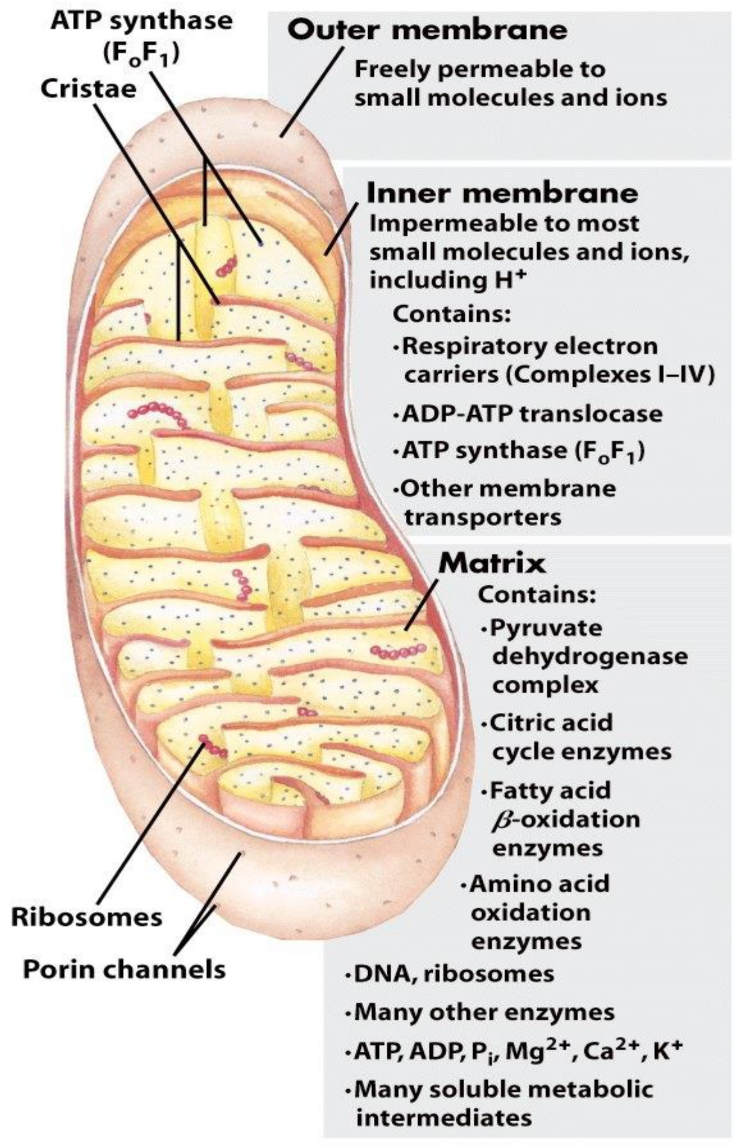
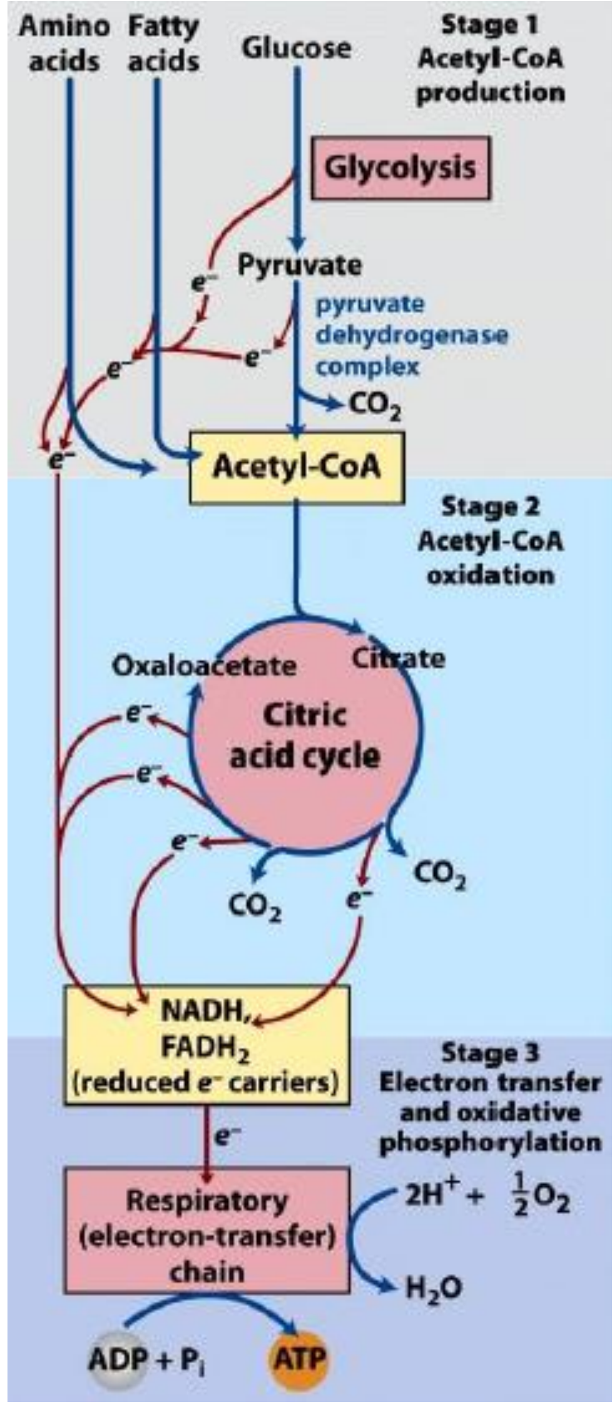
Mitochondrial transport processes, respiratory chain and ATP synthesis

From the Chemistry Exam to the Final Exam in Biochemistry

Dr. Lengyel Anna

Biochemical anatomy of a mitochondrion





Amino acids
Fatty acids
Glucose
Stage 1
Acetyl-CoA production
Glycolysis
Pyruvate
pyruvate dehydrogenase complex
CO_2
e^-
Acetyl-CoA
Stage 2
Acetyl-CoA oxidation
Oxaloacetate
Citrate
Citric acid cycle
CO_2
NADH, $FADH_2$ (reduced e^- carriers)
Stage 3
Electron transfer and oxidative phosphorylation
Respiratory (electron-transfer) chain
$2H^+ + \frac{1}{2}O_2$
H_2O
ADP + P_i
ATP

Question

What phase of cellular respiration has the highest ATP yield?

a) Oxidative phosphorylation
b) Gluconeogenesis
c) Krebs cycle
d) Glycolysis
e) Fermentation

Question

Given a healthy individual with a normal metabolic rate, which of the following compounds is the most energy rich?

A. GTP
B. ATP
C. $FADH_2$
D. NADH

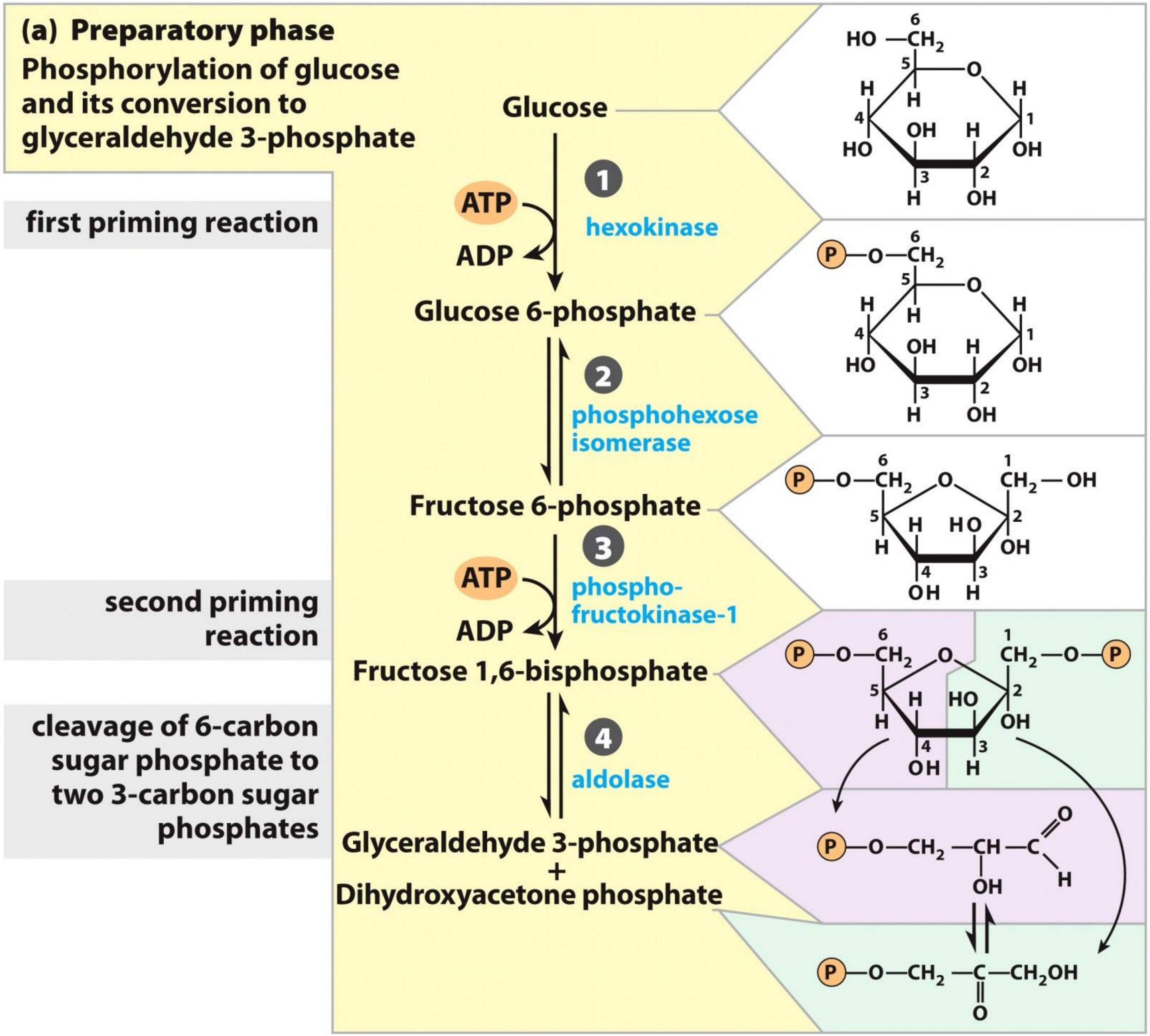


Figure 14-2 part 1

Lehninger Principles of Biochemistry, Sixth Edition

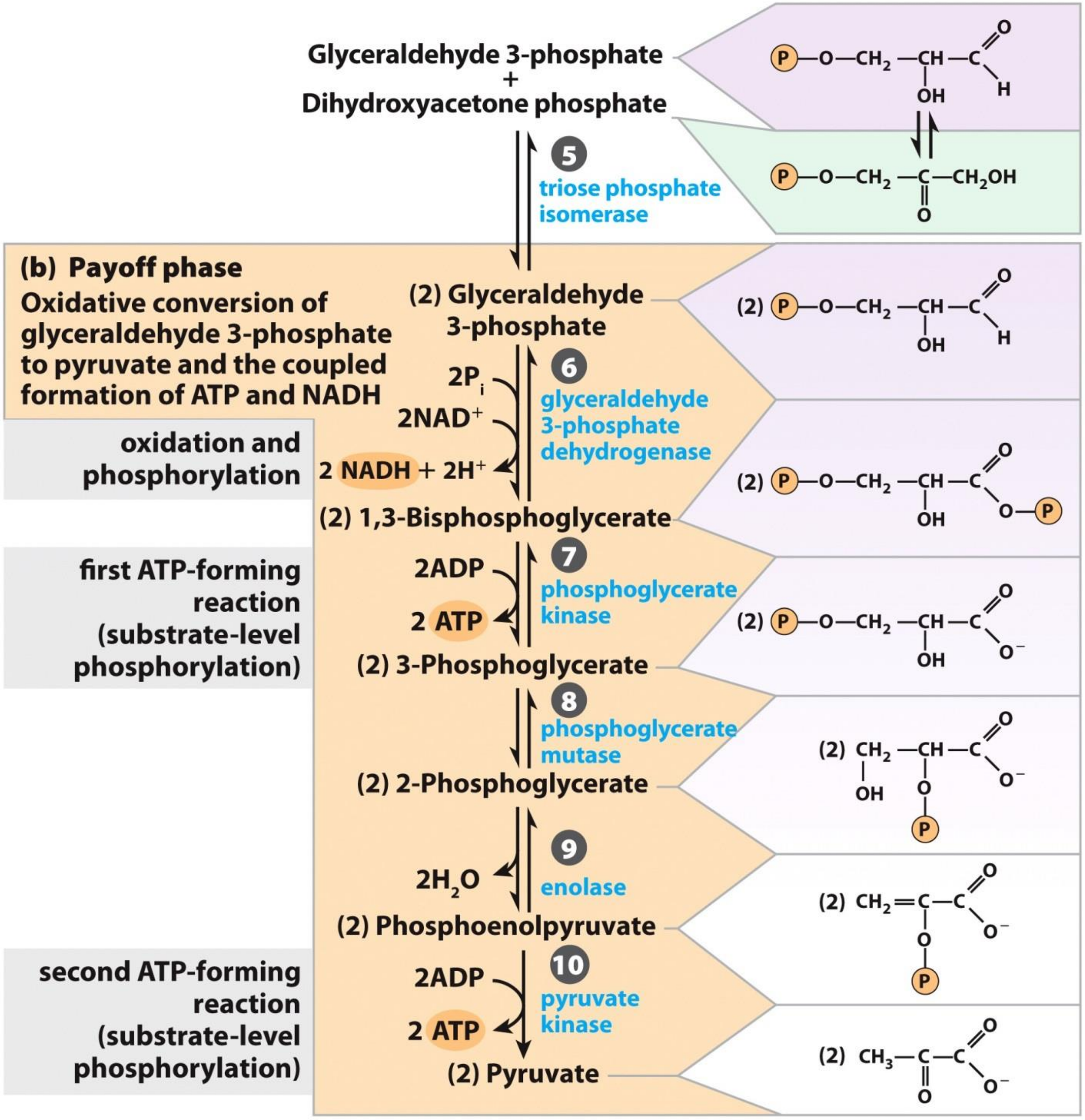


Figure 14-2 part 2

Lehninger Principles of Biochemistry, Sixth Edition

Question

In substrate level phosphorylation…

A. ATP synthesis is linked to dissipation of proton gradient.

B. High energy intermediate compounds cannot be isolated.

C. Oxidation of one molecule of substrate is linked to synthesis of more than one ATP molecule.

D. Only mitochondrial reactions participate in ATP formation.

E. The cleavage of the high-energy bond in the substrate provides the energy required for ATP synthesis.

Question

The energy of oxidation is initially trapped as a high-energy phosphate compound and then used to form ATP. Which of the following intermediates of glycolysis is a high energy compound?

A. Fructose-6-P

B. Glyceraldehyde-3-P

C. Fructose-1,6 bisphosphate

D. Glucose-6-P

E. Phosphoenol pyruvate

Mitochondrial transports

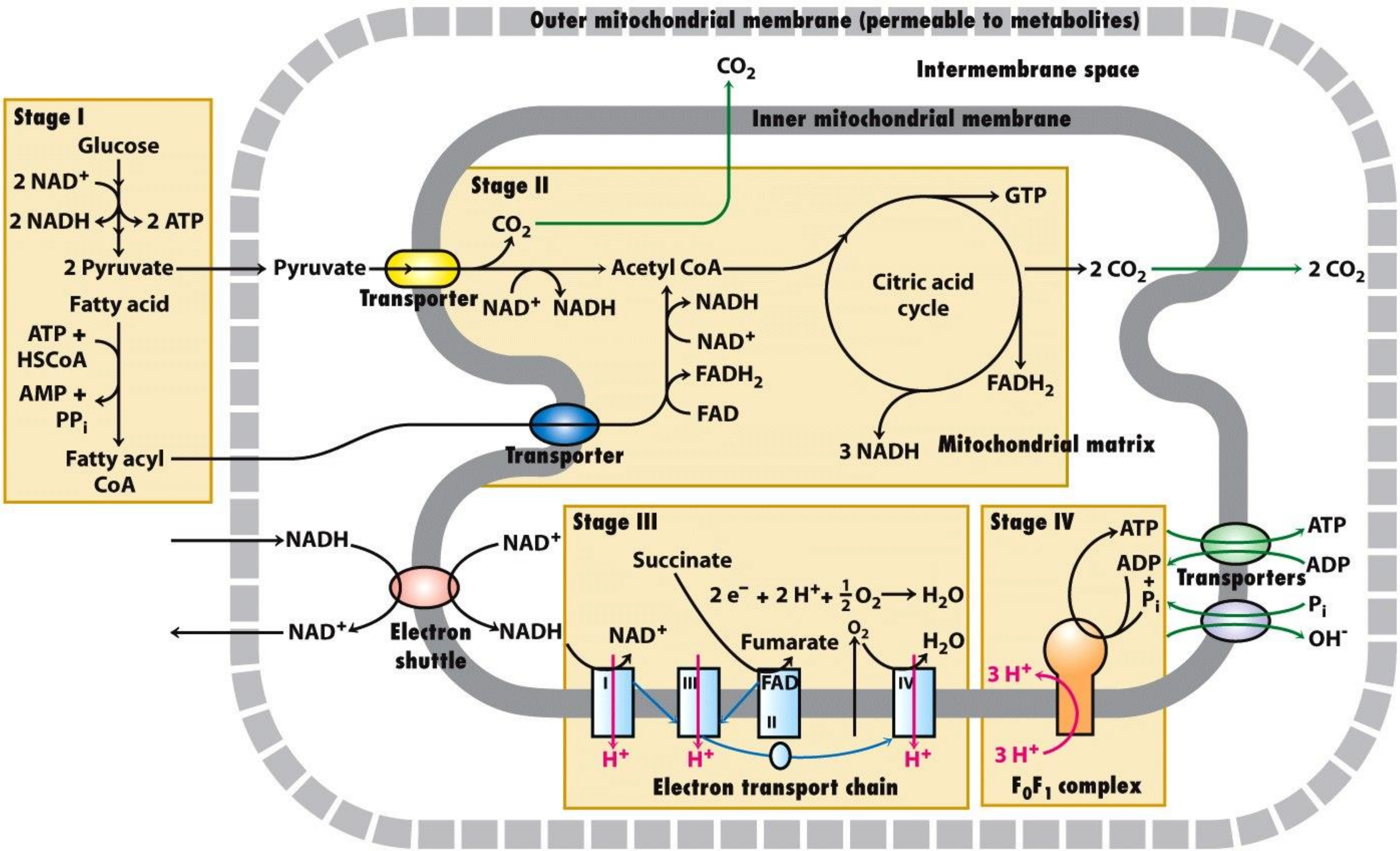
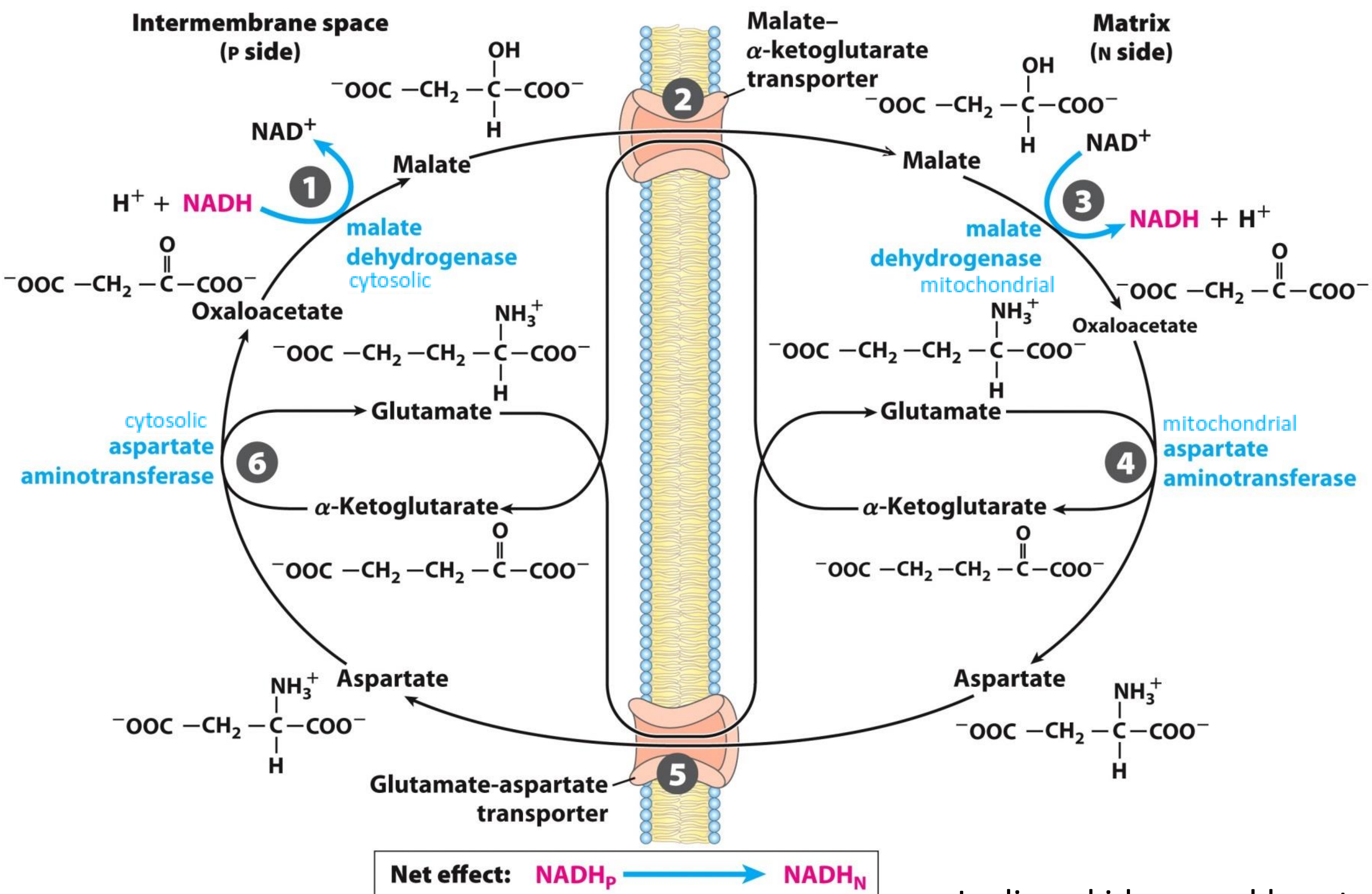


Figure 12-8
Molecular Cell Biology, Sixth Edition

Malat – aspartate shuttle

P/O = 2.5

From cytosolic NADH+H⁺ mitochondrial NADH+H⁺



In liver, kidney and heart

Figure 19-31
Lehninger Principles of Biochemistry, Sixth Edition

Glycerol 3-phosphate shuttle

P/O = 1.5

From cytosolic NADH+H^+ mitochondrial $FADH_2$

NADH + H^+ + E–FAD
cytosolic — mitochondrial

↓

NAD^+ + E–$FADH_2$
cytosolic — mitochondrial

In brain and muscle

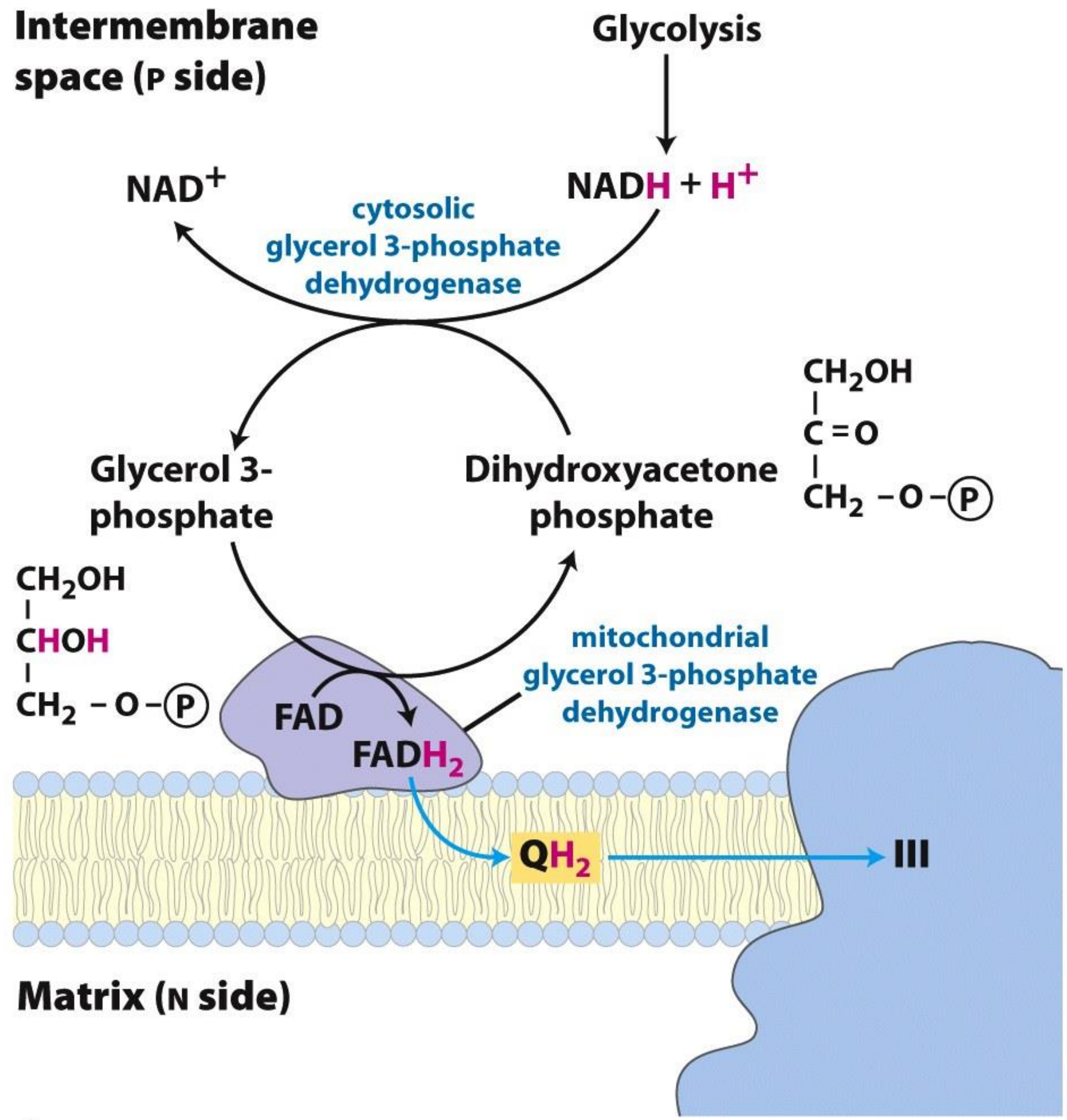
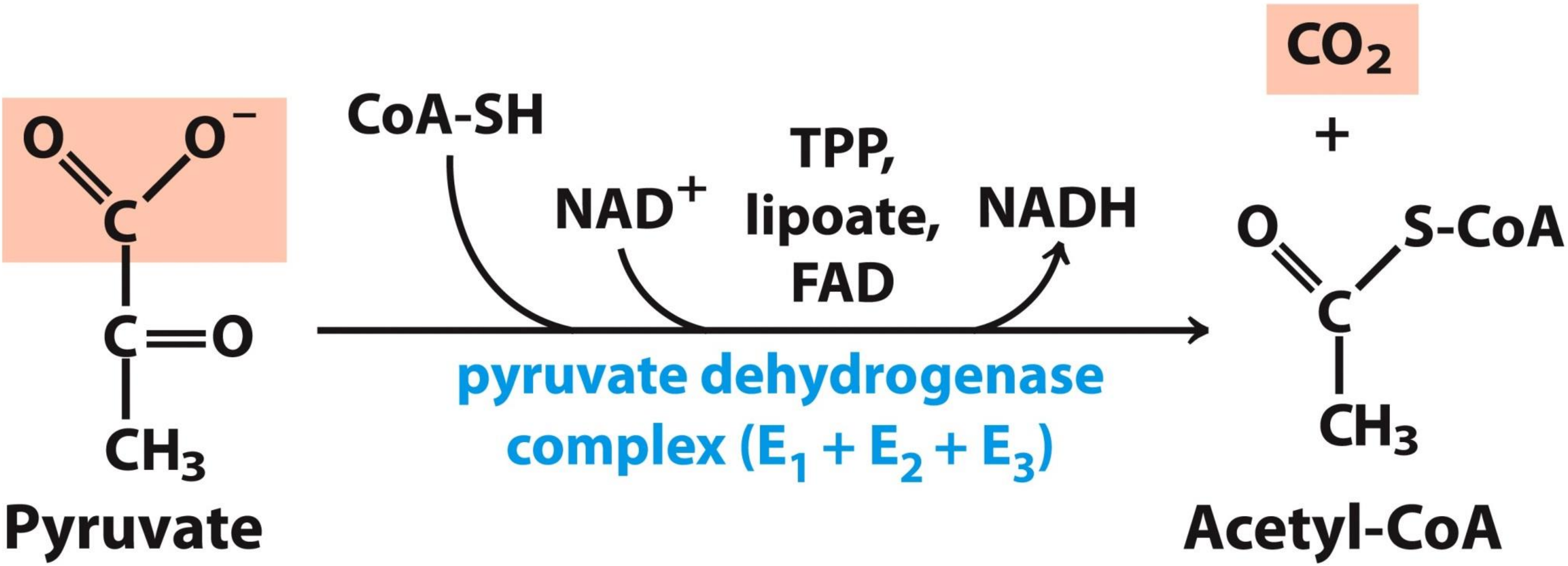


Figure 19-30
Lehninger Principles of Biochemistry, Fifth Edition



$\Delta G'^{\circ} = -33.4$ kJ/mol

Figure 16-2
Lehninger Principles of Biochemistry, Sixth Edition

Question

Which one of the following enzymes catalyzes substrate level phosphorylation in TCA cycle?

A. Malate dehydrogenase

B. Succinyl-CoA synthetase

C. α-ketoglutarate dehydrogenase complex

D. Isocitrate dehydrogenase

E. Succinate dehydrogenase

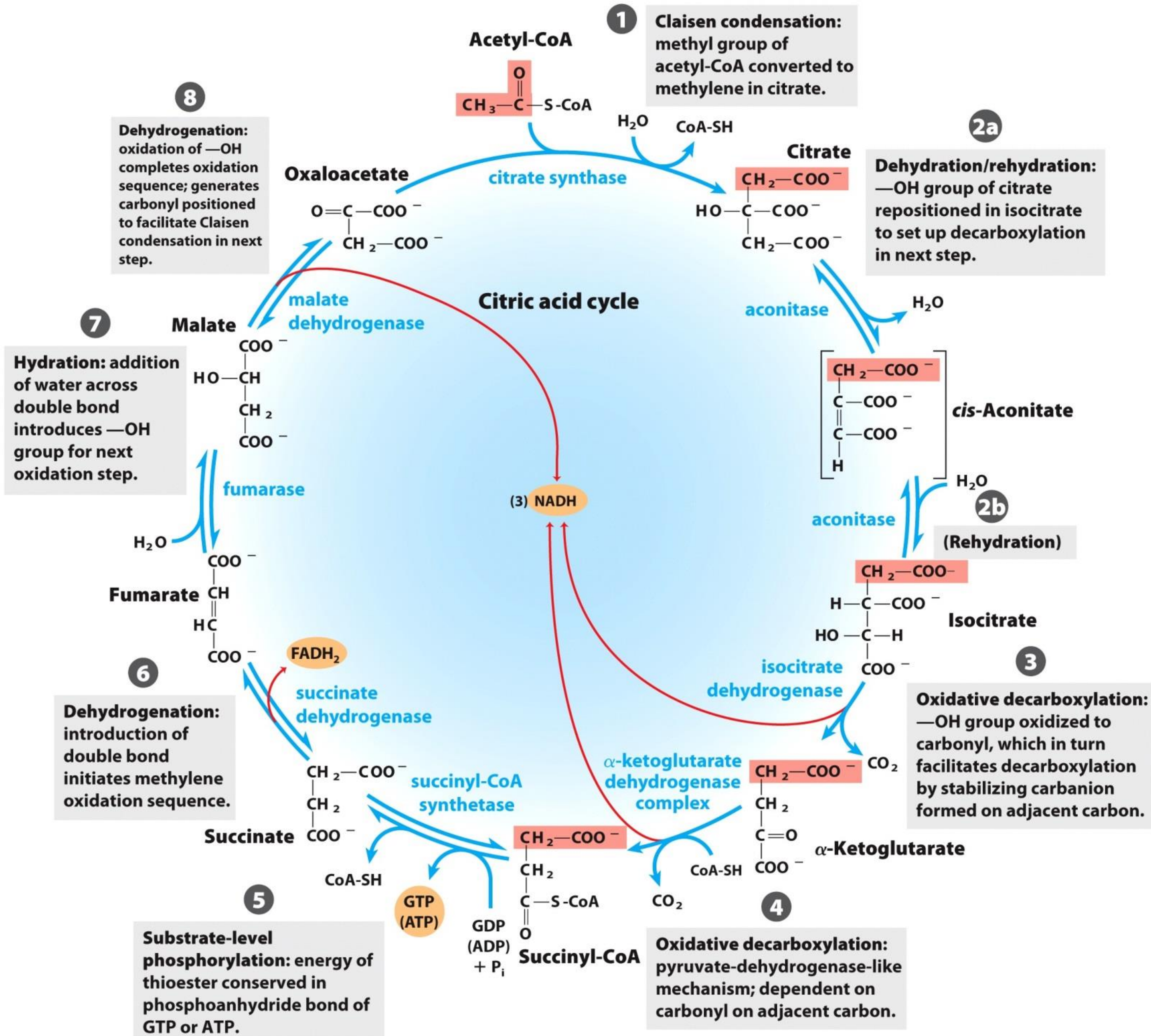


Figure 16-7

Lehninger Principles of Biochemistry, Sixth Edition

Question

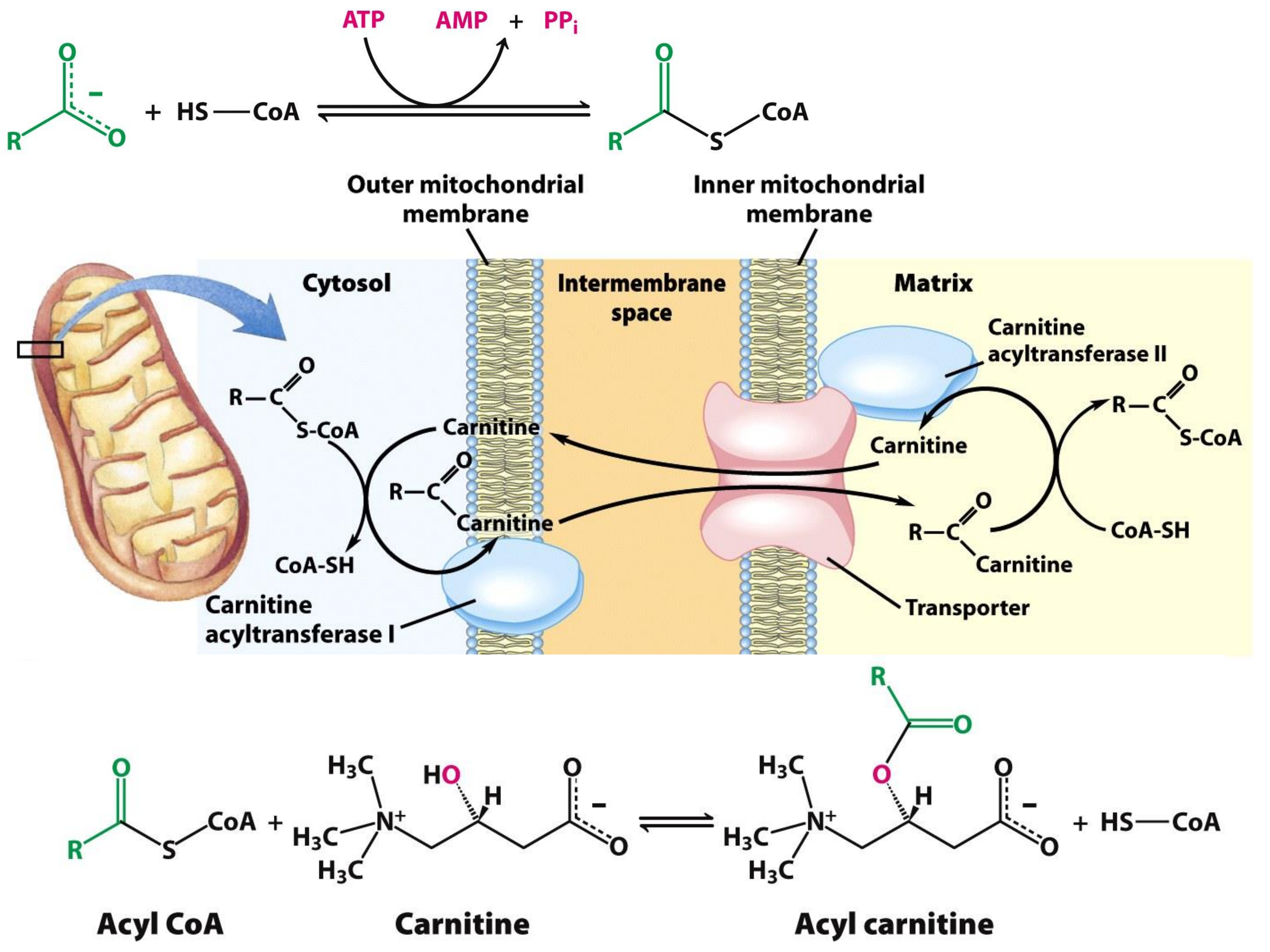
During aerobic respiration, which of the following pathways correctly orders the process of cellular metabolism after glycolysis in eukaryotic cells?

A. Citric acid cycle → Pyruvate decarboxylation → Oxidative phosphorylation

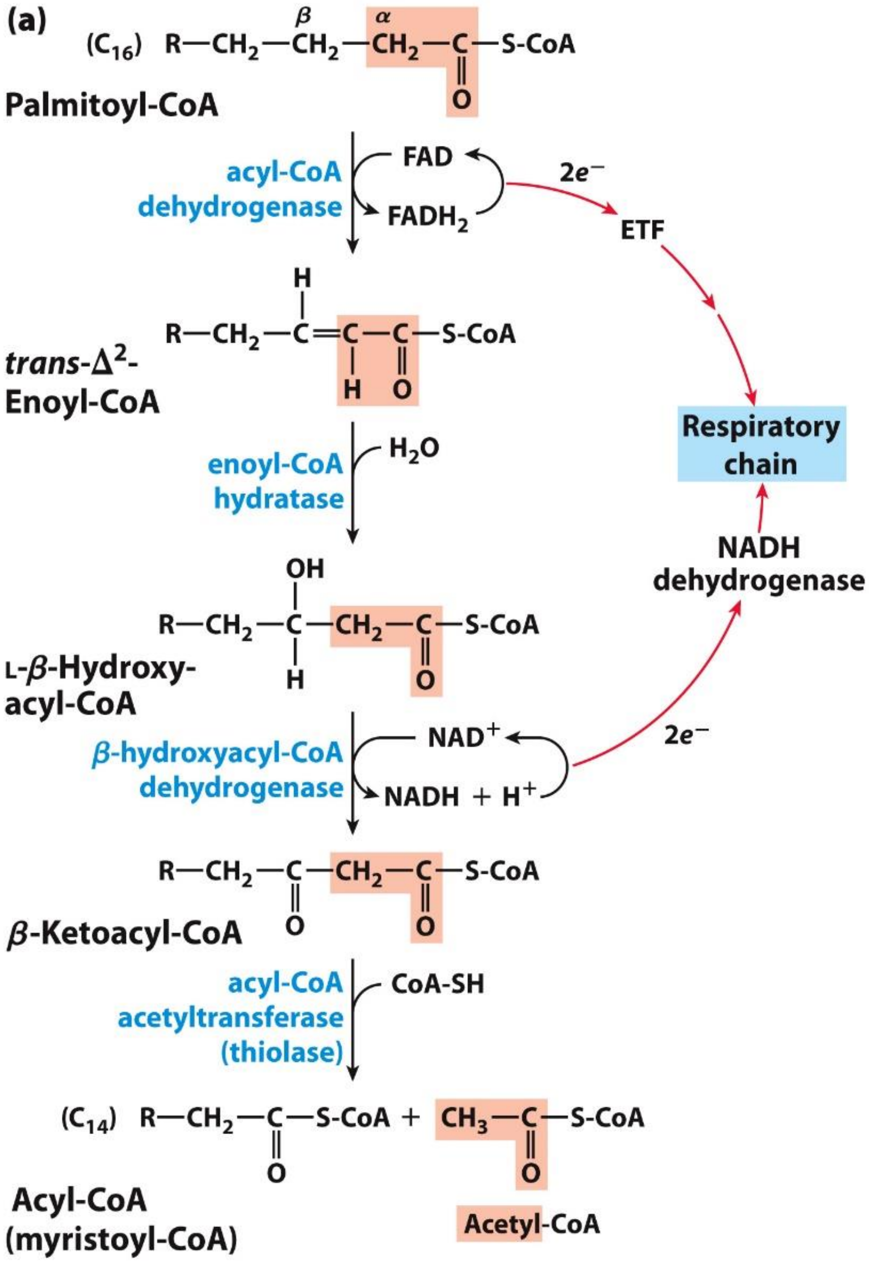
B. Pyruvate decarboxylation → Oxidative phosphorylation → Citric acid cycle

C. Citric acid cycle → Oxidative phosphorylation → Pyruvate decarboxylation

D. Pyruvate decarboxylation → Citric acid cycle → Oxidative phosphorylation



ATP
AMP + PP$_i$
R
O
O
+ HS—CoA
R
O
S
CoA
Outer mitochondrial membrane
Inner mitochondrial membrane
Cytosol
Intermembrane space
Matrix
R—C
O
S-CoA
Carnitine
R—C
O
Carnitine
CoA-SH
Carnitine acyltransferase I
Carnitine acyltransferase II
R—C
O
S-CoA
Carnitine
R—C
O
Carnitine
CoA-SH
Transporter
R
O
S
CoA
+
H$_3$C
H$_3$C
H$_3$C
N$^+$
HO
H
O
O
R
O
O
H$_3$C
H$_3$C
H$_3$C
N$^+$
H
O
O
+ HS—CoA
Acyl CoA
Carnitine
Acyl carnitine



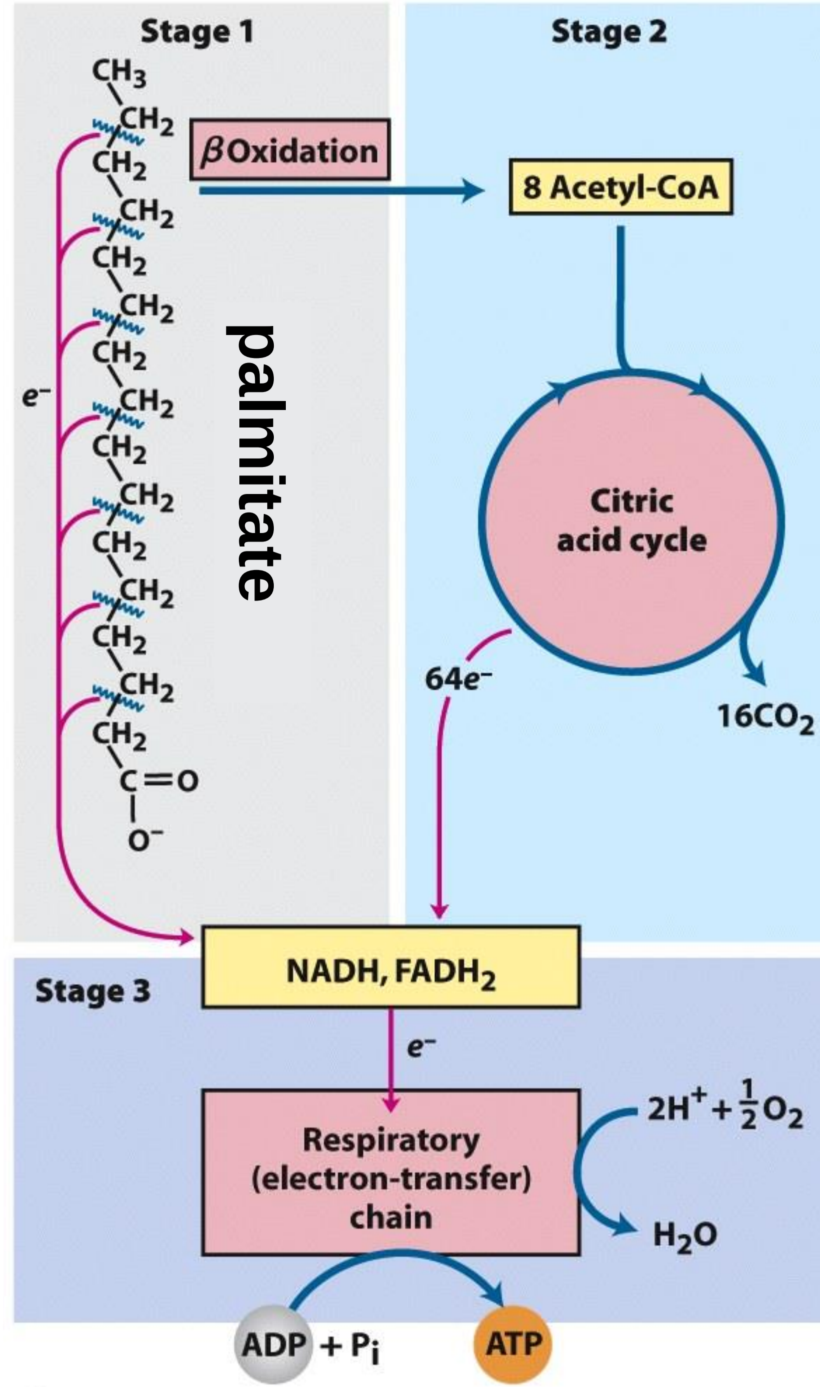


Figure 17-8
Lehninger Principles of Biochemistry, Seventh Edition

Question

All of the following except one are NAD^+ requiring enzymes:

A. Acyl-CoA dehydrogenase

B. Glyceraldehyde-3-P dehydrogenase

C. Pyruvate dehydrogenase complex

D. Malate dehydrogenase

E. Lactate dehydrogenase

The respiratory chain

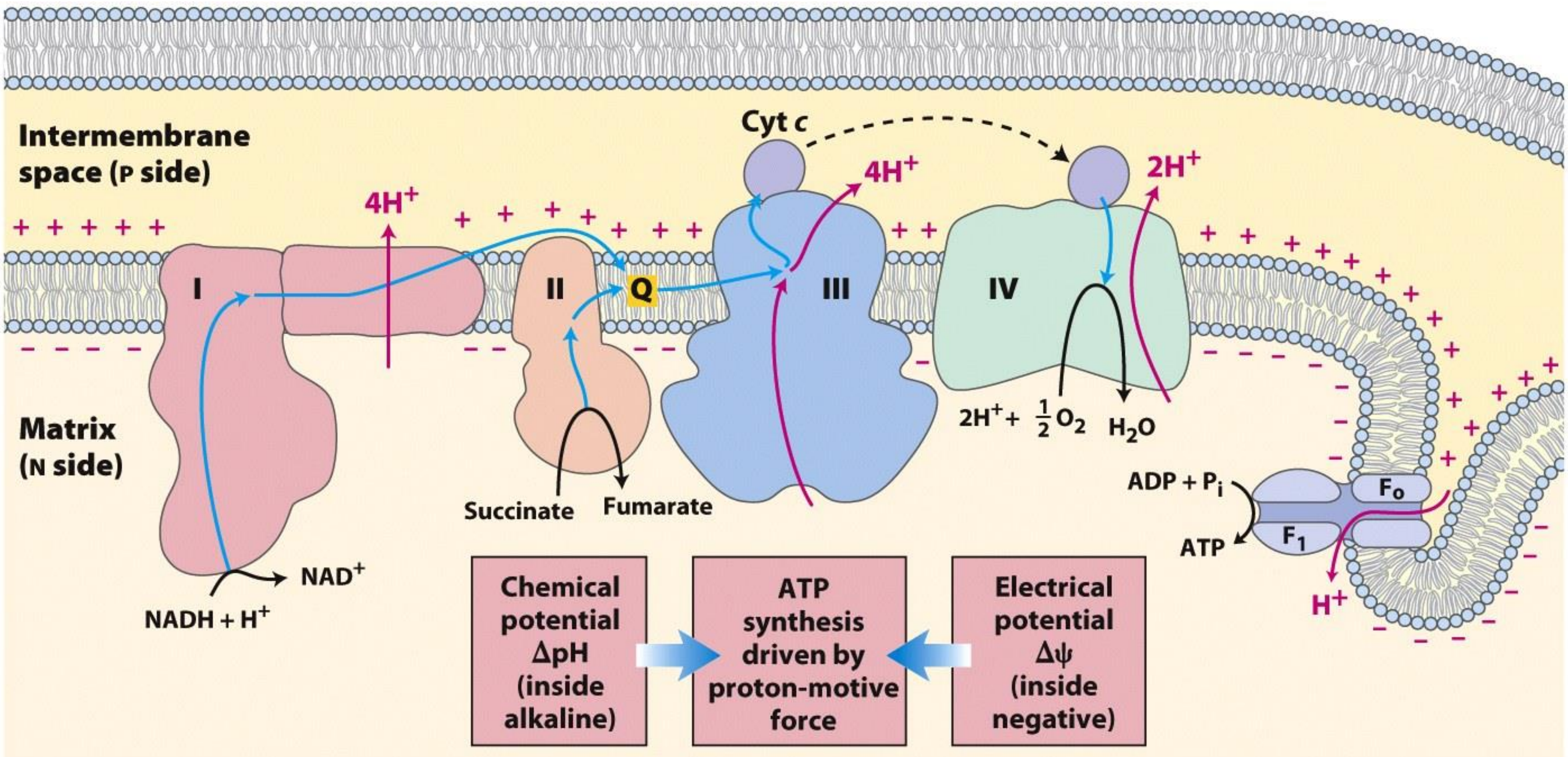


Figure 19-19
Lehninger Principles of Biochemistry, Fifth Edition

The e^- transport is an exergonic process ($\Delta G<0$), it covers the energy requirement of the p^+ transport which is an endergonic process ($\Delta G>0$).

Question

The primary purpose of the electron transport chain of mitochondria is ___________.

a) to directly phosphorylate ADP
b) to synthesize ATP synthase
c) to directly phosphorylate AMP
d) to carry ADP into the mitochondrial matrix
e) the generation of energy to sequester protons in the intermembrane space

Question

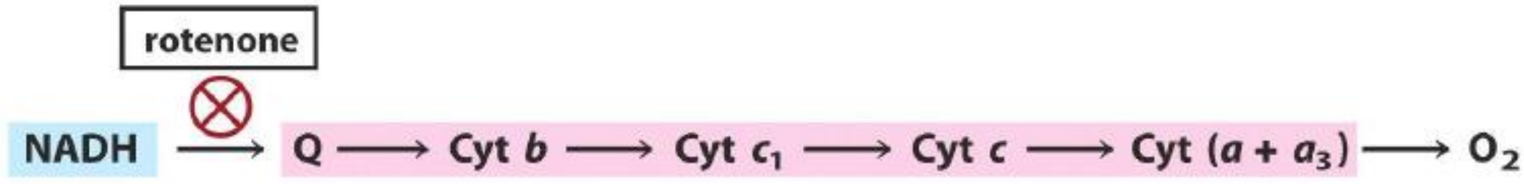
Which of the following areas of the mitochondria has the lowest pH?

A. The mitochondrial matrix
B. The intermembrane space
C. The cytosol
D. The mitochondrial cristae

Question

Why is oxygen necessary in aerobic cellular respiration?

A. It provides the hydrogen nuclei needed to create a proton gradient in the intermembrane space.
B. It is the final electron acceptor in the electron transport chain.
C. It is needed for glycolysis, which begins the process of respiration in cells.
D. It is important in creating oxaloacetate in the Kreb's cycle.

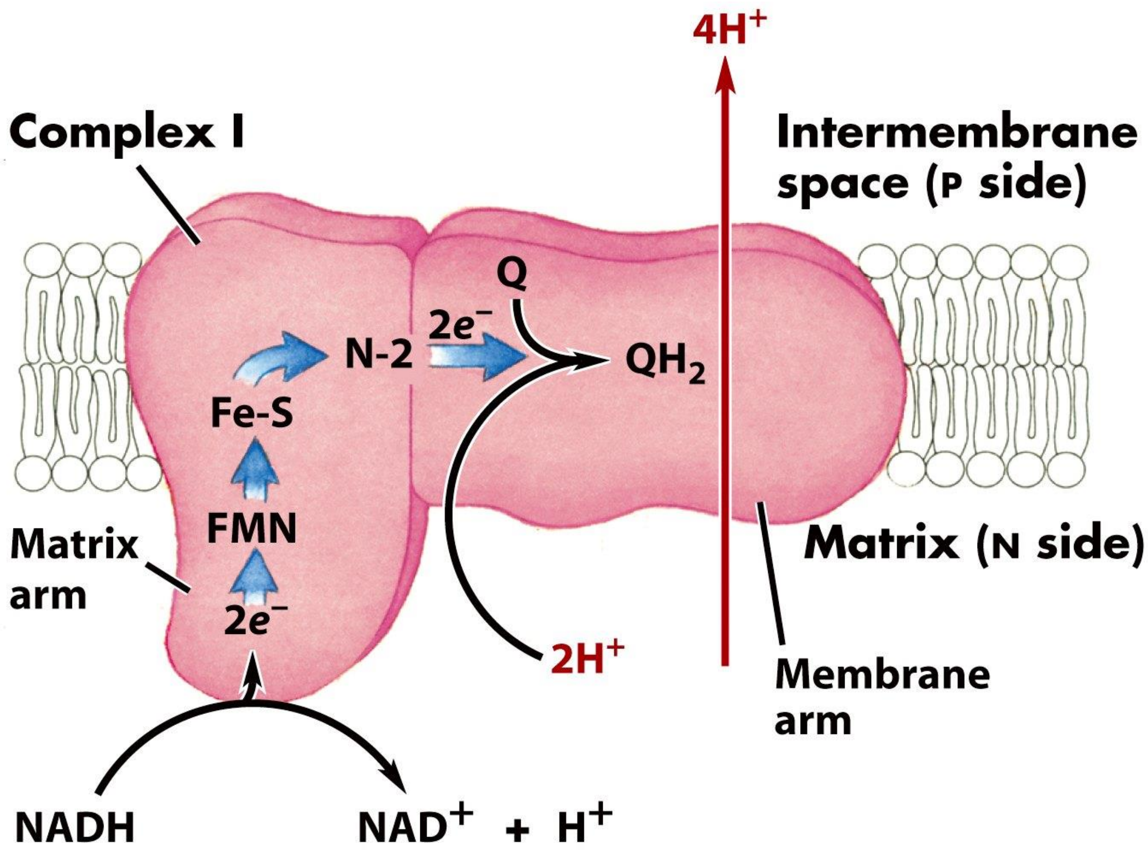


redox process (ox + n e$^-$ → red)	n	ε°' (V)
½ O_2 *(g)* + 2 H^+ + 2 e^- → H_2O *(l)*	2	0.81
cytochrome-a_3 (Fe^{3+}) + e^- → cytochrome-a_3 (Fe^{2+})	1	0.55
cytochrome-a (Fe^{3+}) + e^- → cytochrome-a (Fe^{2+})	1	0.29
cytochrome-c (Fe^{3+}) → cytochrome-c (Fe^{2+})	1	0.25
cytochrome-c_1 (Fe^{3+}) → cytochrome-c_1 (Fe^{2+})	1	0.22
cytochrome-b (Fe^{3+}) → cytochrome-b (Fe^{2+})	1	0.07
ubiquinone + 2H^+ + 2e^- → ubiquinol	2	0.04
NADH dehydrogenase (FMN) + 2H^+ + 2e^- → NADH dehydrogenase ($FMNH_2$)	2	-0.03
$NADP^+$ + H^+ + 2 e^- → NADPH	2	- 0.32
NAD^+ + H^+ + 2 e^- → NADH	2	- 0.32
2 H^+ + 2 e^- → H_2 *(g)* (pH = 7)	2	- 0.41

Complex I. NADH-dehydrogenase
(NADH: ubiquinone oxidoreductase)

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$

inhibitors: amytal, rotenone, piericidin A



Complex II. Succinate-dehydrogenase

$$\text{Succinate} + \text{Q} \xrightleftharpoons{\text{FAD}} \text{Fumarate} + \text{QH}_2$$

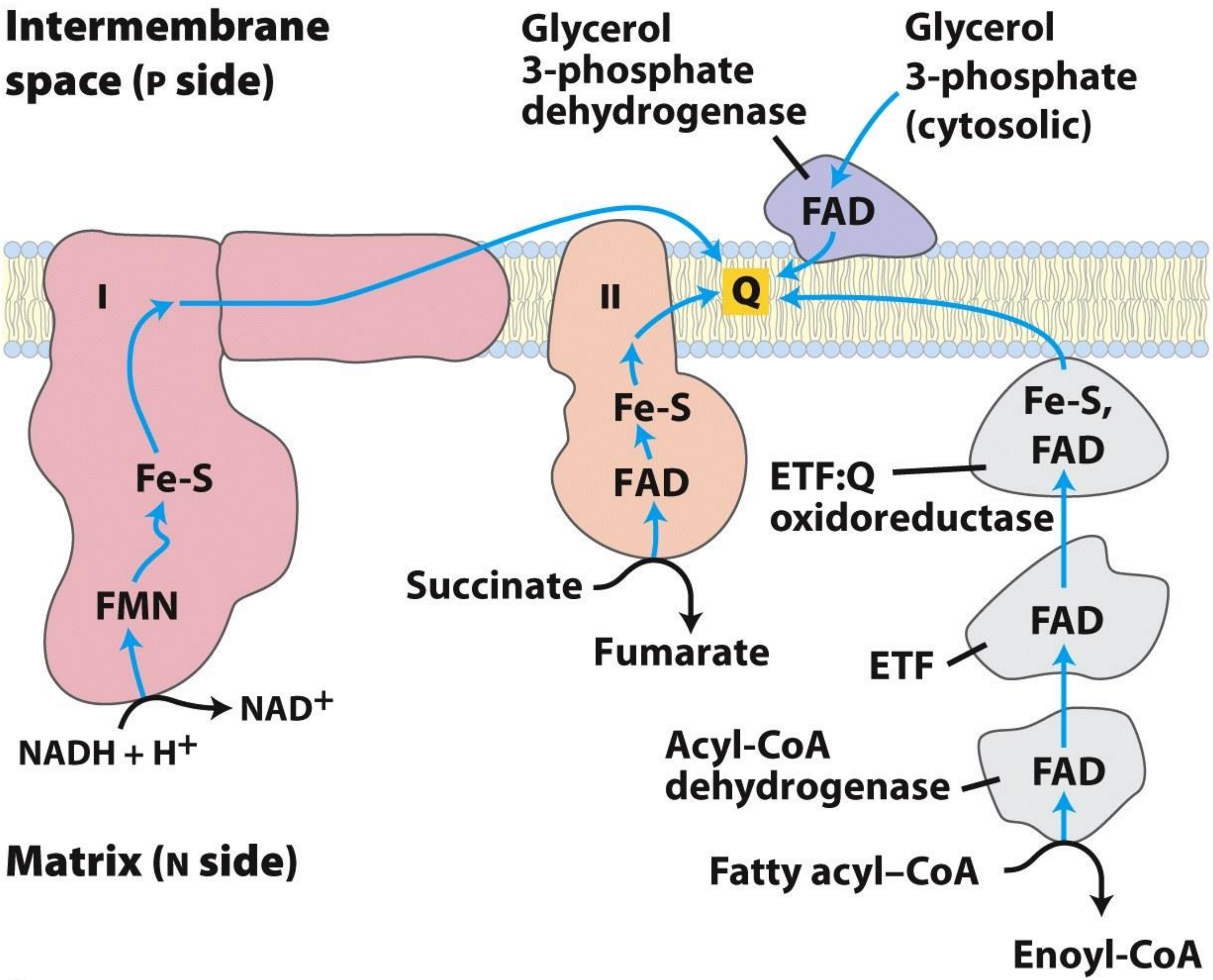
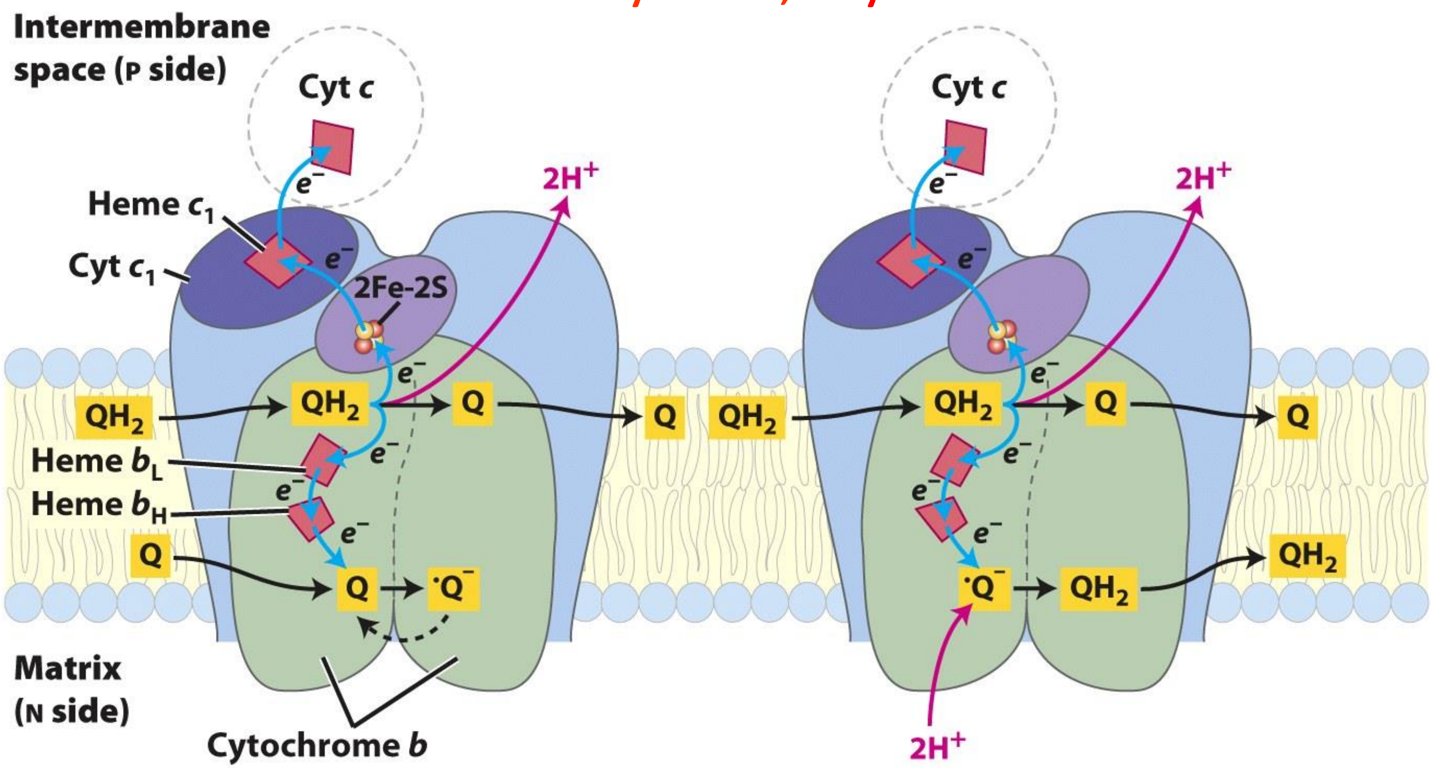


Figure 19-8
Lehninger Principles of Biochemistry, Fifth Edition

Complex III. Ubiquinone-cytochrome c-oxidoreductase

$$QH_2\ (red) + 2\ cyt\ c\ (ox) + 2H^+_N \longrightarrow Q\ (ox) + 2\ cyt\ c\ (red) + 4H^+_P$$

Inhibitor: antimycin A, myxothiazol



$$QH_2 + Q + cyt\ c_1\ (oxidized) \longrightarrow {}^{\cdot}Q^- + Q + 2H^+_P + cyt\ c_1\ (reduced)$$

$$QH_2 + {}^{\cdot}Q^- + 2H^+_N + cyt\ c_1\ (oxidized) \longrightarrow QH_2 + 2H^+_P + Q + cyt\ c_1\ (reduced)$$

Net equation: $QH_2 + 2\ cyt\ c_1\ (oxidized) + 2H^+_N \longrightarrow Q + 2\ cyt\ c_1\ (reduced) + 4H^+_P$

Figure 19-12
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Complex IV. Cytochrome c-oxidase

$$4\ \text{Cyt-c (red.)} + 8H^+(n) + O_2 \longrightarrow 4\ \text{Cyt-c (ox.)} + 4H^+(p) + 2\ H_2O$$

Inhibitors: cyanide, CO, hydrogen sulfide, azides

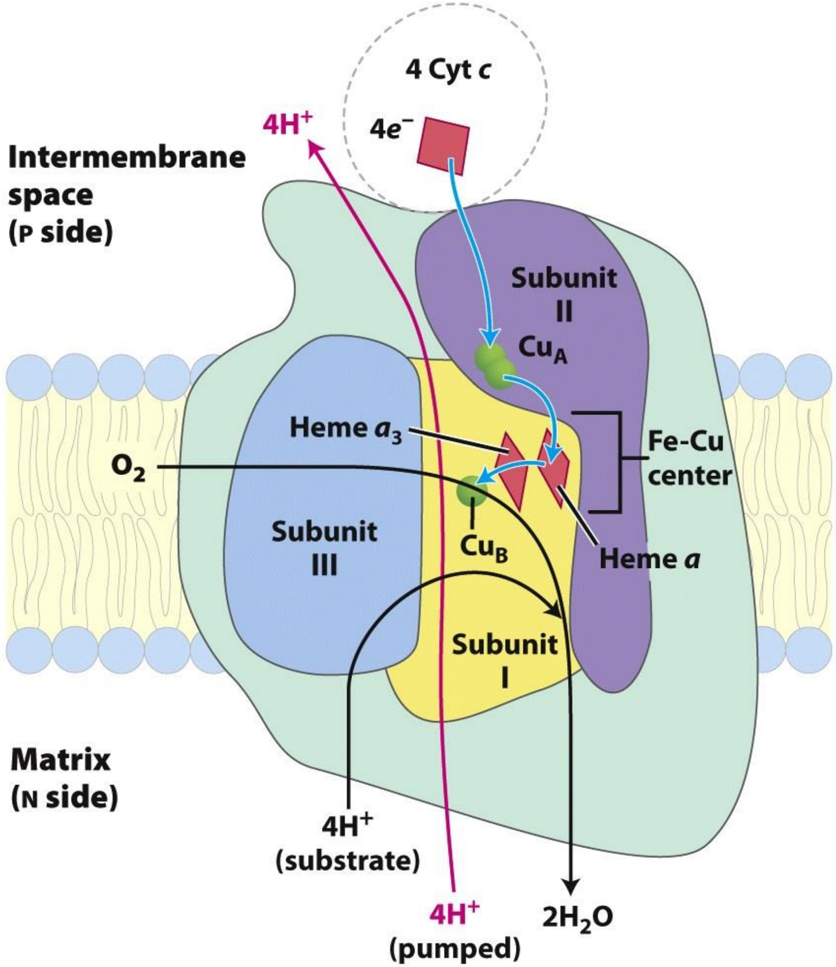

Figure 19-14
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$$2\ \text{cyt c (red)} + 4H_N^+ + \tfrac{1}{2}O_2 \longrightarrow 2\ \text{cyt c (ox)} + 2H_P^+ + H_2O$$

Question

Which of the following components of electron transport chain does not contain iron sulfur center?

A. NADH dehydrogenase complex

B. Ubiquinone-cytochrome c-oxidoreductase

C. Succinate dehydrogenase

D. Cytochrome c-oxidase

The ATP synthase

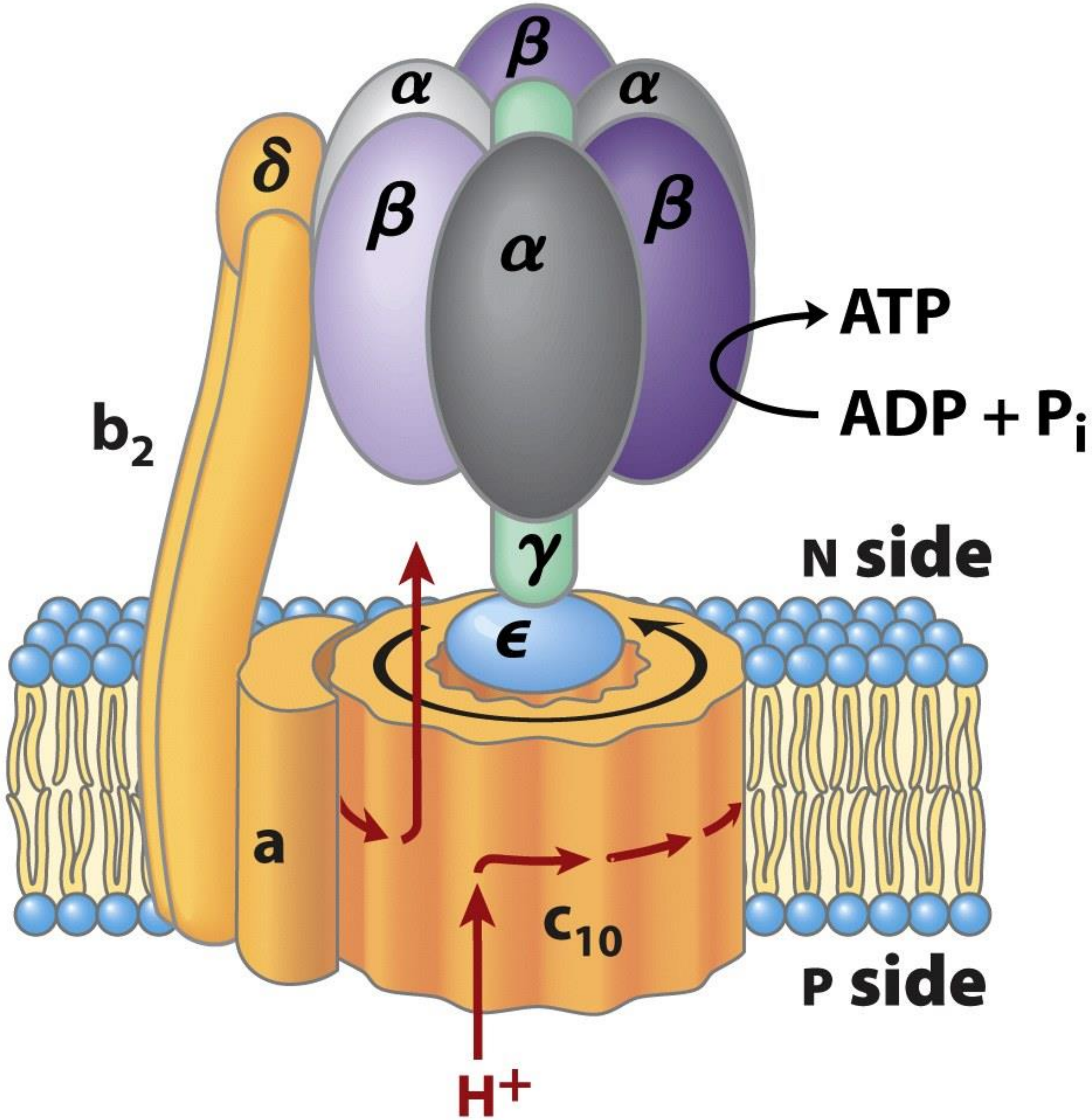

Figure 19-25f
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1 turnaround =

translocation of 10 protons =

synthesis of 3 ATP

Adenine nucletide and phosphate translocases

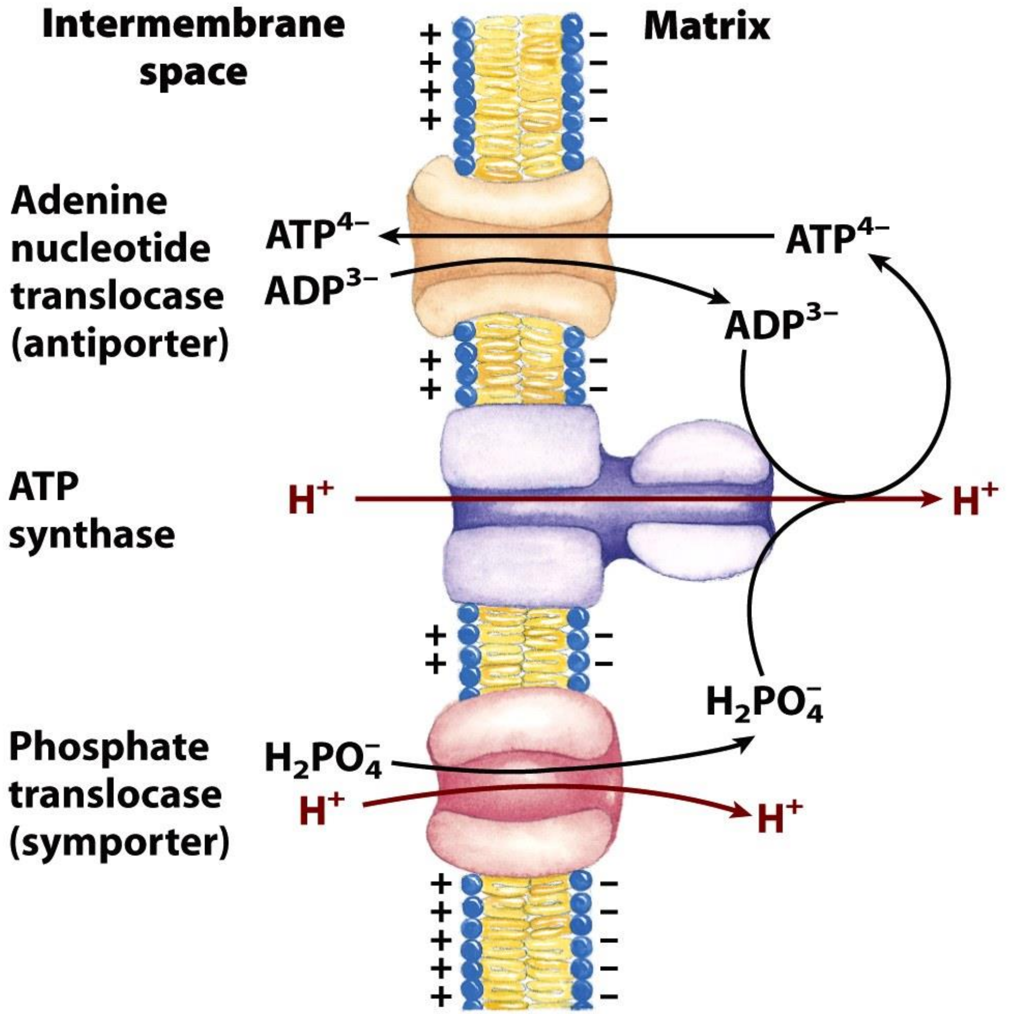

Figure 19-28
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The P/O Ratio:
How many ATP is synthetized from the energy released by the reduction of an oxygen atom?

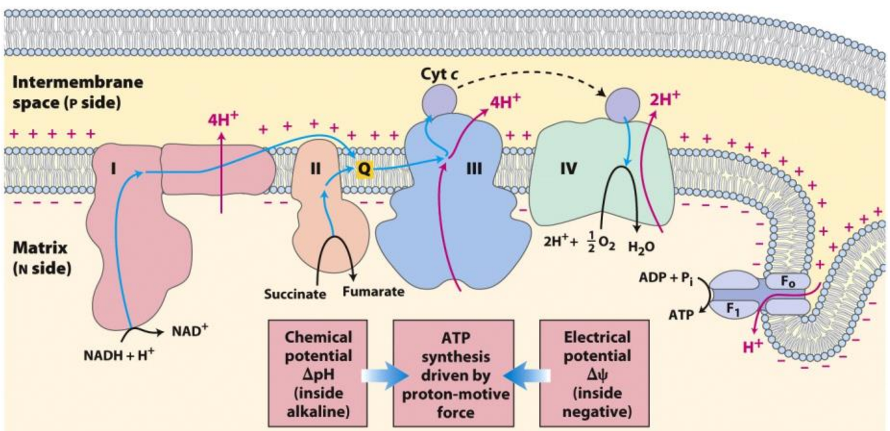

Figure 19-19
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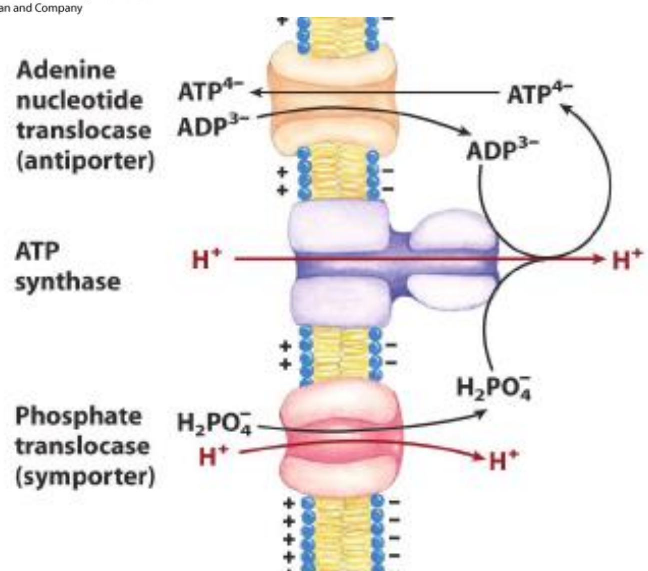
- 1 oxygen atom is reduced by 2 electrons
- Both NADH and $FADH_2$ provide two electrons
- During their oxidation in the respiratory chain:

NADH „pumps” 10 protons into the IMS
$FADH_2$ „pumps” 6 protons into the IMS

- Translocation of 10 protons into the matrix through ATP synthase results in the synthesis of 3 ATP molecules



4 H^+/ ATP is needed:
1 for the transport of P_i, ADP and ATP
and
3 for the production of 1 ATP

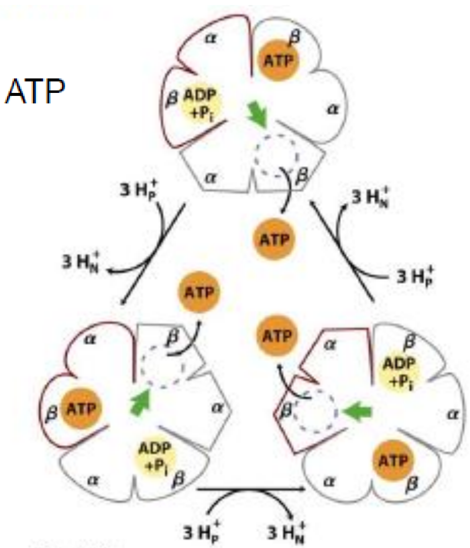


Figure 19-26
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The P/O Ratio: How many ATP is synthetised from the energy released by the reduction of an oxygen atom?

- The P/O ratio was expected to be 3 ATP / NADH and 2 ATP / $FADH_2$ (outdated!!!).
- Today we have experimentally determined results, which show ~**2.5** ATP / NADH and ~**1.5** ATP / $FADH_2$.

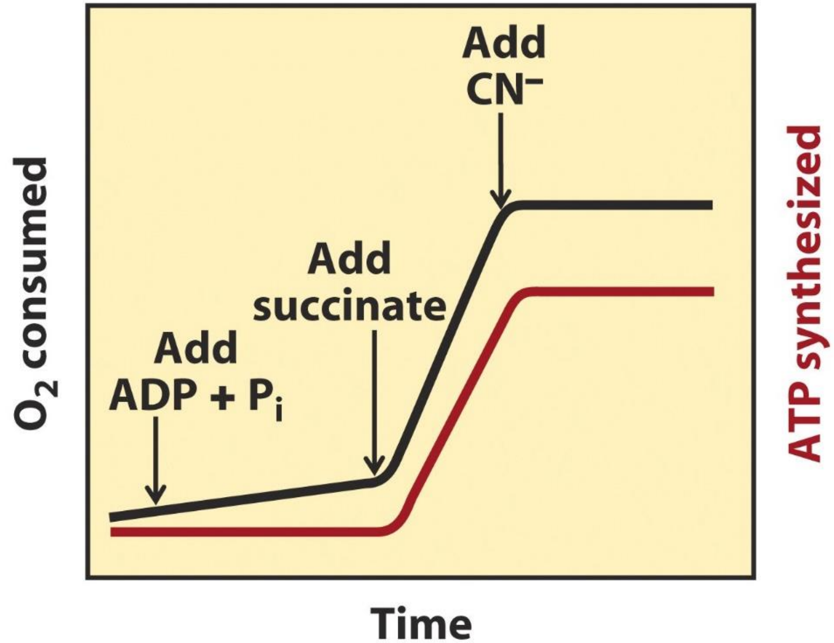
Consider: the pumping of protons into the intermembrane space is NOT a stoichiometric process.

ATP synthesis is coupled with the redox reactions of the respiratory chain (electrontransfer).
In an uncoupled mitochondrium, oxidation of NADH or succinate (without oxidative phophorylation [ATP synthesis]) leads to heat production.
e.g.: **thermogenin (UCP1)** (physiologic uncoupling protein).

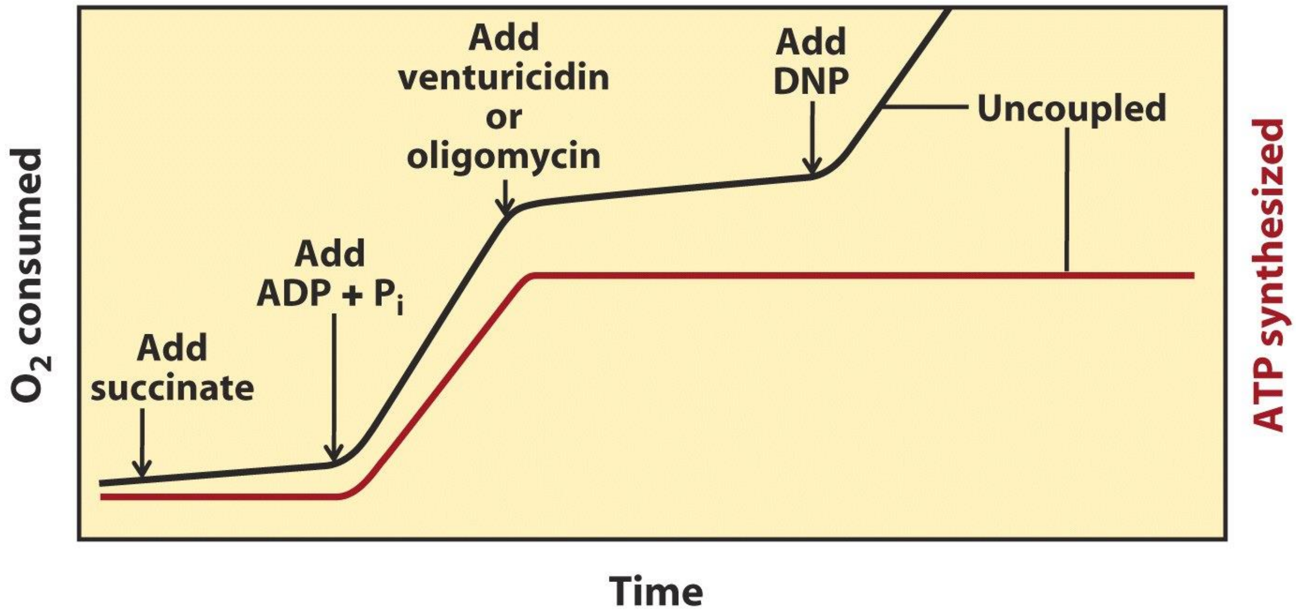
Isolated mitochondria + ADP + Pi + substrate (succinate) + buffer

- Substrate (succinate) is oxidized
- O_2 is consumed
- ATP is synthesized

O_2 consumption in coupled mitochondria can be inhibited by CIII and CIV inhibitors:
Antimycin A, Cyanid, CO



Isolated mitochondria + ADP + Pi + substrate (succinate) + buffer + inhibitors



- **In intact (coupled) mitochondria the inhibition of ATP synthesis *(Fo-F1)* blocks electron transfer.**

  Inhibitors: Venturicidin, Oligomycin, Aurovertin

- **Uncoupling of oxidation and phosphorylation can also be demonstrated using chemical compounds. Respiration increases, but no ATP is produced.**

  Uncoupling with chemicals: 2,4 Dinitrophenol (DNP), FCCP

Summary

- Oxidative phosphorylation occurs in the inner membrane of eukaryotic mitochondrion. Proton pumps of the respiratory chain and the F_OF_1 ATP synthase work in this process together, they are coupled.
- 4 complexes of the respiratory chain (many subunits and redox centers) transfer electrons from reduced coenzymes to the terminal electron acceptor O_2, which will be reduced to water.
- In this process, a proton gradient arises between the outer and inner sides of the membrane. Protons reenter the matrix through the F_OF_1 ATP synthase and drive ATP synthesis.
- Proton gradient arose by the oxidation of NADH produces ~2,5 mol ATP, in the case of $FADH_2$ ~1,5 mol ATP. This is the P/O-ratio.
- Oxidation and phosphorylation can be uncoupled! The uncoupling protein, thermogenin induces heat production instead of ATP synthesis.
- Oxidative phosphorylation is regulated by the ADP level.
- Communication between cytosol and mitochondrium is fullfilled by several transporters located in the inner membrane.

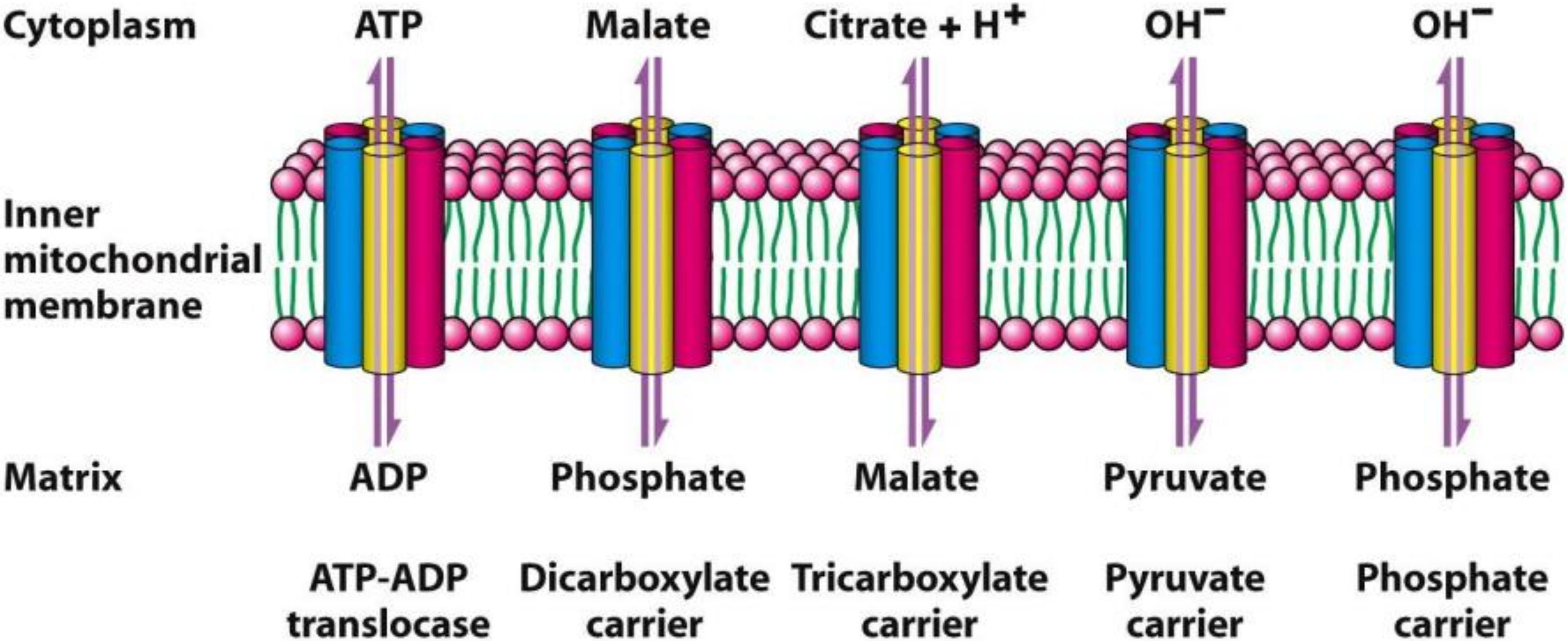
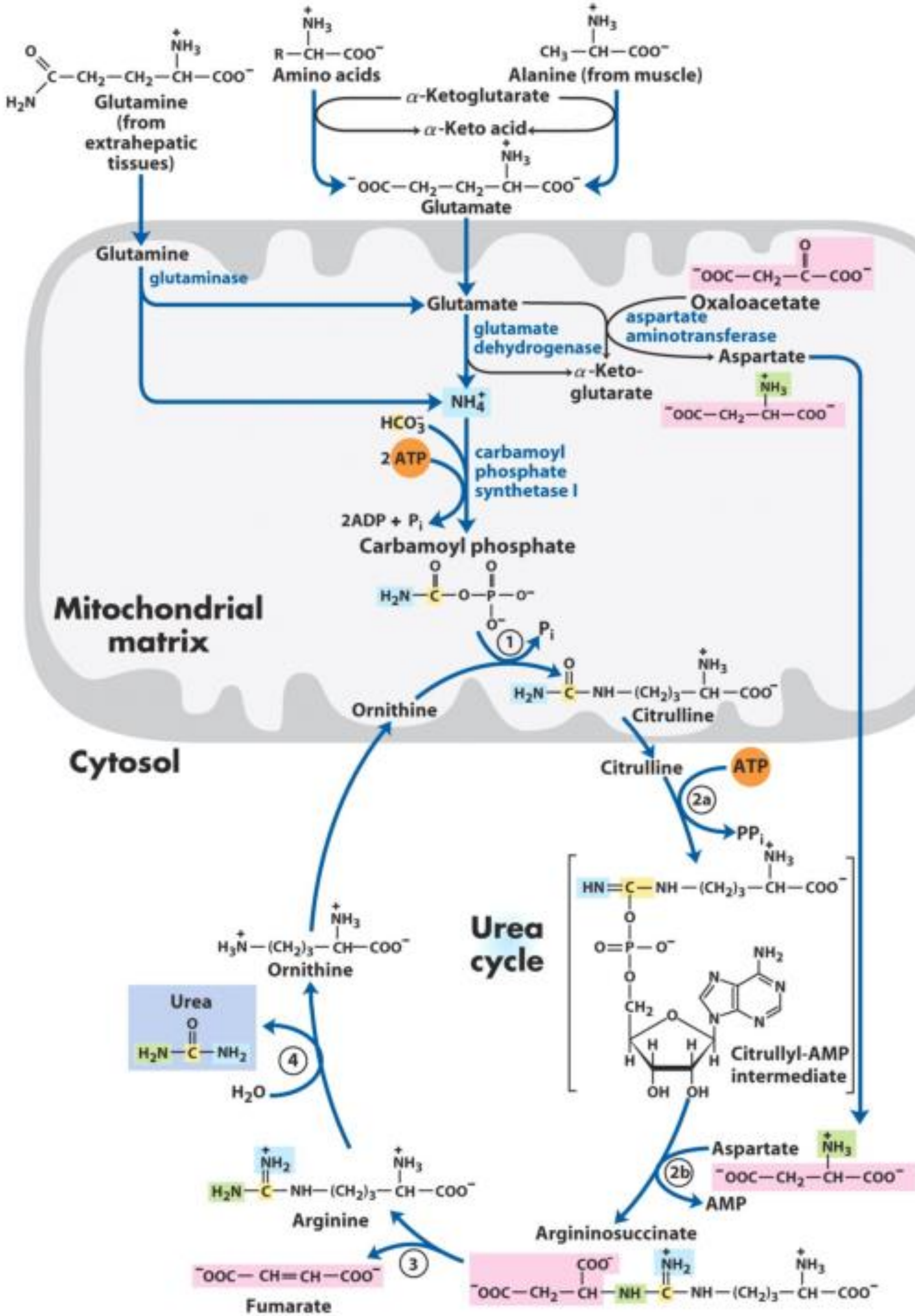
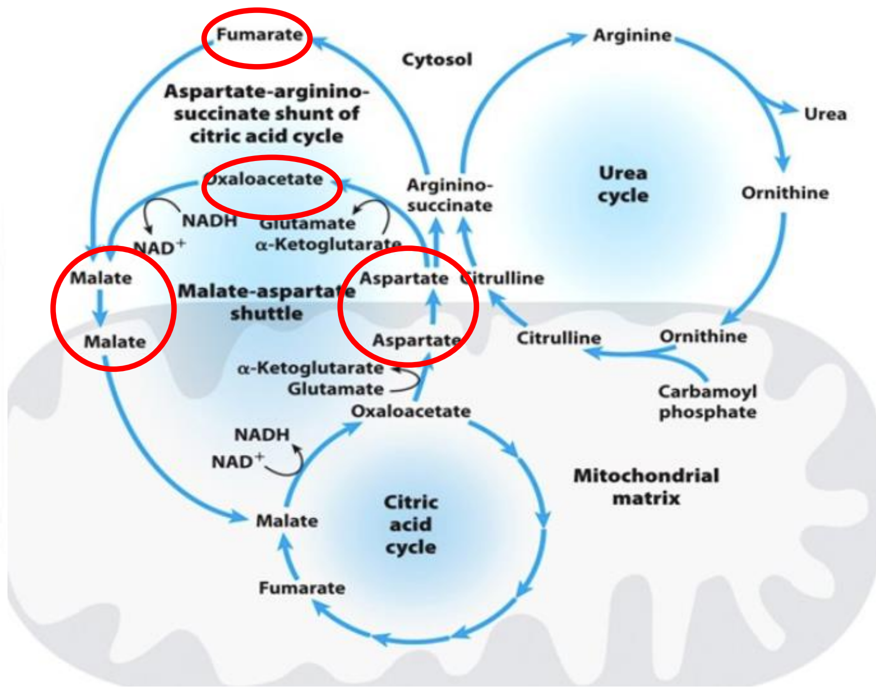


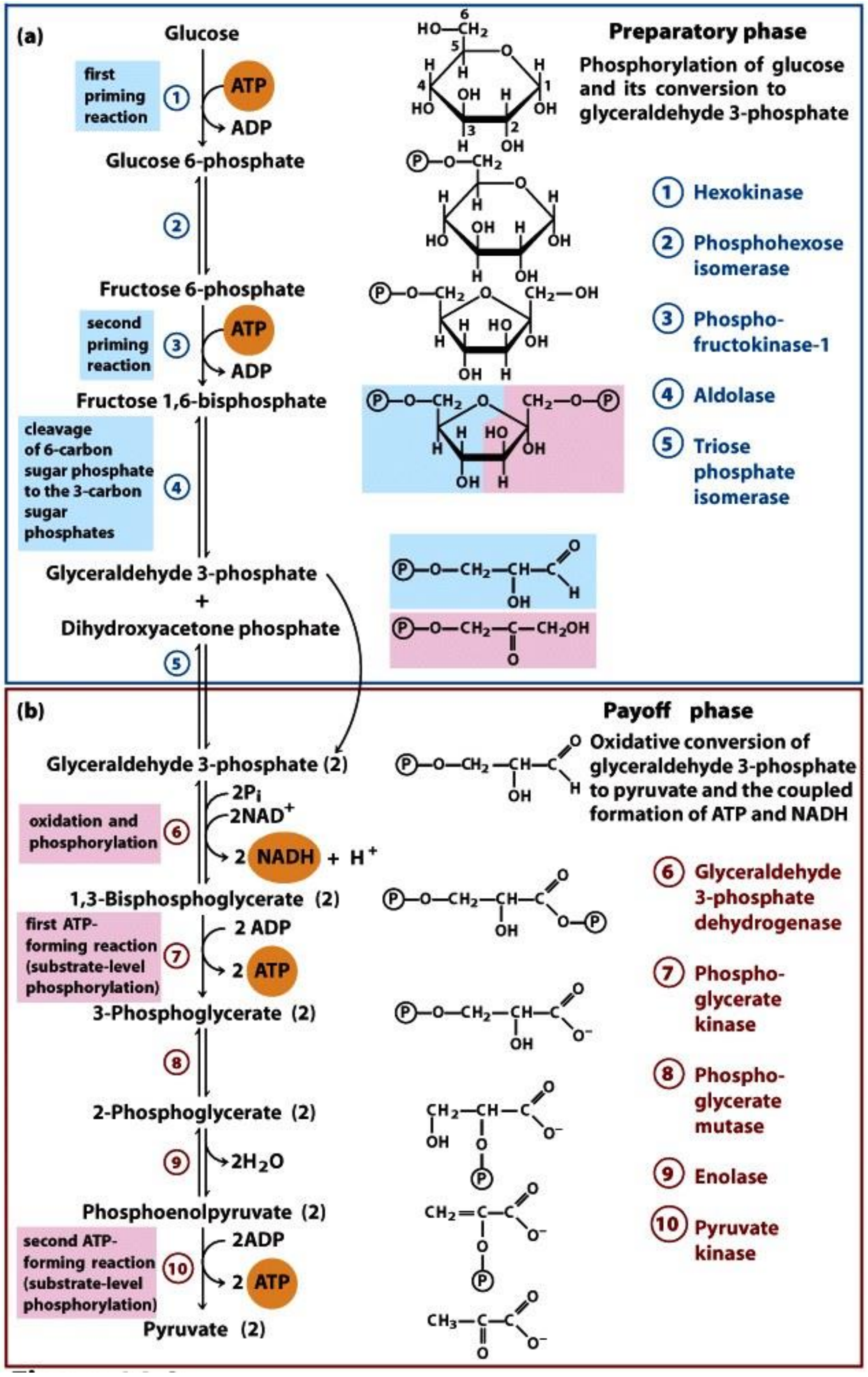
Figure 18.38
***Biochemistry,* Seventh Edition**

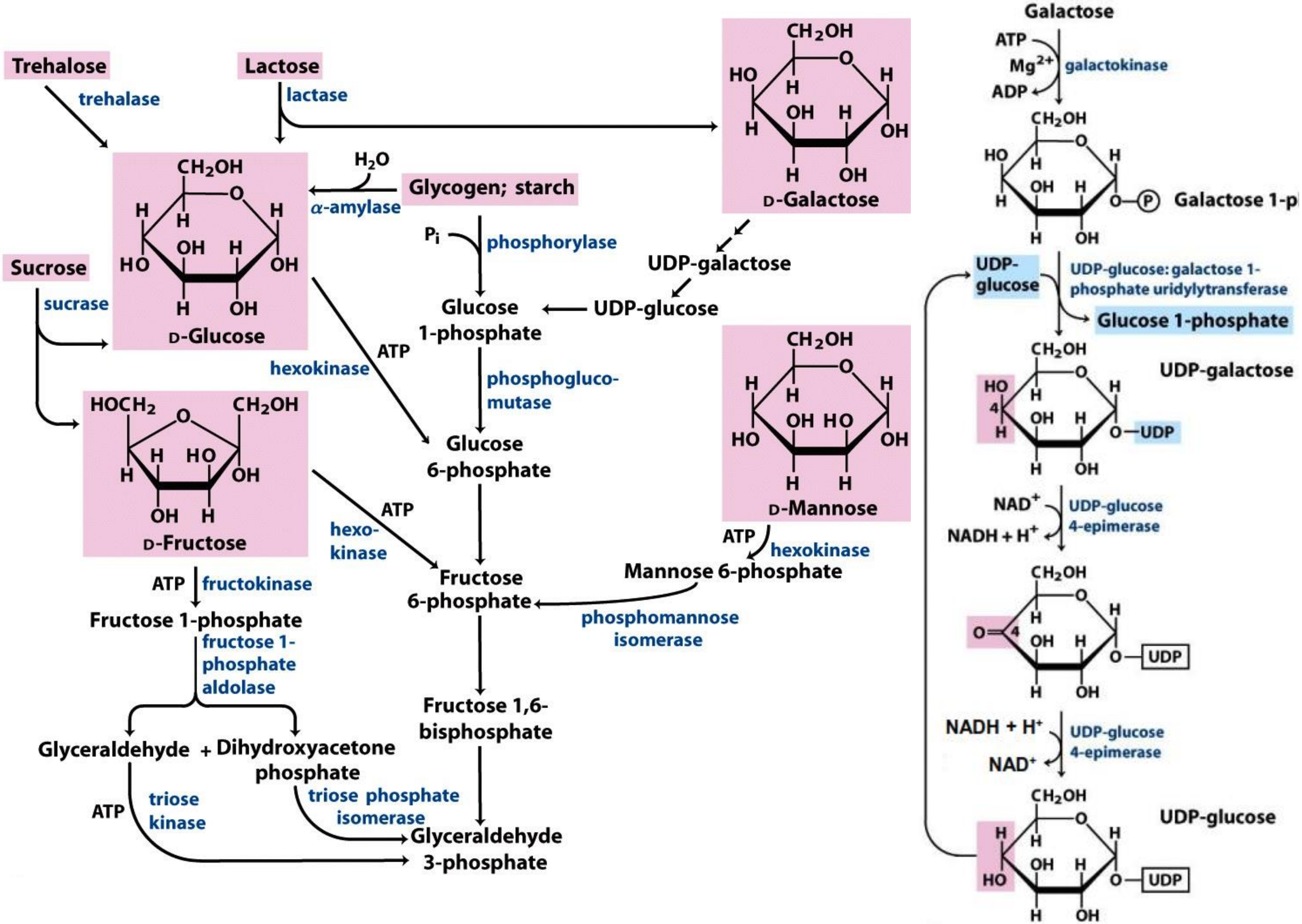


Glutamine (from extrahepatic tissues)
Amino acids
Alanine (from muscle)
α-Ketoglutarate
α-Keto acid
Glutamate
Glutamine
glutaminase
Glutamate
glutamate dehydrogenase
Oxaloacetate
aspartate aminotransferase
Aspartate
α-Ketoglutarate
NH4+
HCO3−
2 ATP
2ADP + Pi
carbamoyl phosphate synthetase I
Carbamoyl phosphate
Mitochondrial matrix
Ornithine
Citrulline
Cytosol
Citrulline
ATP
PPi
Citrullyl-AMP intermediate
Urea cycle
Ornithine
Urea
H2O
Arginine
Aspartate
AMP
Argininosuccinate
Fumarate

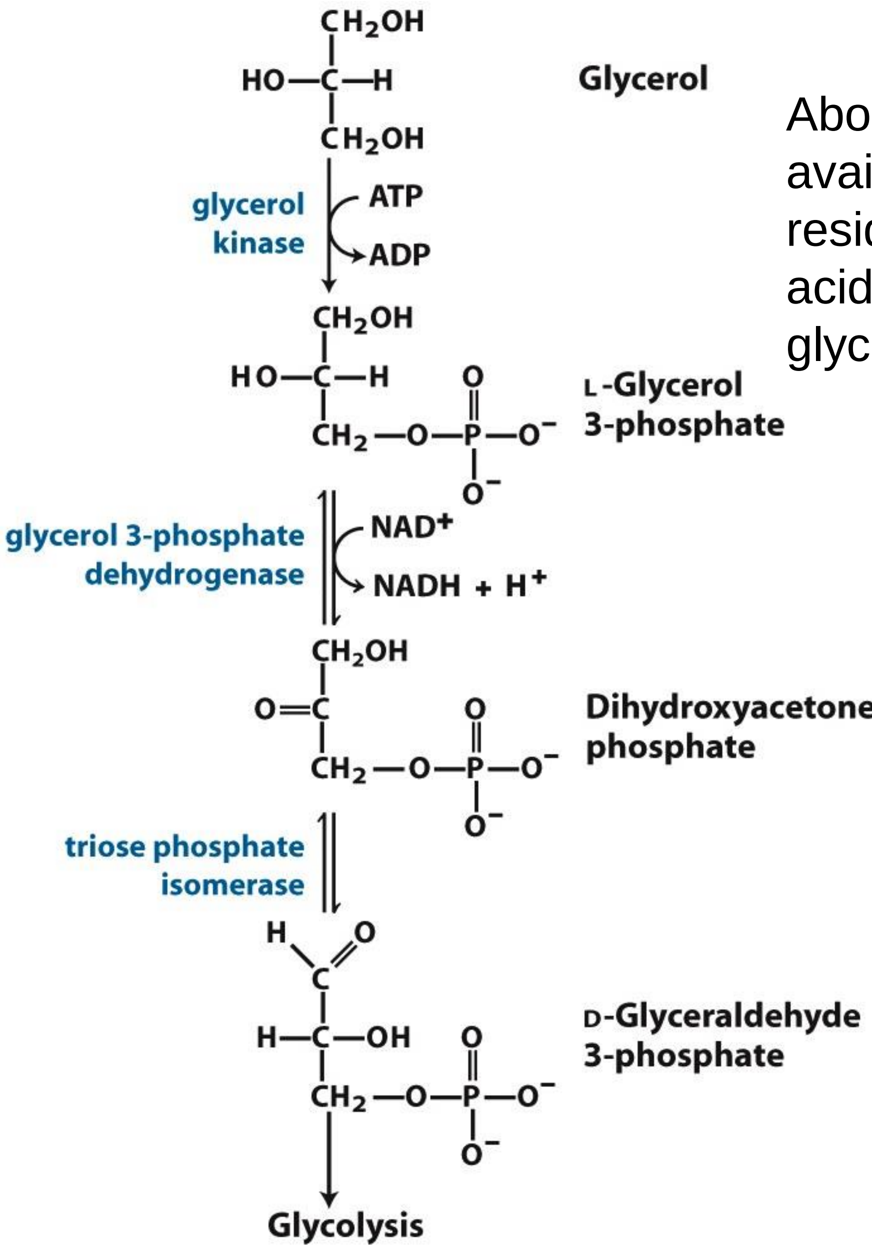


Fumarate
Cytosol
Arginine
Urea
Aspartate-argininosuccinate shunt of citric acid cycle
Oxaloacetate
NADH
NAD+
Glutamate
α-Ketoglutarate
Arginino-succinate
Urea cycle
Ornithine
Malate
Malate-aspartate shuttle
Aspartate
Citrulline
Malate
Aspartate
Citrulline
Ornithine
α-Ketoglutarate
Glutamate
Oxaloacetate
Carbamoyl phosphate
NADH
NAD+
Mitochondrial matrix
Malate
Citric acid cycle
Fumarate

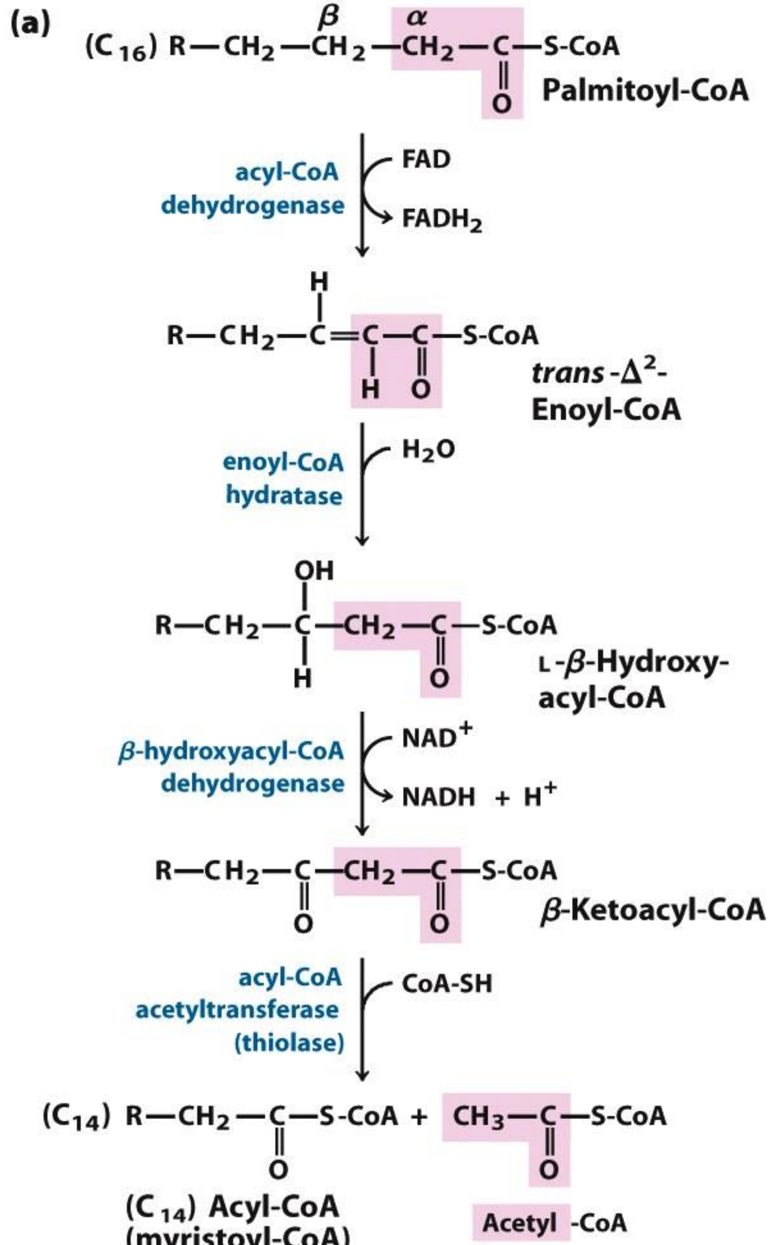

(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to the 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase
(b)
Payoff phase
Glyceraldehyde 3-phosphate (2)
2Pi
2NAD+
oxidation and phosphorylation
6
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2 ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase


Trehalose
trehalase
Lactose
lactase
D-Glucose
H_2O
Glycogen; starch
α-amylase
P_i
phosphorylase
D-Galactose
UDP-galactose
UDP-glucose
Glucose 1-phosphate
Sucrose
sucrase
hexokinase
ATP
phosphogluco-
mutase
Glucose 6-phosphate
D-Fructose
hexo-
kinase
D-Mannose
hexokinase
Mannose 6-phosphate
Fructose 6-phosphate
phosphomannose isomerase
fructokinase
Fructose 1-phosphate
fructose 1-phosphate aldolase
Fructose 1,6-bisphosphate
Glyceraldehyde + Dihydroxyacetone phosphate
triose kinase
triose phosphate isomerase
Glyceraldehyde 3-phosphate
Galactose
ATP
Mg^{2+}
ADP
galactokinase
Galactose 1-p
UDP-glucose
UDP-glucose: galactose 1-phosphate uridylyltransferase
Glucose 1-phosphate
UDP-galactose
NAD^+
$NADH + H^+$
UDP-glucose 4-epimerase
UDP-glucose

Entry of glycerol into glycolysis



About 95% of the biologically available energy of triacylglycerols resides in their three long-chain fatty acids; only 5% is contributed by the glycerol moiety.



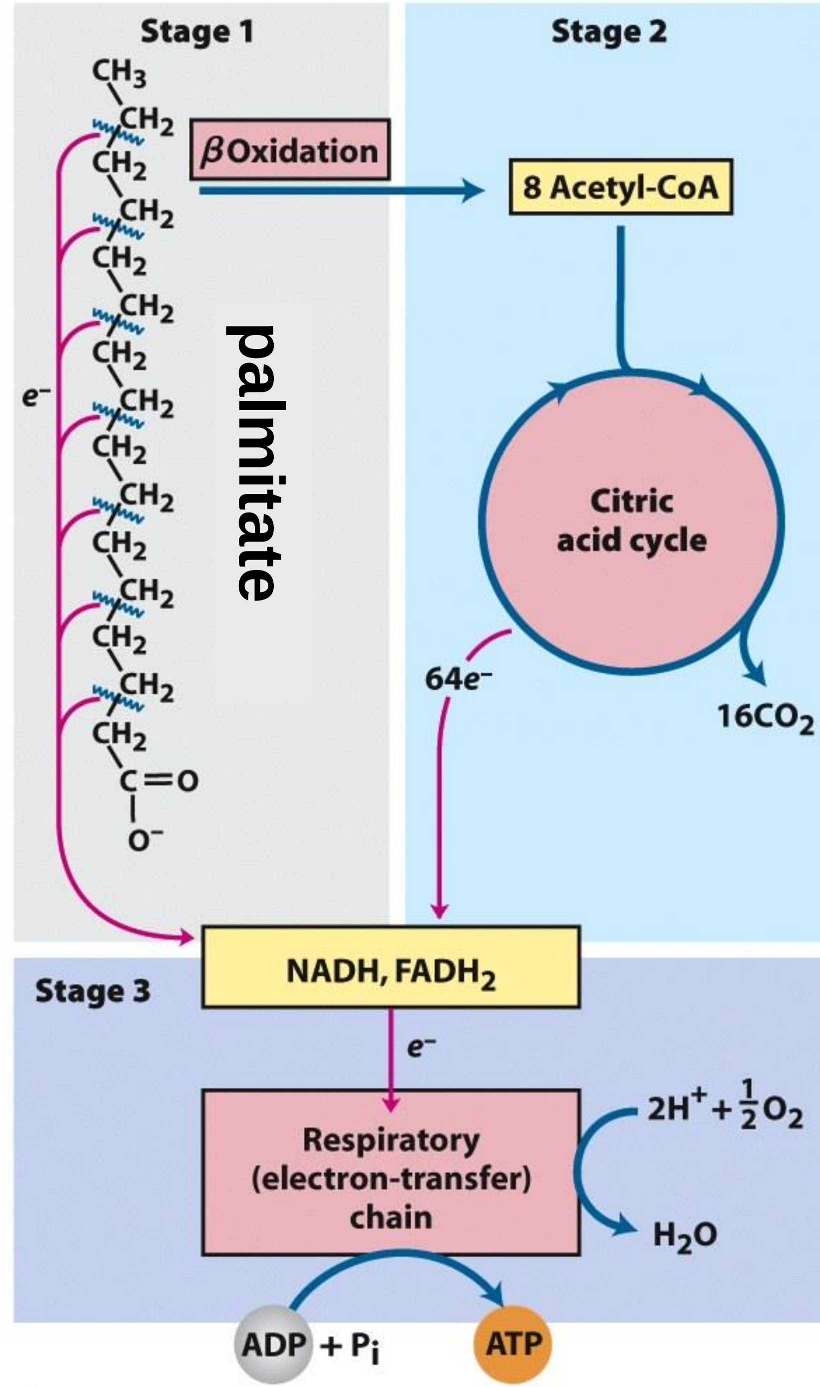


Figure 17-8

Lehninger Principles of Biochemistry, Fifth Edition

The use of ketone bodies as fuel

Ketone bodies are used as fuels in all tissues except liver, which lacks this enzyme.

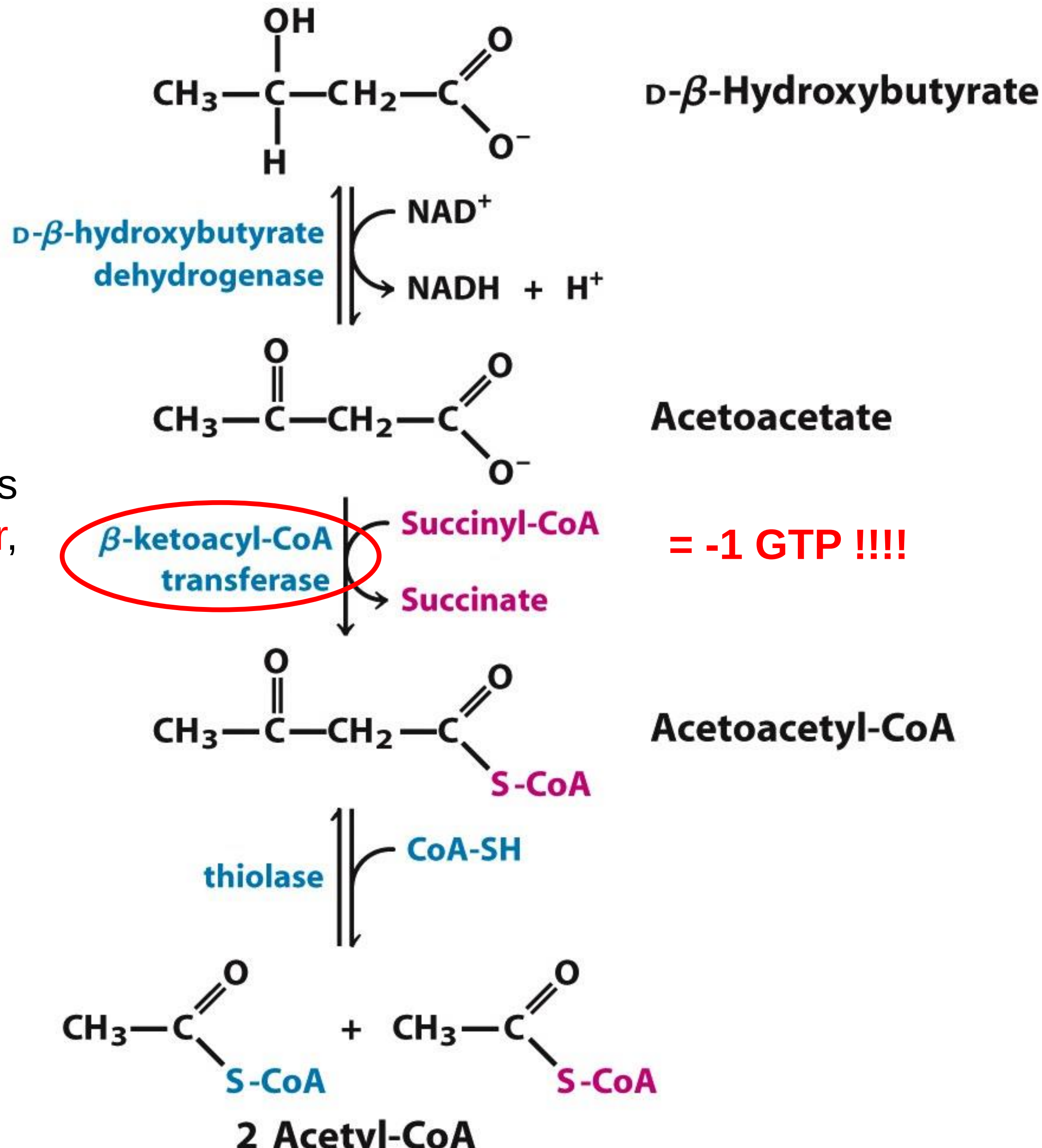


Figure 17-19
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Glycolysis: 2+2x2.5 → Malate –Aspartate-Shuttle 2x2.5=5 / Glycerol-3-Phosphat-Shuttle 2x1.5=3

PDHC 2x2.5

Citric acid cycle: 2x(3x2.5+1.5+1)

} 25

TABLE 16–1	**Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation**	
Reaction	**Number of ATP or reduced coenzyme directly formed**	**Number of ATP ultimately formed***
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

†This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–30 and 19–31.

Table 16-1

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palmitoyl-CoA + 23 O_2 + 108 Pi + 108 ADP → CoA + 108 ATP + 16 CO_2 + 23 H_2O

TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 510).

The energetic cost of activating a fatty acid is equivalent to **2** ATP, and the net gain per molecule of **palmitate** is 106 ATP.

Thank you for your attention!