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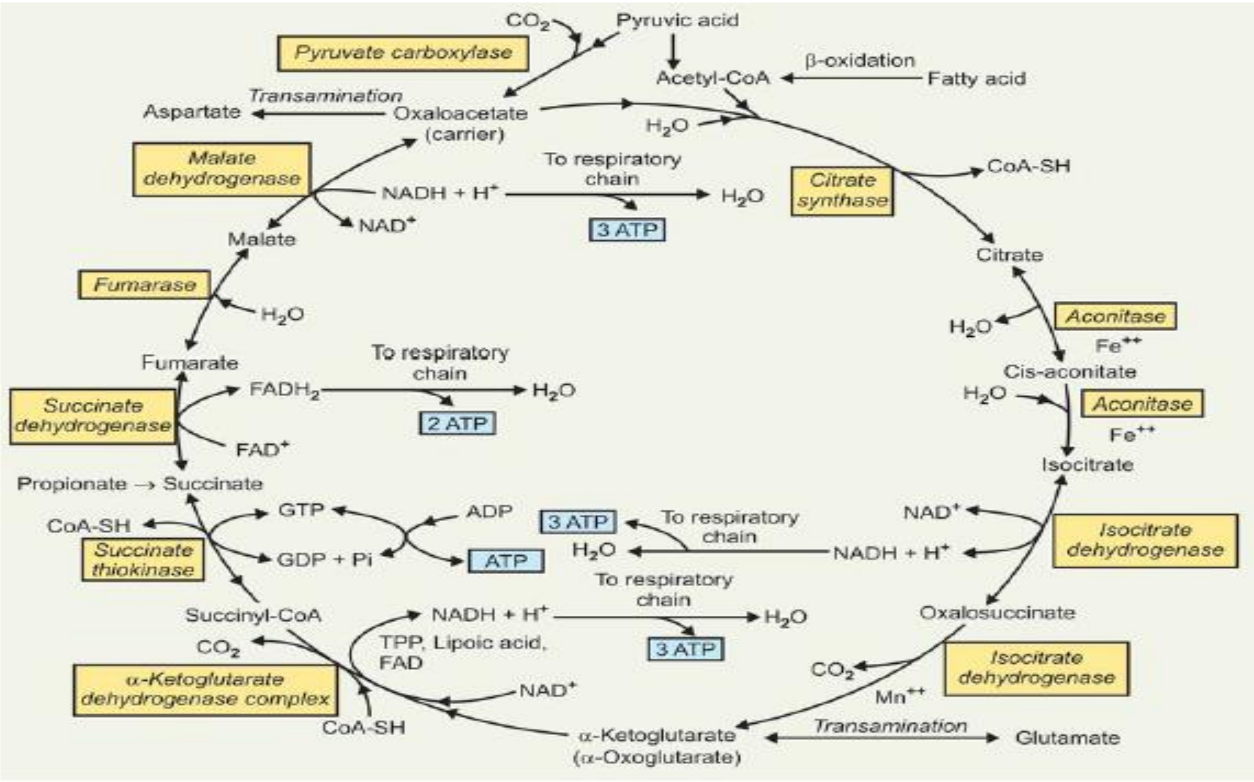
Department of medical chemistry Lecture/

Tricarboxylic Acid Cycle

Introduction:

The tri-carboxylic acid cycle ([TCA cycle] also called the citric acid cycle or the Krebs cycle) plays several roles in metabolism. It is the final pathway where the oxidative catabolism of carbohydrate s , amino acids , acetate , fatty acids and *ketone bodies all generate acetyl CoA, which is the substrate for the TCA cycle. As the activated 2-carbon acetyl group is oxidized to two molecules of CO_2, energy is conserved as NADH, FAD(2H), and GTP. The TCA cycle accounts for over two thirds of the ATP generated from fuel oxidation.*

Citric Acid Cycle



Biomedical Importance of Citric Acid Cycle

• Final common pathway for carbohydrates, proteins and fats, through formation of 2-carbon unit acetyl-CoA.

• Acetyl-CoA is oxidised to CO_2 and H_2O giving out energy **catabolic role.**

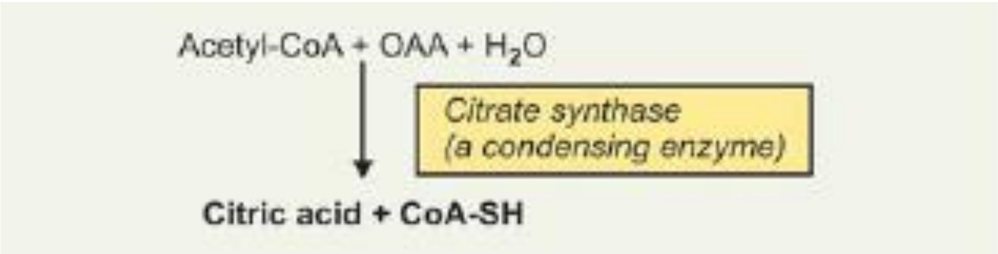
• Intermediates of TCA cycle play a major role in synthesis also like heme formation, formation of non-essential amino acids, FA synthesis, cholesterol and steroid synthesis **anabolic role**.

Reaction of Citric Acid Cycle:

Reactions of citric acid cycle are ***arbitrarily*** divided into **four stages** for discussion:

Stage I

1. ***Formation of Citric Acid from Acetyl-CoA and OAA***



• An ***irreversible*** reaction and an exergonic reaction gives out 7.8 Kcal.

• Acetyl group of acetyl-CoA is transferred to OAA, no oxidation or decarboxylation is involved.

• A molecule of H_2O is required to hydrolyse the “high energy” bond linkage between the acetyl group and CoA, the energy released is used for citrate condensation. ***No ATP is required.***

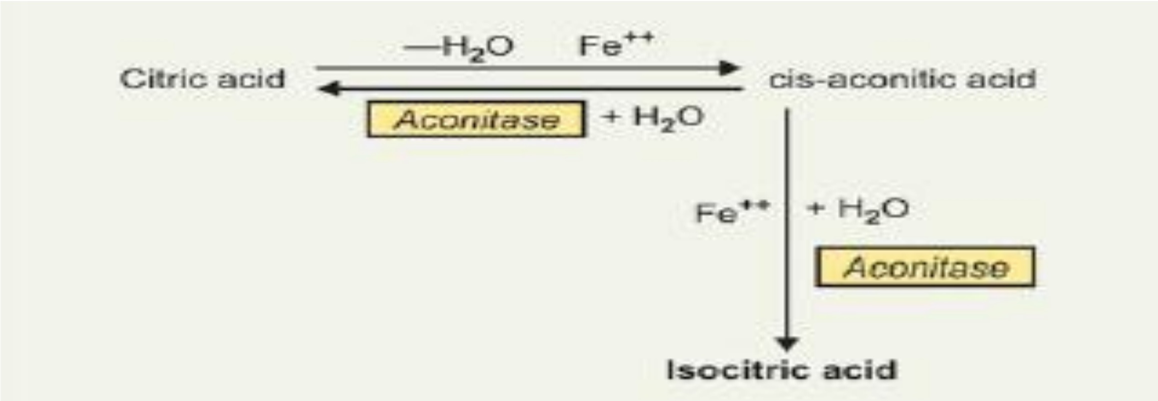
• COA-SH released is reutilized for oxidative decarboxylation of PA.

2. *Formation of cis-aconitic acid and isocitric acid from citric acid:*

Citric acid is converted to isocitric acid by the enzyme ***aconitase***. This conversion takes place in **two steps:**

• Formation of cis-aconitic acid from citric acid as a result of ***asymmetric dehydration,*** and

• Formation of isocitric acid from cis-aconitic acid as a result of ***stereospecific rehydration.*** Both processes are catalyzed by the same enzyme ***Aconitase*** which requires Fe^{++}.



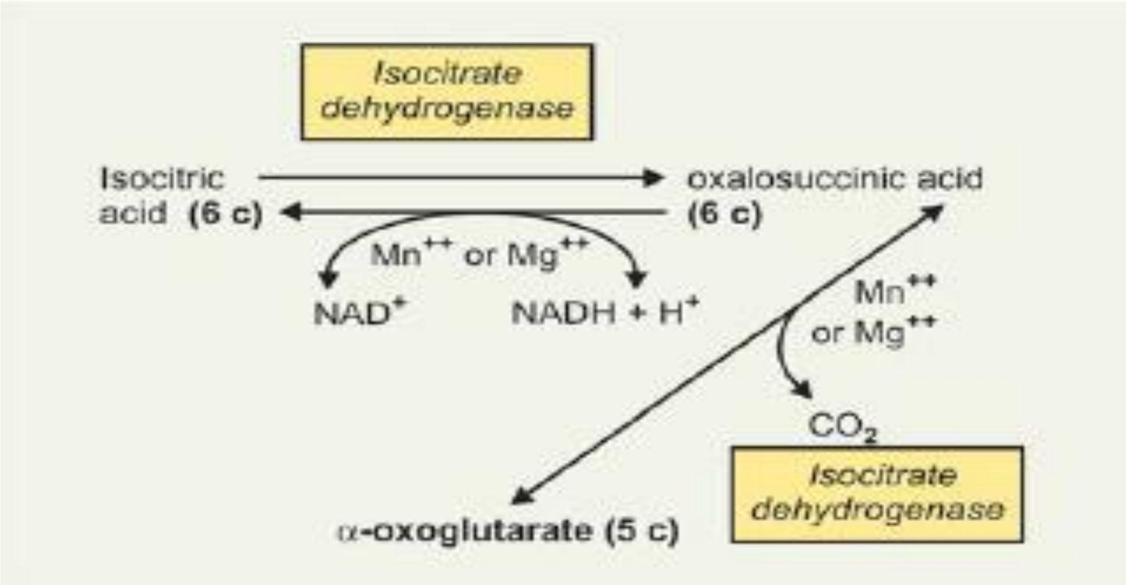
Inhibitor: *Fluoroacetate* is inhibitor of *aconitase*. Fluoroacetate, in the form of fluoroacetyl-CoA condenses with OAA to form fluorocitrate, which in turn inhibits the enzyme *aconitase* and allows citrate to accumulate.

Energetics: *No ATP formation at this stage*

Stage II:

The six-carbon isocitric acid is converted to a derivative of the ***four carbon succinyl-CoA***. The isocitric acid undergoes oxidation followed by decarboxylation to give α-oxoglutarate (5 C) (α-ketoglutarate).

1. Formation of oxalosuccinic acid and α-oxo-glutarate from isocitric acid: Since it is not possible to separate the dehydrogenase from the decarboxylase activity, it is concluded that these two reactions are catalyzed by a single enzyme. It is believed that oxalo-succinate is not a free intermediate but rather exists bound to the enzyme.



Isocitrate dehydrogenase (ICD) enzyme: Three types described:

- One NADP dependant ICD found in cytosol.
- Another NADP dependant ICD exists in mitochondria, greater activity and more widely distributed.
- One NAD^+ dependant ICD, found only in mitochondria. Respiratory chain-linked oxidation of isocitrate proceeds almost completely through the NAD^+ dependant ICD in mitochondrion.

2. Oxidative decarboxylation of α-oxoglutarate to succinyl-CoA: This reaction is analogous to oxidative decarboxylation of Pyruvic acid to acetyl-

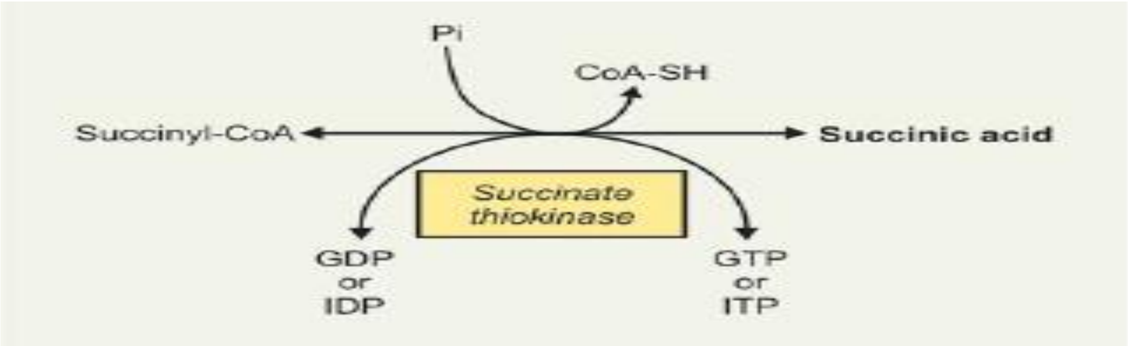
CoA. Enzyme is α-Ketoglutarate dehydrogenase complex It requires identical coenzymes and cofactors: **TPP, Lipoic acid, CoASH, FAD, NAD+ and Mg++.** Reaction steps are similar to PDH reaction. The reaction is ***irreversible***

Inhibitor: **Arsenite** inhibits the reaction causing the substrate α-oxoglutarate to accumulate.

Energetics: NADH produced is oxidised in respiratory chain yielding 3 ATP, from 2 NADH → 6 ATP will be produced **+ 6 ATP**

Stage III

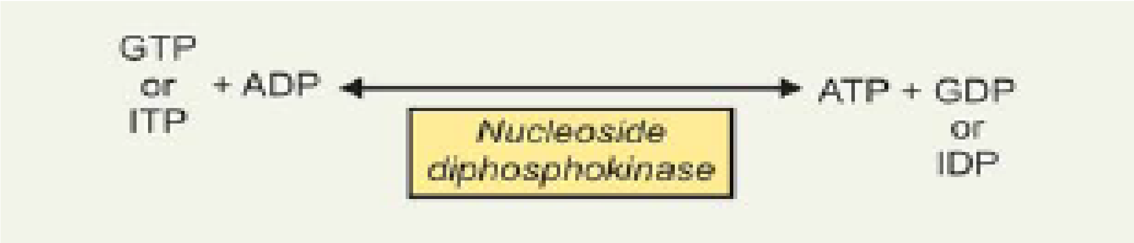
The product of preceding stage succinyl-CoA is converted to succinic acid to continue the cycle. Enzyme catalyzing this reaction is ***succinate thiokinase*** (also called as ***succinyl-CoA synthase***).



• Reaction requires GDP or IDP, which is converted in presence of Pi to either GTP or ITP.

• The release of free energy from oxidative decarboxylation of α-oxoglutarate is sufficient to generate a high energy bond in addition to the formation of NADH.

• In presence of enzyme ***nucleoside diphosphate kinase***, ATP is produced either from GTP or ITP.



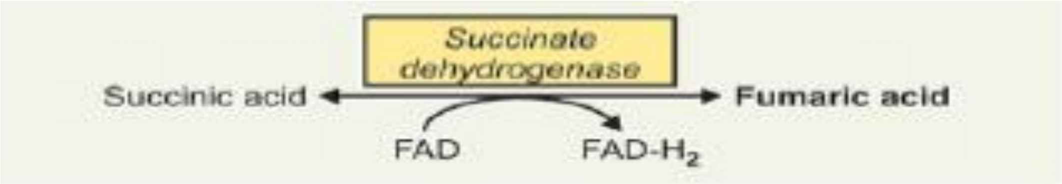
Thus, **ATP is produced at substrate level** without participation of electron transport chain. ***This is the only example of* substrate level Phosphorylation *in TCA cycle.***

Energetic: One ATP is produced in this reaction at substrate level. So from two succinyl-CoA → 2 ATP will be produced **+ 2 ATP**

Stage IV

This involves **three successive reactions** in which succinic acid is oxidised to oxaloacetate (OAA).

1. **Oxidation of succinic acid to fumaric acid:** It is a dehydrogenation reaction catalyzed by the enzyme ***succinate dehydrogenase***, hydrogen acceptor is FAD. The enzyme is **Ferri-flavoprotein**, mol. wt = 200,000 containing FAD and Iron-sulphur (Fe: S), contains 4 atoms of non-haem Fe and one FAD per mol. Of enzyme. In contrast to other enzymes of TCA cycle, this enzyme is bound to the inner surface of the inner mitochondrial membrane.



This is the only dehydrogenation in citric acid cycle which involves direct transfer of H from substrate to a flavoprotein without the participation of NAD^+.

Inhibitor: Addition of ***Malonate***/or ***OAA*** inhibits succinate dehydrogenase competitively resulting in accumulation of succinate.

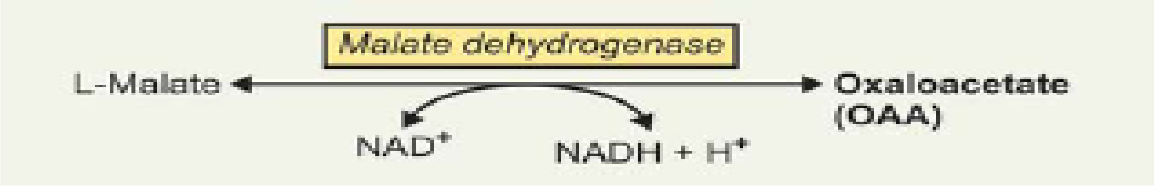
Energetic: Oxidation of FAD.H2 through ETC yields 2 ATP. Hence 2 molecules of succinic acid will give 4 ATP **+ 4 ATP**

2. *Formation of Malic Acid from Fumaric Acid*

In addition to being specific for the L-isomer of malonate, fumarase catalyses the addition of the elements of water to the double bond of fumarate in the ‘trans’ configuration.

3. Oxidation of malic acid to oxaloacetate (OAA): The reaction is catalyzed by the enzyme ***Malate dehydrogenase*** which requires NAD^+ as H-acceptor.

OAA produced acts ‘Catalytically’, combines with a fresh molecule of acetyl-CoA and the whole process is repeated.



Energetic: Oxidation of $NADH_2$ through ETC produces 3 ATP. Hence two molecules of NADH+ will give 6 ATP **+ 6 ATP**

Role of Vitamins in TCA Cycle:

Five B vitamins are associated with TCA cycle essential for yielding energy.

• Riboflavin: In the form of flavin adenine dinuleotide (FAD) a cofactor for succinate dehydrogenase enzyme.

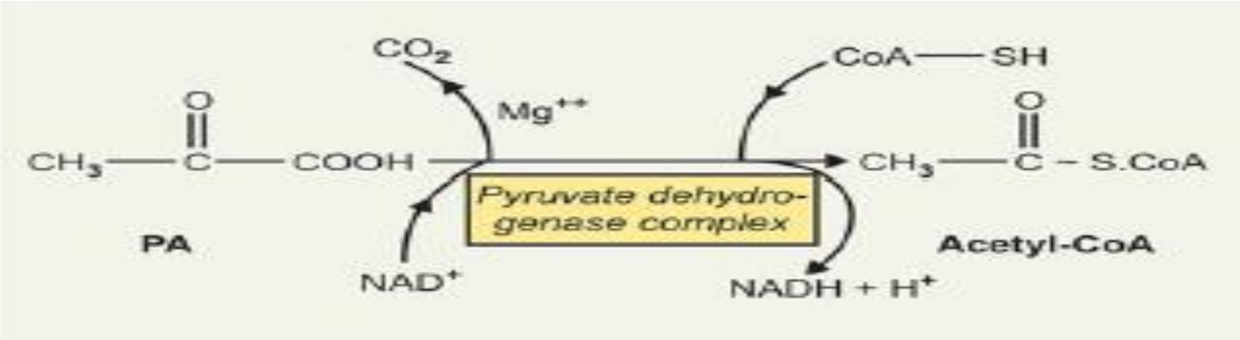
• Niacin: In the form of nicotinamide adenine dinucleotide (NAD) the electron acceptor for isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase.

• Thiamine: As "thiamine diphosphate"—required as coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction.

• Lipoic acid: It is required as coenzyme for α-ketoglutarate dehydrogenase reaction.

• Pantothenic acid: As part of coenzyme A, the cofactor attached to "active" carboxylic acid residues such as acety CoA and succinyl-CoA.

Oxidative decarboxylation of pyruvic acid



A. Glycolysis	*ATP yield per hexose unit*
1. Glycogen → Fructose-1, 6 bi — P	–1 ATP
2. Glucose → Fructose-1, 6 bi — P	–2 ATP
3. Glyceraldehyde-3P-dehydrogenase ($2\ NADH \rightarrow 2\ NAD^+$)	+ 6 ATP
4. Substrate level Phosphorylation	
(a) 2-phosphoglycerate kinase	+ 2 ATP
(b) Pyruvate kinase	+ 2 ATP
Net gain	
• For glucose =	+ 8 ATP
• For glycogen =	+ 9 ATP
B. Oxidative decarboxylation of PA	
1. Pyruvate dehydrogenase complex ($2\ NADH \rightarrow 2\ NAD^+$)	+ 6 ATP

C. *TCA Cycle*

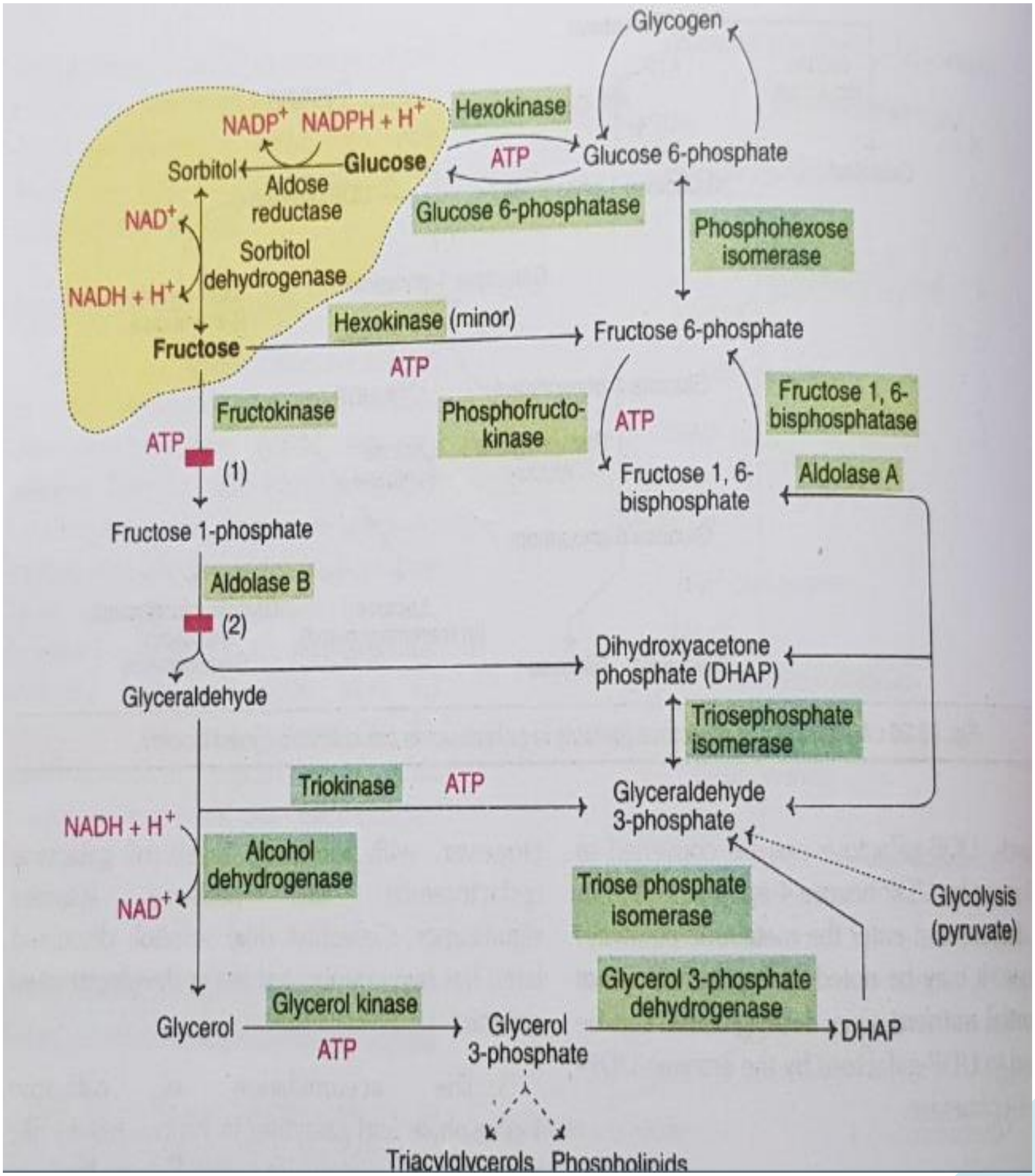
1. Isocitrate dehydrogenase
   2 ($NADH \rightarrow NAD^+$) + 6 ATP
2. α-Ketoglutarate dehydrogenase complex
   2 ($NADH \rightarrow NAD^+$) + 6 ATP
3. Substrate level phosphorylation
   succinate thiokinase
   2 GTP or 2 ITP → 2 ATP + 2 ATP
4. Succinate dehydrogenase
   2($FAD.H_2 \rightarrow FAD$) + 4 ATP
5. Malate dehydrogenase
   2($NADH \rightarrow NAD^+$) + 6 ATP

Total per mol. of glucose = 30 + 8
(under aerobic condition) = 38 ATP

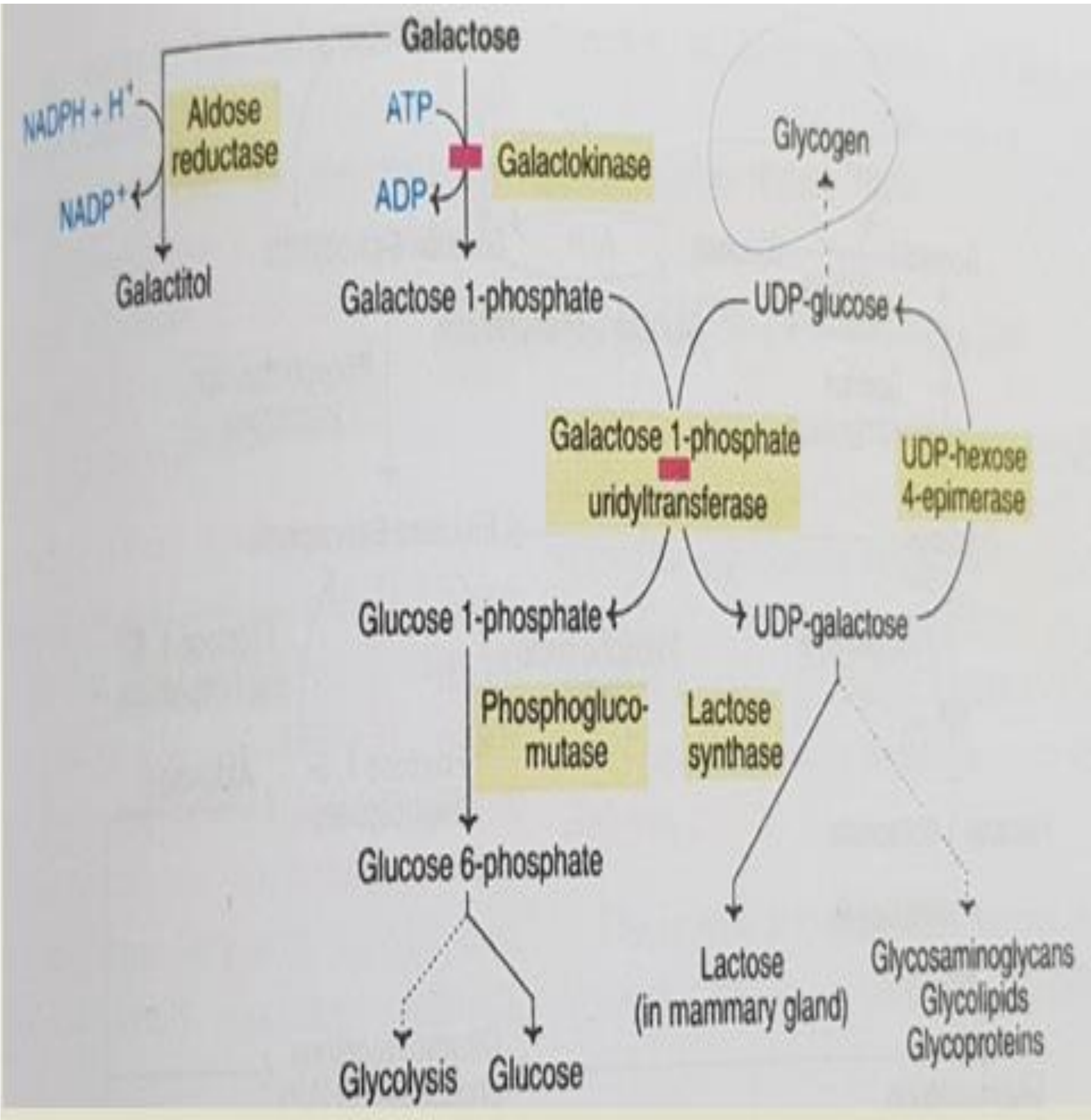
Total per mol. of glycogen
(glycogenolysis provides G-1-P 30 + 9
(G-1-P → G-6-P) = 39 ATP

Total per mol. of glucose
(under anaerobic condition) = +2ATP
(only in anaerobic glycolysis)

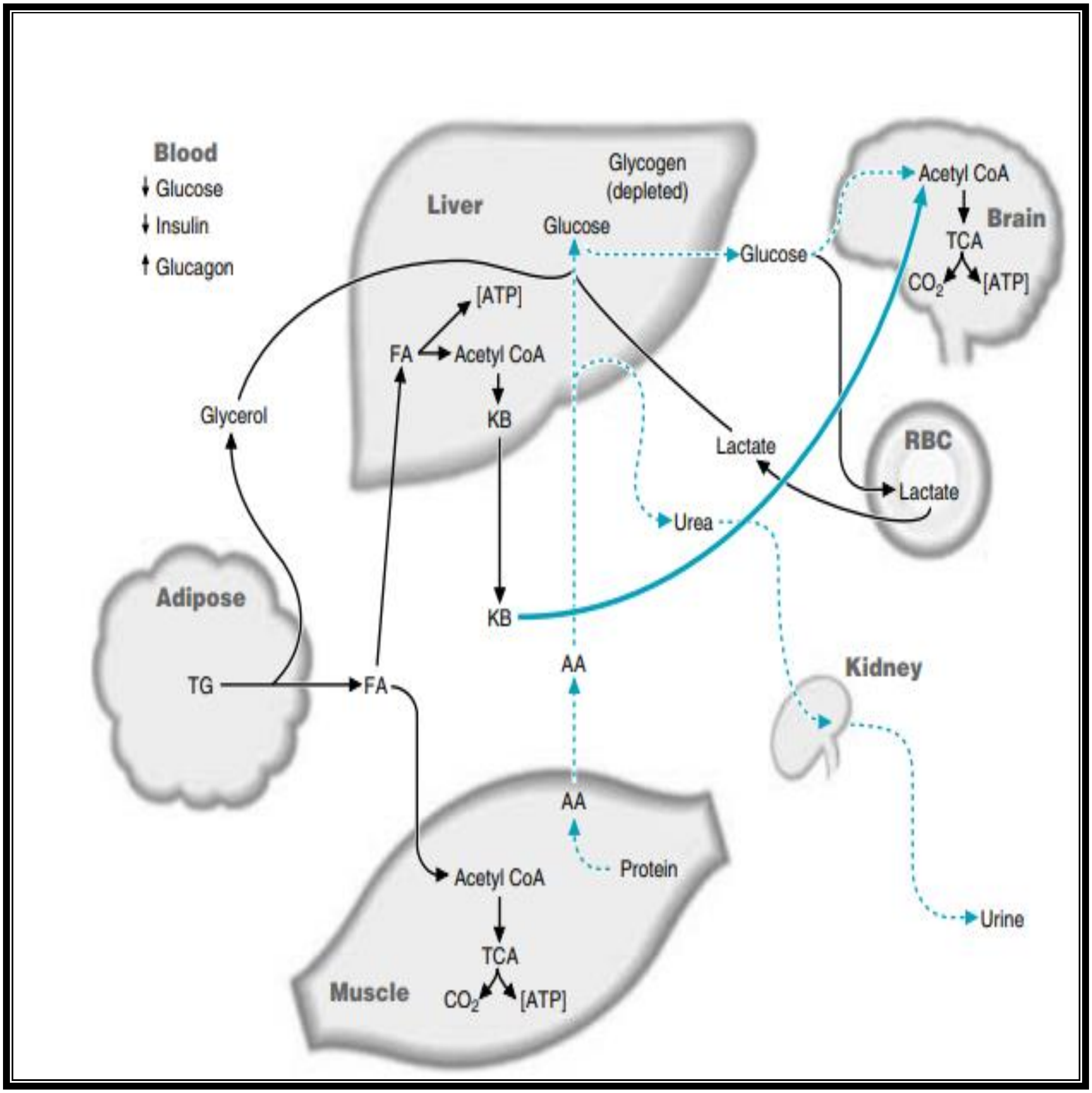
Fructose metabolism:



Galactose Metabolism:



Starved state:



Cori Cycle:

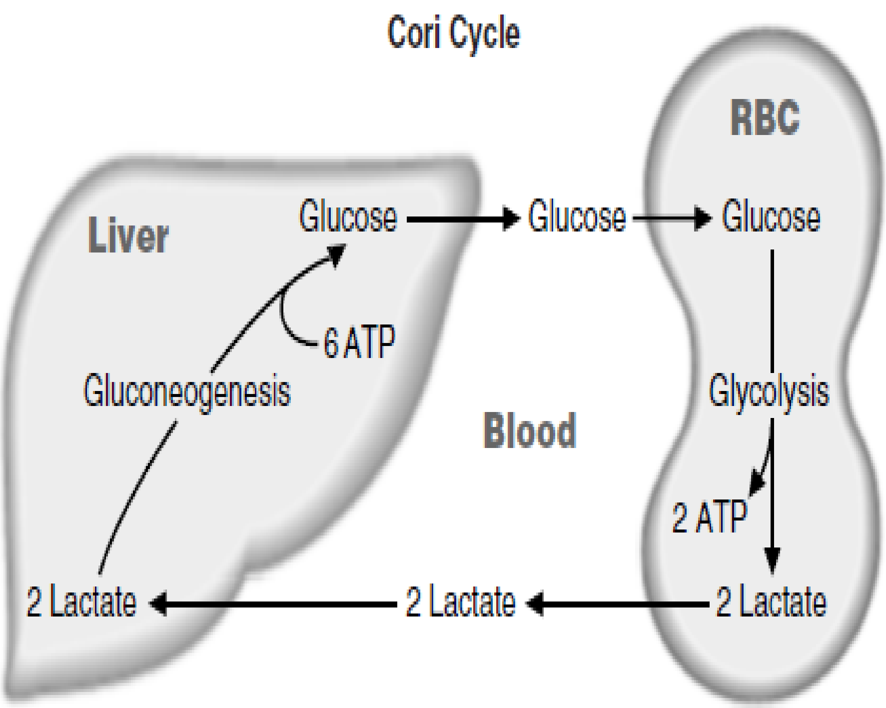


Fig. 22.10. Cori cycle. Glucose, produced in the liver by gluconeogenesis, is converted by glycolysis in muscle, red blood cells, and many other cells, to lactate. Lactate returns to the liver and is reconverted to glucose by gluconeogenesis.