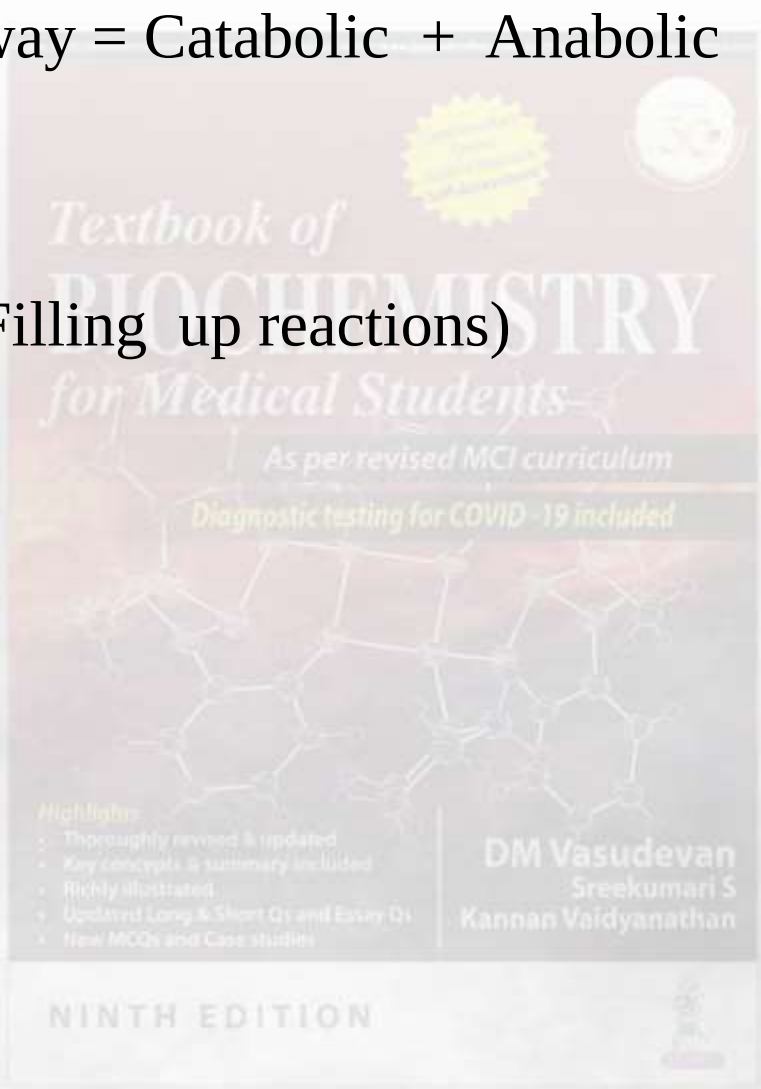
Influx of TCA cycle intermediates. Glucogenic amino acids entering the cycle are shown in violet boxes



Efflux of TCA cycle intermediates

Amphibolic pathway = Catabolic + Anabolic

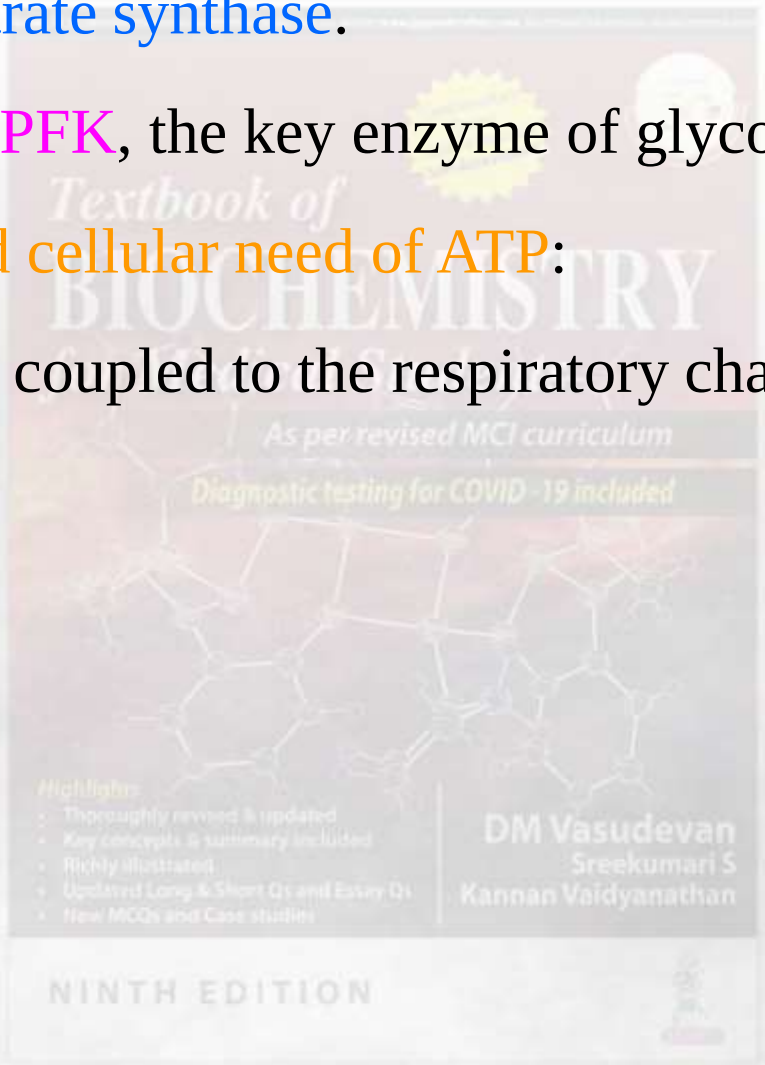
Anaplerotic role (Filling up reactions)



Regulation of the Citric Acid Cycle

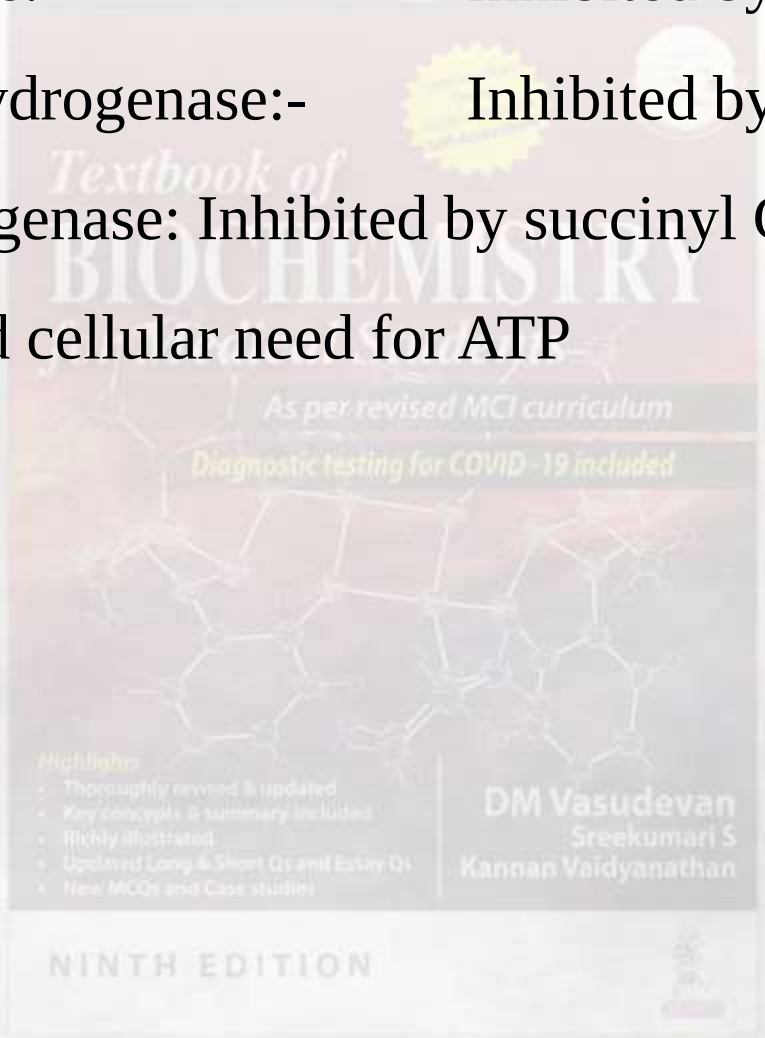
1. ATP inhibits citrate synthase.
2. Citrate inhibits PFK, the key enzyme of glycolysis.
3. Availability and cellular need of ATP:

The cycle is tightly coupled to the respiratory chain providing ATP.



Regulation of TCA Cycle

1. Citrate Synthase:- Inhibited by citrate; ATP
2. IsoCitrate Dehydrogenase:- Inhibited by NADH
3. α-KG Dehydrogenase: Inhibited by succinyl Co-A and NADH.
4. Availability and cellular need for ATP



Inhibitors of TCA Cycle

- Fluoroacetate : non-competitive type

  (Enzyme inhibited :Aconitase)

- Arsenite : non-competitive type

  (Enzyme inhibited:α-KG dehydrogenase)

- Malonate : competitive type

  (Enzyme inhibited :Succinate dehydrogenase)

  Malonate has structural similarity to succinate

Metabolic Defects of Oxidative Metabolism

Enzymes	Reactions catalysed	Abnormalities
Pyruvate dehydrogenase	Pyruvate →acetyl CoA	Lactic acidosis Neurological disorders
Acyl CoA-dehydrogenase	Fatty acyl CoA → alpha, beta-unsaturated fatty acyl CoA	Organic aciduria, glutaric aciduria, acidosis, hypoglycemia. Electron flow from FAD → CoQ is affected
Pyruvate carboxylase	Pyruvate →Oxaloacetate	Oxaloacetate needed for sparking TCA cycle is deficient. Lactic acidosis, hyperammonemia and hyperalaninemia

Summary of Bioenergetics

1. **Free energy** is a measure of the energy available to perform useful work.
2. ΔG can predict the direction of a chemical reaction.
3. Chemical reactions can be **coupled** which allows an energetically unfavorable reaction to proceed to conclusion.
4. ΔG measured under physiological conditions may be different from that at a standard state.

Redox Potentials

Redox potential of a system is the electron transfer potential E0′.

Oxidation is defined as the loss of electrons and reduction as the gain in electrons.

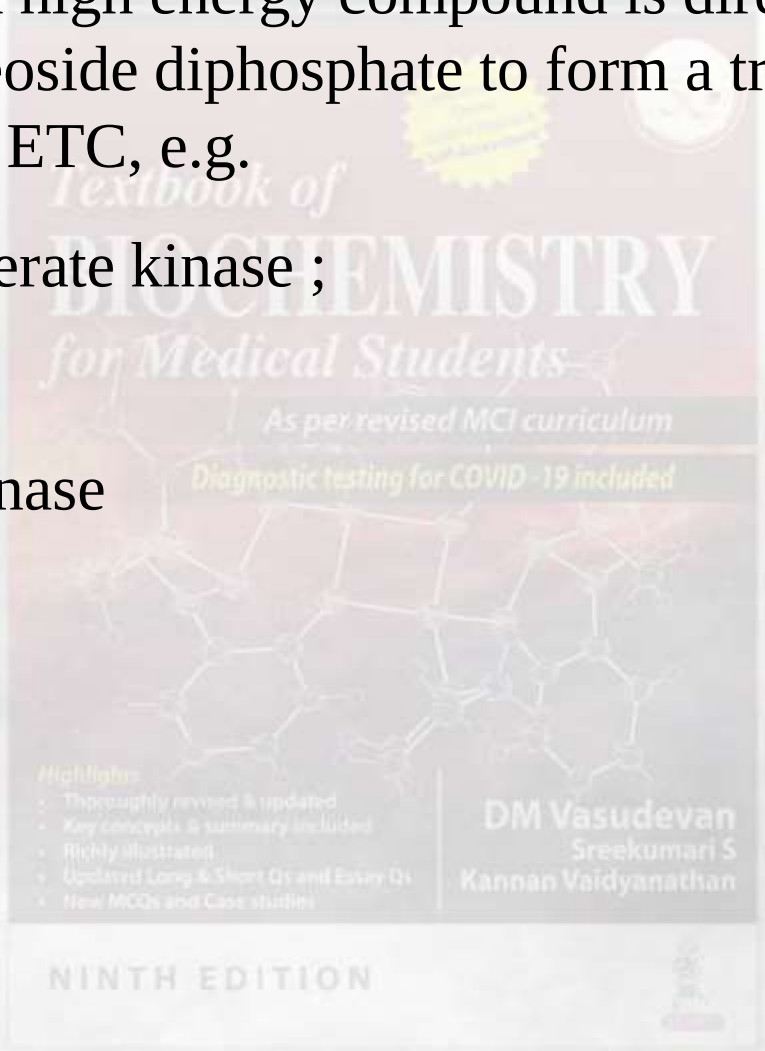
When a substance exists both in the reduced state and in the oxidised state, the pair is called a **redox couple**.

A redox couple is a pair of reductant (electron carrying reductant) and oxidant with a difference in the reduction potential between them denoted by E_0'.

Substrate Level Phosphorylation

Here energy from a high energy compound is directly transferred to nucleoside diphosphate to form a triphosphate without the help of ETC, e.g.

- Bisphosphoglycerate kinase ;
- Pyruvate kinase
- Succinate thiokinase



Redox Potentials

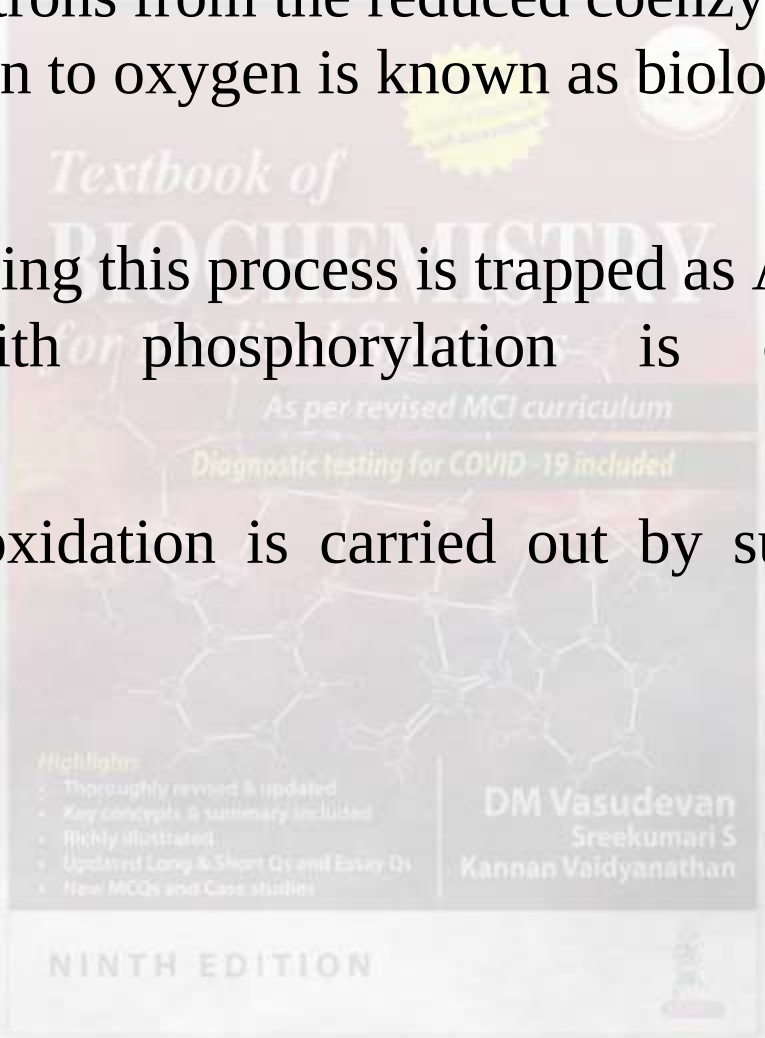
Oxidant	Reductant	E0' (in V)
NAD^+	NADH + H+	**– 0.32**
Cytochrome b+++	Cytochrome b++	**+ 0.07**
Coenzyme Q	Coenzyme QH2	**+ 0.10**
Cytochrome c+++	Cytochrome c++	**+ 0.22**
Cytochrome a+++	Cytochrome a++	**+ 0.29**
½ O2 + 2H	**H2O**	**+ 0.82**

Biological Oxidation

The transfer of electrons from the reduced coenzymes through the respiratory chain to oxygen is known as biological oxidation.

Energy released during this process is trapped as ATP. This coupling of oxidation with phosphorylation is called **oxidative phosphorylation**.

In the body, this oxidation is carried out by successive steps of **dehydrogenations**.



Electron Transport Chain

The electron flow occurs through successive dehydrogenase enzymes, together known as electron transport chain. **The electrons flow from electronegative potential (–0.32) to electropositive potential (+ 0.82).**

Energetics of Oxidative Phosphorylation

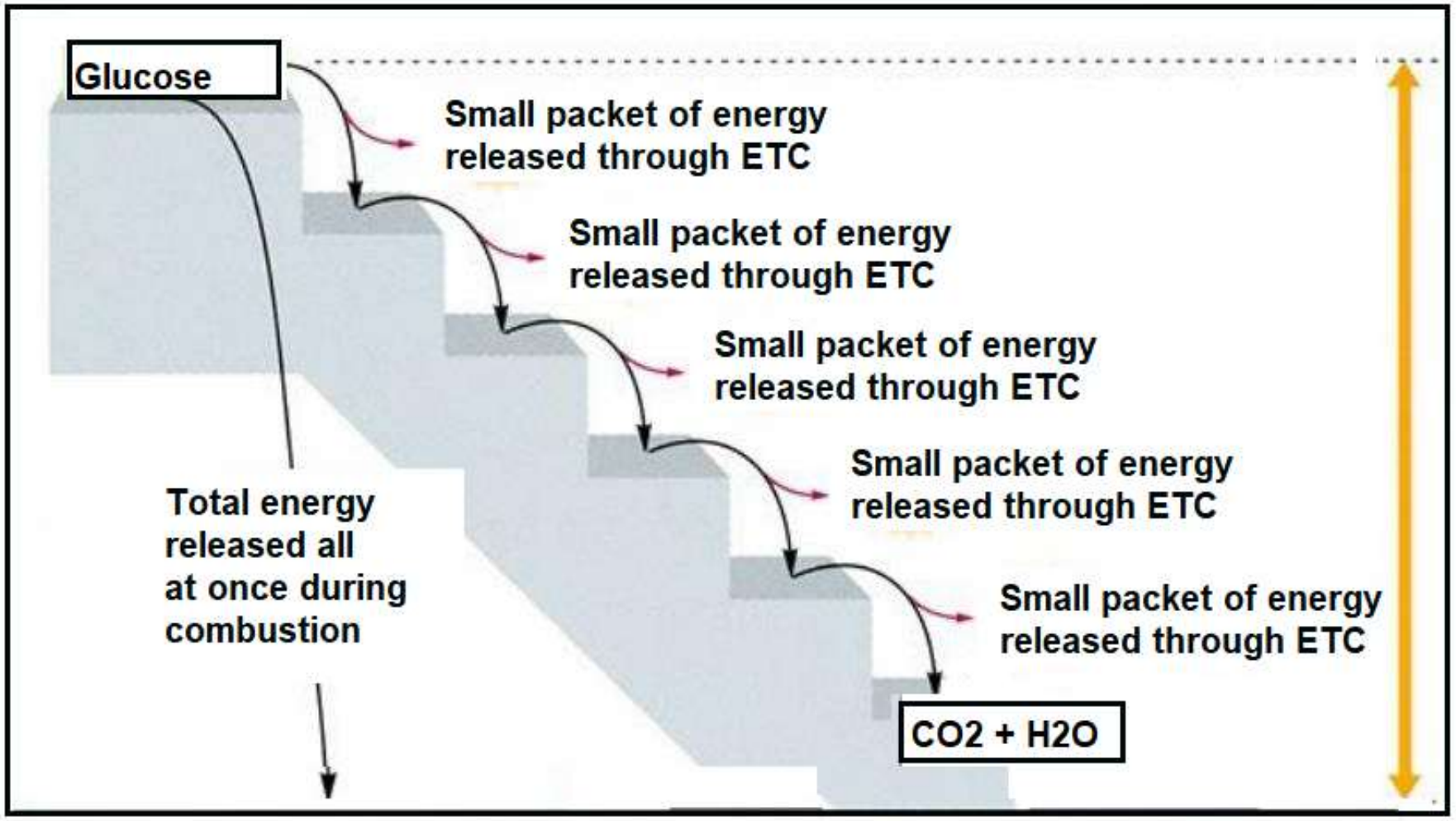
It is seen that there is an overall difference of 1.14V between NADH and oxygen

$NADH \rightarrow NAD + 2H + 2e$ (–0.32)

$1/2\ O_2 + 2H + 2e \rightarrow H_2O$ (+0.82).

When the ΔG0' is calculated, the electron transport is an exergonic process. The free energy change between NAD+ and water is equal to 53 kcal/mol. This is so great that, if this much energy is released at one stretch, body cannot utilize it. Hence with the help of ETC assembly, the total energy change is released in small increments so that energy can be trappedas **chemical bond energy, ATP.** The free energy change required to produce one ATP is only –7.5 kcal/mol. The total energy change is broken up into 3 parcels each contributing to the synthesis of ATP.

Body traps energy with small increments



The NAD^+ linked dehydrogenases are:

Glyceraldehyde-3-phosphate dehydrogenase

Isocitrate dehydrogenase

Malate dehydrogenase

Glutamate dehydrogenase

Beta hydroxy acyl CoA dehydrogenase

Pyruvate dehydrogenase

Alpha keto glutarate dehydrogenase

FAD-linked dehydrogenases are:

Succinate dehydrogenase

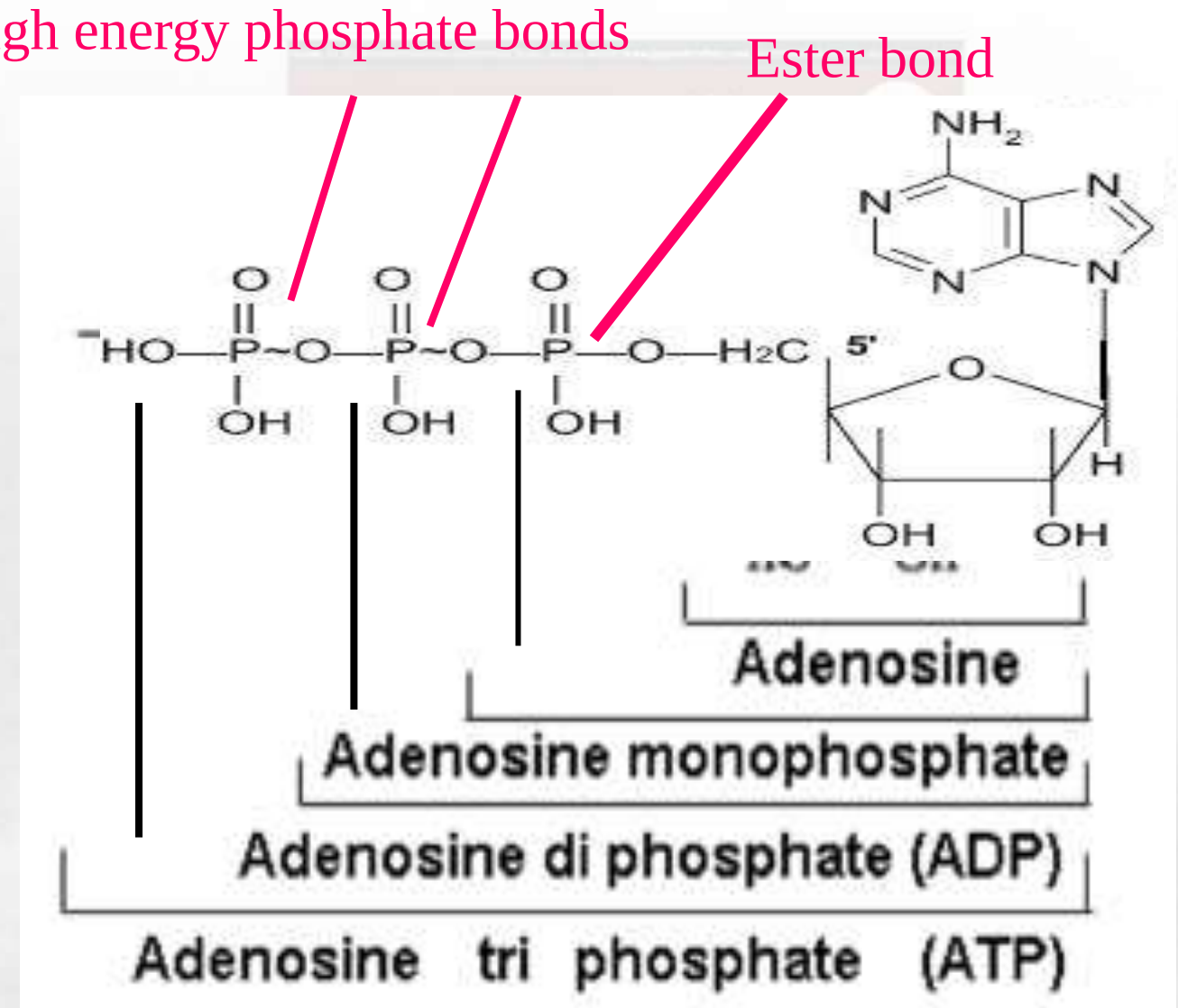
Fatty acyl CoA dehydrogenase

Glycerolphosphate dehydrogenase

High-energy Compounds

Energy rich compound	ΔG0´ in kcal/mol
1. Nucleotides: (ATP, GTP, UTP, UDP-glucose)	
ATP to AMP + PPi	–10.7 kcal
ATP to ADP + Pi	– 7.3
2. Creatine phosphate	–10.5
3. Arginine phosphate	
4. 1,3-bisphospho glycerate	–10.1
5. Phosphoenol pyruvate	–14.8
6. Inorganic pyrophosphate	
7. Carbamoyl phosphate	–12.3
8. Acetyl CoA	– 7.5
9. S-adenosyl methionine (SAM)	-7.1

ATP

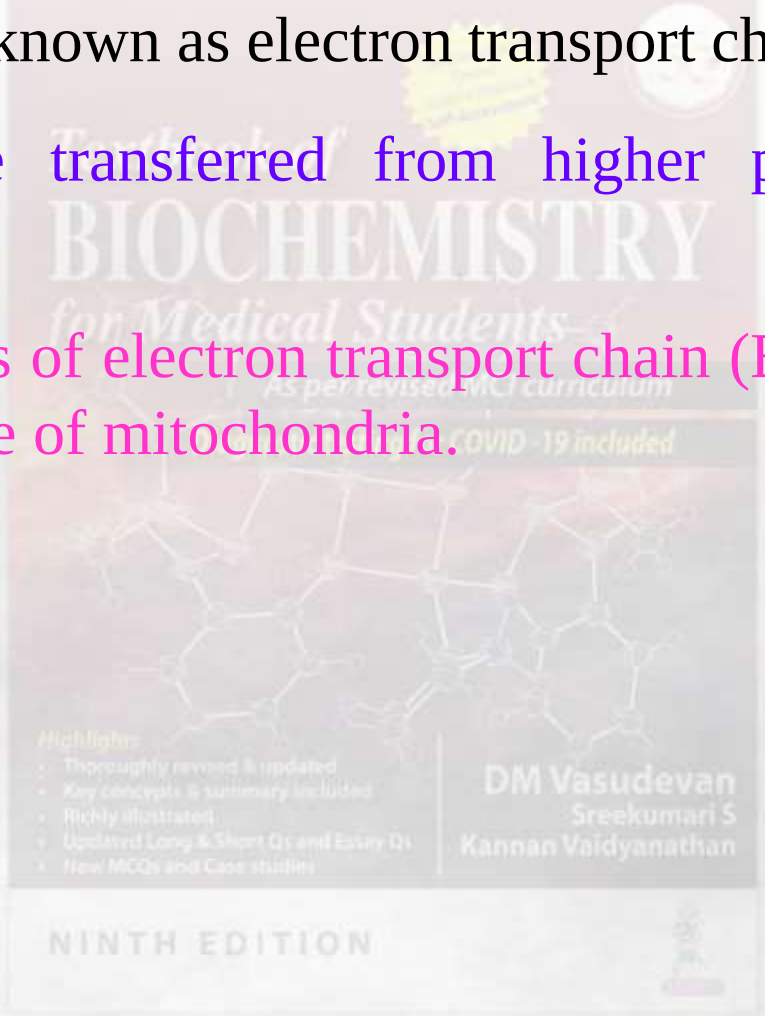

High energy phosphate bonds
Ester bond
NH2
N
N
N
N
O
O
O
HO
P~O
P~O
P
O
H2C
5'
OH
OH
OH
O
H
OH
OH
Adenosine
Adenosine monophosphate
Adenosine di phosphate (ADP)
Adenosine tri phosphate (ATP)

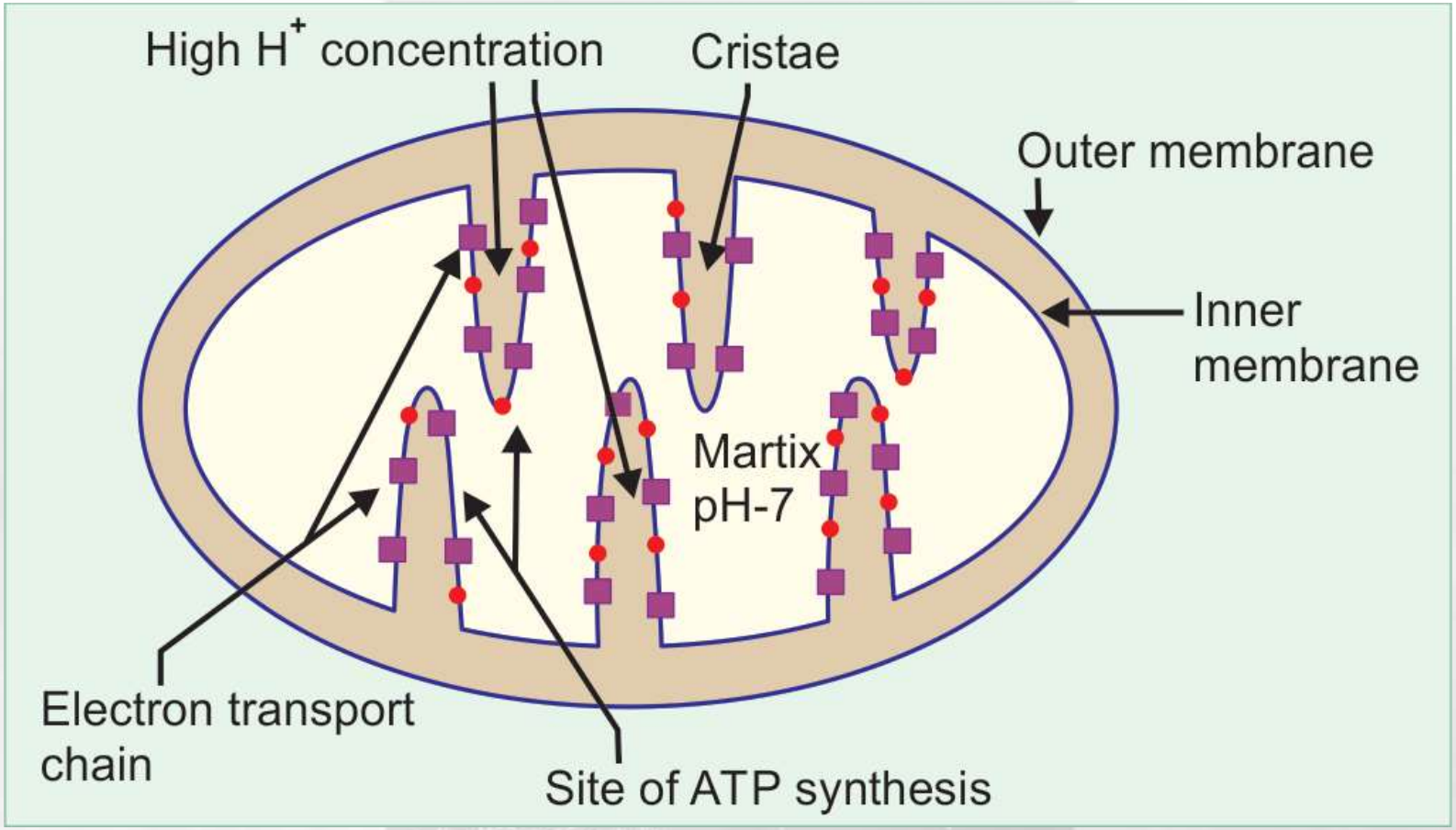
Electron Transport Chain

The electron flow occurs through successive dehydrogenase enzymes, together known as electron transport chain (ETC).

The electrons are transferred from higher potential to lower potential.

All the components of electron transport chain (ETC) are located in the inner membrane of mitochondria.





Mitochondria

Location of Enzymes in Mitochondria.

Mitochondria, outer membrane:

- Mono amino oxidase
- Acyl-CoA synthetase
- Phospholipase A2

In between outer and inner membrane:

- Adenylate kinase
- Creatine kinase

Inner membrane, outer surface:

- Glycerol-3-phosphate dehydrogenase

Inner membrane, inner surface:

- Succinate dehydrogenase
- Enzymes of respiratory chain

Soluble matrix:

- Enzymes of citric acid cycle
- Enzymes of beta oxidation of fatty acid

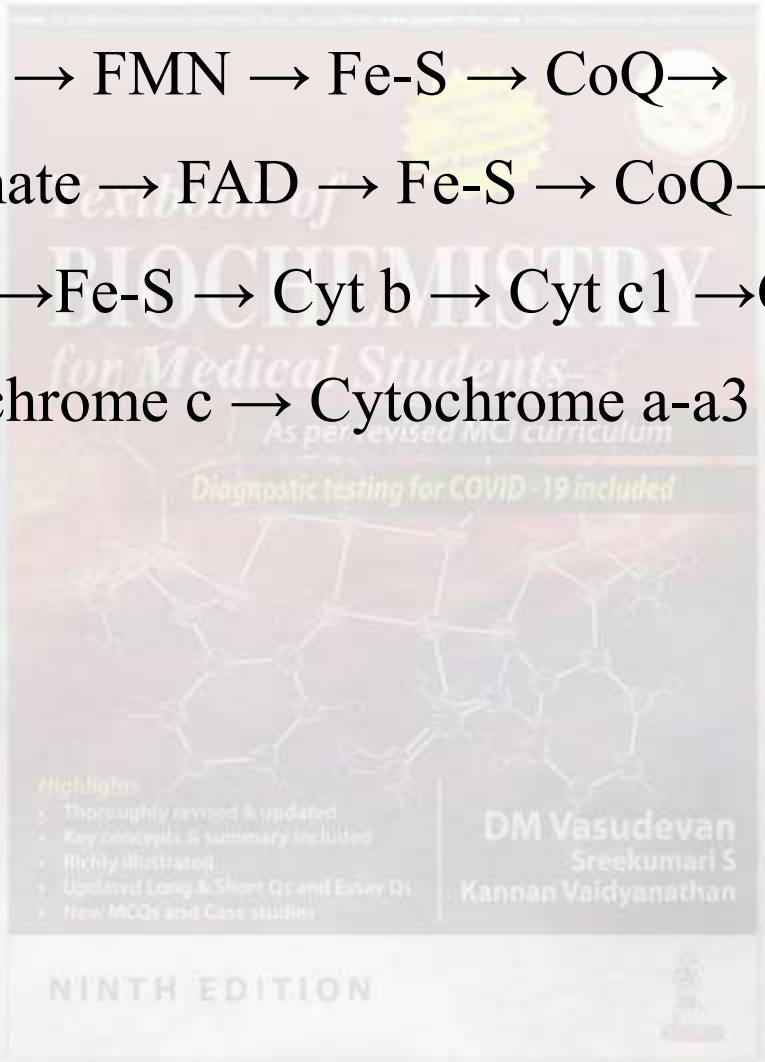
Summary of Electron Flow in Electron Transport Chain (ETC)

Complex I: NADH → FMN → Fe-S → CoQ→

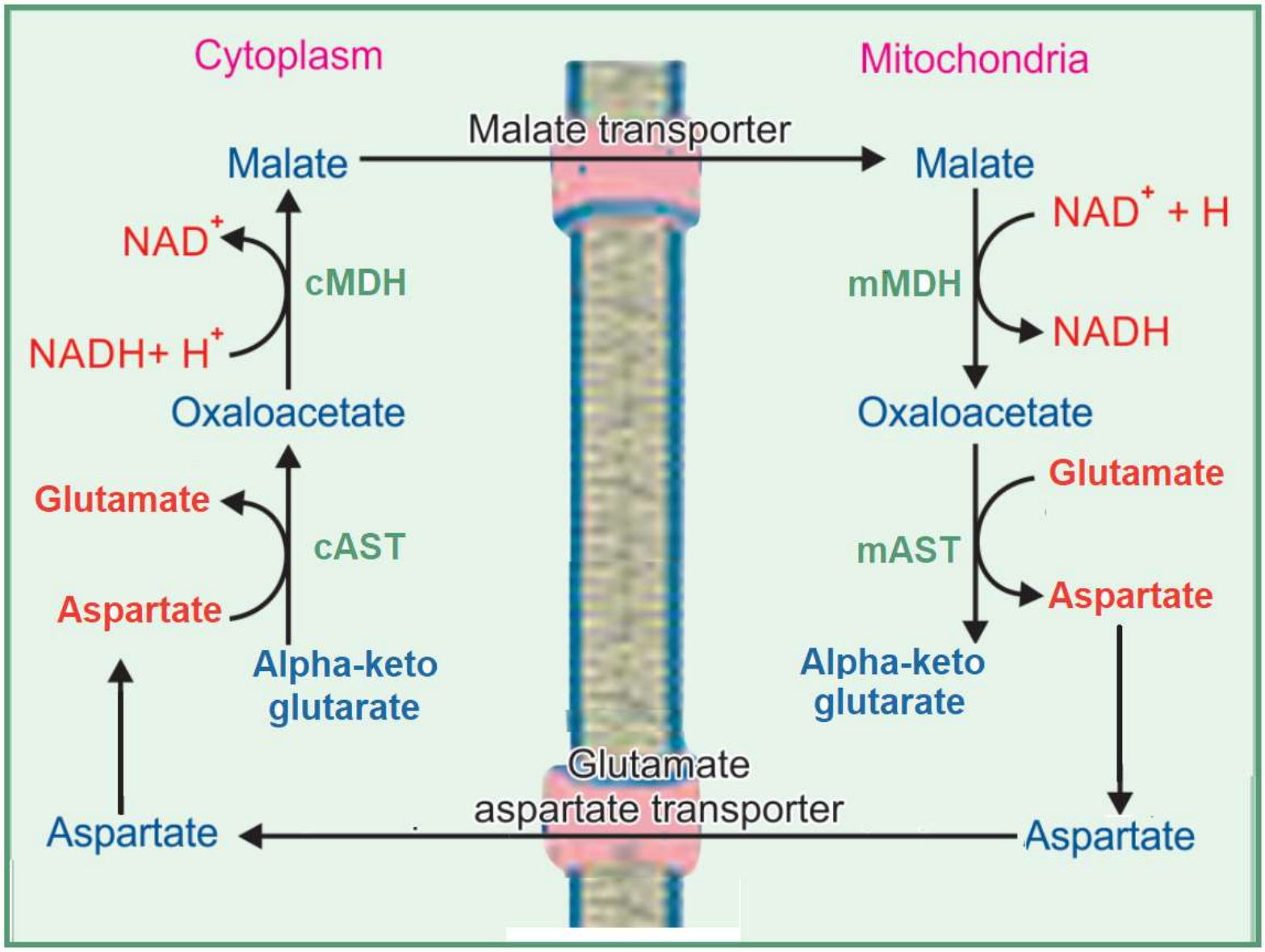
Complex II: Succinate → FAD → Fe-S → CoQ→

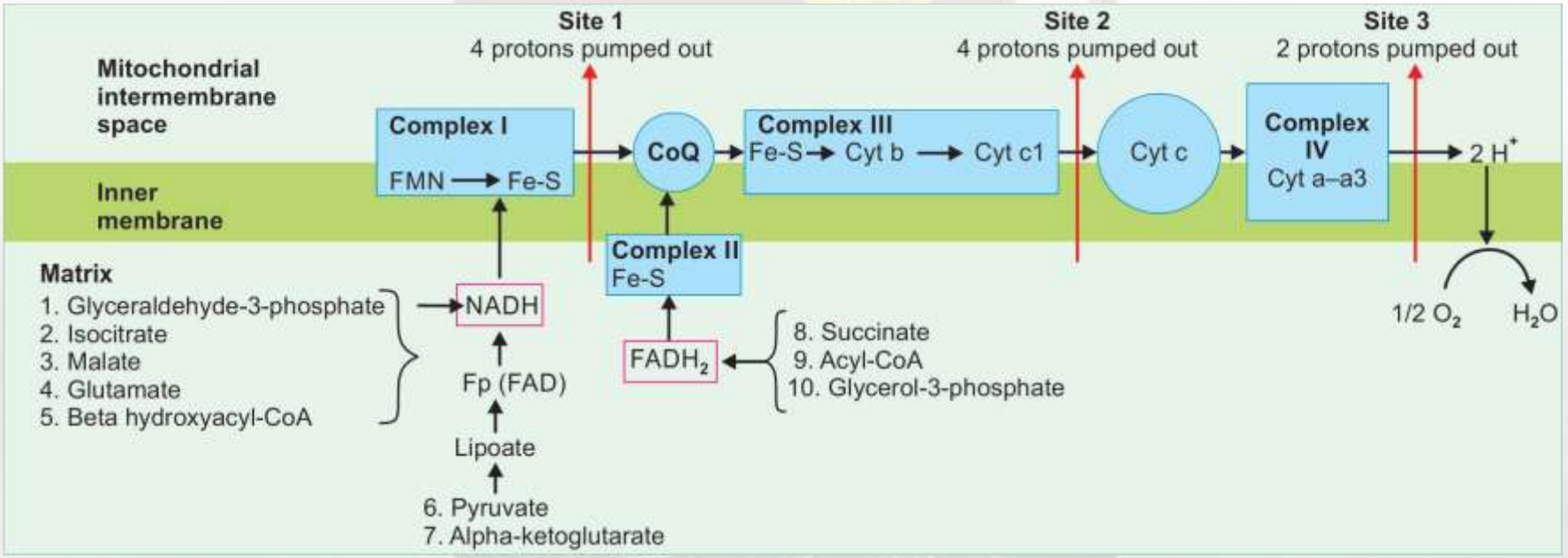
Complex III: CoQ →Fe-S → Cyt b → Cyt c1 →Cyt c

Complex IV: Cytochrome c → Cytochrome a-a3 → O



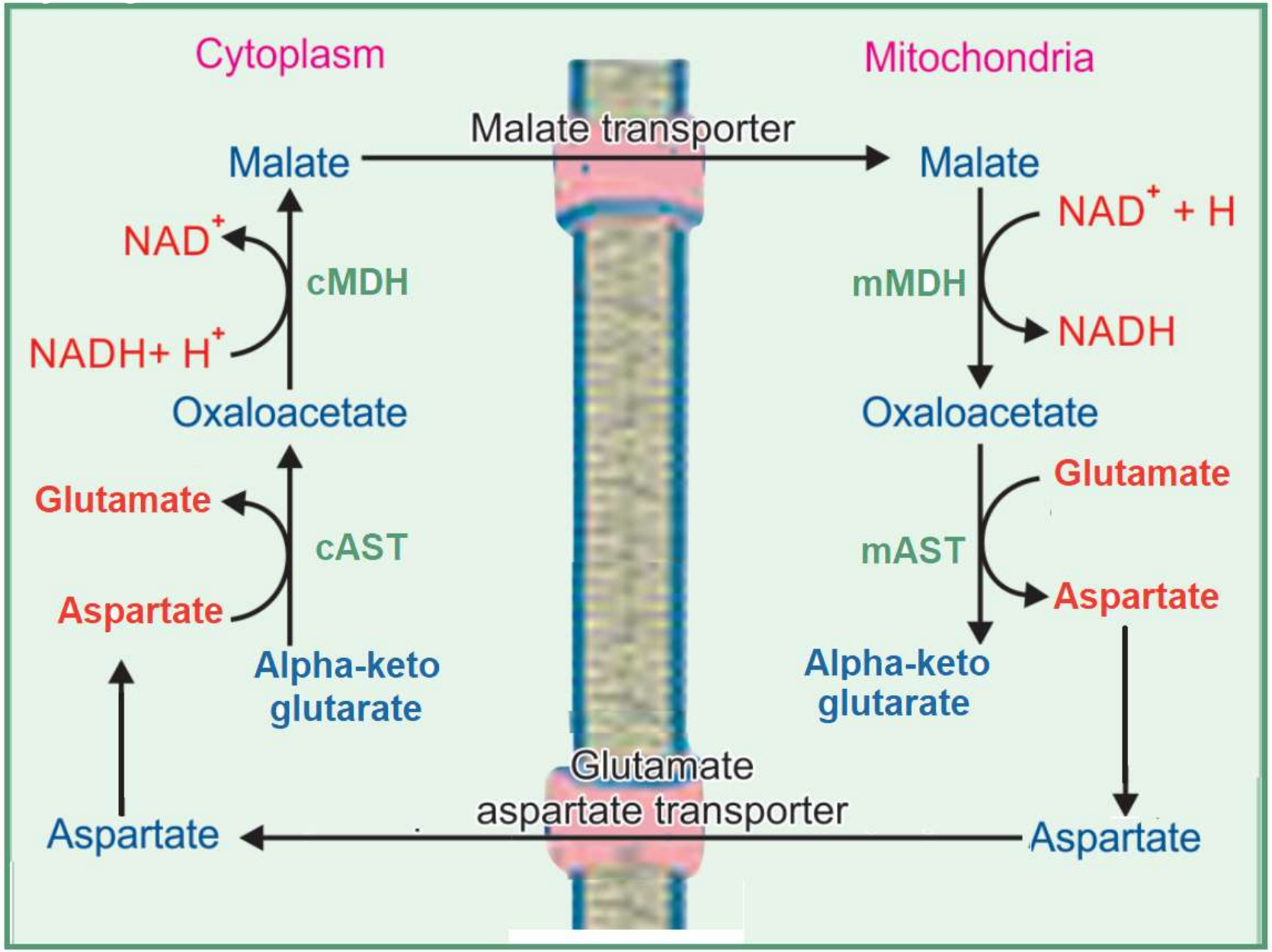
Mitochondrial transport of NADH by malate-aspartate shuttle.

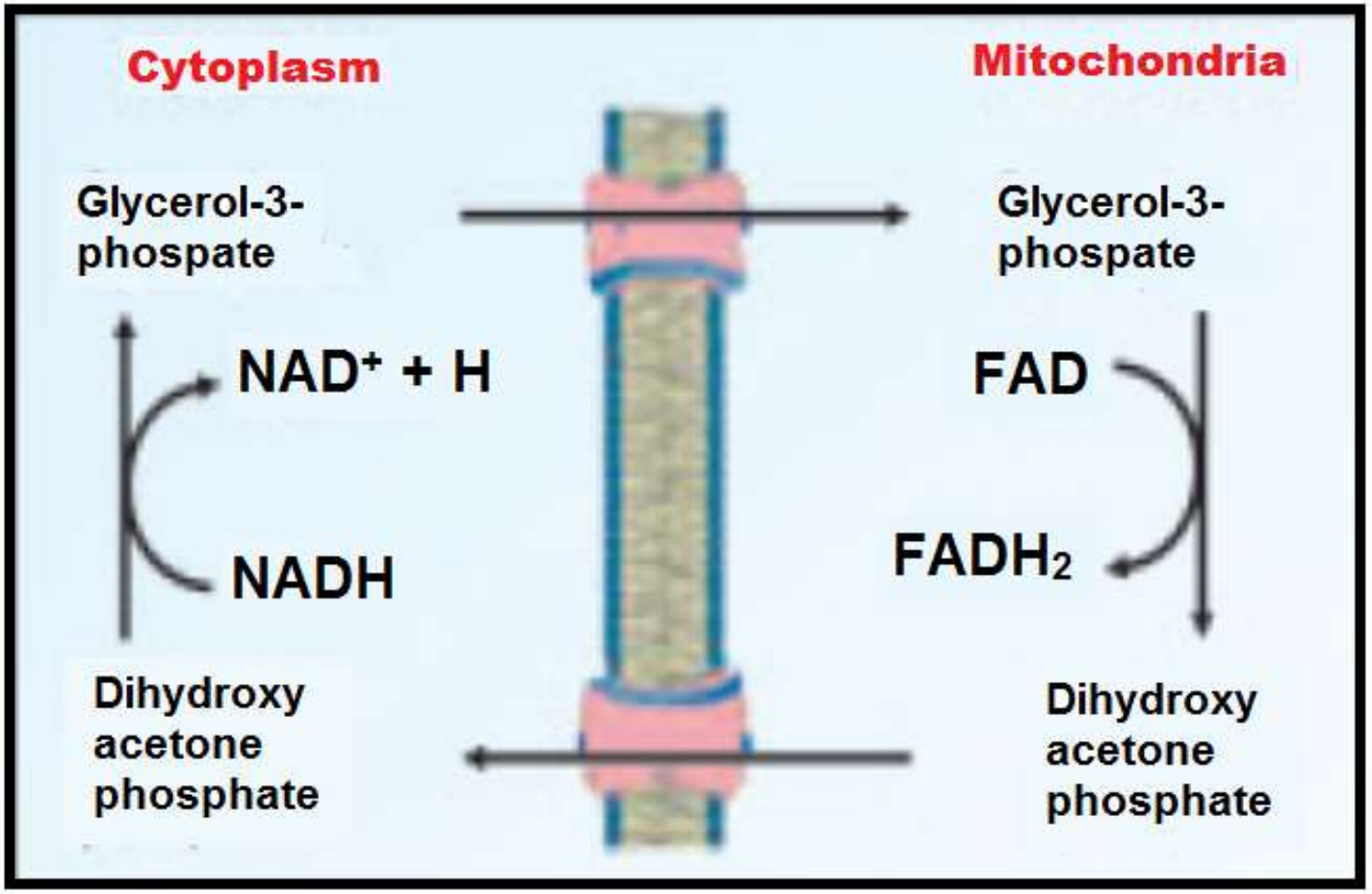




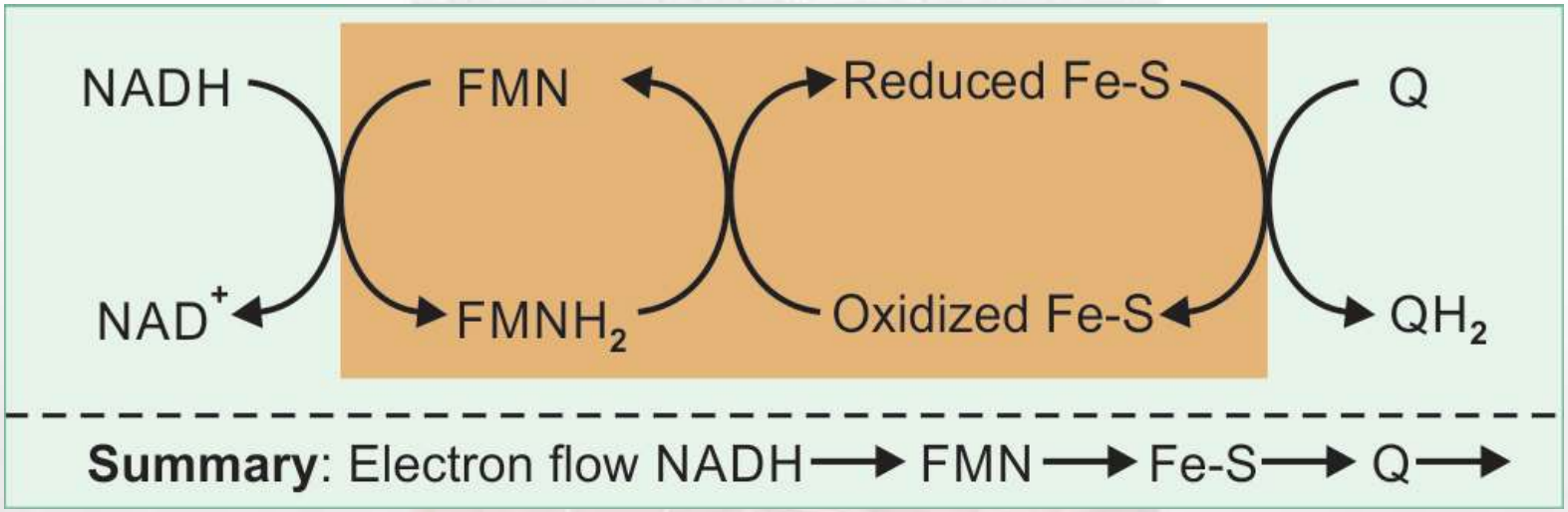
Components and sequence of reactions of electron transport chain.

Mitochondrial transport of NADH by malate-aspartate shuttle. cMDH = cytoplasmic malate dehydrogenase. mMDH = mitochondrial malate dehydrogenase

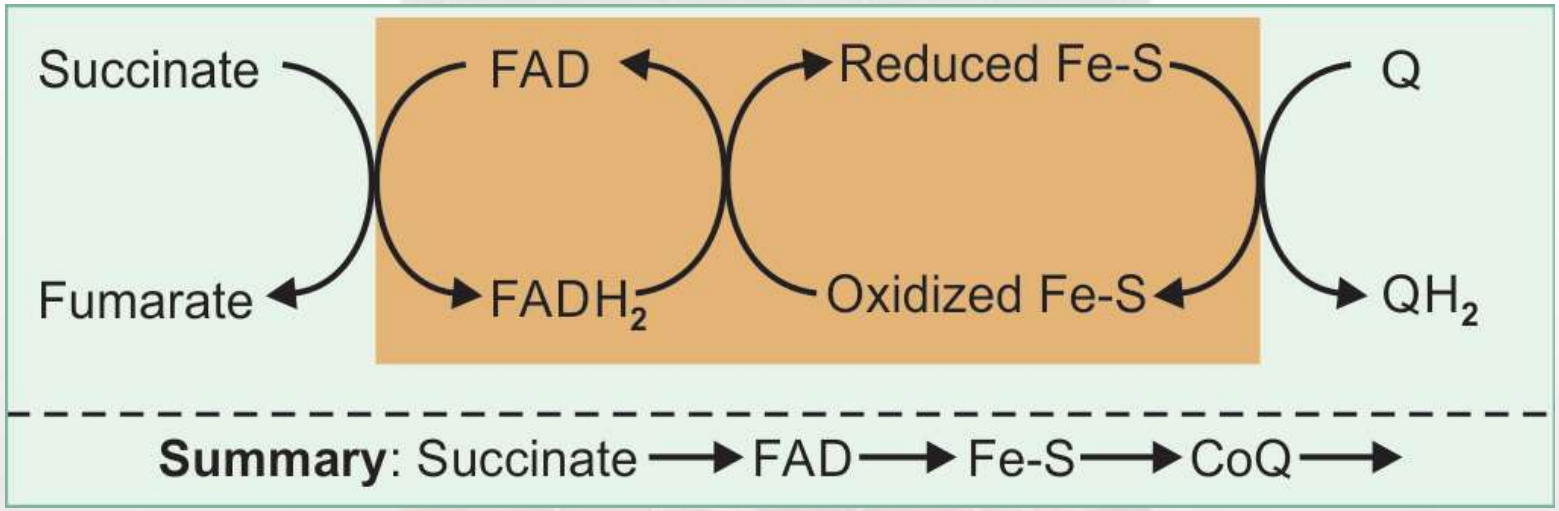




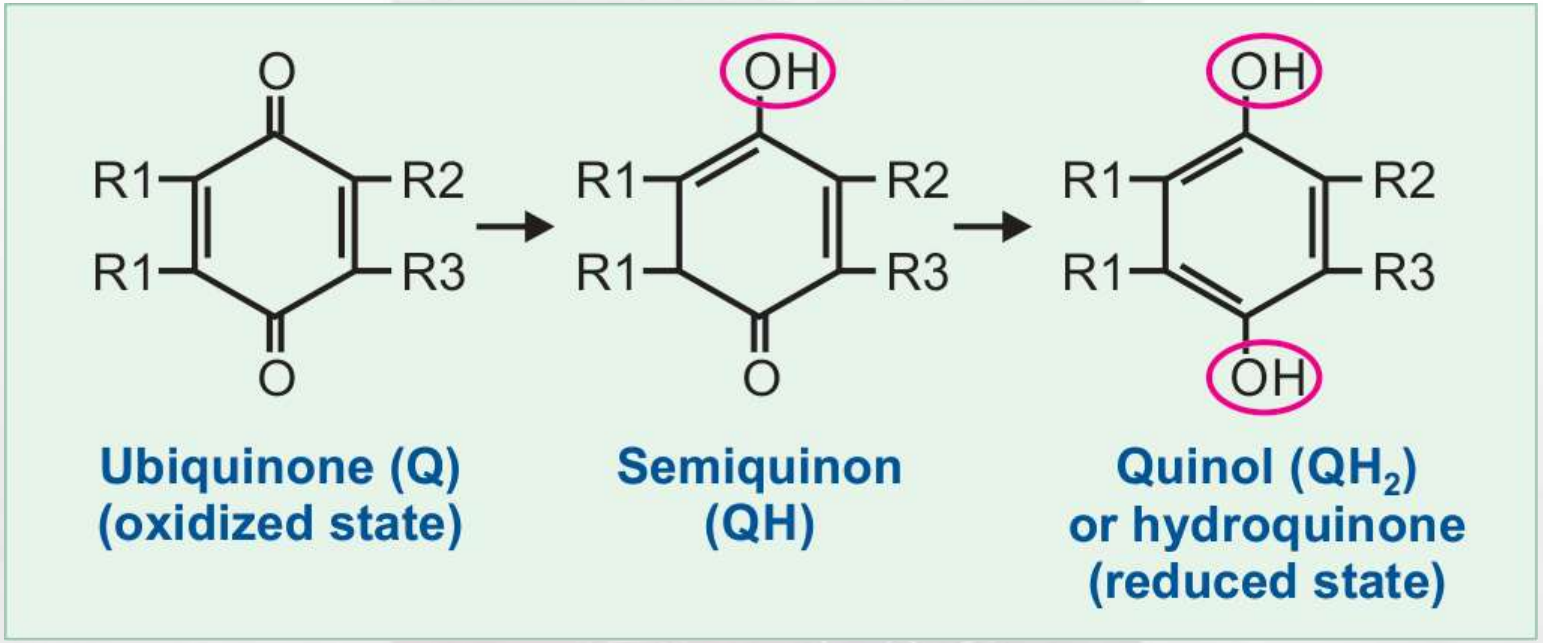
Glycerol-3-phosphate shuttle in muscle and brain.



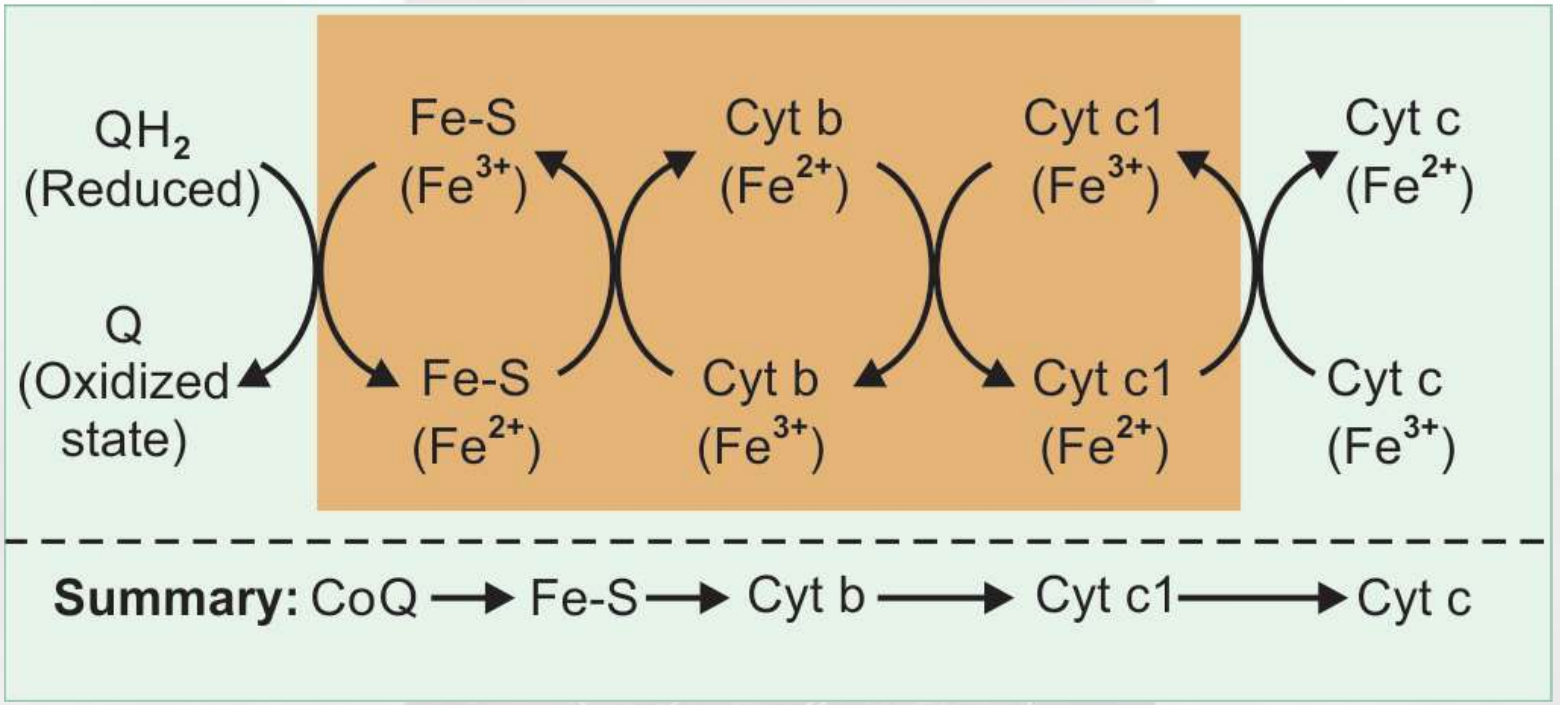
Complex I or NADH-CoQ reductase (NADH dehydrogenase complex).



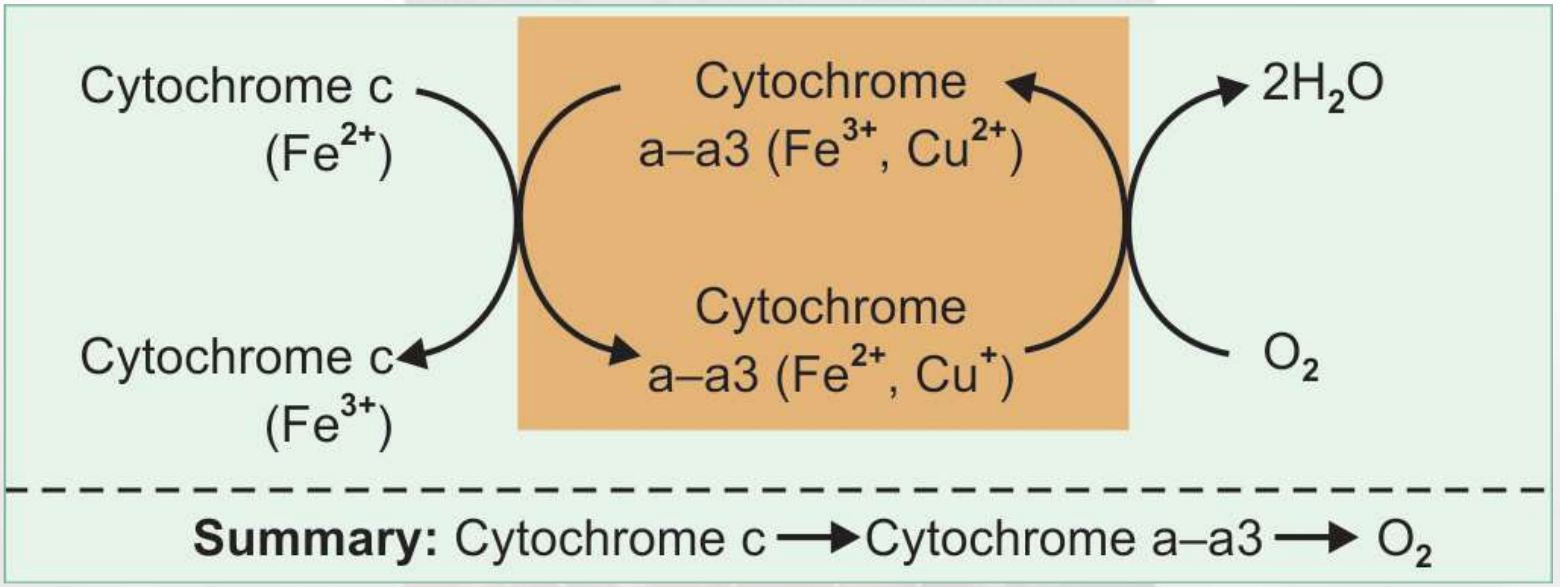
Complex II; Succinate-Q-reductase.



Addition of H+ to coenzyme Q. R1 = CH3O group. R2 = CH3- group. R3 = 10 isoprene units (one isoprene unit contains 5 carbon atoms)



Complex III or cytochrome reductase
(Cytochrome b-c1 complex) of respiratory chain.

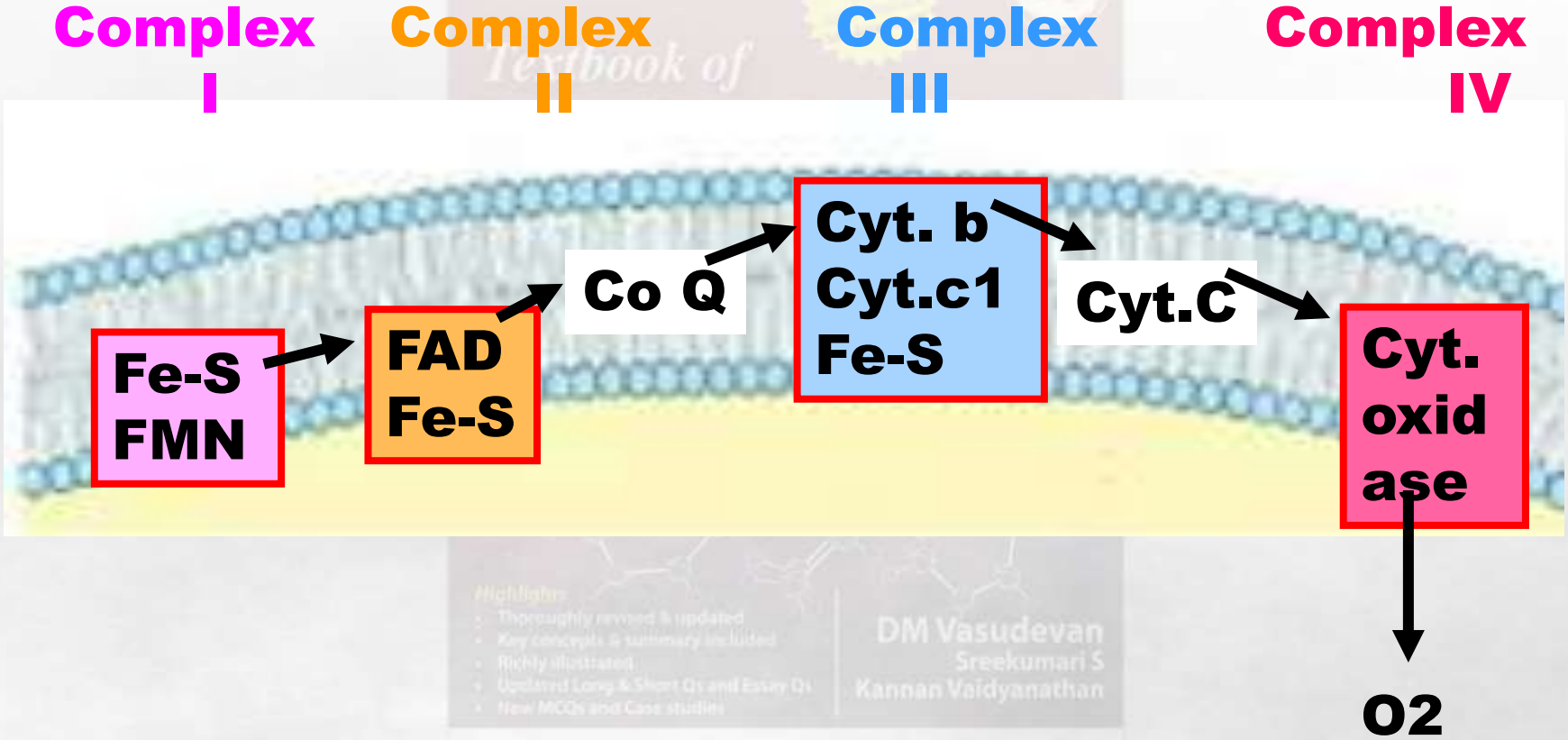


Complex IV (cytochrome oxidase) of respiratory chain.

Complex I

NADH ←

1. Glyceraldehyde-3-phosphate
2. Isocitrate
3. Malate
4. Glutamate
5. Beta hydroxy acyl CoA

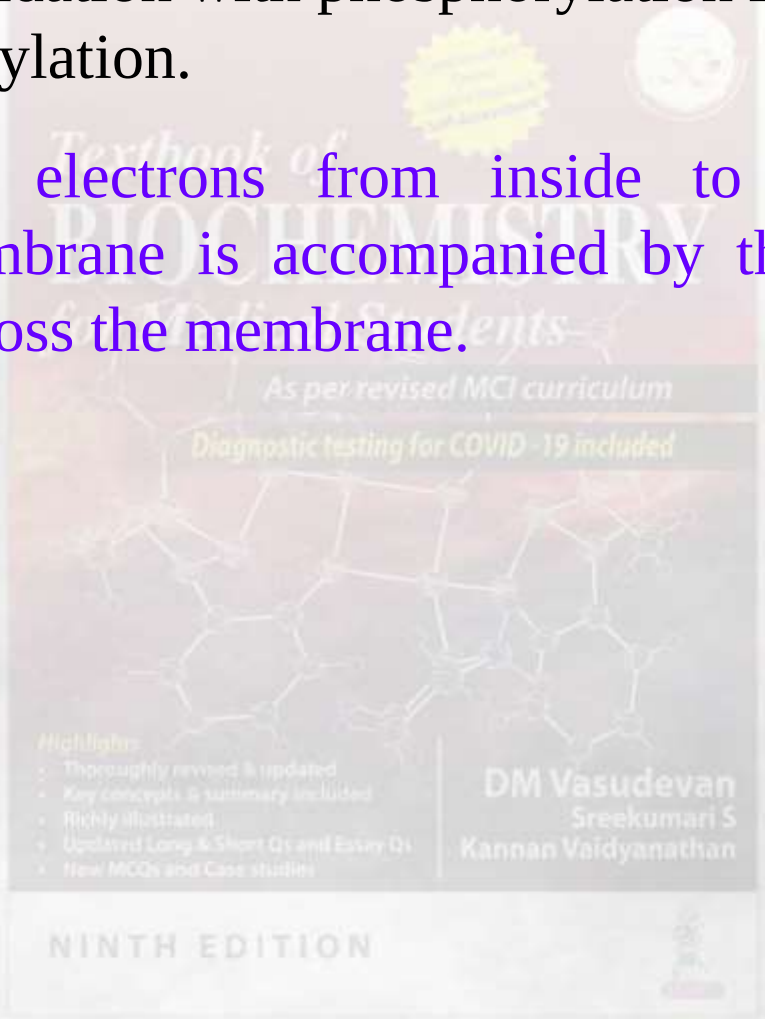

Complex I
Complex II
Complex III
Complex IV
Fe-S
FMN
FAD
Fe-S
Co Q
Cyt. b
Cyt.c1
Fe-S
Cyt.C
Cyt. oxid ase
O2

ATP generated by oxidation of	Presently Accepted value
NADH	2.5
FADH	1.5
Glucose	32
Acetyl CoA	10
Palmitate	106

Chemi-osmotic Theory

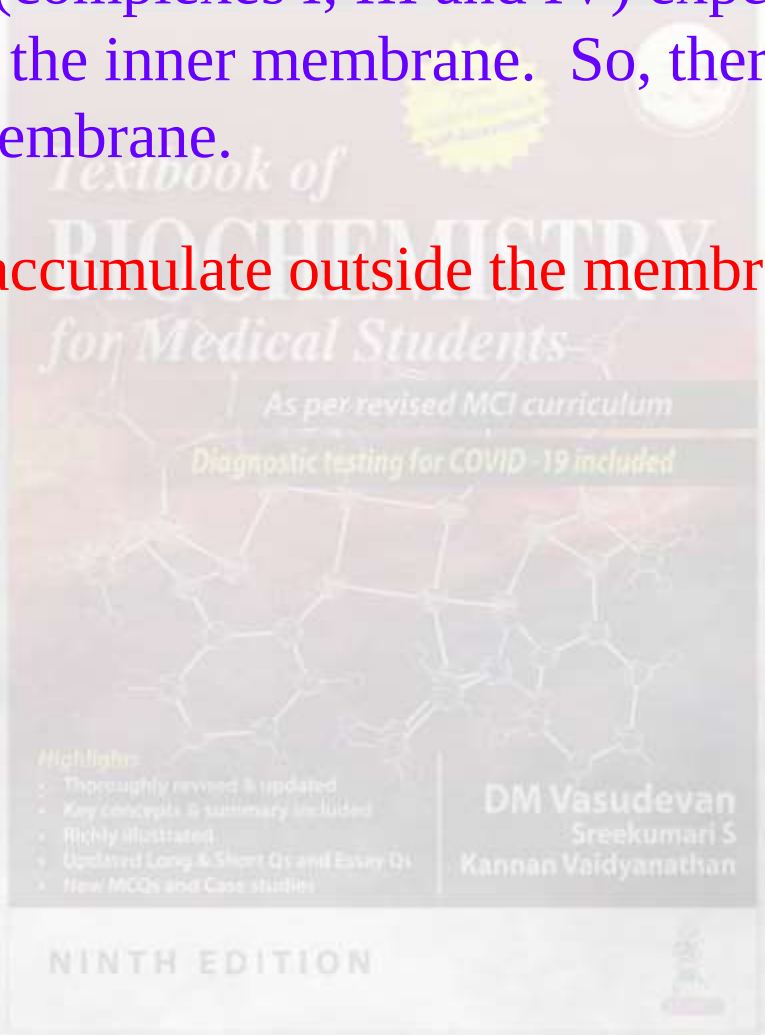
The coupling of oxidation with phosphorylation is termed oxidative phosphorylation.

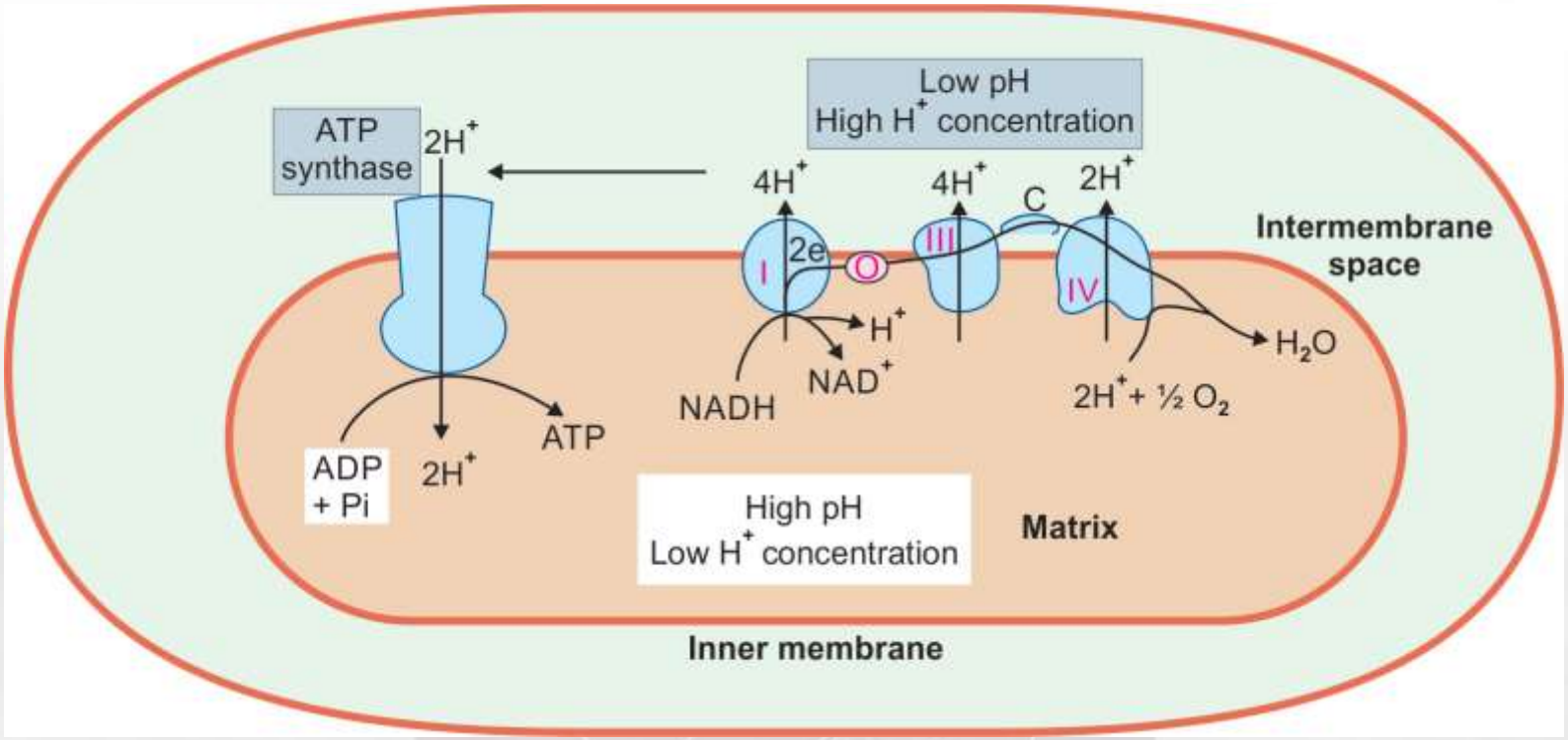
The transport of electrons from inside to outside of inner mitochondrial membrane is accompanied by the generation of a proton gradient across the membrane.



The proton pumps (complexes I, III and IV) expel H+ from inside to outside of the inner membrane. So, there is high H+ outside the inner membrane.

Protons (H+ ions) accumulate outside the membrane.





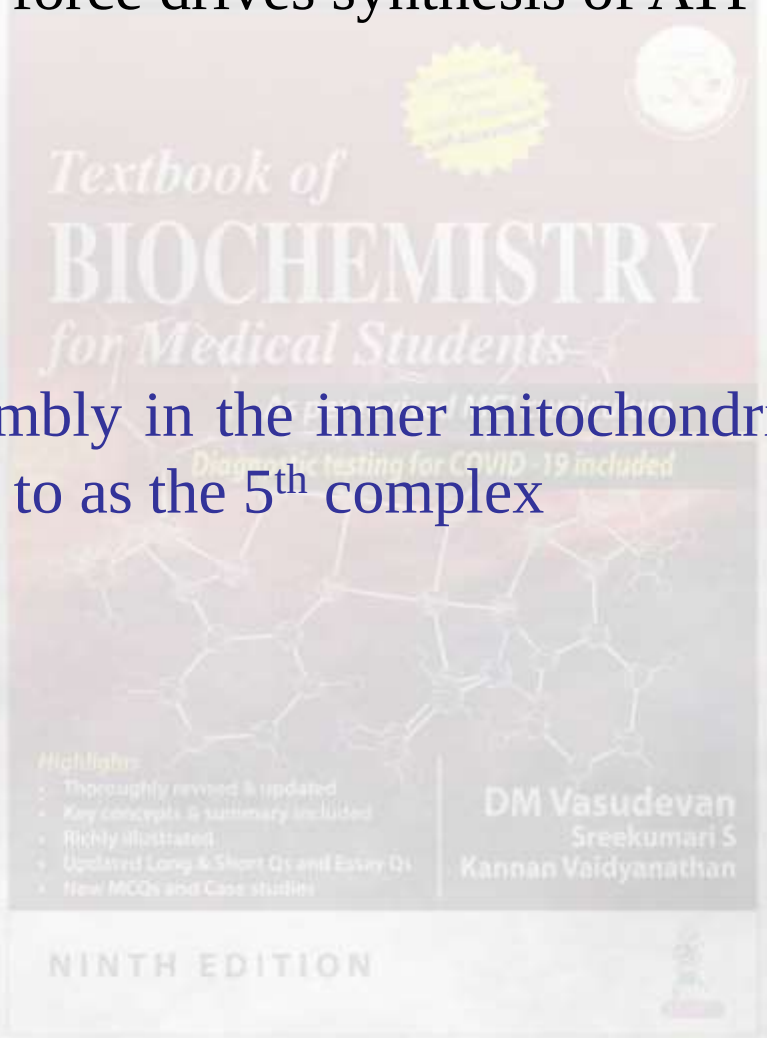
Summary of ATP synthesis. One mitochondrion is depicted, with inner and outer membranes. ETC complexes will push hydrogen ions from matrix into the inter membrane space. So, intermediate space has more H+ (highly acidic) than matrix. So,hydrogen ions tend to leak into matrix through Fo. Then ATPs are synthesized. I, II, III, IV = components of ETC.

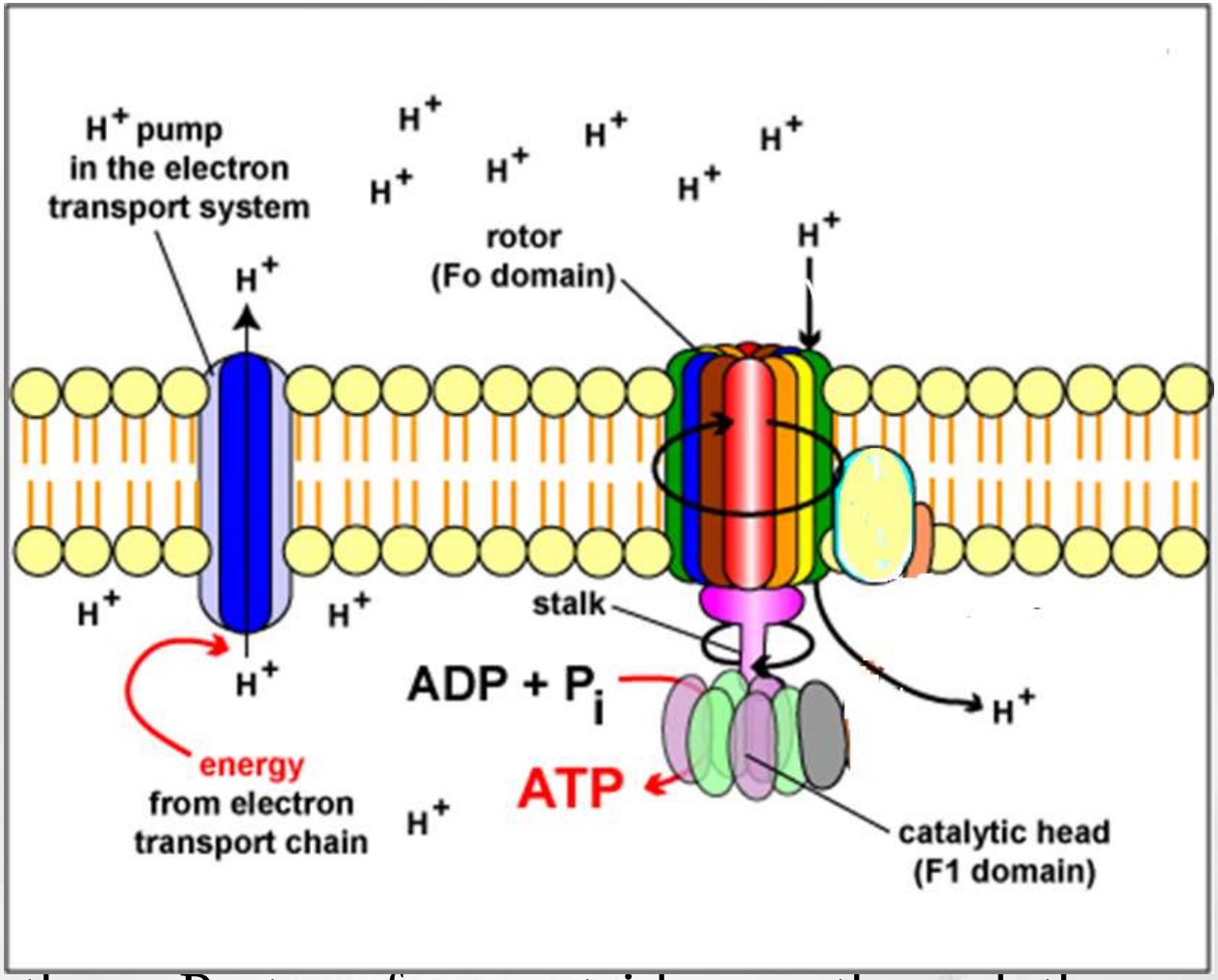
This proton motive force drives synthesis of ATP by ATP synthase complex.

ATP Synthase (5th Complex)

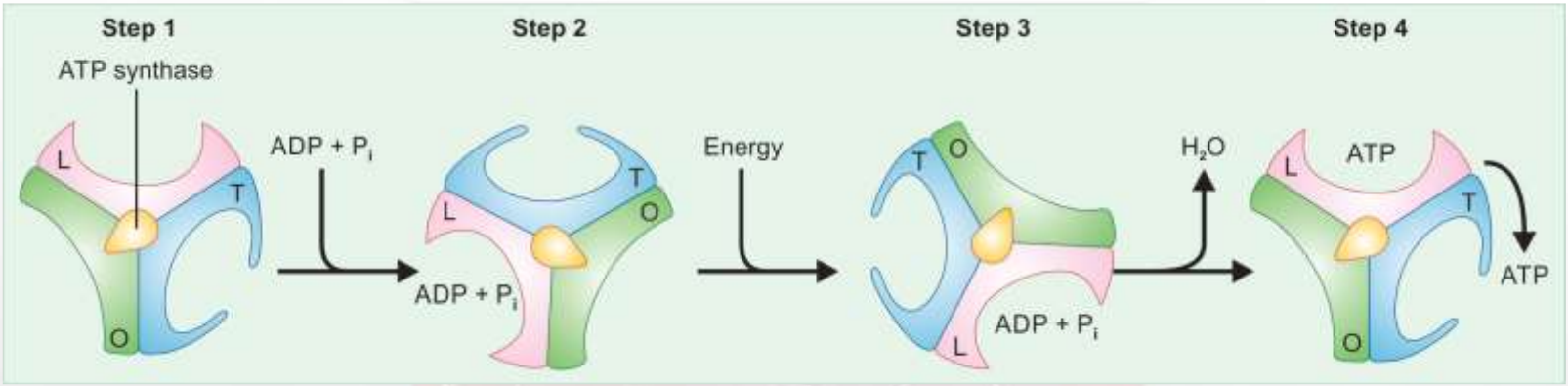
5th Complex

It is a protein assembly in the inner mitochondrial membrane. It is sometimes referred to as the 5th complex





ATP synthase. Protons from outside pass through the pore of Fo into the matrix, when ATP is synthesized.



The binding change mechanism for ATP synthase. F1 has 3 chemically identical but conformationally distinct functional states. "O" means open conformation with no affinity for substrates and is catalytically inactive. "L" binds ligands loosely and catalytically sluggish. "T" binds ligands tightly and catalytically active. Step 1 = Old confirmation regained and cycle continues. Step 2 = ADP and Pi binds to "L" site. Then energy dependent conformational change occurs. Step 3 = ATP is synthesized; conformation is again changed. Step 4 = ATP is released with confirmation change.

Compounds which affect electron transport chain and oxidative phosphorylation

1. Site-1 (complex I to Co-Q) specific inhibitors

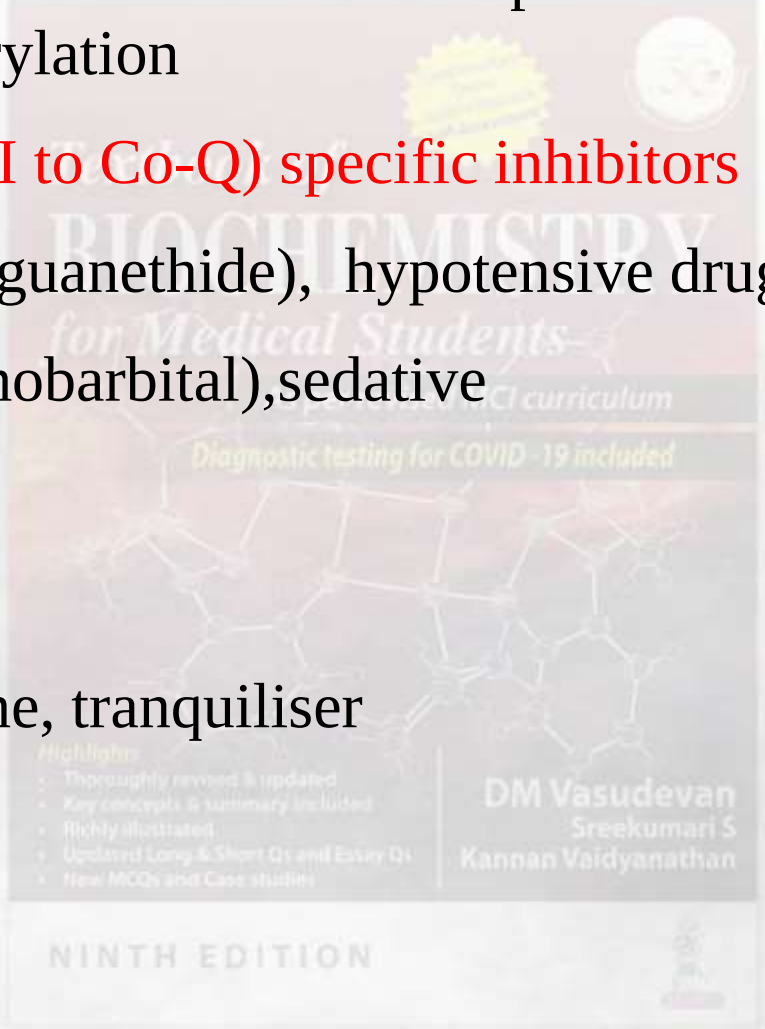
i. Alkylguanides (guanethide), hypotensive drug

ii. Barbiturates (amobarbital),sedative

iii -Piericidin A

-Rotenone

-Chlorpromazine, tranquiliser



2. **Complex II to Co-Q**

Carboxin

3. Complex III to cytochrome c inhibitors

BAL (British anti lewisite), antidote of war gas

Antimycin A

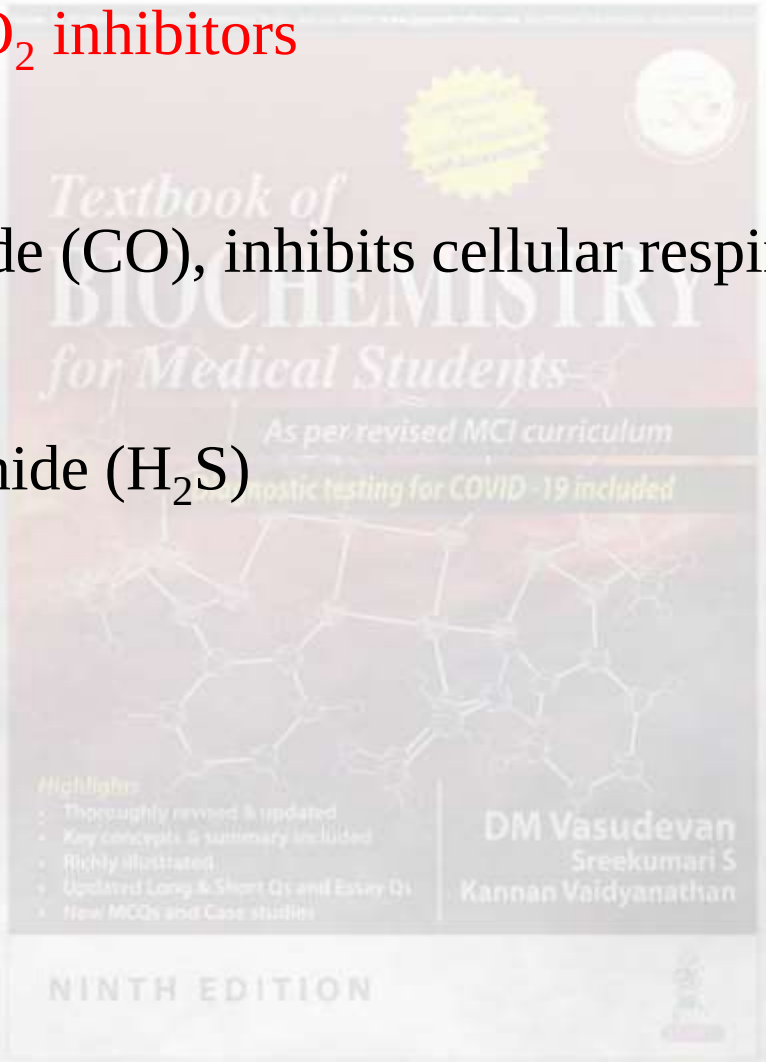
4. Complex IV to O_2 inhibitors

i. Cyanide (CN–)

ii. Carbon monoxide (CO), inhibits cellular respiration

iii. Azide ($N3^-$)

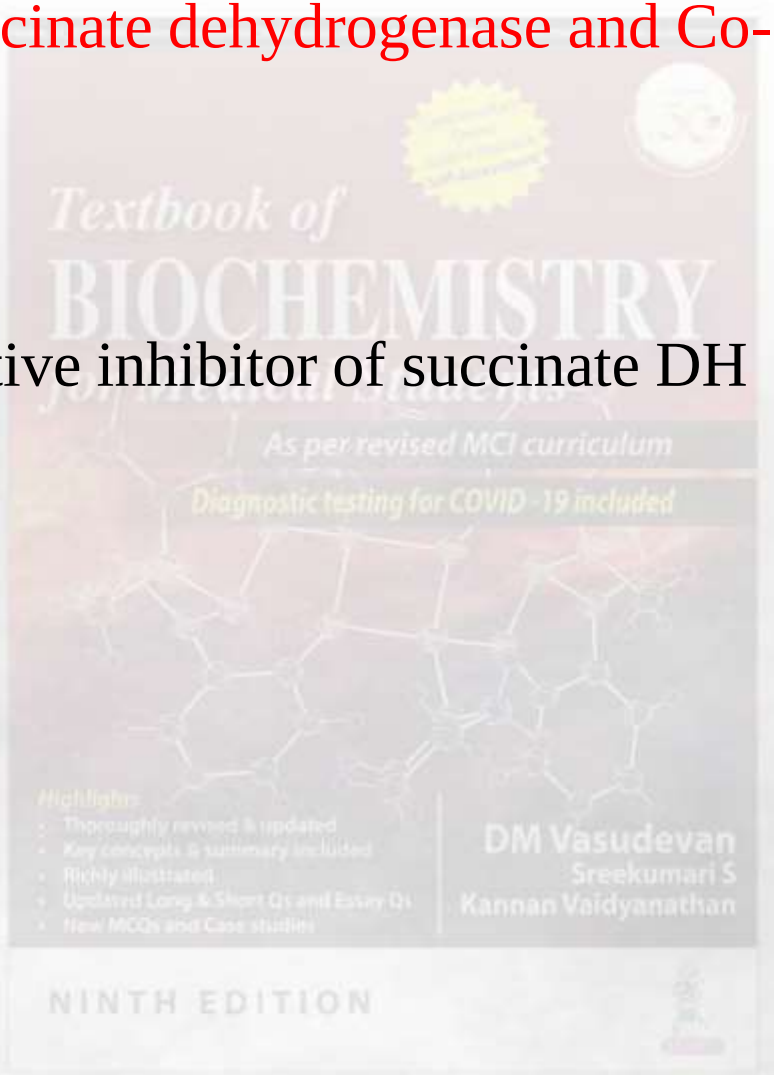
iv. Hydrogen sulphide (H_2S)



5. Site between succinate dehydrogenase and Co-Q

Carboxin

Malonate, competitive inhibitor of succinate DH



6. Inhibitors of oxidative phosphorylation

i. Atractyloside, inhibits translocase

ii. Oligomycin, inhibits flow of protons through Fo

iii. Ionophores, e.g., Valinomycin

7. Uncouplers

i. 2,4-dinitro phenol (2,4-DNP)

ii. 2,4-dinitro cresol (2,4-DNC)

iii. CCCP (Chloro carbonyl cyanide phenyl hydrazone)

8. Physiological uncouplers

i. Thyroxine, in high doses

ii. Thermogenin in brown adipose tissue

8. Ionophores

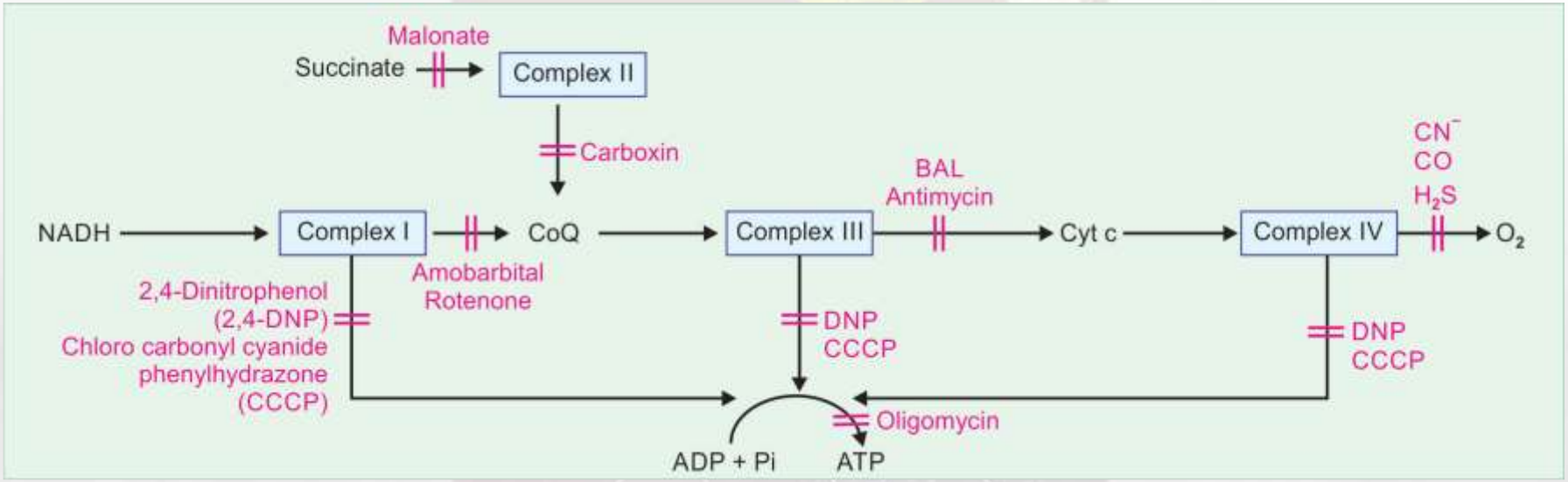
8-A)Mobile ion carriers:

Eg: Valinomycin: allows passage of K+; decrease the net positive charge on the outside of inner mitochondrial membrane

8-B)Channel formers:

Eg: Gramicidin.

Inhibitors of Electron Transport Chain and Oxidative Phosphorylation.



Inherited Disorders of Oxidative Phosphorylation (OXPHOS Diseases)

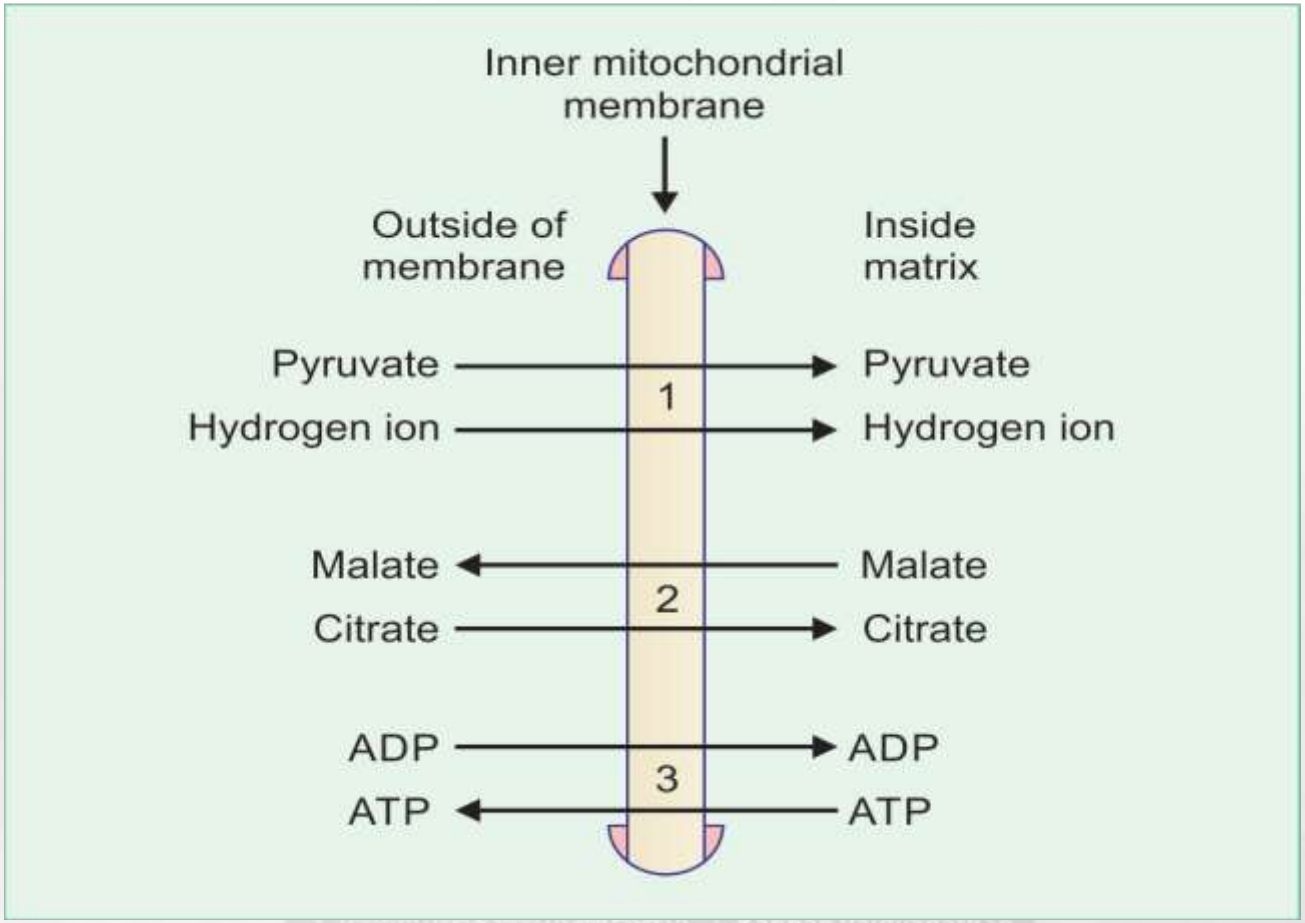
Cause : Defects in mitochondrial DNA

1) Leber's hereditary optic neuropathy (LHON)

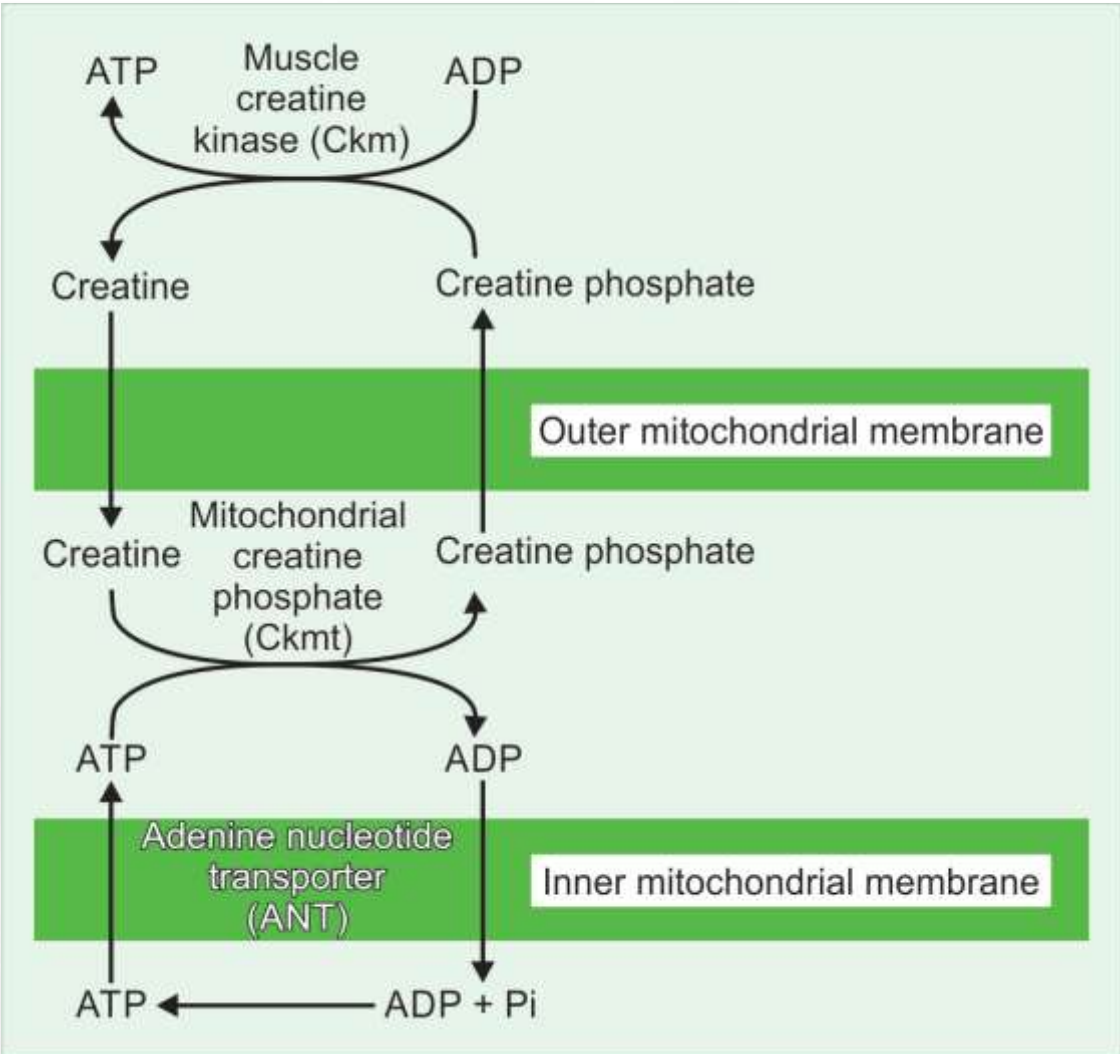
2) Mitochondrial myopathies:

-Fatal infantile mitochondrial myopathy.

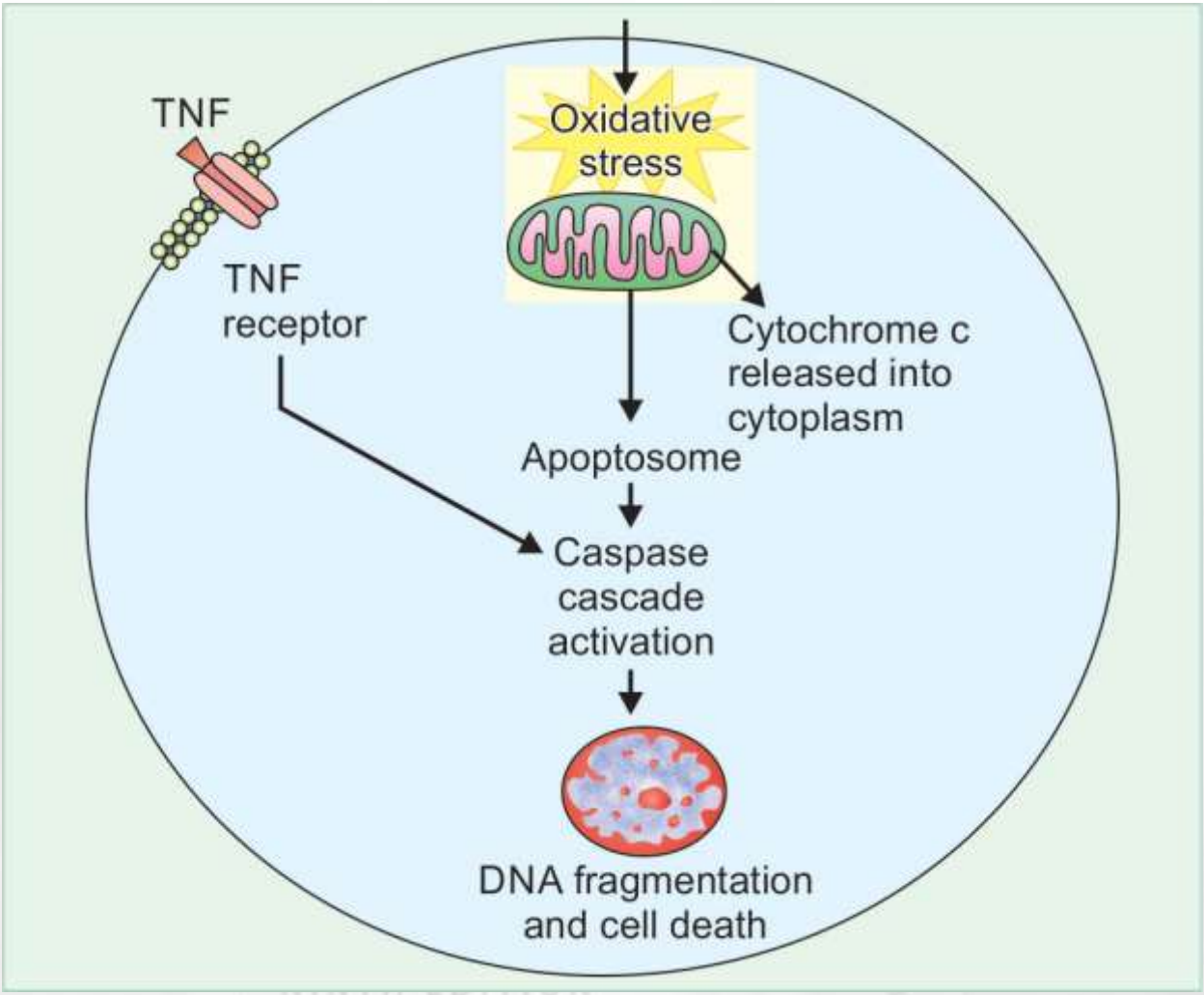
-Mitochondrial Encephalopathy Lactic acidosis and Stroke-like disorder (MELAS)



Important mitochondrial membrane transporters. 1 = PYT (pyruvate transporter); 2 = TCT (tricarboxylate transporter); 3 = ANT (adenine nucleotide transporter)



Creatine phosphate shuttle.



Role of mitochondria in apoptosis.