VU Medical Zone

Admin: Amaan Khan

BIO 303- Biochemistry II

Lecture 64 to 125

Final term ppt

Mechanism of Enzyme Action

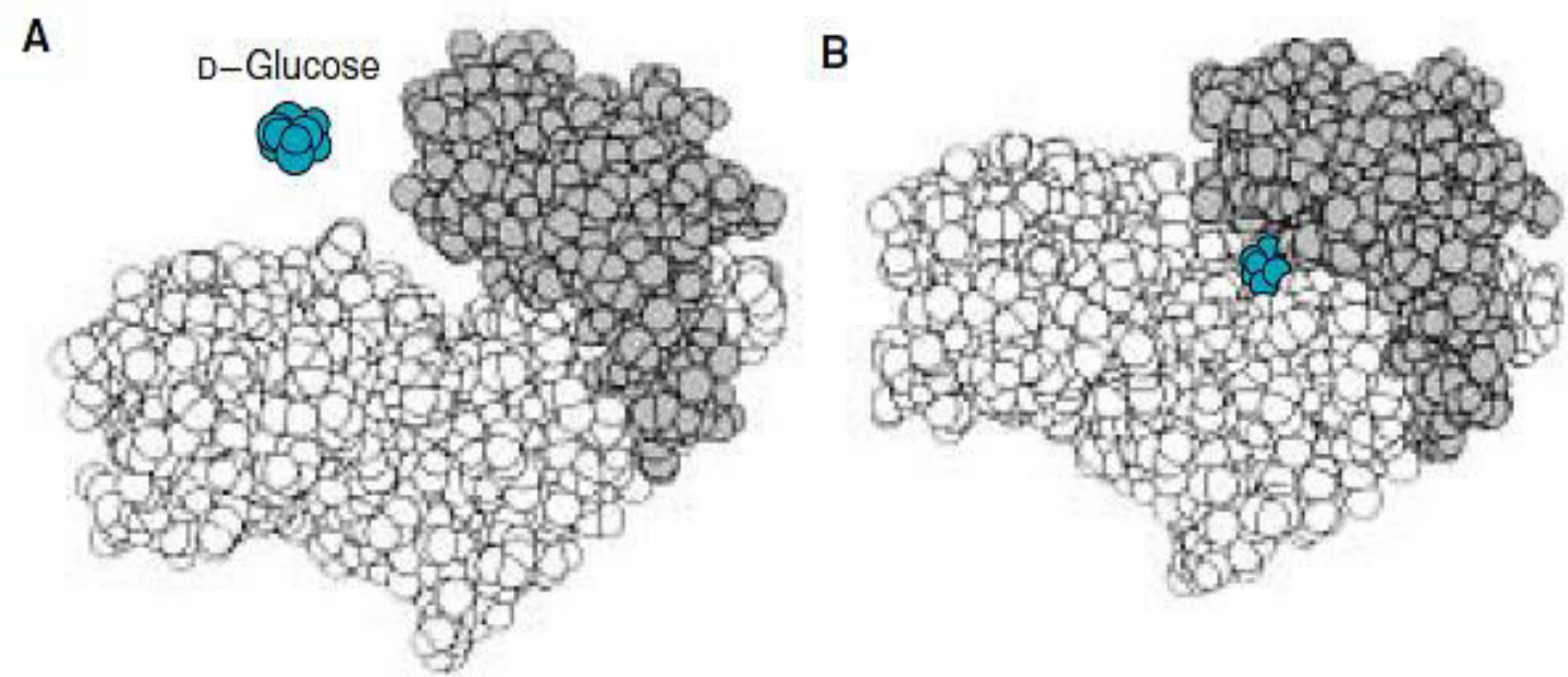
Enzymes bind and chemically transform other molecules— they catalyze reactions.

- The molecules acted upon by enzymes are called reaction **substrates** rather than ligands.

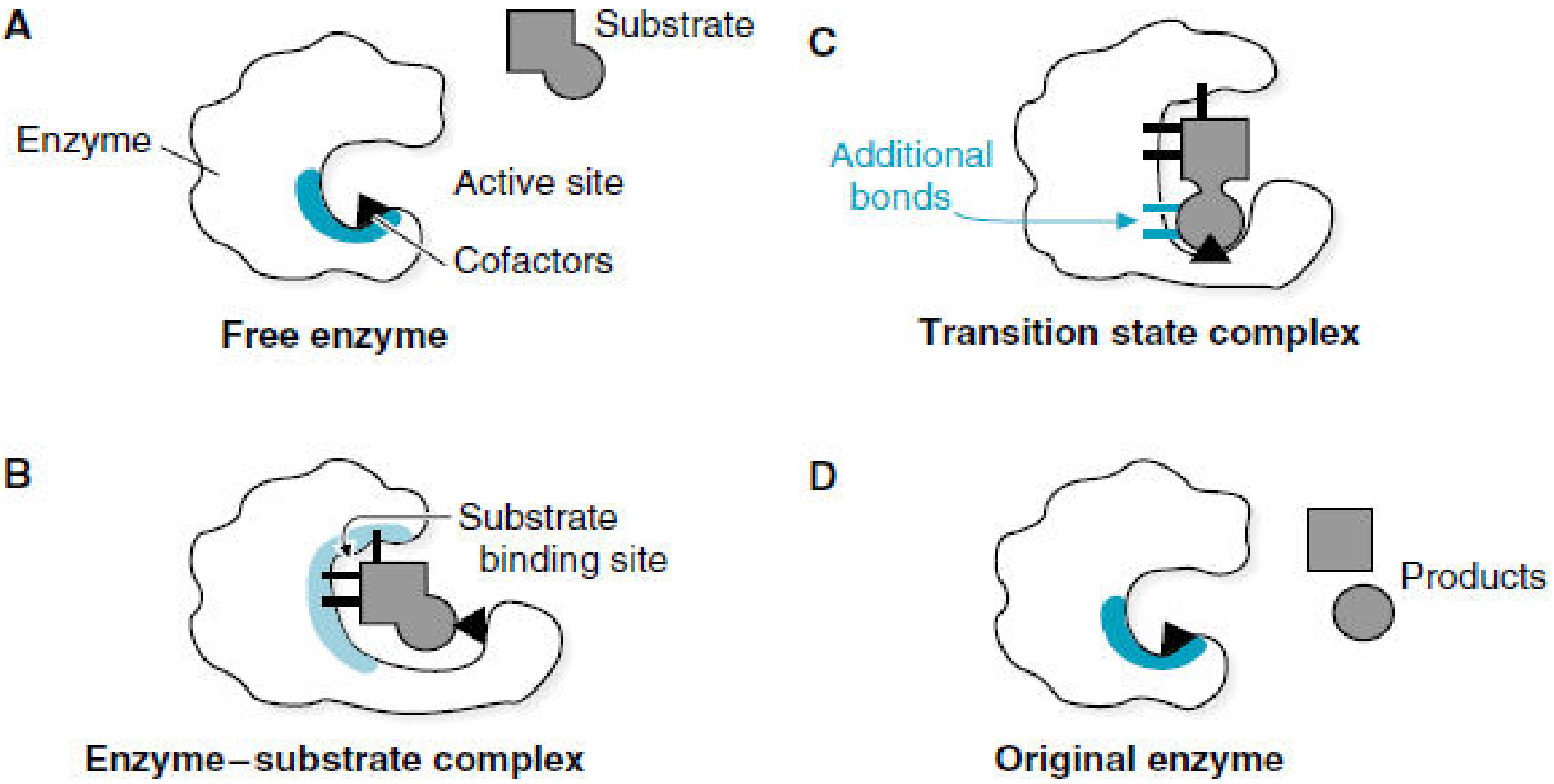
- Enzymes are highly effective catalysts, commonly enhancing reaction rates by a factor of 10^5 to 10^{17}.
- The distinguishing feature of an enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site.**

- The surface of the active site is lined with **amino acid** residues with side chains that complement and bind the substrate and catalyze its chemical transformation.

- Often, the active site encloses a substrate, sequestering it completely from solution.
- The enzyme substrate complex, whose existence is central to the action of enzymes.


A
D–Glucose
B

- Enzyme-catalyzed reactions have three basic steps:
- binding of substrate: **E+S↔ES**
- conversion of bound substrate to bound product: **ES ↔ EP**
- release of product : **EP ↔ E+P**


A
Substrate
Enzyme
Active site
Cofactors
Free enzyme
B
Substrate
binding site
Enzyme–substrate complex
C
Additional
bonds
Transition state complex
D
Products
Original enzyme

- To understand catalysis, we must first appreciate the important distinction between
- reaction equilibria and
- reaction rates.

END

Mechanism of Enzyme Action (Contd.)

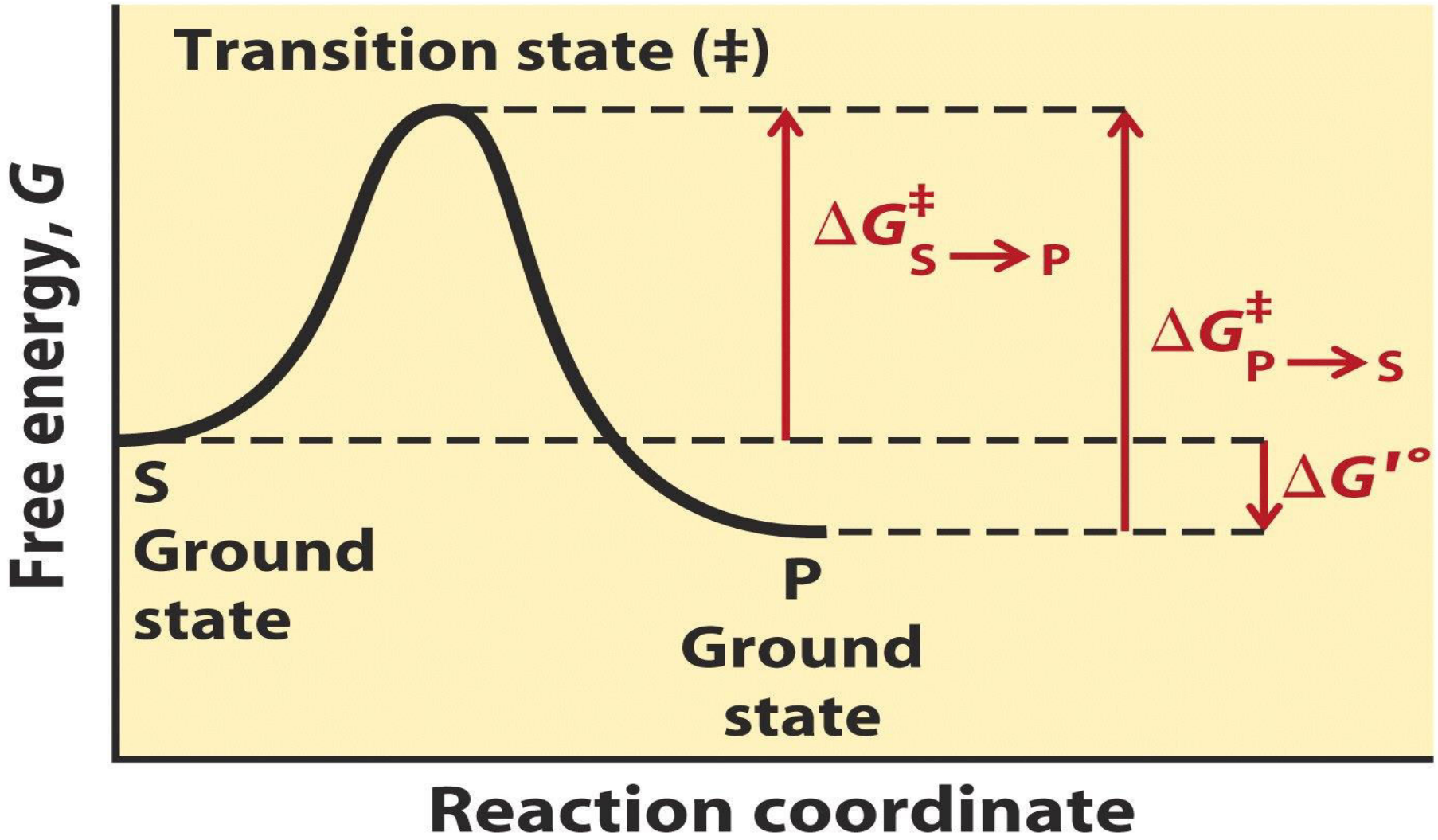
Reaction Equilibria

- The function of a catalyst is to increase the rate of a reaction.
- Catalysts *do not affect reaction equilibria*
- Any reaction, such as S $\leftrightarrow$ P, can be described by a reaction coordinate diagram

- The free energy of the system is plotted against the progress of the reaction **S → P**.

- A diagram of this kind is a description of the energy changes during the reaction.

- The horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P.


Transition state (‡)
$\Delta G^{\ddagger}_{S \rightarrow P}$
$\Delta G^{\ddagger}_{P \rightarrow S}$
$\Delta G'^{\circ}$
S
Ground state
P
Ground state
Free energy, G
Reaction coordinate

Activation energies, *G‡,*

- The activation energies, *G‡, for* the
- **S → P and P → S** reactions are indicated.
- *G'^{o} is the overall standard* free-energy change in the direction **S → P**

Double dagger . $\Delta G^{\ddagger}$.

Reaction coordinate diagram for a chemical reaction.
The free energy of the system is plotted against the progress of the reaction S→P. A diagram of this kind is a description of the energy changes during the reaction, and the horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P. The activation energies, $G‡$, for the S→P and P→S reactions are indicated. G is the overall standard free-energy change in the direction S→P.

Free-energy change ΔG

- When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force
- the magnitude of which can be expressed as the free-energy change for the reaction, ΔG.

Standard free-energy change, ΔG^{o}

- Under standard conditions (298 K = 25 C)
- when reactants and products are initially present at 1 M concentrations or
- for gases, at partial pressures of 101.3 (kPa), or 1 atm

- the force driving the system toward equilibrium is defined as the standard free-energy change, ΔG^{o}
- However, because the conditions in the body systems are different from standard conditions
- energy in biological systems is described in terms of free energy, G'^{o}.

END

Mechanism of Enzyme Action (Contd.)

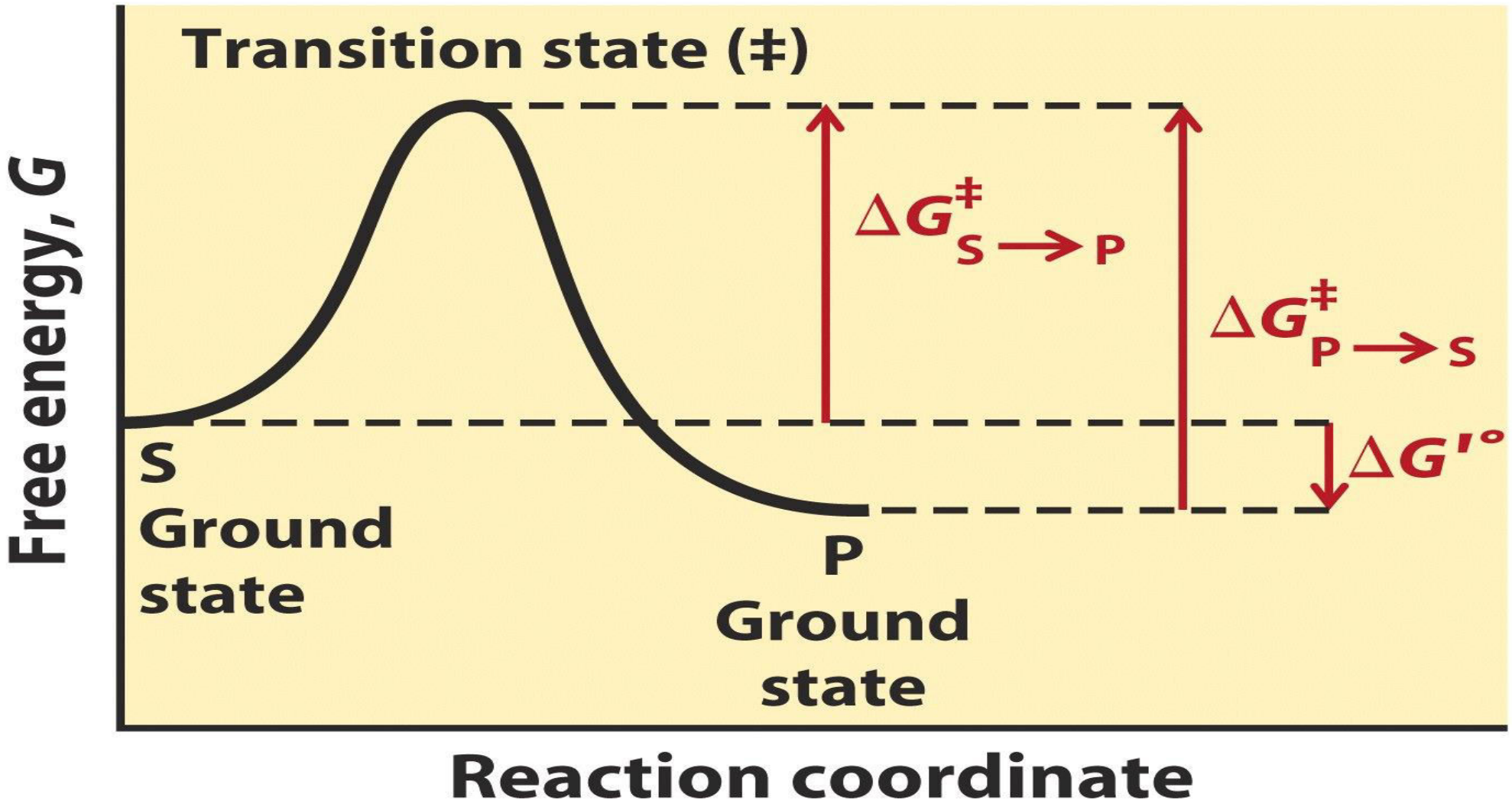
Ground State

&

Transition State

- The starting point for either the forward or the reverse reaction is called the **ground state**
- The equilibrium between S and P reflects the difference in the free energies of their ground states.

- The free energy of the ground state of P is **lower** than that of S
- So G'^{o} *for the* reaction is negative and the equilibrium favors P.
- The position and *direction* of equilibrium are *not affected by* any catalyst.


Transition state (‡)
$\Delta G^{\ddagger}_{S \rightarrow P}$
$\Delta G^{\ddagger}_{P \rightarrow S}$
$\Delta G'^{\circ}$
S
Ground
state
P
Ground
state
Free energy, G
Reaction coordinate

- But there is an **energy barrier** between S and P**:** The energy required for
- alignment of reacting groups
- formation of transient unstable charges
- bond rearrangements
- and other transformations

- This is illustrated by the energy “hill”
- To undergo reaction, the molecules must overcome this barrier and therefore must be raised to a higher energy level
- This is called the **transition state.**

- It is simply a fleeting molecular moment in which events such as
- bond breakage,
- bond formation, and
- charge development
- have proceeded to the precise point at which decay to either substrate or product is equally likely.

- The difference between the energy levels of the ground state and the transition state is the **activation energy, *G*‡.**

- At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way)

- A substance that modifies the transition state to lower the activation energy is termed a catalyst; a biological catalyst is termed an enzyme.

END

Mechanism of Enzyme Action (Contd.)

Activation energy $\Delta G^{\ddagger}$.

- The difference between the energy levels of the ground state and the transition state is the **activation energy $\Delta G^{\ddagger}$.**

$\Delta G^{\ddagger}$. Double dagger. The least amount of energy needed for a chemical reaction to take plac e. Some elements and compounds react together naturally just by being close to each other, and their activation energy is zero. Others will react together only after a certain amount of energy is added to them.

- The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction.

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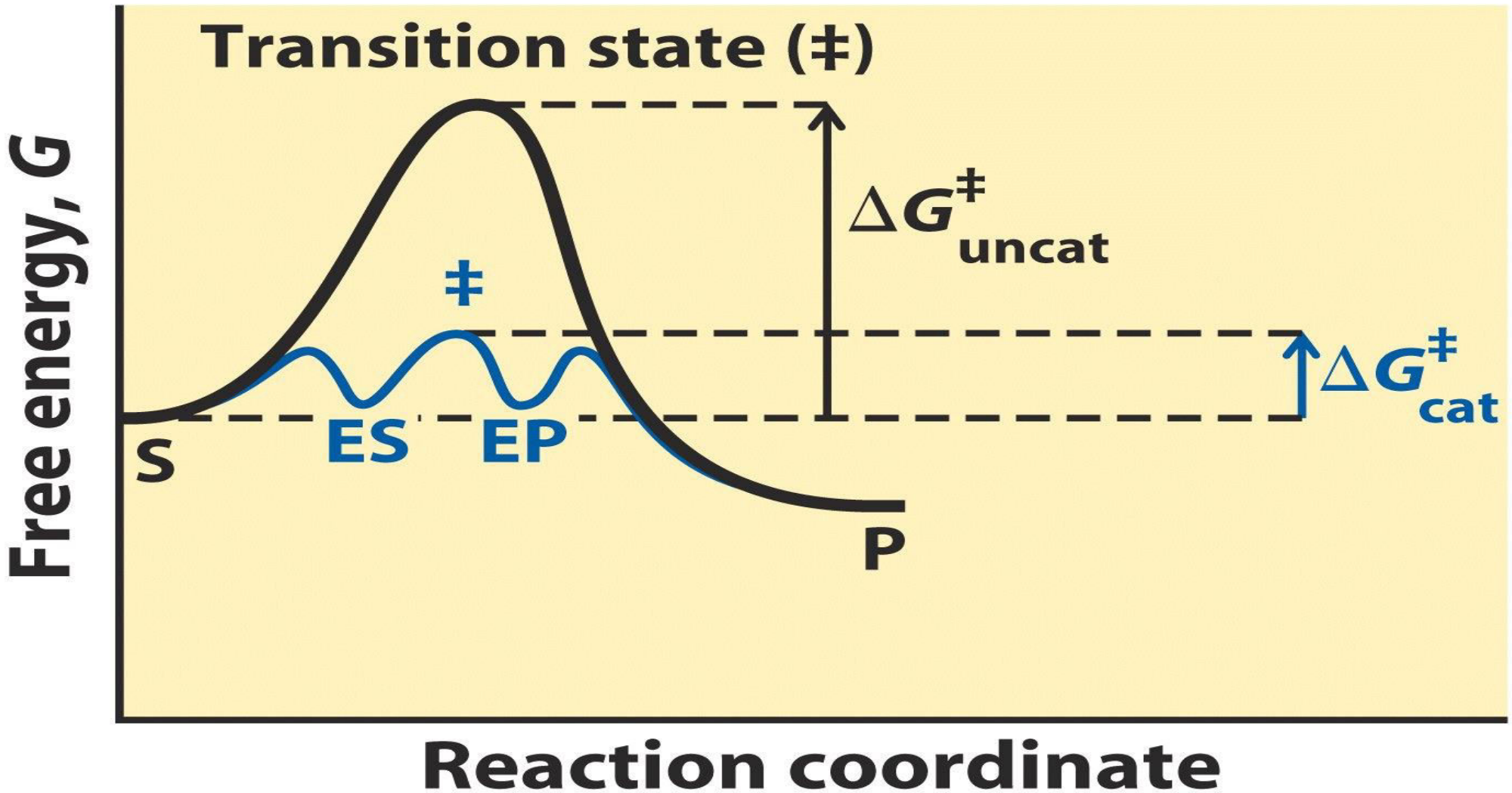
- Reaction rates can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier.

- Alternatively, the activation energy can be lowered by adding a catalyst.

- Catalysts enhance reaction rates by lowering activation energies.


Transition state (‡)
Free energy, G
$\Delta G^{‡}_{uncat}$
‡
$\Delta G^{‡}_{cat}$
S
ES
EP
P
Reaction coordinate

Reaction coordinate diagram comparing enzyme catalyzed and uncatalyzed reactions. In the reaction S→P, the ES and EP intermediates occupy minima in the energy progress curve of the enzyme-catalyzed reaction. The terms $G‡_{uncat}$ and $G‡_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction, respectively. The activation energy is lower when the enzyme catalyzes the reaction.

- The role of enzymes is to accelerate the inter-conversion of S and P.

- i.e ***enzymes lower the energy of activation, $\Delta G^{\ddagger}$, of a reaction.***

- The enzyme is not used up in the process, and the equilibrium point is unaffected.

- However, the reaction reaches equilibrium much faster when the appropriate enzyme is present,
- because the rate of the reaction is increased.

END

The Induced Fit Hypothesis

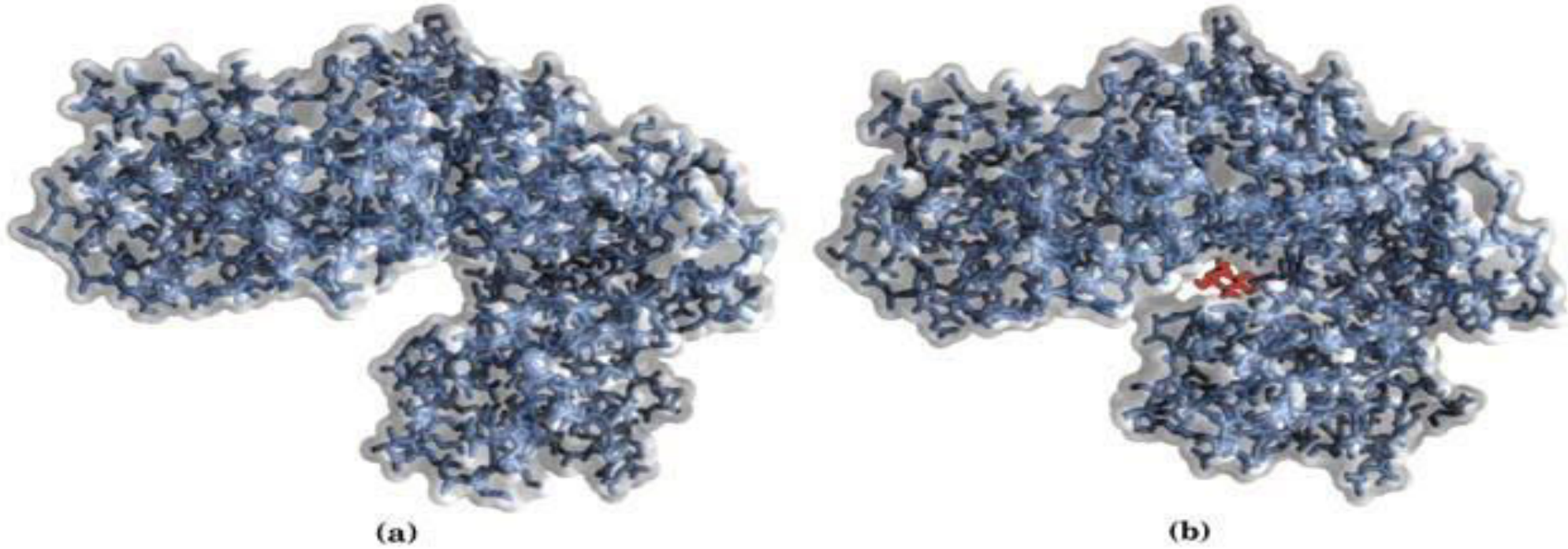
- Some proteins can change their shape (conformation)

- When a substrate combines with an enzyme, it induces a change in the enzyme's conformation

- This change in conformation when the substrate binds is induced by multiple weak interactions with the substrate.
- There may also be rearrangements of covalent bonds during an enzyme-catalyzed reaction.

- This conformational change is referred to as **induced fit.**

The Induced Fit Hypothesis



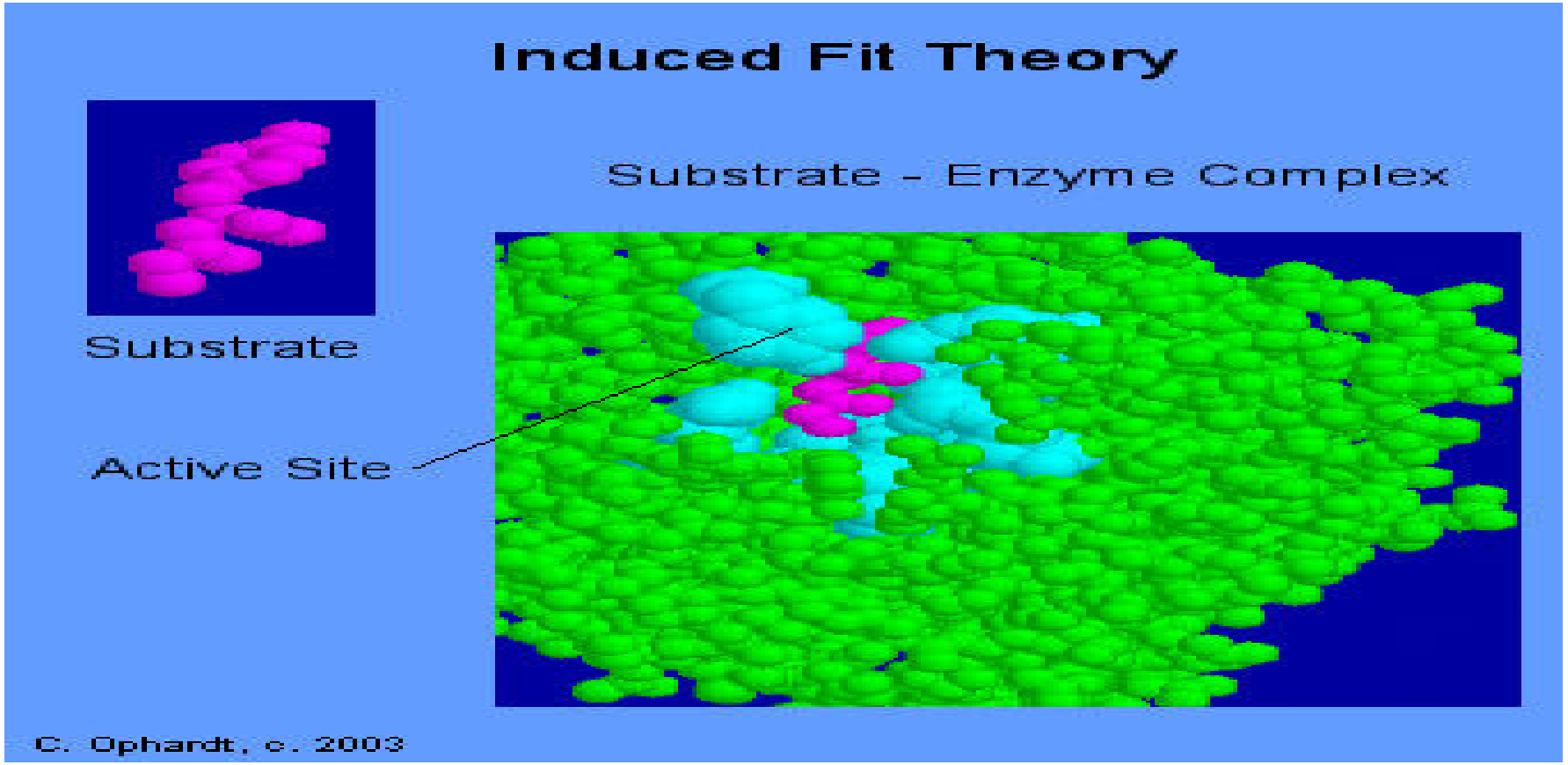
Hexokinase (a) without (b) with glucose substrate

- Chemical reactions of many types take place between substrates and enzyme’s functional groups (specific amino acid side chains, metal ions, and coenzymes

- Induced fit serves to bring specific functional groups on the enzyme into the proper position to catalyze the reaction.

Induced Fit Theory

- Enzyme is not rigid, changes shape with substrate.



- The active site is also moulded into a precise conformation
- Making the chemical environment suitable for the reaction

END

Cofactors, Coenzymes and Prosthetic groups

- Some enzymes require no chemical groups for activity other than their amino acid residues.
- Whereas some enzymes require molecules other than proteins for enzymic activity.

- If the non-protein moiety is a metal ion such as Zn^{2+} or Fe^{2+}, it is called a cofactor.

- If it is a complex organic molecule or metallo-organic compound it is termed a coenzyme.

Some Coenzymes That Serve as Transient Carriers

Coenzyme	*Examples of chemical groups transferred*
Biocytin	CO_2
Coenzyme A	Acyl groups
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups
Flavin adenine dinucleotide	Electrons
Lipoate	Electrons and acyl groups
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)

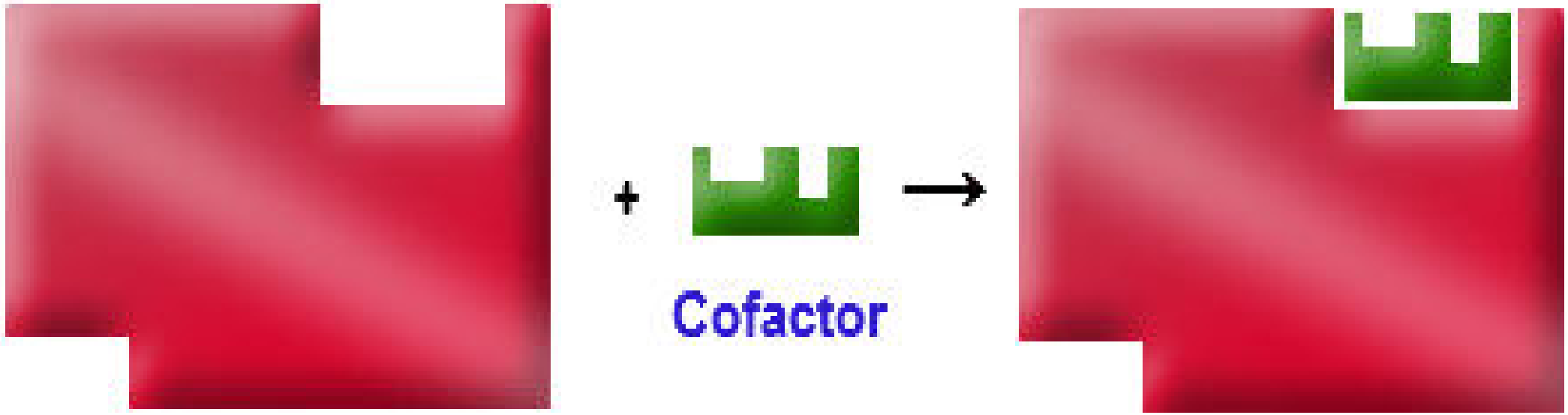
Some Inorganic Elements That Serve as Cofactors for Enzymes

Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase

- A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group.

- The term holoenzyme refers to the active enzyme with its non-protein component,

- whereas the enzyme without its non-protein moiety is termed an apoenzyme (apoprotein) and is inactive.



Apoenzyme

Holoenzyme

- Coenzymes serve as recyclable shuttles that transport many substrates from one point within the cell to another.
- The function of these shuttles is twofold.

- First, they stabilize species
- such as hydrogen atoms (FADH) or hydride ions (NADH)
- that are too reactive to persist for any significant time in the presence of the water or organic molecules that permeate cells.

- Second, they serve as an adaptor or handle that facilitates the
- recognition and binding of small chemical groups, such as acetate (coenzyme A) or glucose (UDP), by their target enzymes

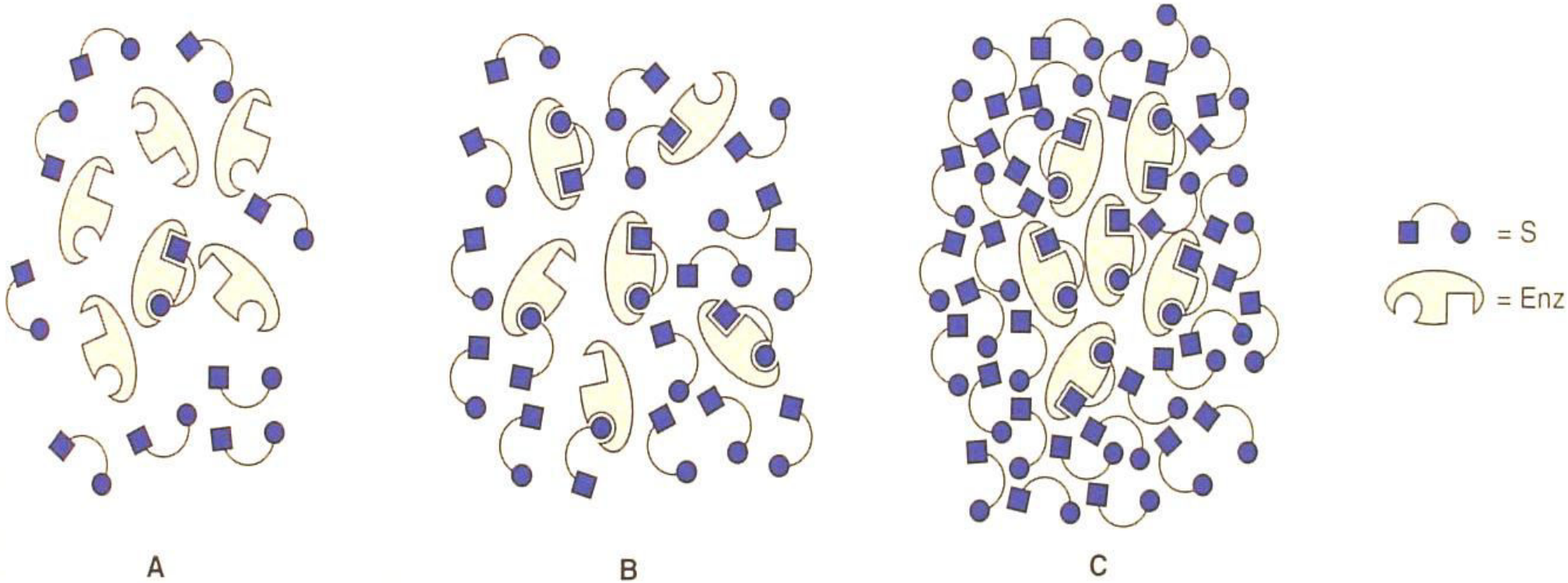
END

Reaction Rates and Order of Reactions

Reaction Velocity (v)

- The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time;
- Velocity is usually expressed as **µmol of product** formed **per minute**.

- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached- reflecting the **saturation** with substrate of **all available binding sites are occupied** on the enzyme molecules present.



A

B

C

- The rate of any reaction is determined by the concentration of the reactant (or reactants) and by a **rate constant,** usually denoted by ***k.***

- For the uni molecular reaction S → P, the rate (or velocity) of the reaction, V—representing the amount of S that reacts per unit time—is expressed by a **rate equation:** $V = k[S]$.

- An equilibrium such as S $\leftrightarrow$ P is described by an **equilibrium constant, Keq**, or simply **K.**
- Under the standard conditions used to compare biochemical processes, an equilibrium constant is denoted ***K´eq*** (or ***K´*)*.***

- In this reaction, the rate depends only on the concentration of S.
- This is called a first-order reaction.
- The factor k is a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature, and so forth).

- If a reaction rate depends on the concentration of two different compounds.
- or if the reaction is between two molecules of the same compound, the reaction is second order.

- The rate equation then becomes

- **$V = k[S_1][S_2]$**

END

Factors Affecting Enzymatic Activity

- Enzymes can be isolated from cells, and their properties studied in a test tube (that is, in vitro).

Factors Affecting Enzymatic Activity

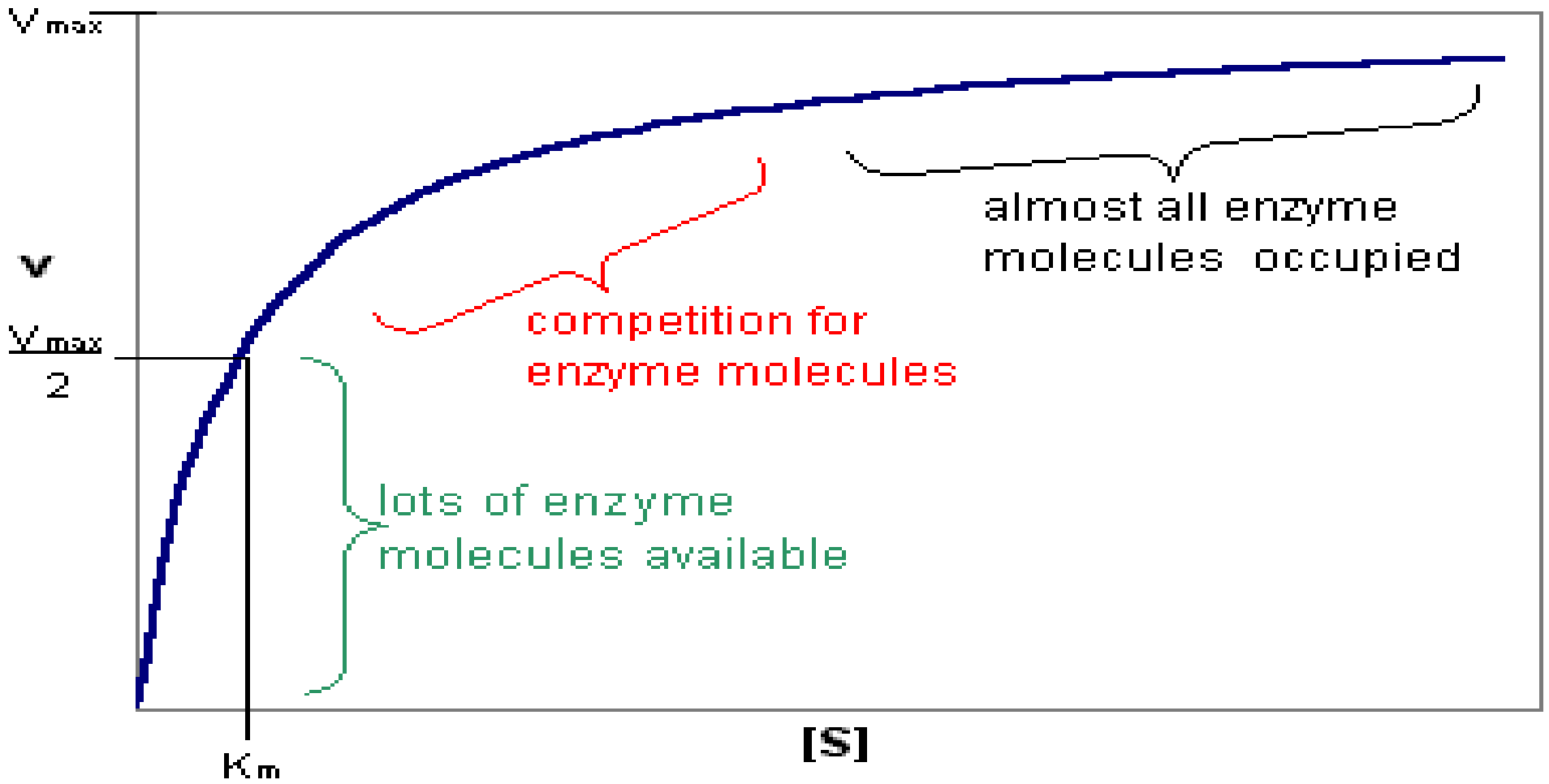
- Different enzymes show different responses to changes in;
- substrate concentration
- temperature, and
- pH.

- Enzymic responses to these factors give us valuable clues as to how enzymes function in living cells (that is, in vivo)

Substrate concentration

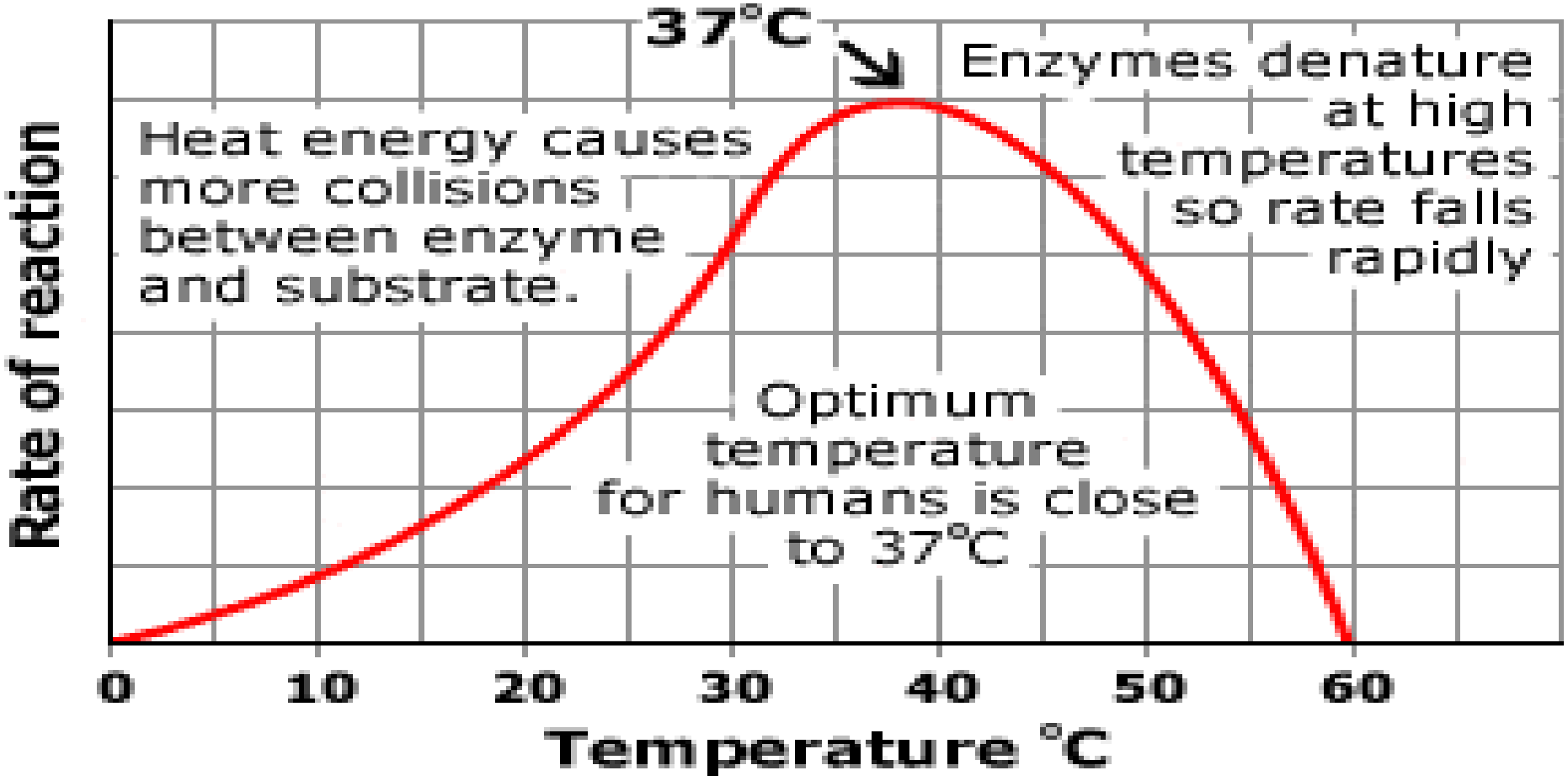
- The rate of an enzyme-catalyzed reaction increases with substrate concentration until maximal velocity (Vmax) is reached

- The leveling off of the reaction rate at high substrate concentrations
- reflects the saturation with substrate
- of all available binding sites on the enzyme molecules present.


V max
V
V max
2
almost all enzyme
molecules occupied
competition for
enzyme molecules
lots of enzyme
molecules available
Km
[S]

Temperature

- The reaction velocity increases with temperature until a peak velocity is reached
- This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products.


37°C
Enzymes denature at high temperatures so rate falls rapidly
Heat energy causes more collisions between enzyme and substrate.
Optimum temperature for humans is close to 37°C
Rate of reaction
0
10
20
30
40
50
60
Temperature °C

- Further elevation
- of the temperature results in a decrease in reaction velocity as a
- result of temperature-induced denaturation of the enzyme

END

Factors Affecting Enzymatic Activity

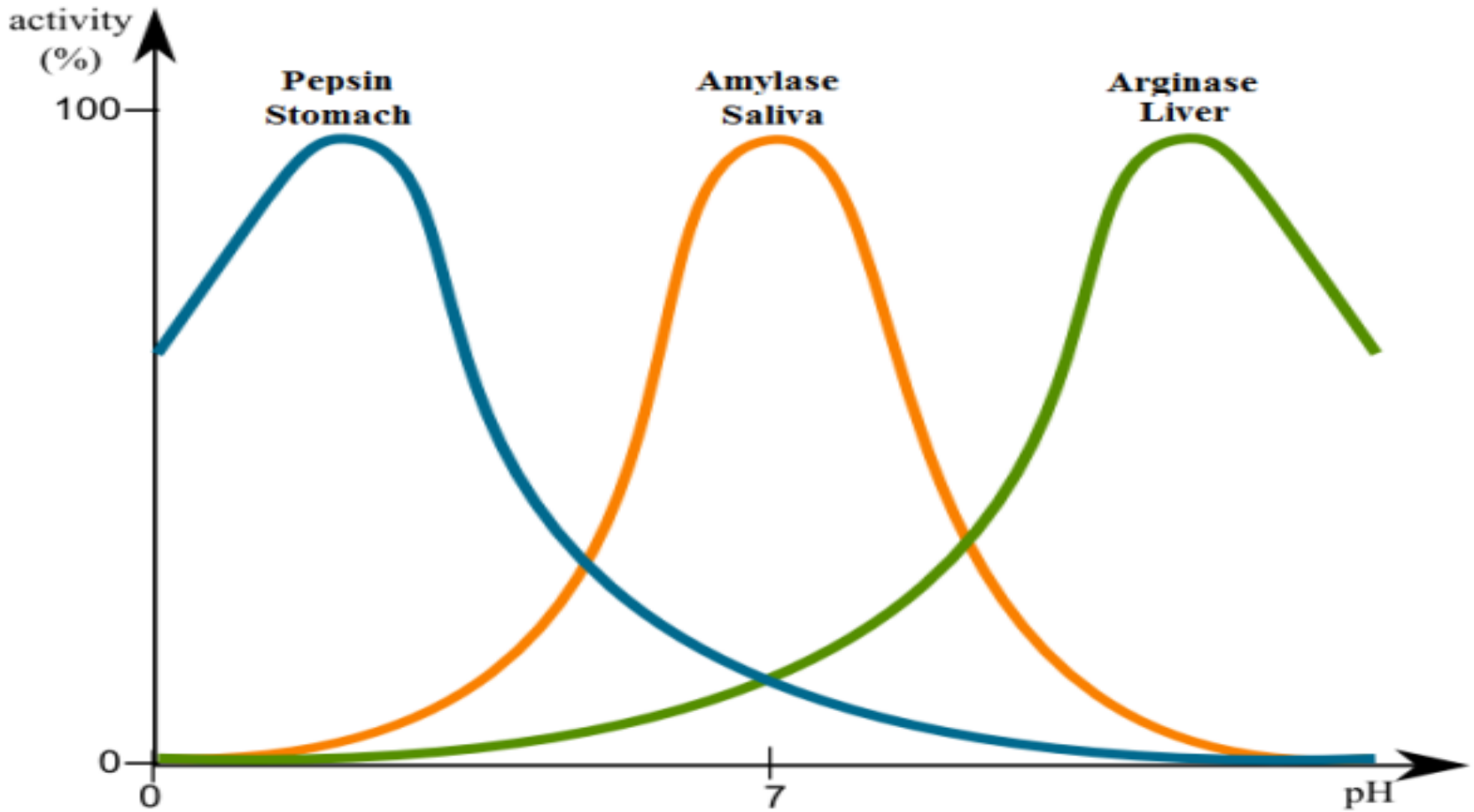
(Contd.)

Effect of pH

- **The pH optimum varies for different enzymes:**
- The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the [H+] at which the enzyme functions in the body.

- For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2.
- Whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment

- Another examples is that there are two types of phosphatases in the body.
- The one that acts in the alkaline pH is called alkaline phosphatase and
- the other which acts at acidic pH is known as acid phosphatase.


activity
(%)
100
0
0
7
pH
Pepsin
Stomach
Amylase
Saliva
Arginase
Liver

- Effect of pH on the ionization of the active site:
- The concentration of H+ affects reaction velocity in several ways.

- First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact.

- For example, catalytic activity may require that an amino group of the enzyme be in the protonated form

  (–NH3 +).
- At alkaline pH, this group is deprotonated, and the rate of the reaction, therefore, declines.

- Extremes of pH can also lead to denaturation of the enzyme.

END

Enzyme Kinetics

Substrate Concentration Affects the Rate of Enzyme-Catalyzed Reactions

$$E + S \leftrightarrow ES \leftrightarrow EP$$

$$\leftrightarrow E + P$$

- A key factor affecting the rate of a reaction catalyzed by an enzyme is the concentration of substrate, [S].

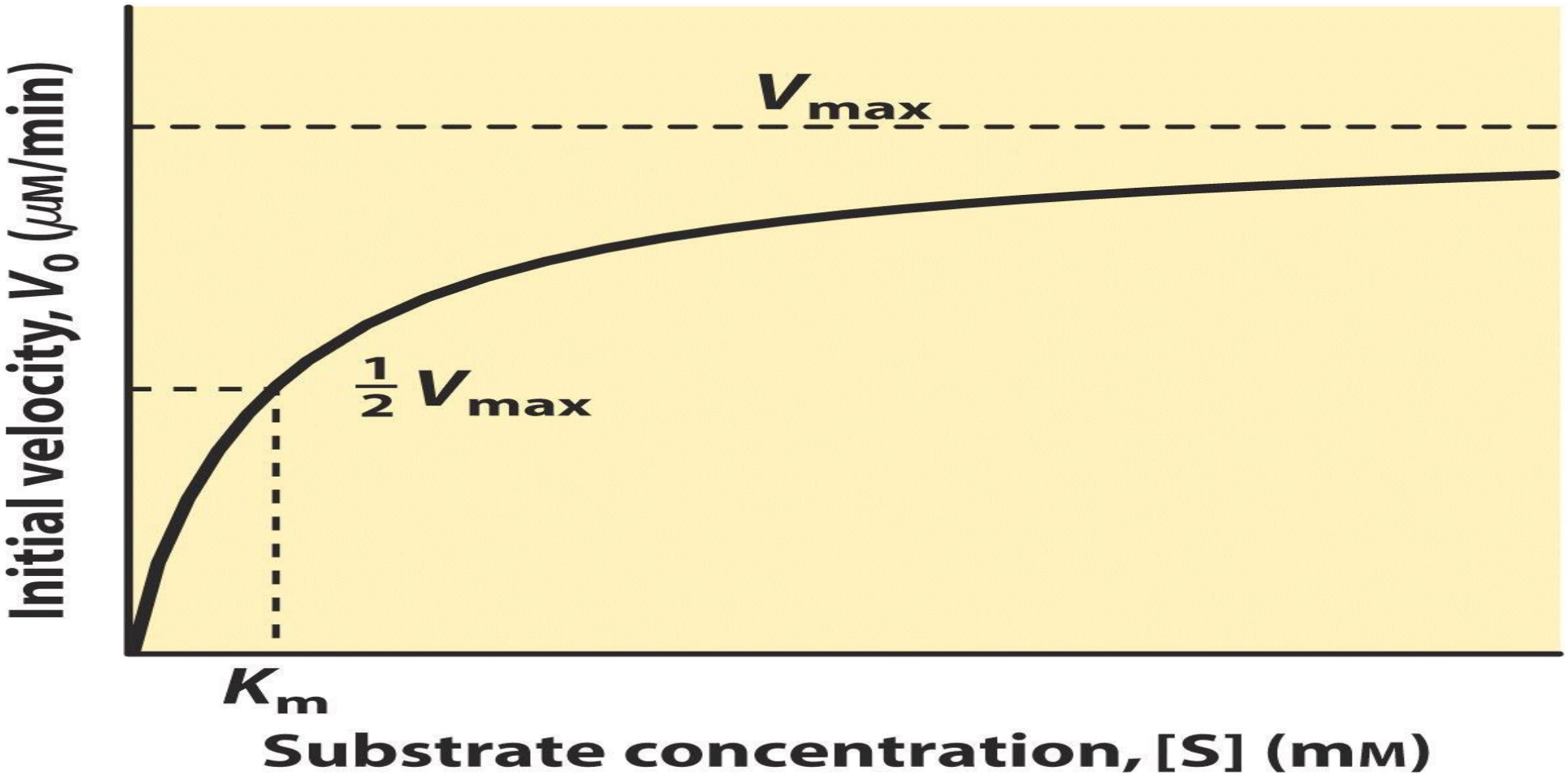
- However, studying the effects of substrate concentration is complicated because substrate is converted to product
- and because of reversibility of reactions, e.g. conversion of product back to substrate.

- One simplifying approach in kinetics experiments is to measure the **initial rate (or initial velocity), designated V_0,** when [S] is much greater than the concentration of enzyme, [E].

- The velocity (v) of a reaction is the rate of product formation
- Whereas V_0 is the initial velocity and is measured as soon as the reactants and enzymes are mixed.

- At the start of a reaction, [S] is in large excess of [P].
- Thus the initial velocity of the reaction will be dependent on substrate concentration

- At that time, the concentration of product is very small and, therefore, the rate of the back reaction from P to S can be ignored.


Initial velocity, V_0 (μM/min)
V_{max}
$\frac{1}{2} V_{max}$
K_m
Substrate concentration, [S] (mM)

- Increase in substrate concentration increases V_0 i.e.
- initial velocity is increased whenever a fixed concentration of enzyme is mixed with an increased concentration of substrate.

END

Enzyme Kinetics (Contd.)

Maximal Velocity V_{max}

- When initial velocity **(V_o)** is plotted against [S],
- a **hyperbolic curve** results,
- where V_{max} represents the maximum reaction velocity.

We have already defined the V and **v_o.**

A hyperbola is an open curve, meaning that it continues indefinitely to infinity, rather than closing on itself as an ellipse does

Enzyme Kinetics (Contd.)

Maximal Velocity V_{max}

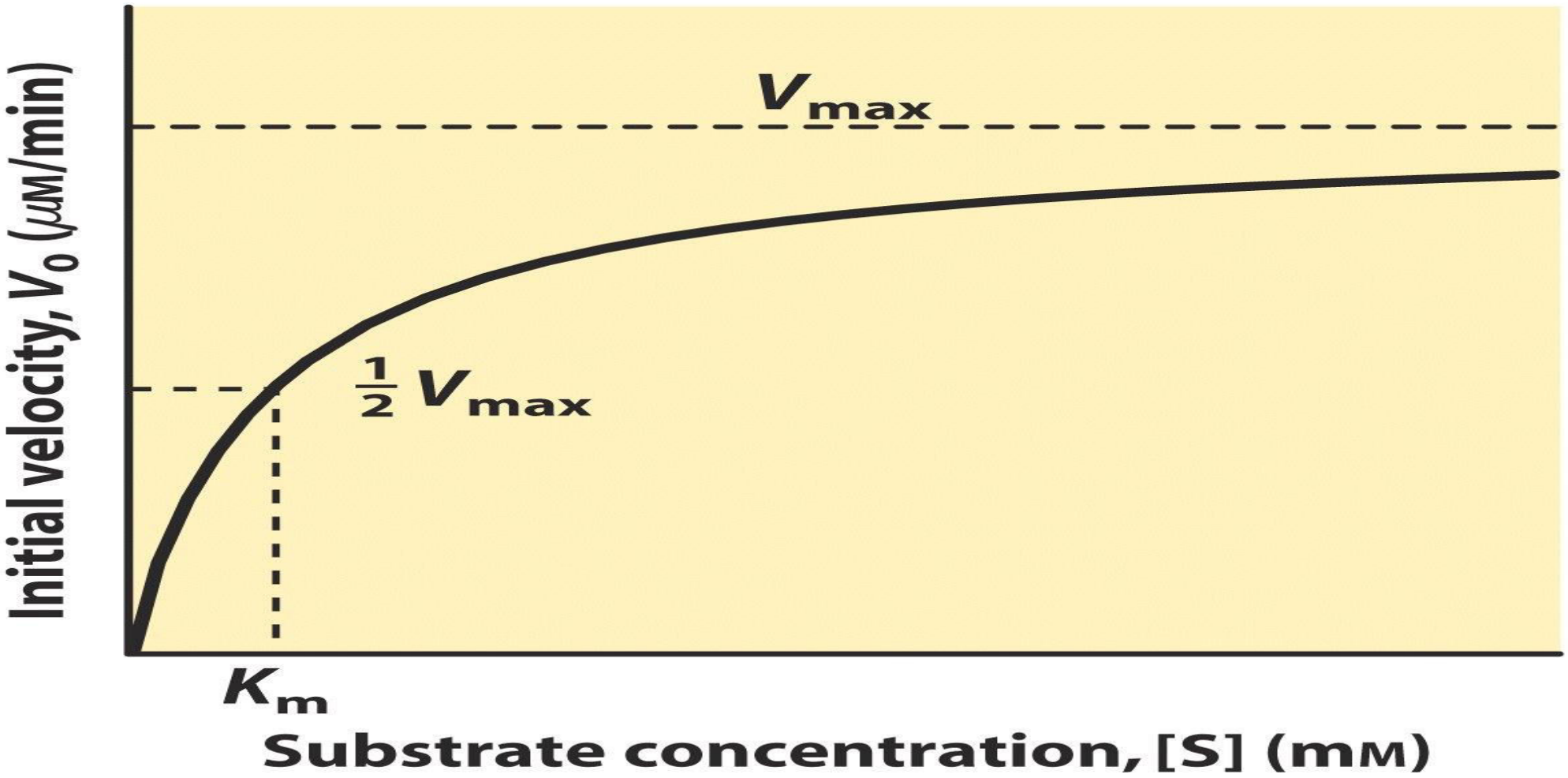
- When initial velocity **(V_o)** is plotted against [S],
- a **hyperbolic curve** results,
- where V_{max} represents the maximum reaction velocity.

The effect of varying [S], on $V_{0,}$ when the **enzyme concentration is held constant.**

At relatively low concentrations of substrate, V_o increases almost linearly.

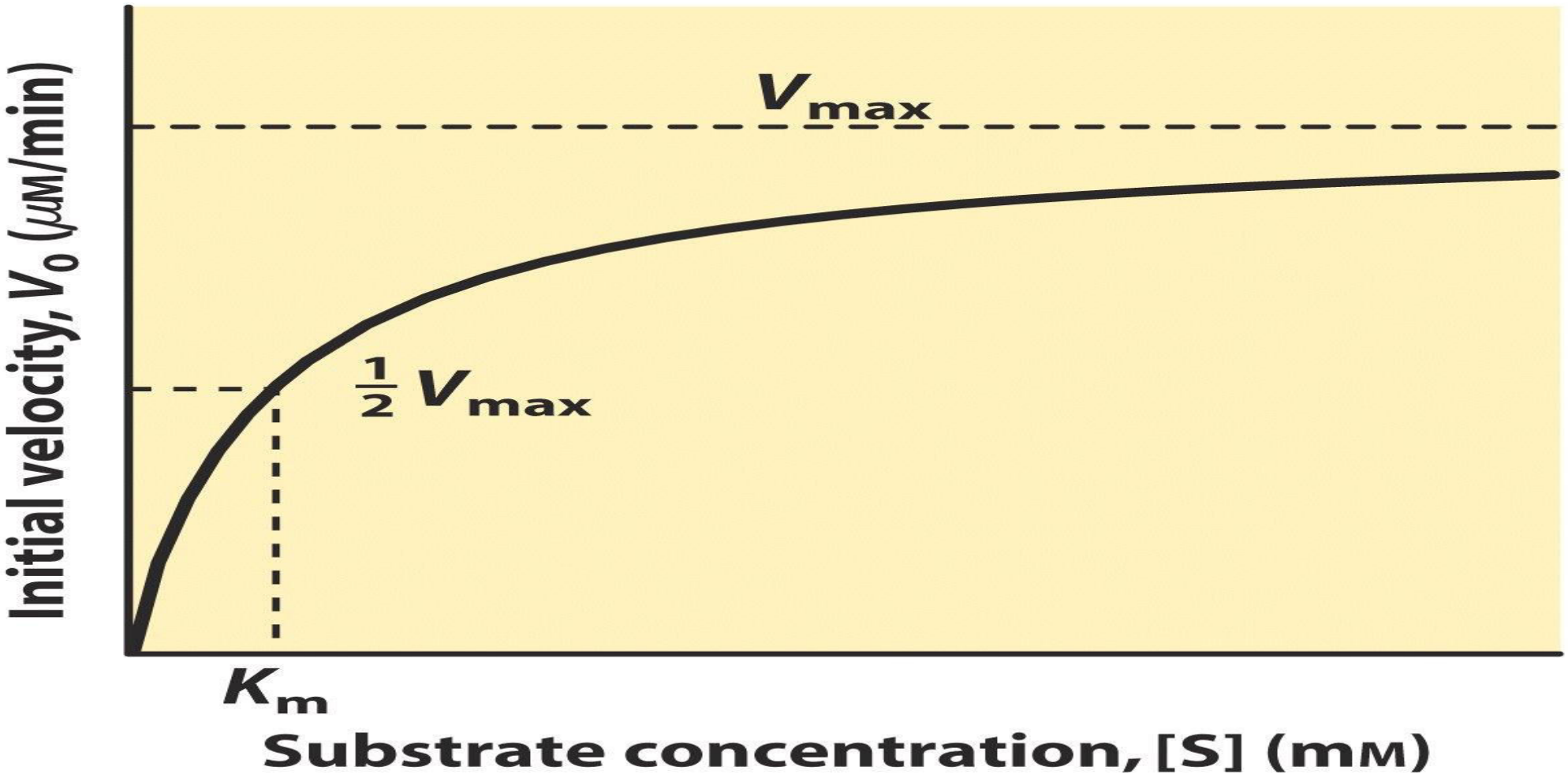
At higher substrate concentrations, V_o *increases by smaller and smaller amounts in* response to increases in [S].

A hyperbola is an open curve, meaning that it continues indefinitely to infinity, rather than closing on itself as an ellipse does

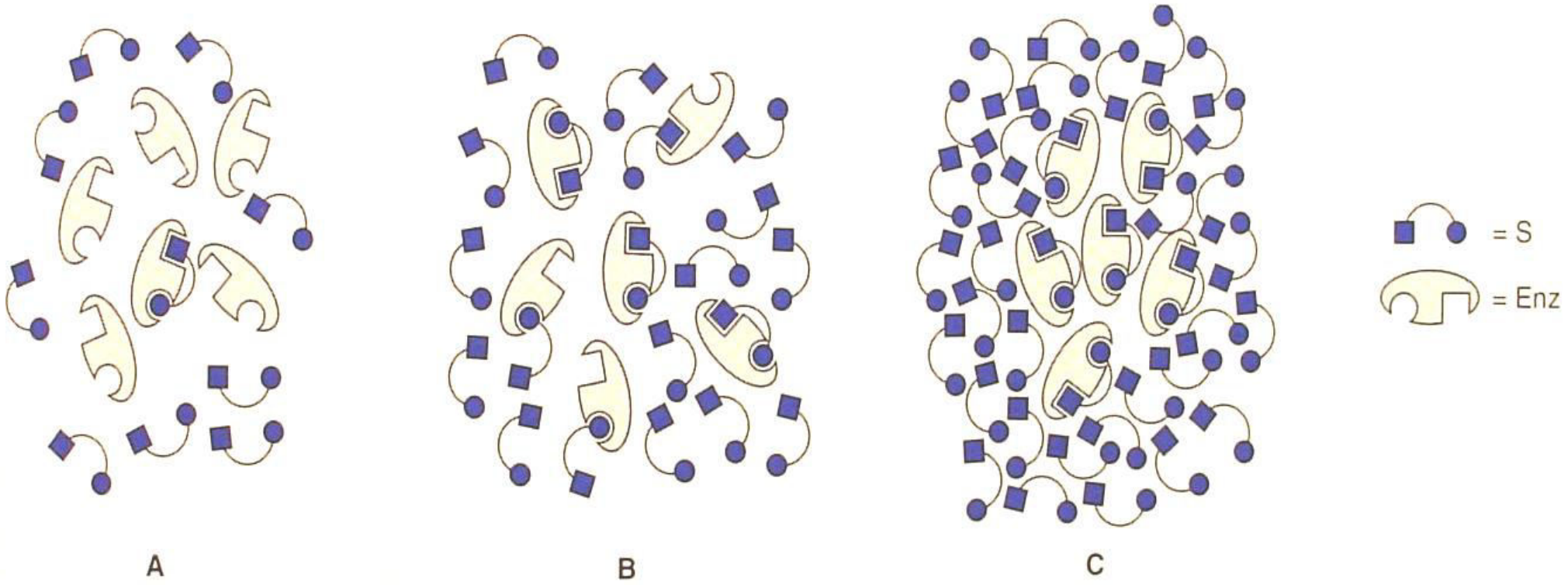

Initial velocity, V_0 (μM/min)
V_{max}
$\frac{1}{2} V_{max}$
K_m
Substrate concentration, [S] (mM)

- At relatively low concentrations of substrate, V_o increases almost linearly.
- At higher substrate concentrations, V_o *increases by smaller and smaller amounts in* response to increases in [S].

- This plateau-like V_o *region is close to the* **maximal velocity, V_{max}.**
- V_{max} *is extrapolated from the* plot, because V_o *approaches but never quite reaches* V_{max}.


Initial velocity, V_0 (μM/min)
V_{max}
$\frac{1}{2} V_{max}$
K_m
Substrate concentration, [S] (mM)

- At this point in the reaction, if **[S] >> E**, all available enzyme is "saturated" with bound substrate, meaning only the **ES complex** is present.
- This dictates that further increasing the substrate concentration will not result in increased V_0



A. Low [S] B. 50% [S] or K_m C. High, saturating [S]

- The leveling off of the reaction rate at high substrate concentrations
- reflects the saturation with substrate
- of all available binding sites on the enzyme molecules present.

- This finite limit of V_{max} is called saturation kinetics.
- Saturation kinetics is a characteristic property of all rate processes dependent on the binding of a ligand to a protein e.g. membrane transporter proteins.

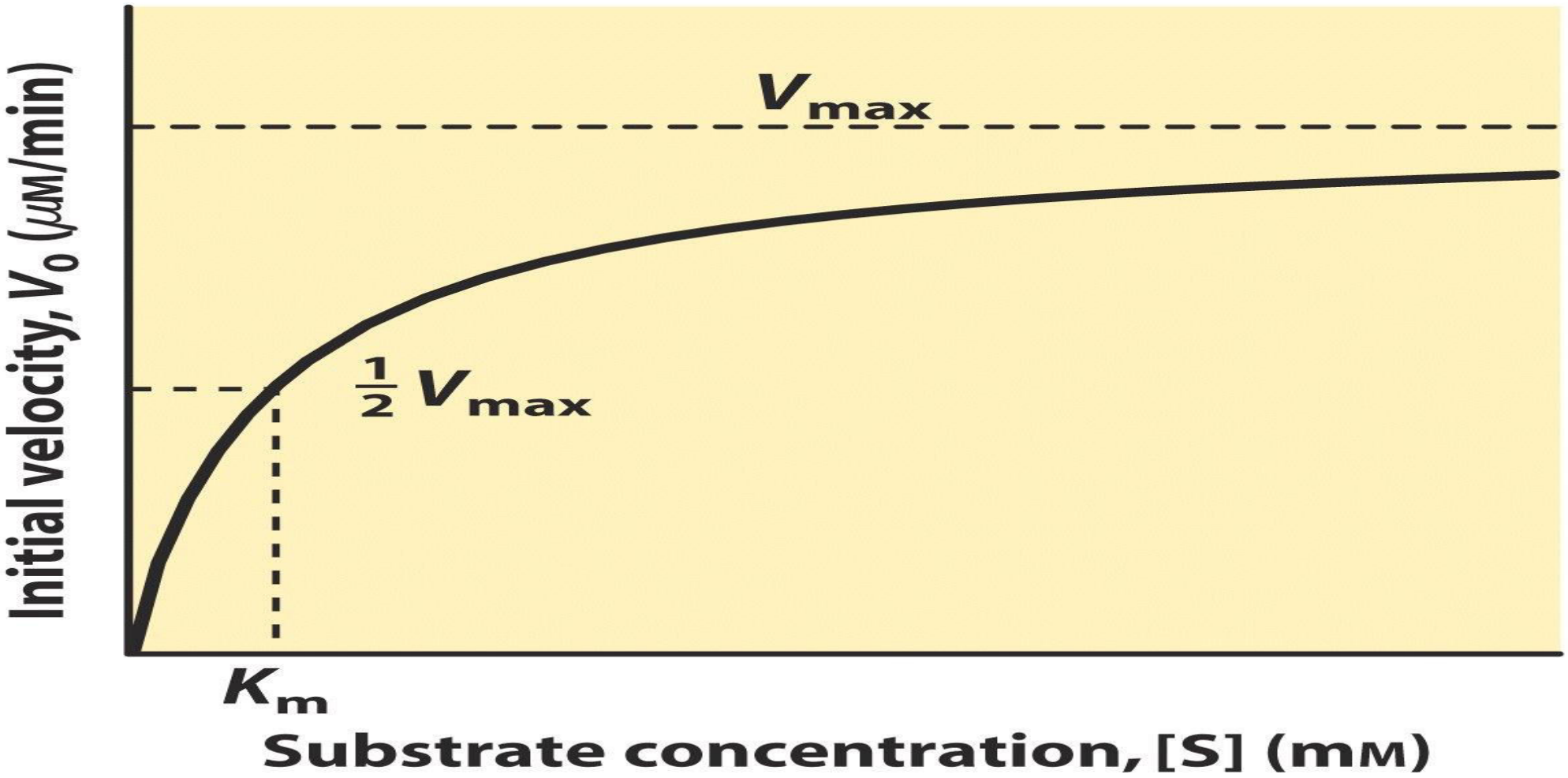
END

The process by which a carrier protein transfers a solute molecule across the lipid bilayer resembles an enzyme-substrate reaction, and in many ways carriers behave like enzymes. In contrast to ordinary enzyme-substrate reactions, however, the transported solute is not covalently modified by the carrier protein, but instead is delivered unchanged to the other side of the membrane.

Enzyme Kinetics (Contd.)

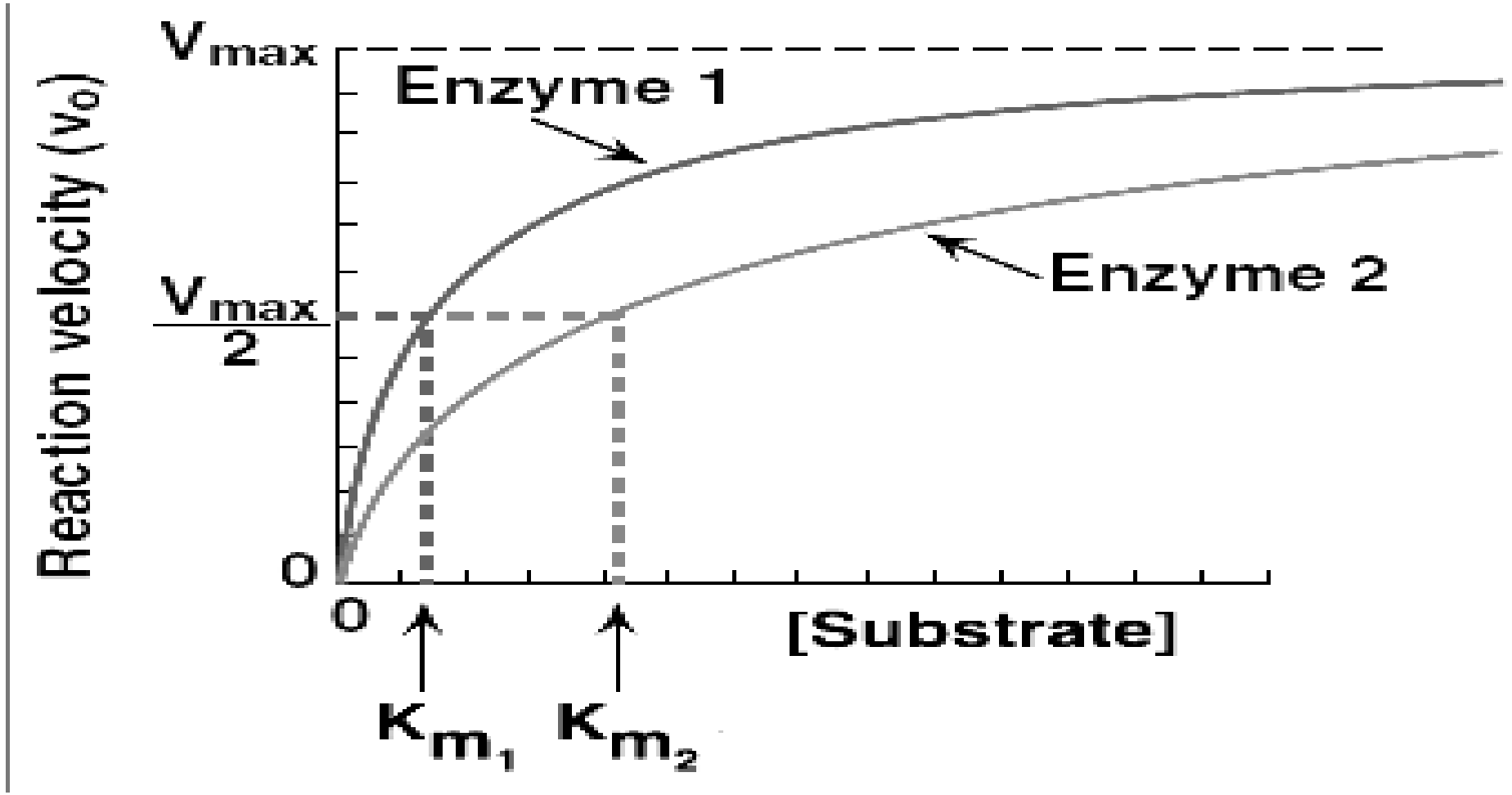
Michaelis constant K_m

- The substrate concentration at which V_o is half maximal is K_m, the Michaelis constant.


Initial velocity, V_0 (μM/min)
V_{max}
$\frac{1}{2} V_{max}$
K_m
Substrate concentration, [S] (mM)

- K_m reflects the affinity of the enzyme for the substrate.
- Small K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme i.e. to reach a velocity that is $1/2V_{max}$.

- A numerically large (high) Km reflects a low affinity of enzyme for substrate because
- a high concentration of substrate is needed to half-saturate the enzyme.


Reaction velocity (v_o)
V_{max}
$\frac{V_{max}}{2}$
Enzyme 1
Enzyme 2
0
0
[Substrate]
K_{m_1}
K_{m_2}

a. **Small K_m:** A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is 1⁄2 V_{max}

Small K_{m1}: A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is 1⁄2 V_{max}.
Large Km2: A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme

b. Large Km: A numerically large (high) Km reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme

- The velocity of an enzyme is most sensitive to changes in substrate concentration over a concentration range below its Km.
- At substrate concentrations less than 1⁄10th of the Km, a doubling of substrate concentration nearly doubles the velocity of the reaction

- At substrate concentrations 10 times the Km, doubling the substrate concentration has little effect on the velocity.

END

- A comparison between the isozymes of hexokinase illustrates the significance of the K_m.
- Hexokinase catalyses the first step in glucose metabolism in most cells, the transfer of a phosphate from ATP to glucose to form glucose 6-phosphate.

- Hexokinase I, the isozyme in red blood cells has a low K_m for glucose of approximately 0.05 mM- helpful in utilizing blood glucose even when the blood glucose concentration is very low.

- The isozyme of hexokinase, called glucokinase, which is found in the liver has a much higher K_m of approximately 5 to 6 mM- helpful in storing large amounts of "excess" glucose as glycogen or converting it to fat after a carbohydrate meal.

Michaelis-Menten equation, the rate equation

- Leonor Michaelis and Maud Menten in 1913, proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions.

- They postulated that the enzyme first combines reversibly with its substrate to form an enzyme-substrate complex in a relatively fast reversible step:

$$E + S \underset{K\text{-}1}{\overset{k1}{\leftrightarrow}} ES$$

- The ES complex then breaks down in a slower second step to yield the free enzyme (E) and the reaction product (P):

$$\mathbf{ES} \underset{K\text{-}2}{\overset{K2}{\leftrightarrow}} \mathbf{E + P}$$

- Early in the reaction, the concentration of the product, **[P], is negligible**, and we make the simplifying assumption that the reverse reaction, P → S (described by *k-2), can be ignored*

- This assumption is not critical but it simplifies our task.
- The overall reaction then reduces to

$$E + S \underset{K\text{-}I}{\overset{k1}{\leftrightarrow}} ES \xrightarrow{K2} E + P$$

E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex

k_1 rate constant for the forward reaction

k_{-1} = rate constant for the breakdown of the ES to substrate

k_2 = rate constant for the formation of the products

- Because the slower second reaction must limit the rate of the overall reaction.
- The overall rate must be proportional to the concentration of the species that react in the second step, that is, ES.

- The maximum initial rate of the catalyzed reaction (*Vmax) is observed when virtually all the* enzyme is present as the ES complex and [E] is vanishingly small.

- Under these conditions, the enzyme is “saturated” with its substrate, so that further increases in [S] have no effect on rate.
- This condition exists when [S] is sufficiently high that essentially all the free enzyme has been converted to the ES form.

- After the ES complex breaks down to yield the product P,
- the enzyme is free to catalyze reaction of another molecule of substrate.

END

Michaelis-Menten equation, the rate equation (Contd.)

$$V_o = \frac{Vmax\ [S]}{Km + [S]}$$

- Where;
- V_o = initial reaction velocity

- Vmax = maximal velocity
- Km = Michaelis constant
- = K1+K2 / K-1
- [S] = substrate concentration

- This is the **Michaelis-Menten equation**-statement of the quantitative relationship between the;
- initial velocity V_o,
- *the maximum velocity Vmax, and*
- *the initial substrate concentration [S], all related* through the Michaelis constant *Km*

- An important numerical relationship emerges from the Michaelis-Menten equation in the special case;
- when V_o *is exactly one-half Vmax*

$$V_o = \frac{Vmax\ [S]}{Km + [S]}$$

$$\frac{Vmax}{2} = \frac{Vmax\ [S]}{Km + [S]}$$

- On dividing by *Vmax, we obtain*
- 1/2 = [S] / Km + [S]
- Solving for *Km, we get*
- *Km + [S] = 2[S], or*
- *Km = [S], when* V_o = 1/2 Vmax

- This is a very useful, practical definition of *Km: Km is* equivalent to the substrate concentration at which V_o *is* one-half *Vmax*.

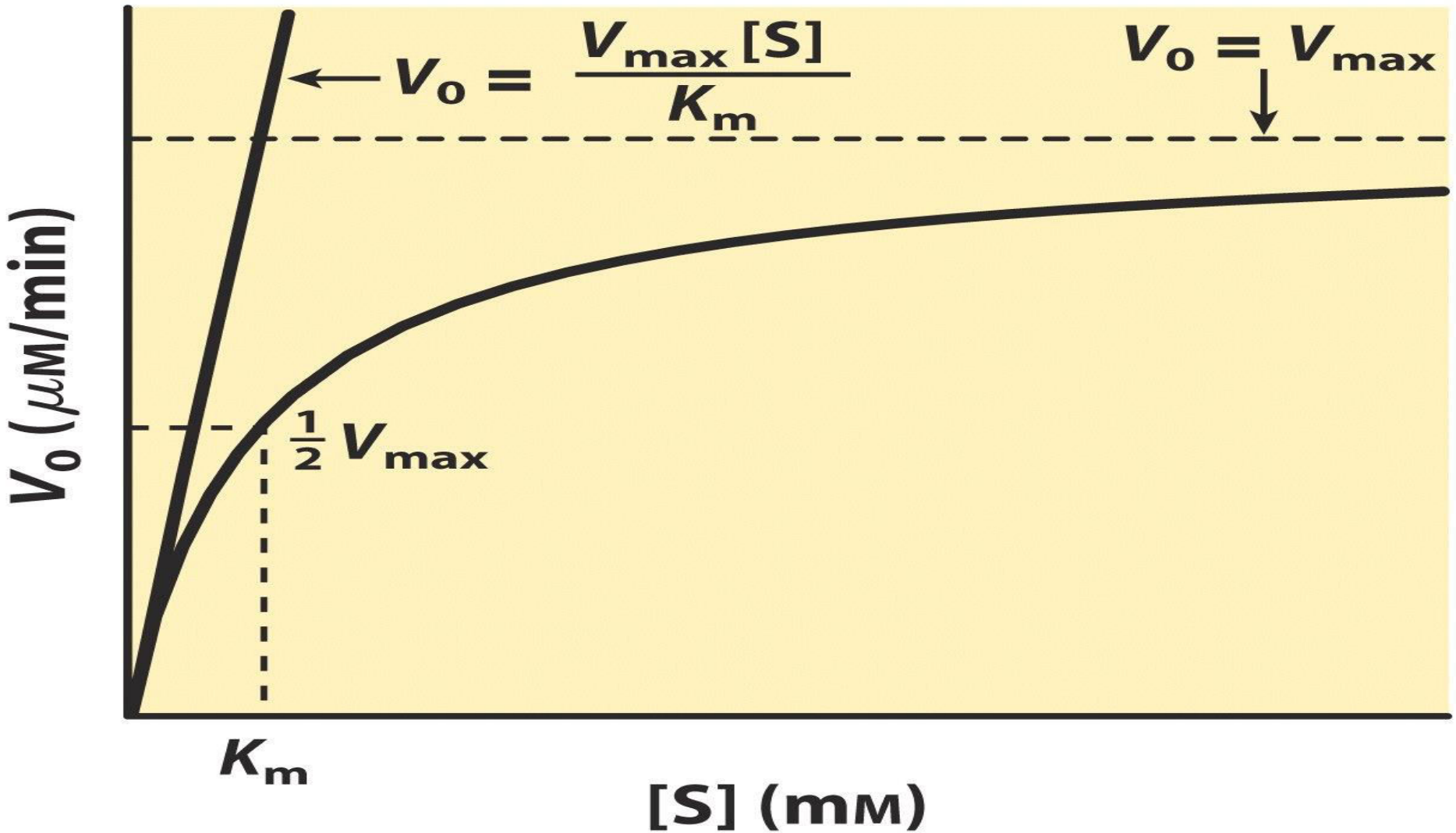
- The equation describes the kinetic behavior of a great many enzymes, and all enzymes that exhibit a hyperbolic dependence of *V_0 on [S] are said to follow* ***Michaelis*- Menten kinetics.**

- The practical rule that *Km= [S] when V0 =1⁄2Vmax holds for all enzymes* that follow Michaelis-Menten kinetics.
- The most important exceptions to Michaelis-Menten kinetics are the regulatory enzymes.

END

The Michaelis-Menton Equation at low [S]

- Interpreting Vmax and Km shows a simple graphical method for obtaining an approximate value for *Km.*
- This graph shows the kinetic parameters that define the limits of the curve at high and low [S].


$V_o = \frac{V_{max}[S]}{K_m}$
$V_o = V_{max}$
V_o (μM/min)
$\frac{1}{2} V_{max}$
K_m
[S] (mM)

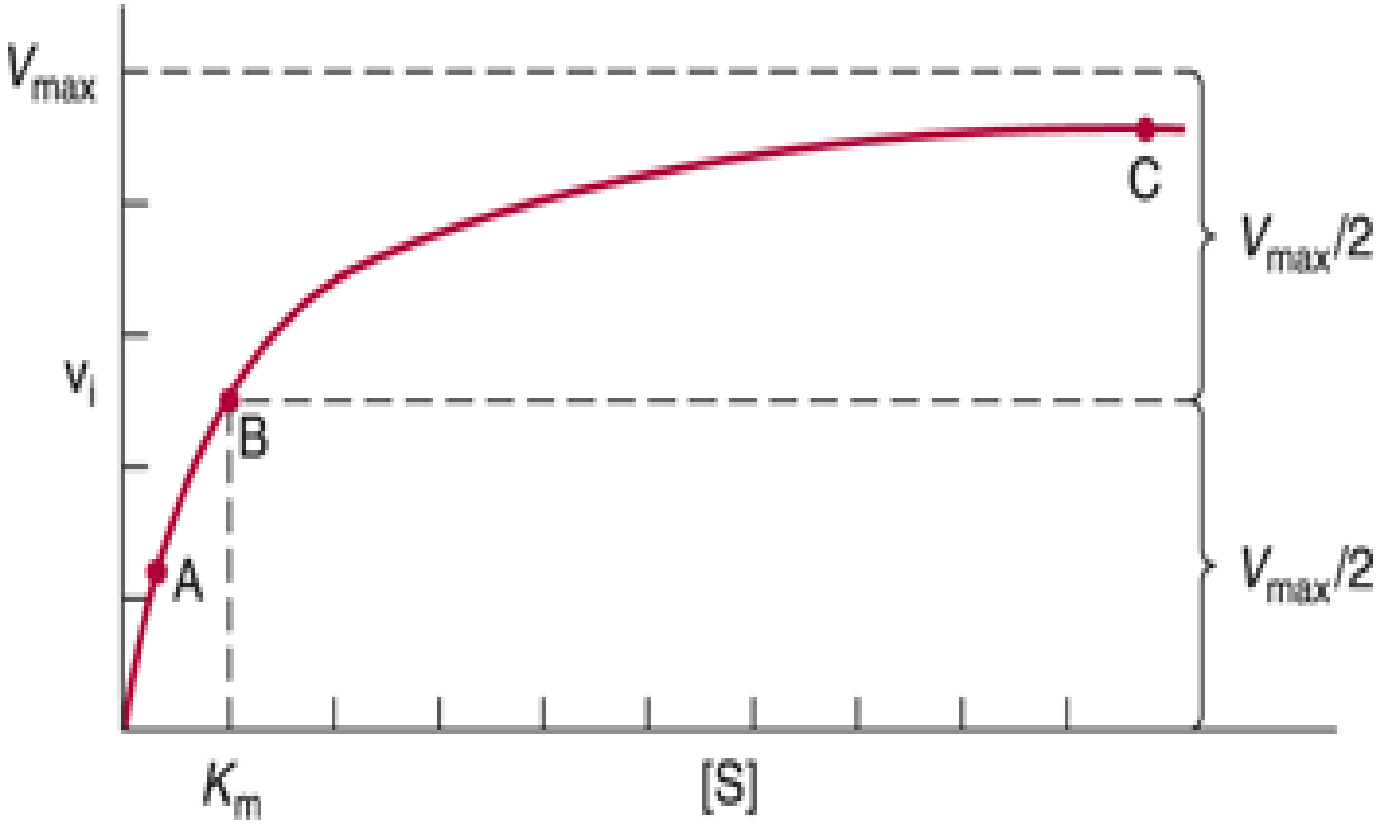
- At low [S], *Km>>[S]* and the *[S]* term in the denominator of the Michaelis-Menten equation becomes insignificant.

- low [S]; $K_m >> [S]$
- $V_o = \frac{Vmax\ [S]}{K^m + [S]}$
- $V_o = \frac{Vmax\ [S]}{K^m}$

- Since V_{max} and K_m are both constants, their ratio is a constant.
- In other words, when [S] is considerably below K_m,
- V_0 is proportionate to k[S].

- The initial reaction velocity, $V_{0,}$ therefore is directly proportionate to [S].

- V_0 exhibits a linear dependence on [S]*, as observed here.*
- *(First order Reaction)*



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 28th Edition:* http://www.accessmedicine.com

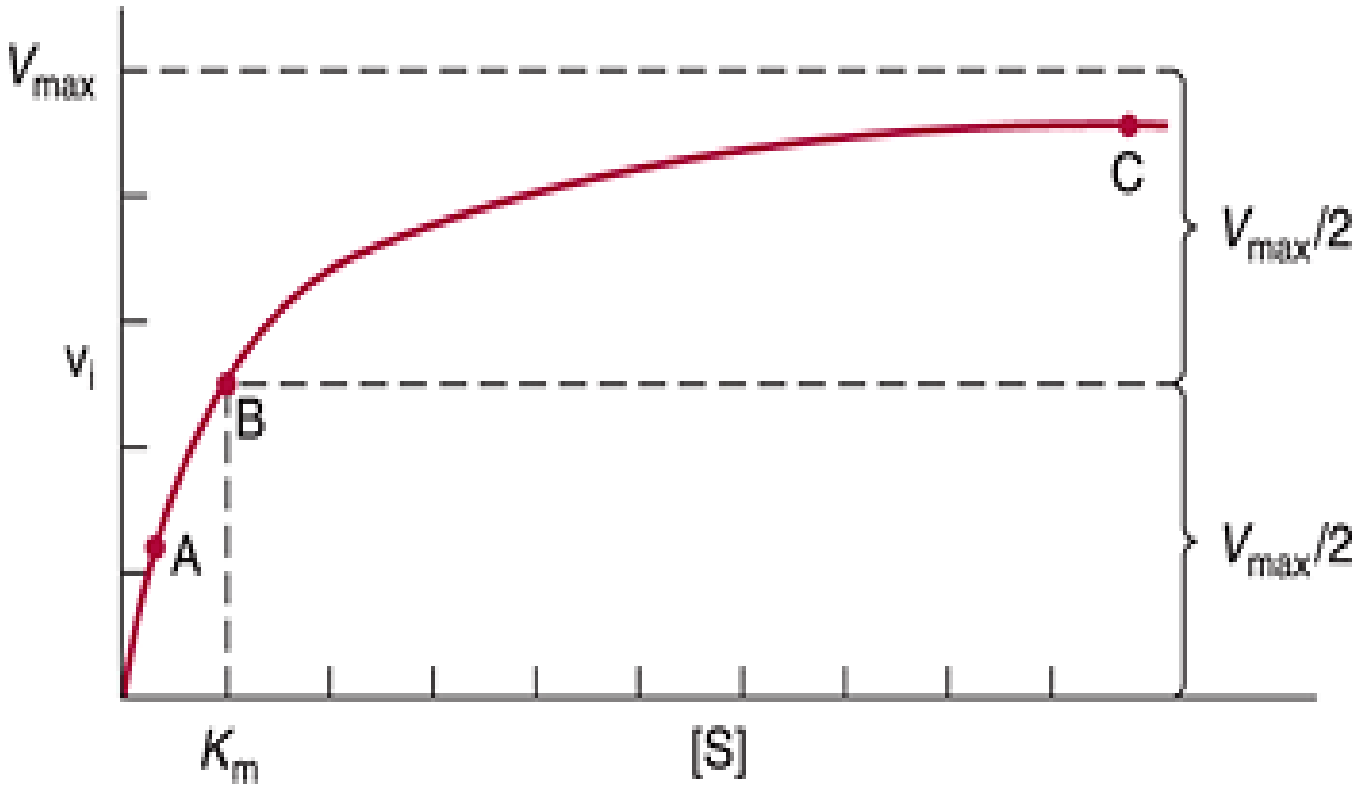
- Therefore at concentrations below K_m reaction rate is first order i.e. it is directly proportional to the concentration of the substrate.

END

The Michaelis-Menton Equation at high [S]

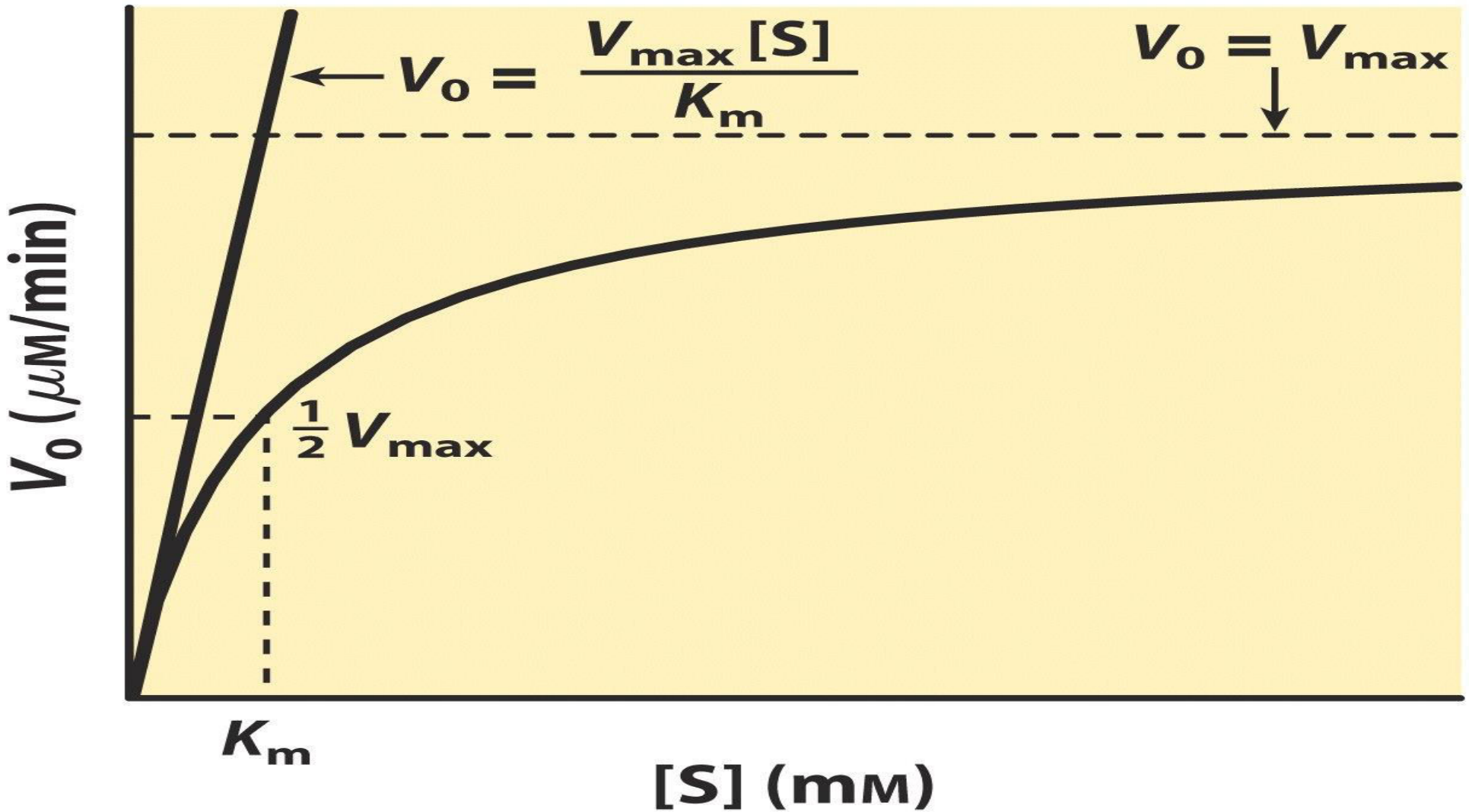
- At high [S] [S] >> K^m
- The term K_m + [S] is essentially equal to [S].
- *The Km term in the denominator of the Michaelis-* Menten equation becomes insignificant
- Replacing K_m + [S] with [S] reduces equation

- high [S] [S] >> K^m
- V_0 = Vmax [S] / K_m + [S]
- Ignoring K_m
- V_0 = Vmax [S] / [S]
- V_0 = Vmax.
- *This is consistent with the plateau observed at high [S]. (Zero Order Reaction)*



Source: Murray RK, Bender DA, Botham KM, Kennelly PJ, Rodwell VW, Weil PA: *Harper's Illustrated Biochemistry, 28th Edition:* http://www.accessmedicine.com

- The rate of reaction is then independent of substrate concentration ***[S]***, and is said to be **zero order** with respect to substrate concentration.


$V_o = \frac{V_{max}[S]}{K_m}$
$V_o = V_{max}$
V_o (μM/min)
$\frac{1}{2} V_{max}$
K_m
[S] (mM)

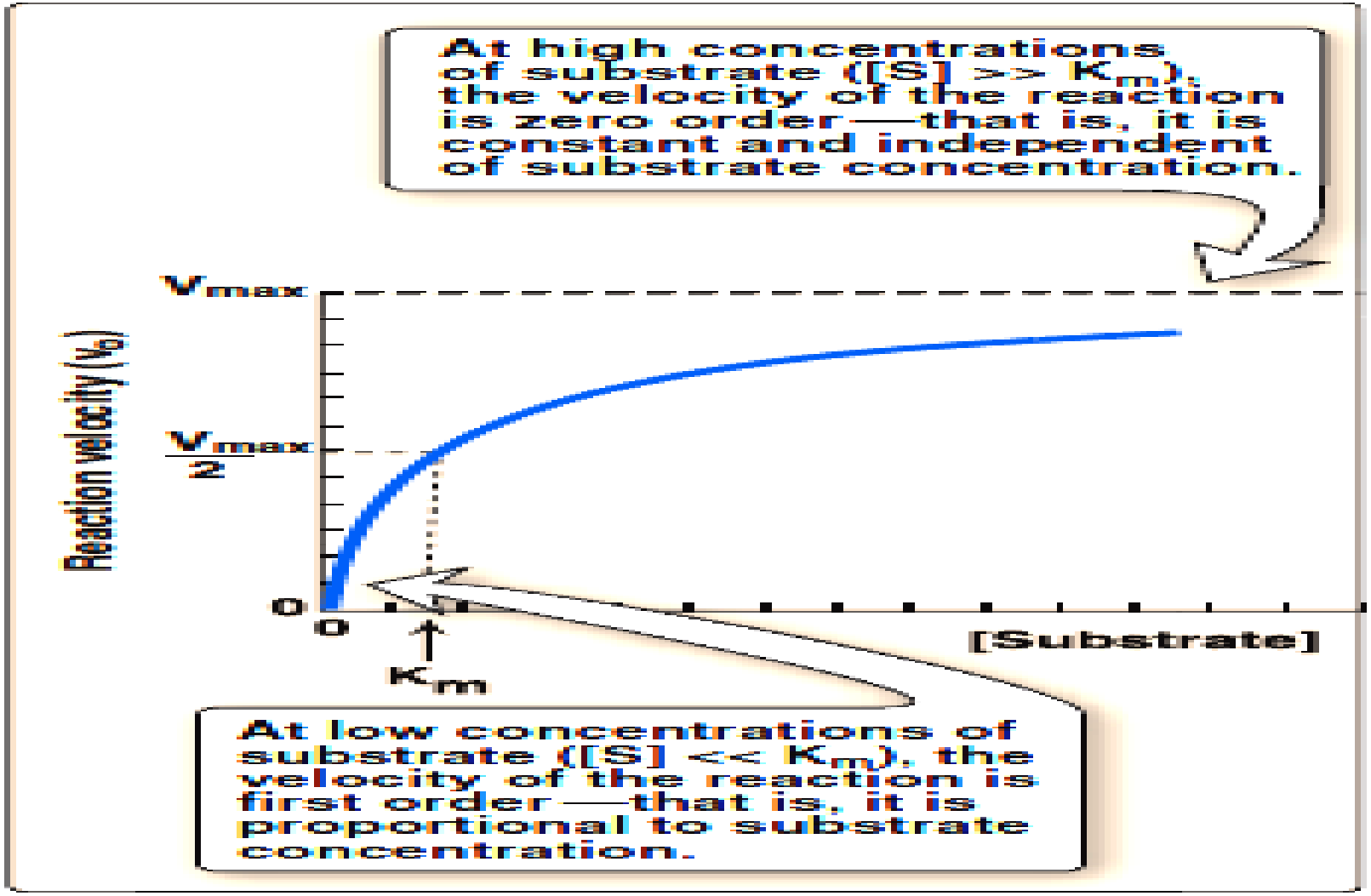
- The Michaelis-Menten equation is therefore consistent with the observed dependence of V_0 on [S], and the shape of the curve is defined by the terms;
- *V_{max}/K_m at low [S] and*
- *V_{max} at high [S].*

END

Order of Reaction

- When **[S]** is much less, then the velocity of the reaction is approximately proportional to the substrate concentration.
- The rate of reaction is then said to be first order with respect to substrate.

- When **[S]** is much greater than Km the velocity is constant and equal to V_{max}.
- The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to substrate concentration

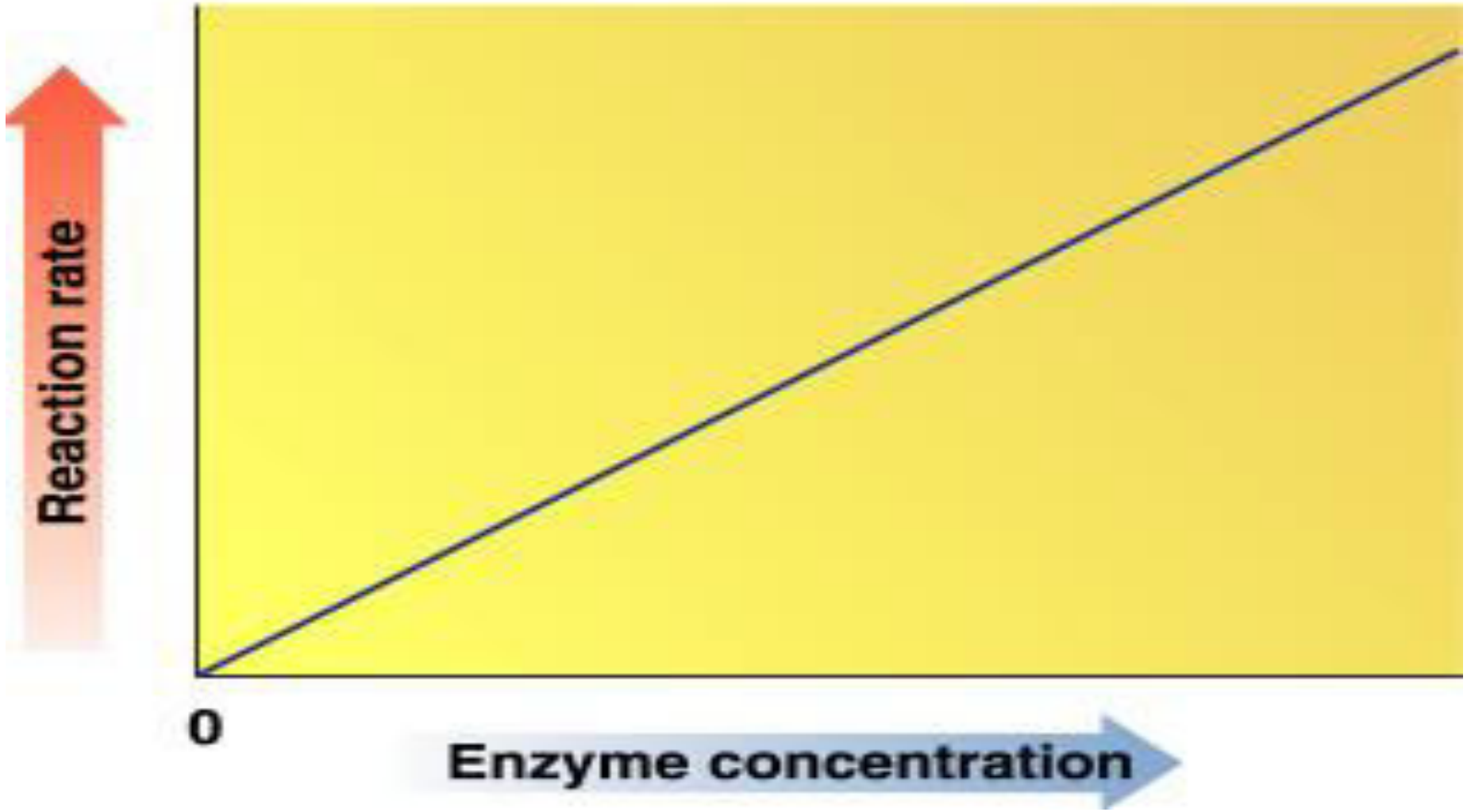

At high concentrations of substrate ([S] >> K_m), the velocity of the reaction is zero order—that is, it is constant and independent of substrate concentration.
V_{max}
$\frac{V_{max}}{2}$
Reaction velocity (V_o)
0
0
K_m
[Substrate]
At low concentrations of substrate ([S] << K_m), the velocity of the reaction is first order—that is, it is proportional to substrate concentration.

Reaction Orders with Respect to Substrate Concentration

Order	Rate Equation	Comments
Zero	Rate = k	Rate is independent of substrate concentration
First	First rate = k[S]	Rate is proportional to the first power of substrate concentration
Second	Rate = k[S1][S2]	Rate is proportional to the first power of each of two reactants

Relationship of velocity to enzyme concentration

- The rate of the reaction is directly proportional to the enzyme concentration.
- There is a linear relationship between reaction rate and enzyme concentration (at constant substrate concentration)

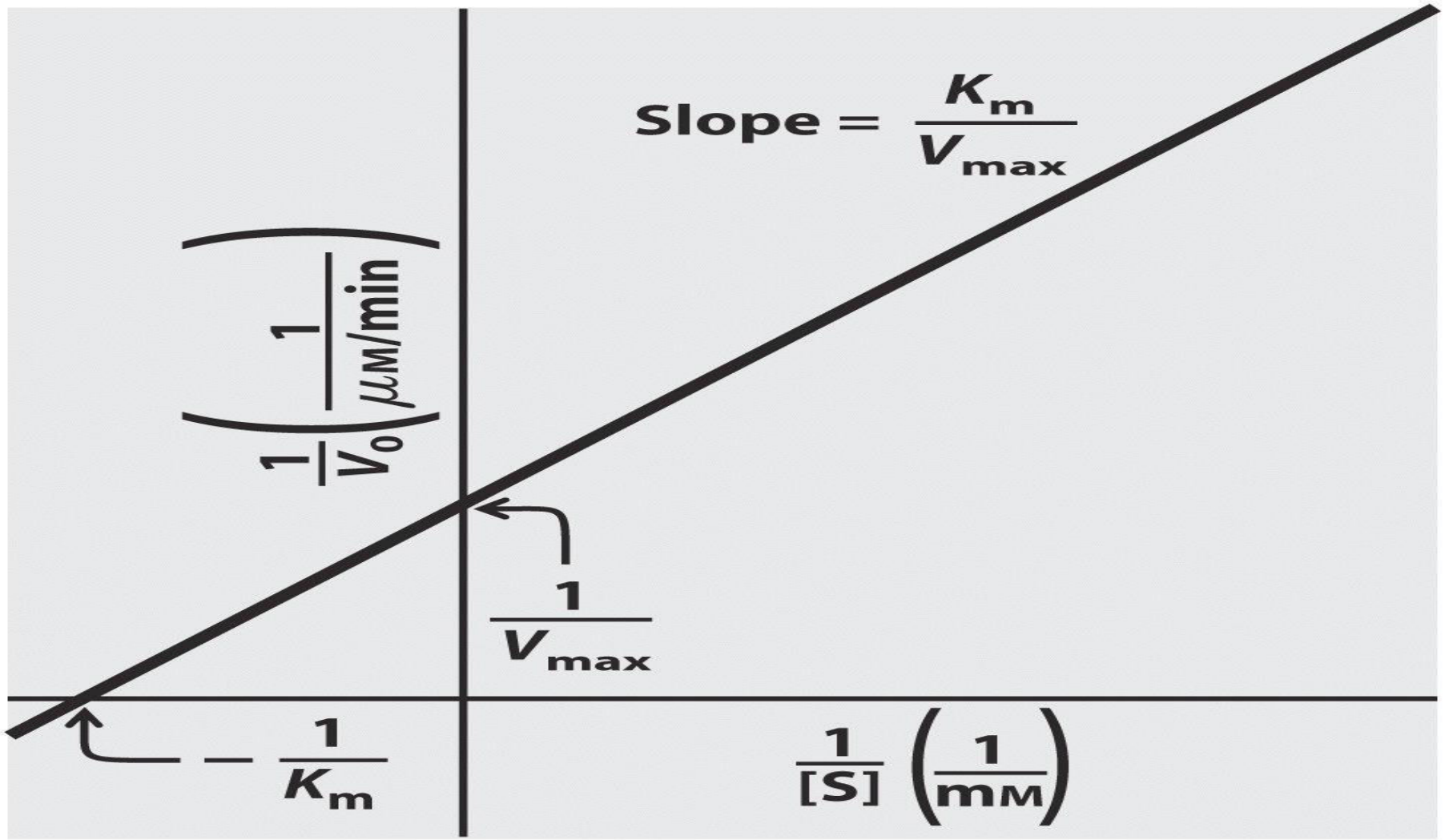

Reaction rate
0
Enzyme concentration

- For example,
- if the enzyme concentration is halved, the initial rate of the reaction (V_o) as well as that of Vmax are reduced to one half that of the original.

END

Lineweaver-Burke plot

- The Michaelis-Menten equation can be algebraically transformed into **Lineweaver-Burke plot,** a *Double Reciprocal Plot*, that is useful in the practical determination of K_m and $V_{max.}$

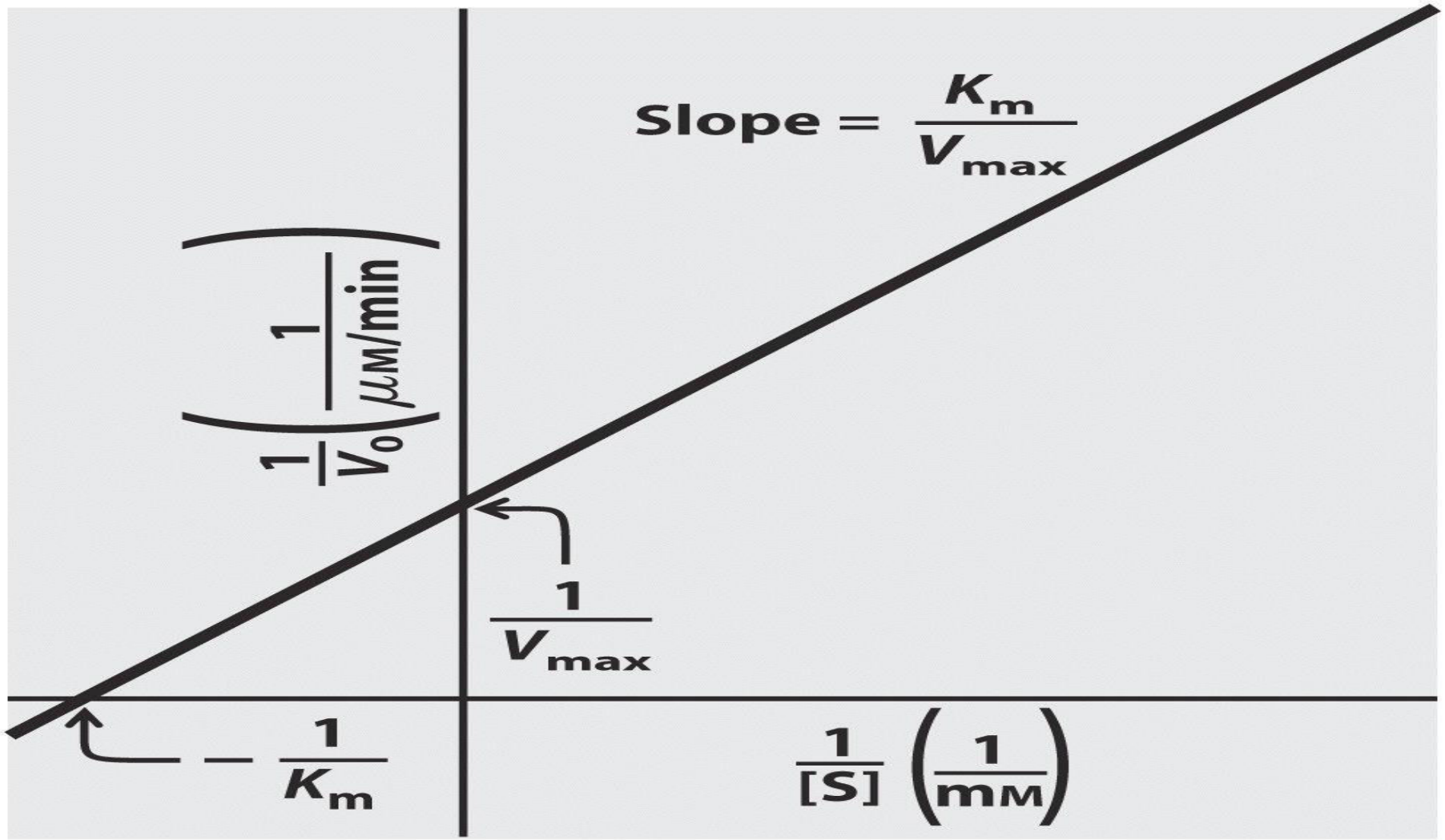

Slope = $\frac{K_m}{V_{max}}$
$\frac{1}{V_o}\left(\frac{1}{\mu M/min}\right)$
$\frac{1}{V_{max}}$
$-\frac{1}{K_m}$
$\frac{1}{[S]}\left(\frac{1}{mM}\right)$

- $V^o = \frac{Vmax\ [S]}{K^m + [S]}$
- Lineweaver-Burke transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:
- $\frac{1}{V^o} = \frac{K^m + [S]}{Vmax\ [S]}$

- $\frac{1}{V_o} = \frac{K^m + [S]}{Vmax\,[S]}$

- $\frac{1}{V_o} = \frac{Km}{Vmax[S]} + \frac{[S]}{Vmax[S]}$

- $\frac{1}{V_o} = \frac{K^m}{Vmax[S]} + \frac{1}{Vmax}$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$-\frac{1}{K_m}$
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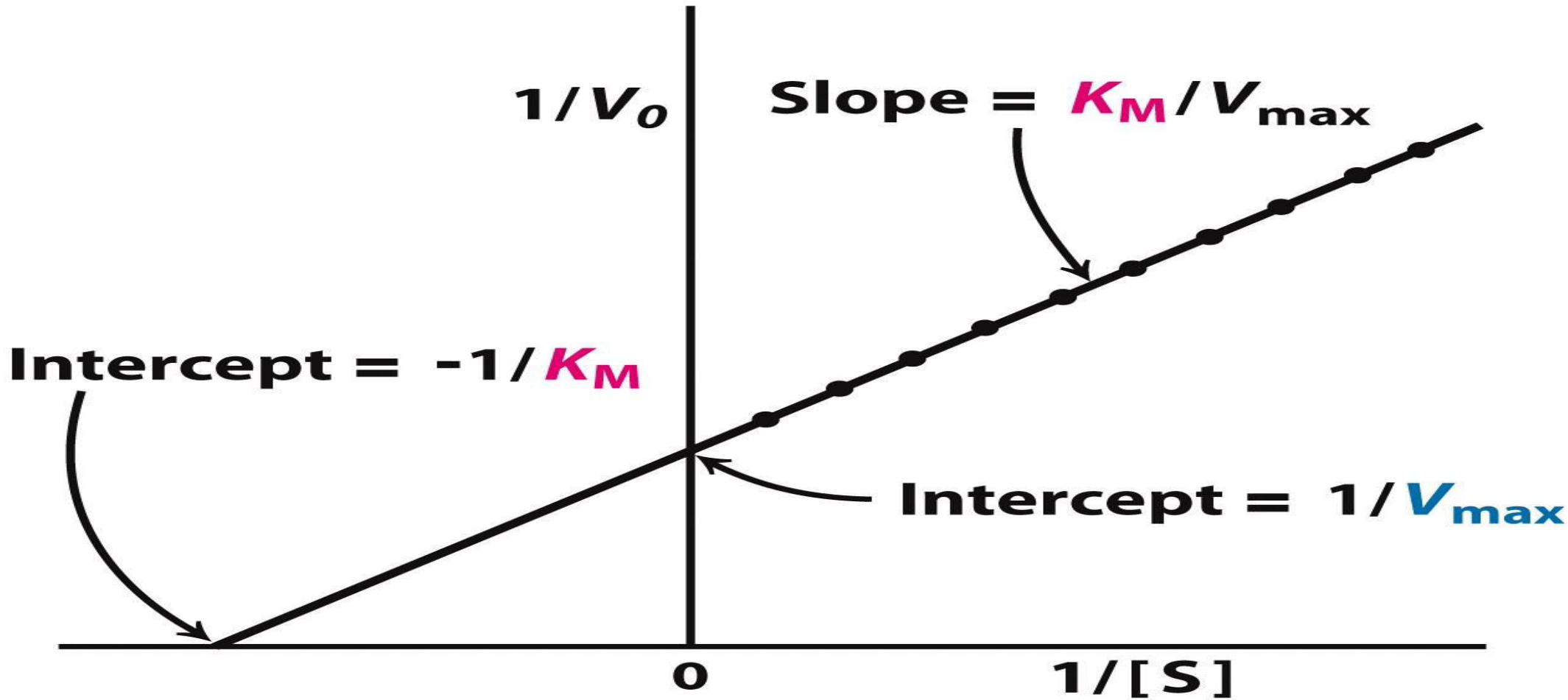


Figure 8.12
***Biochemistry,* Seventh Edition**

- Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of V_{max},
- *Which can only be approximated from* a simple plot of V_o *versus [S].*

- The double-reciprocal plot of enzyme reaction rates is very useful in distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition.

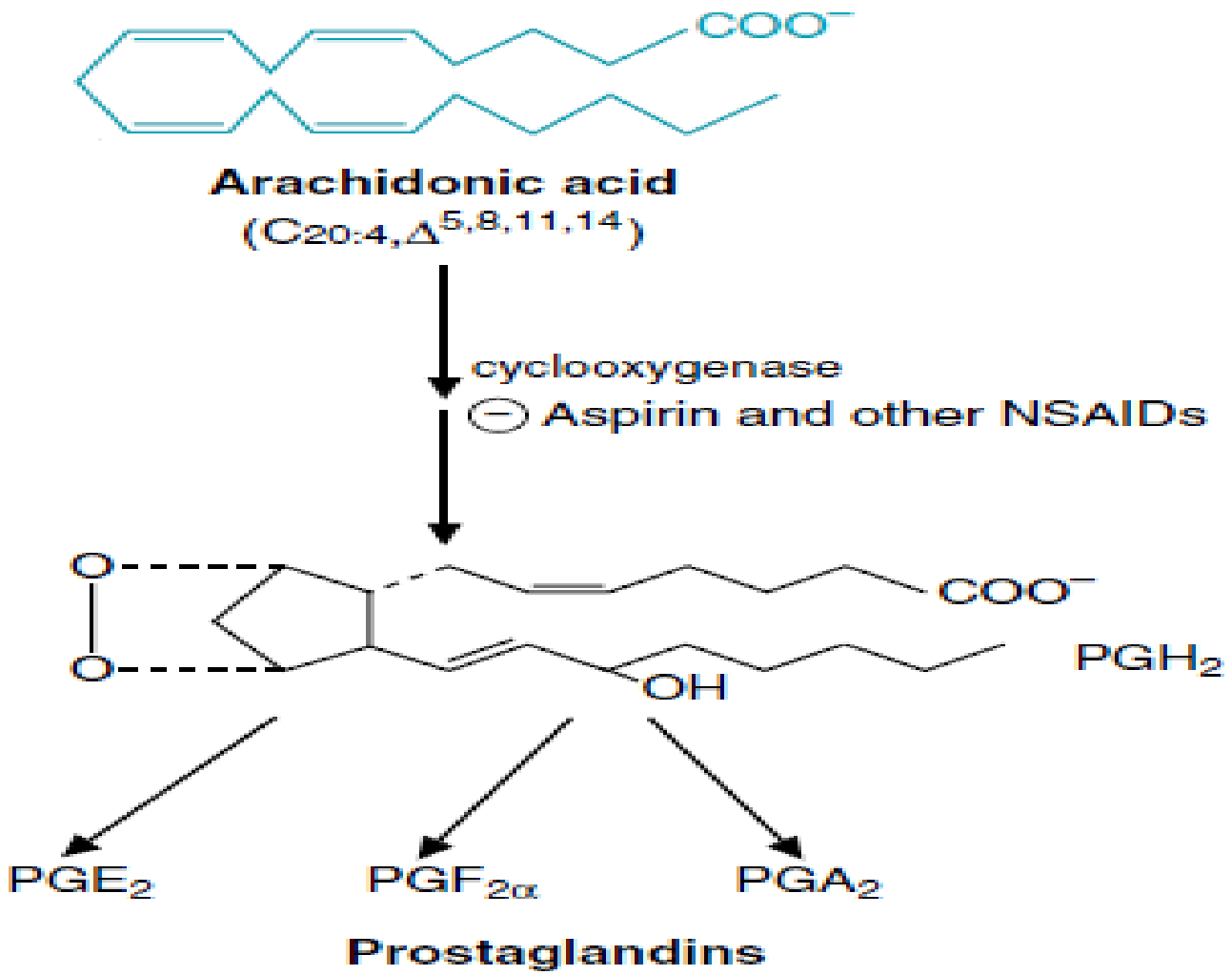
END

INHIBITION OF ENZYME ACTIVITY

- Enzyme inhibitors are molecular agents that interfere with catalysis, slowing or halting enzymatic reactions.
- Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor.

- Enzymes catalyze virtually all cellular processes, so it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known.

For example, aspirin inhibits the enzyme (cyclooxygenase) that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain.


COO⁻
Arachidonic acid
(C20:4,Δ5,8,11,14)
cyclooxygenase
⊖ Aspirin and other NSAIDs
O
O
COO⁻
PGH2
OH
PGE2
PGF2α
PGA2
Prostaglandins

- Inhibitors can be classified on the basis of their site of action on the enzyme,
- on whether they chemically modify the enzyme, or on the kinetic parameters they influence.
- Two broad classes of enzyme inhibitors:
- **1. Reversible**
- **2. Irreversible**.

- In general, irreversible inhibitors bind to enzymes through covalent bonds.
- Reversible inhibitors typically bind to enzymes through noncovalent bonds,

dilution of the enzyme–inhibitor complex results in dissociation of the **reversibly**
bound inhibitor, and recovery of enzyme activity.

- The two most commonly encountered types of reversible inhibition are;
- competitive and
- noncompetitive.

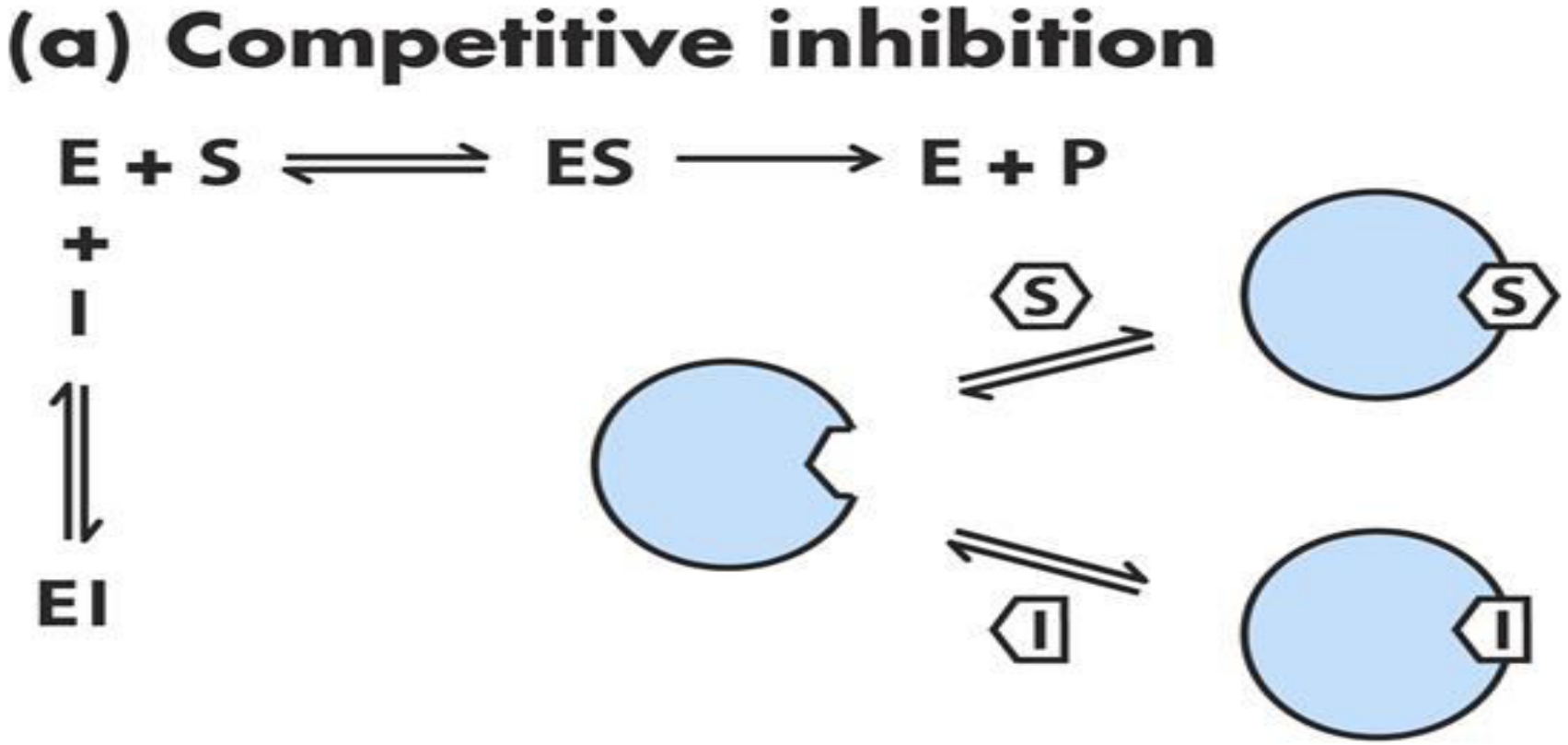
- Competitive inhibitors resemble the substrate and compete for binding to the active site of the enzyme.
- Noncompetitive inhibitors do not bind at the active site. They bind either free enzyme at a site other than active site or the ES complex.

END

Competitive Inhibition

- This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy i.e. active site and, therefore, competes with the substrate for that site.

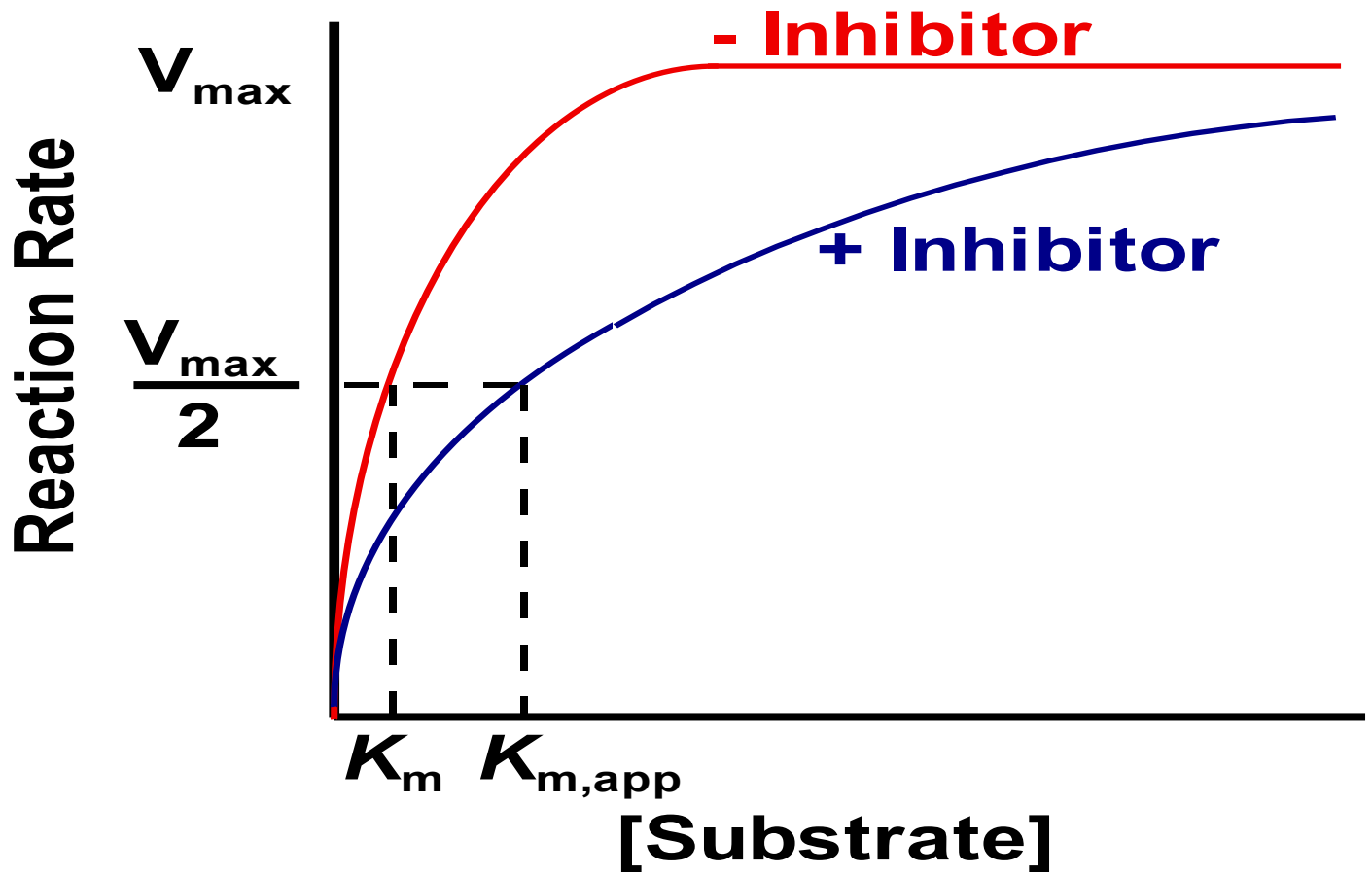
- **Competitive inhibitors bind to the enzyme's active site.**



- Reversible inhibitors bind to enzymes through non covalent bonds.
- Dilution of the enzyme-inhibitor complex results in dissociation of the reversibly bound inhibitor, and recovery of enzyme activity.

1. Effect on Vmax

- The effect of a competitive inhibitor is reversed by increasing [S].
- At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} as observed in the absence of inhibitor.



$V_{max,app} = V_{max}$ $K_{m,app} > K_m$

- When [S] far exceeds [I], the probability that an inhibitor molecule will bind to the enzyme is minimized and the reaction exhibits a normal V_{max}.
- Therefore, a competitive inhibitor does not decrease V_{max}

END

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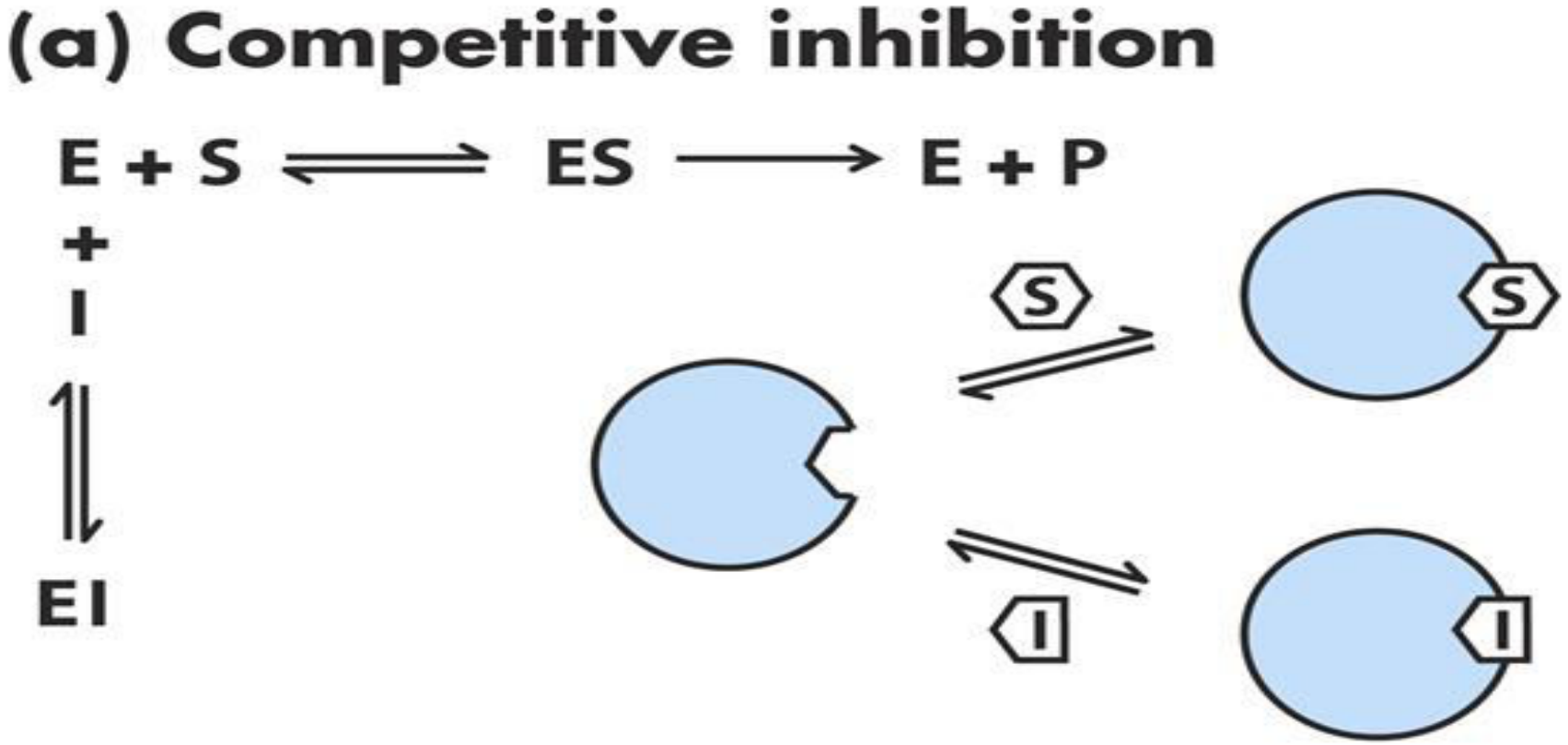

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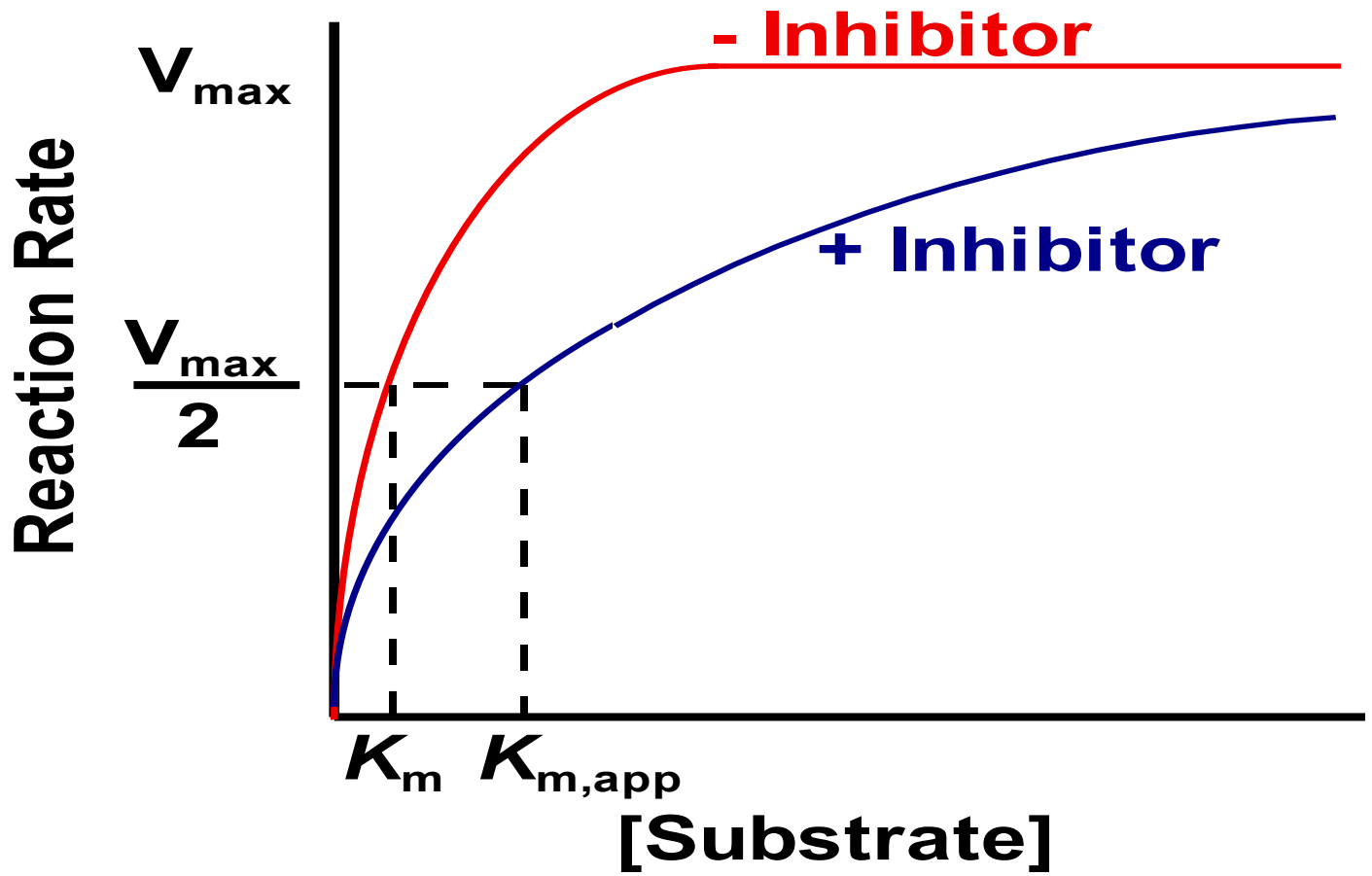

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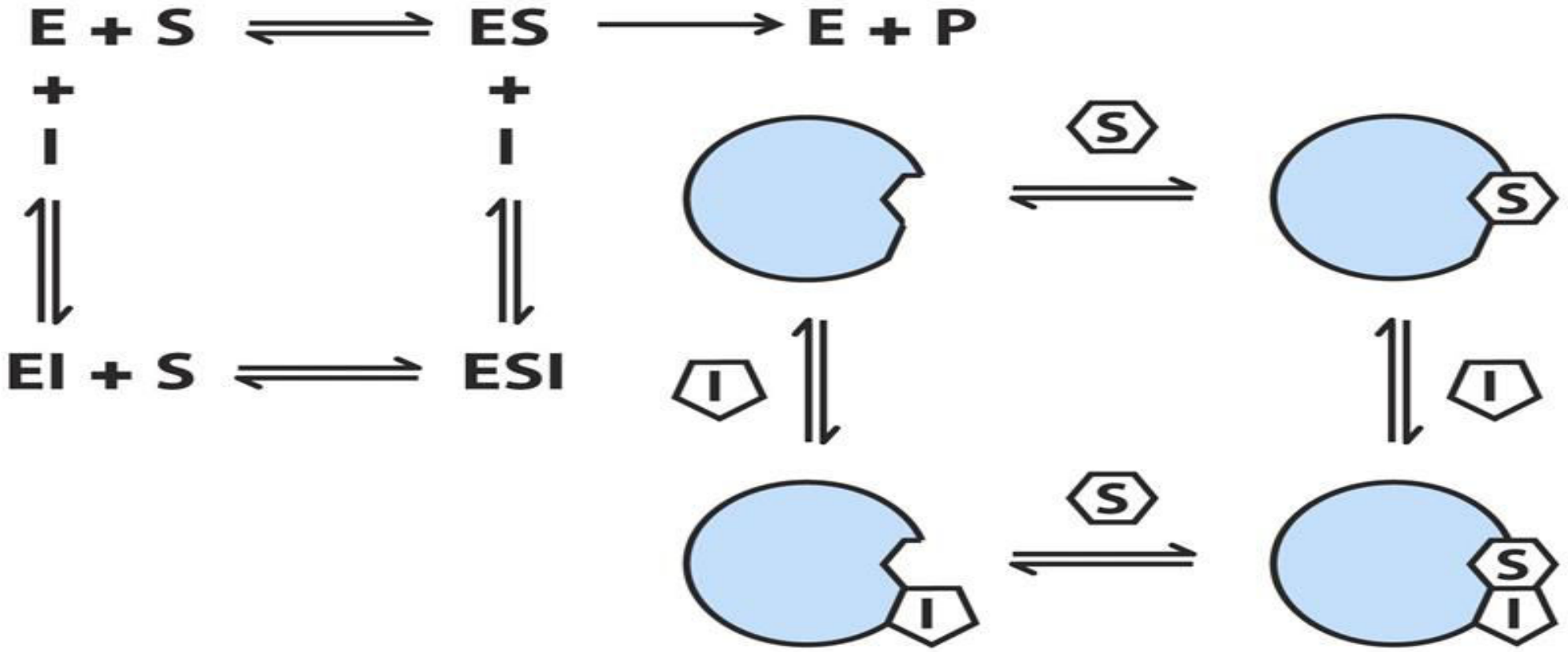
- Therefore, a competitive inhibitor does not decrease V_{max}

END

Noncompetitive Inhibition

- Inhibitors bind enzymes at sites distinct from the substrate-binding site and
- generally bear little or no structural resemblance to the substrate.

Noncompetitive inhibitors bind at a separate site, but may bind to either E or ES.

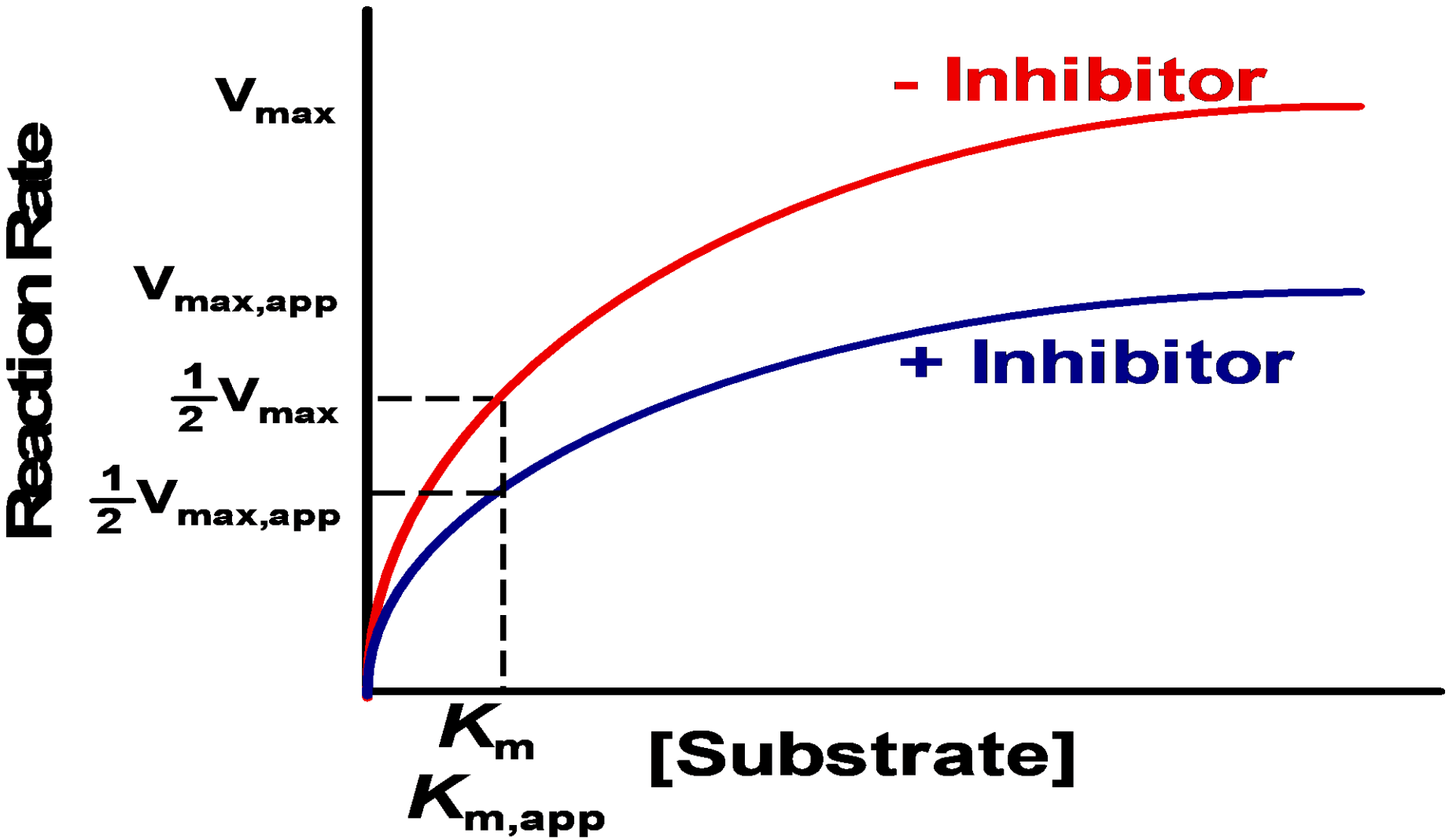


- Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.

1. Effect on V_{max}

- the apparent V_{max} changes, because the inhibitor is capable of preventing catalysis regardless of whether the substrate is bound to the enzyme.

- Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate.
- Thus, noncompetitive inhibitors decrease the V_{max} of the reaction.


Reaction Rate
V_{max}
$V_{max,app}$
$\frac{1}{2}V_{max}$
$\frac{1}{2}V_{max,app}$
- Inhibitor
+ Inhibitor
K_m
$K_{m,app}$
[Substrate]

- The noncompetitive inhibitor, in effect, lowers the concentration of the active enzyme and therefore decreases the V_{max} of the enzyme.

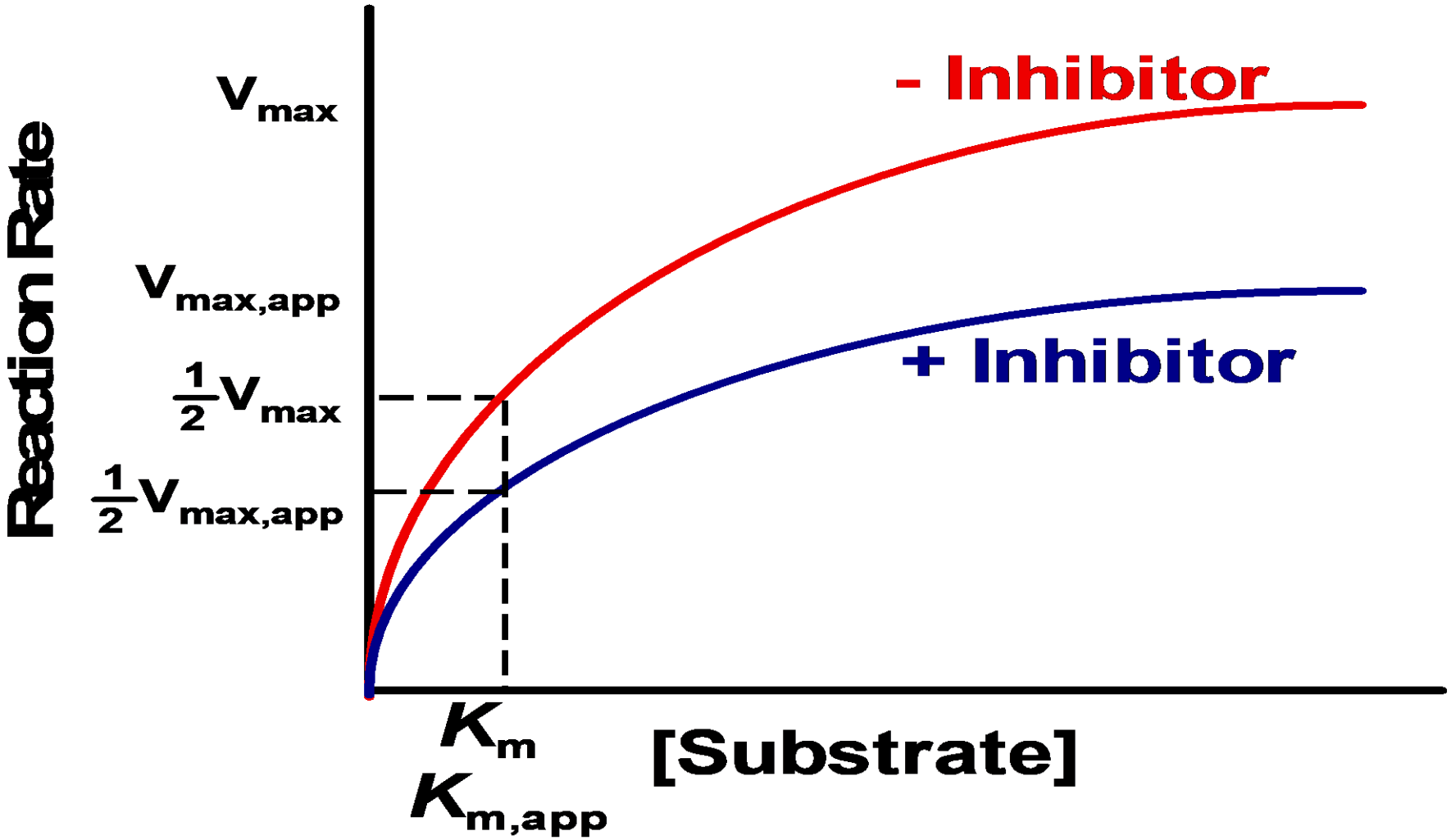
END

Noncompetitive Inhibition

(Contd.)

2. Effect on Km

- Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme.
- Thus, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.

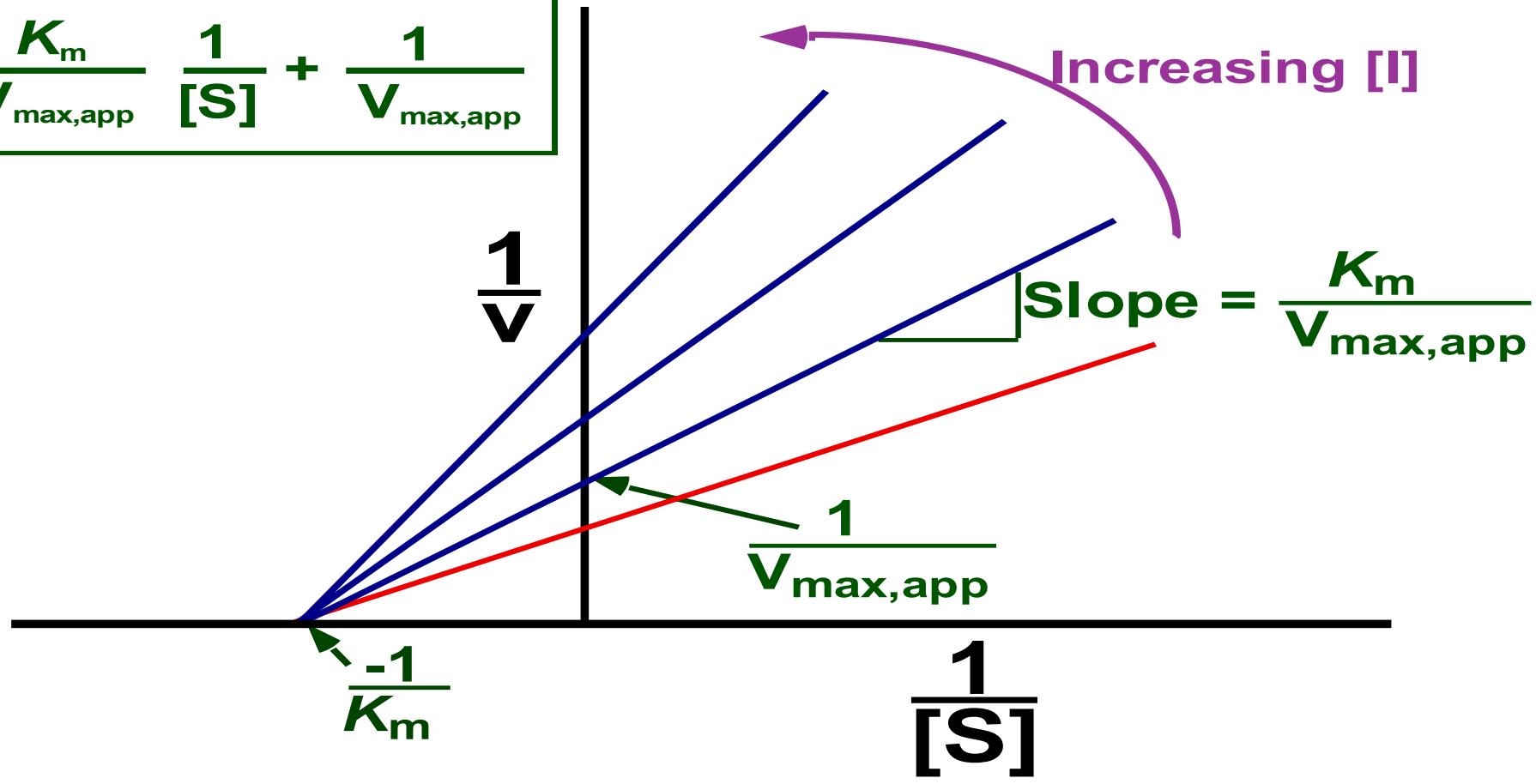

Reaction Rate
V_{max}
$V_{max,app}$
$\frac{1}{2}V_{max}$
$\frac{1}{2}V_{max,app}$
- Inhibitor
+ Inhibitor
K_m
$K_{m,app}$
[Substrate]

3. Effect on Lineweaver-Burk plot

- Noncompetitive inhibition shows a characteristic Lineweaver-Burke plot
- the plots of the inhibited and uninhibited reactions intersect at a single point on the x-axis at K_m
- K_m is unchanged

- However, the inhibited and uninhibited reactions show; different y axis intercepts
- indicating the deccrease in V_{max} in the presence of the competitive inhibitor.

$$\frac{1}{v} = \frac{K_m}{V_{max,app}} \frac{1}{[S]} + \frac{1}{V_{max,app}}$$



- While certain inhibitors exhibit characteristics of a mixture of competitive and noncompetitive inhibition, the evaluation of these inhibitors exceeds the scope of our level of study.

END

Water ,pH &
Buffer Systems

Water

- Water is the most abundant substance in living systems, making up 70% or more of the weight of most organisms

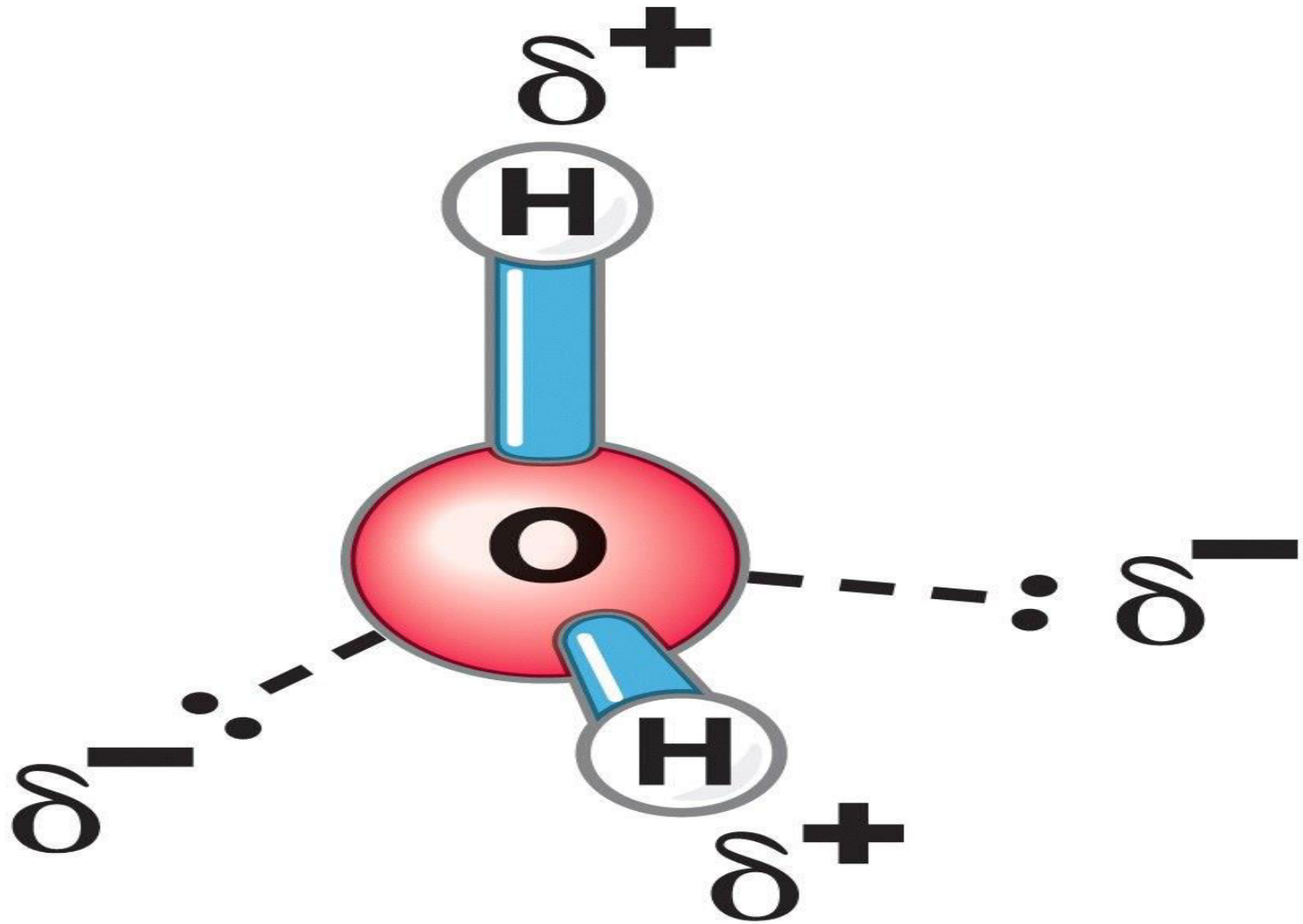
- The hydrogen bonding between water molecules and the slight tendency of water to ionize are of crucial importance to the structure and function of biomolecules

Hydrogen Bonding gives Water its Unusual Properties

- Water has a higher
- melting point,
- boiling point, and
- heat of vaporization than most other common solvents

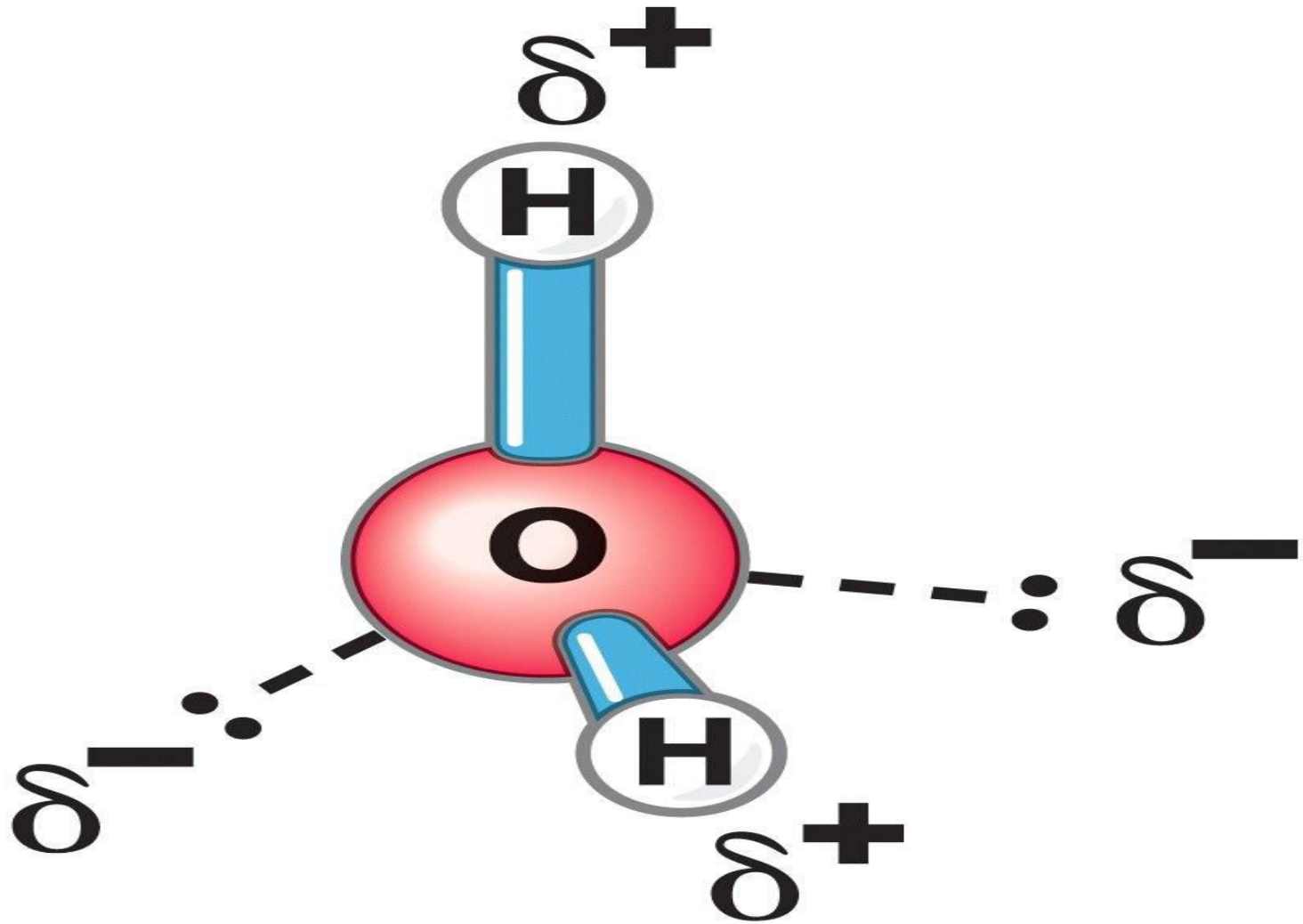
- Each hydrogen atom

  of a water molecule shares an electron pair with the central oxygen atom
- The geometry of the molecule is dictated by the shapes of the outer electron orbitals of the oxygen atom

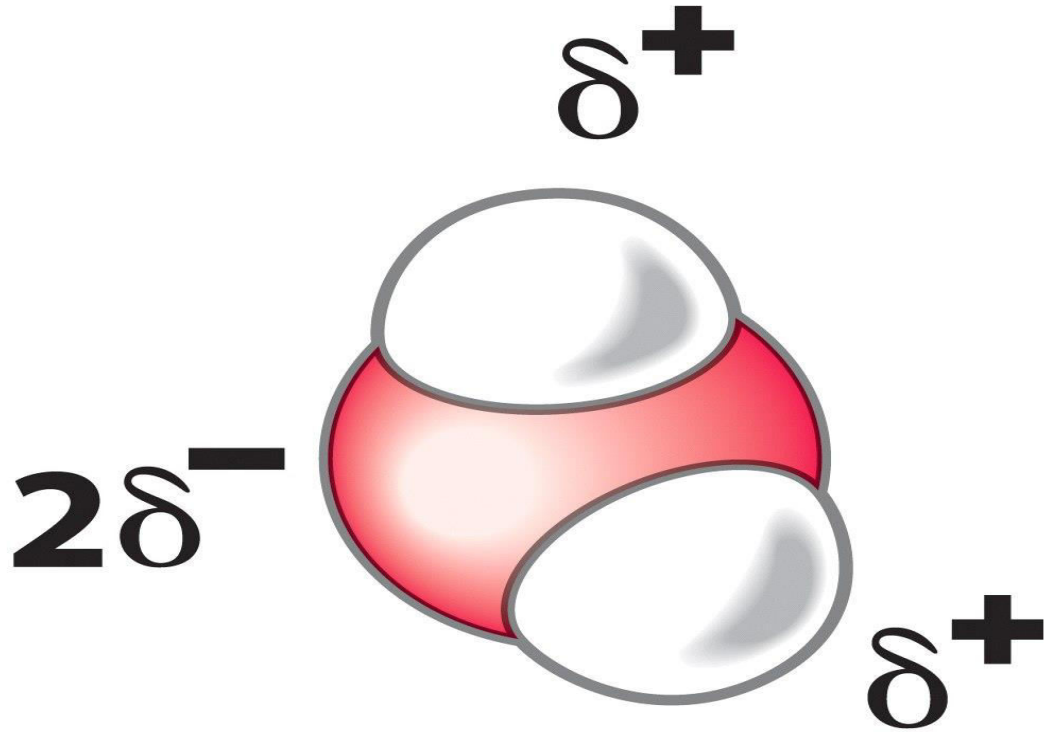

δ+
H
O
δ−
δ−
H
δ+

- Each hydrogen atom

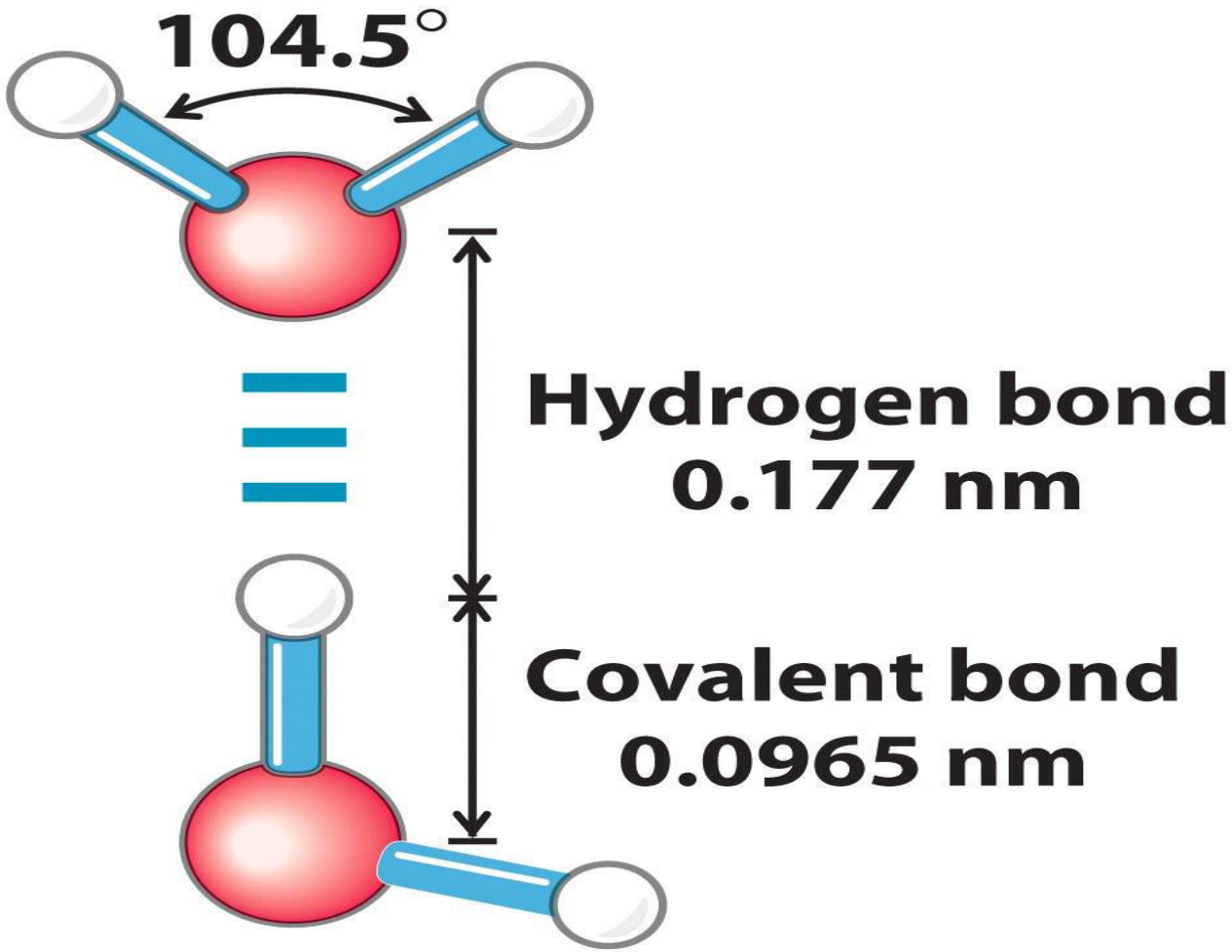
  of a water molecule shares an electron pair with the central oxygen atom
- The geometry of the molecule is dictated by the shapes of the outer electron orbitals of the oxygen atom

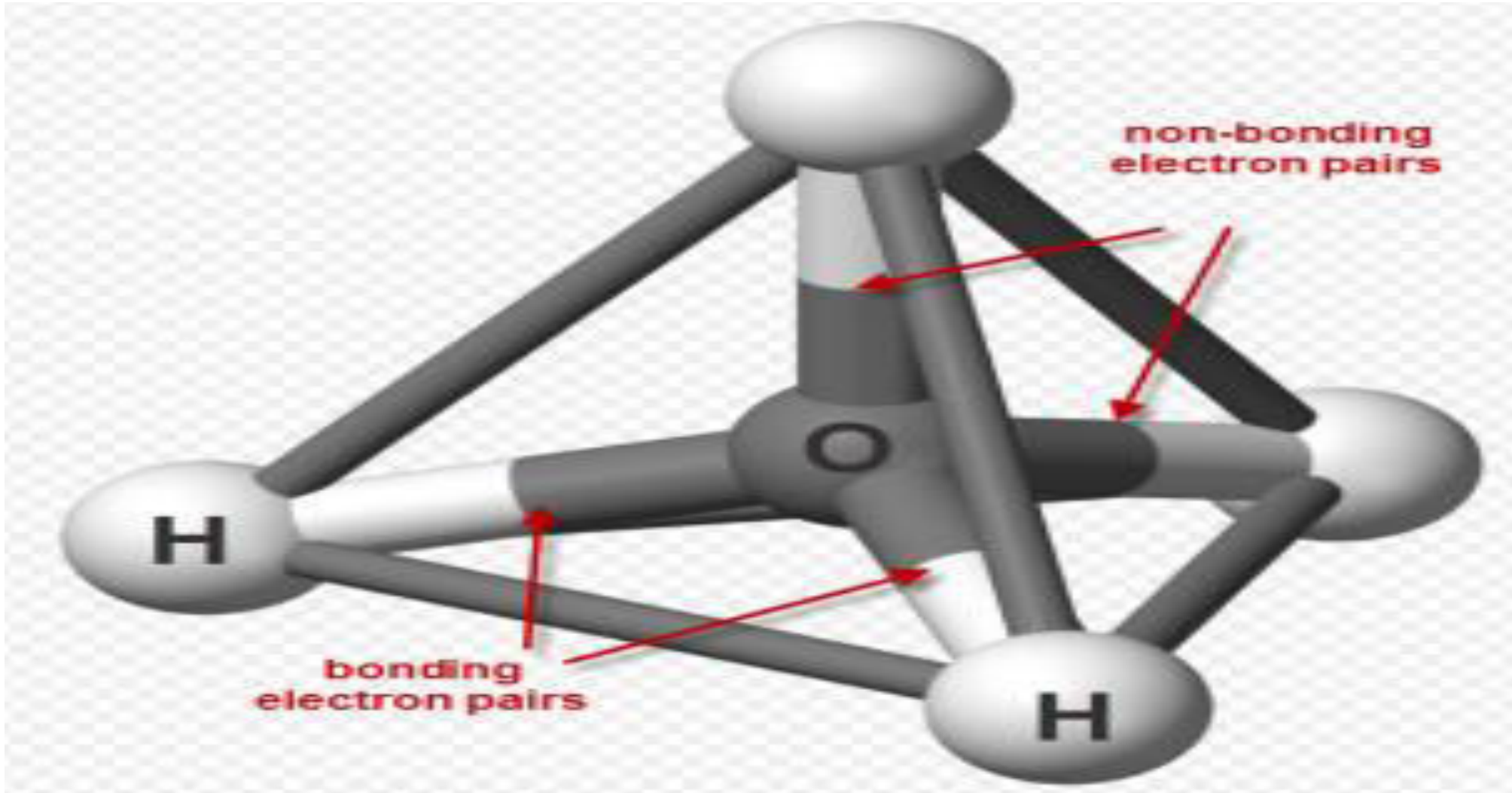

δ+
H
O
δ−
δ−
H
δ+

- The result of this unequal electron sharing is two electric dipoles in the water molecule
- Each hydrogen bears a partial positive charge (δ+), and
- the oxygen atom bears a partial negative charge equal to the sum of the two partial positives (2δ-)


δ+
2δ−
δ+

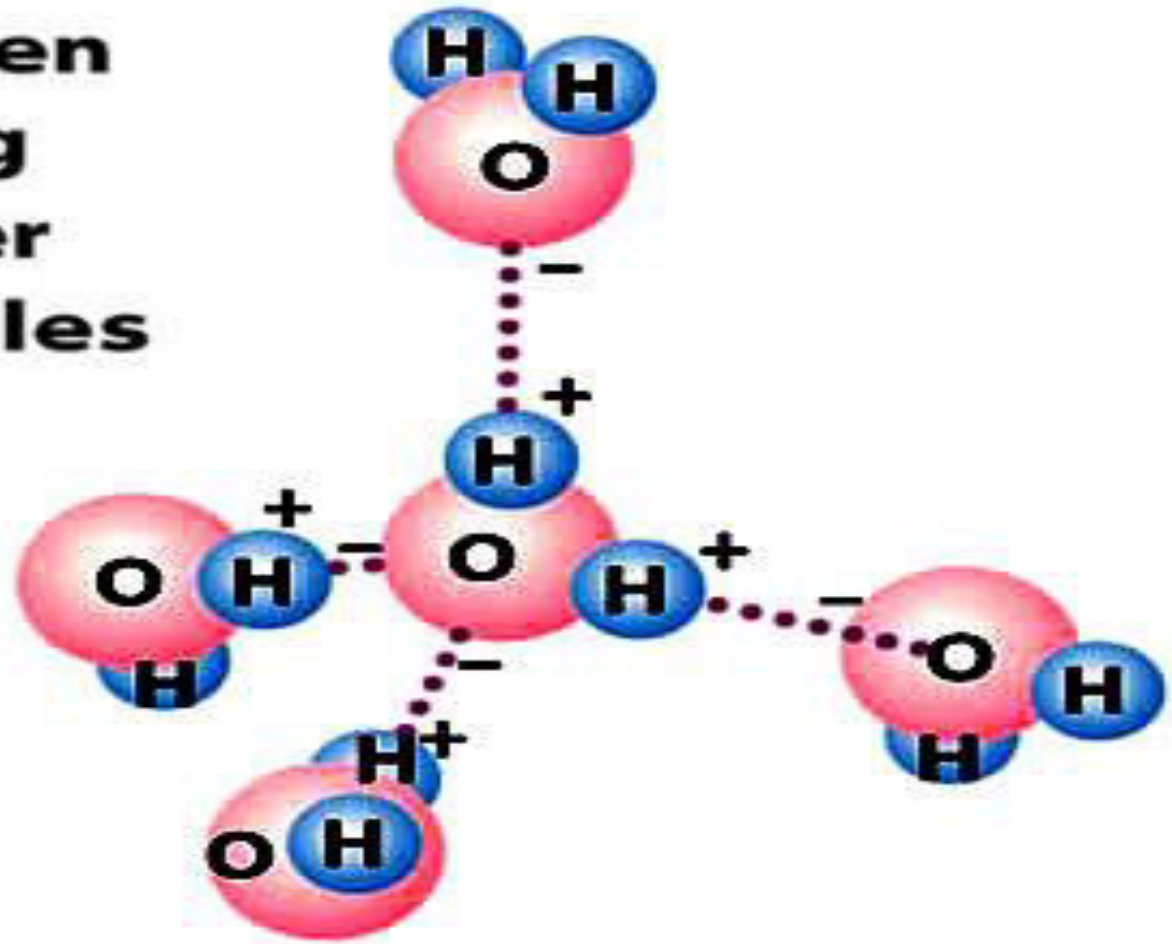
- As a result, there is an electrostatic attraction between the oxygen atom of one water molecule and the hydrogen of another, called a hydrogen bond


104.5°
Hydrogen bond
0.177 nm
Covalent bond
0.0965 nm


non-bonding
electron pairs
O
H
H
bonding
electron pairs

- The nearly tetrahedral arrangement of the orbitals about the oxygen atom allows each water molecule to form hydrogen bonds with as many as four neighboring water molecules

Hydrogen bonding of water molecules



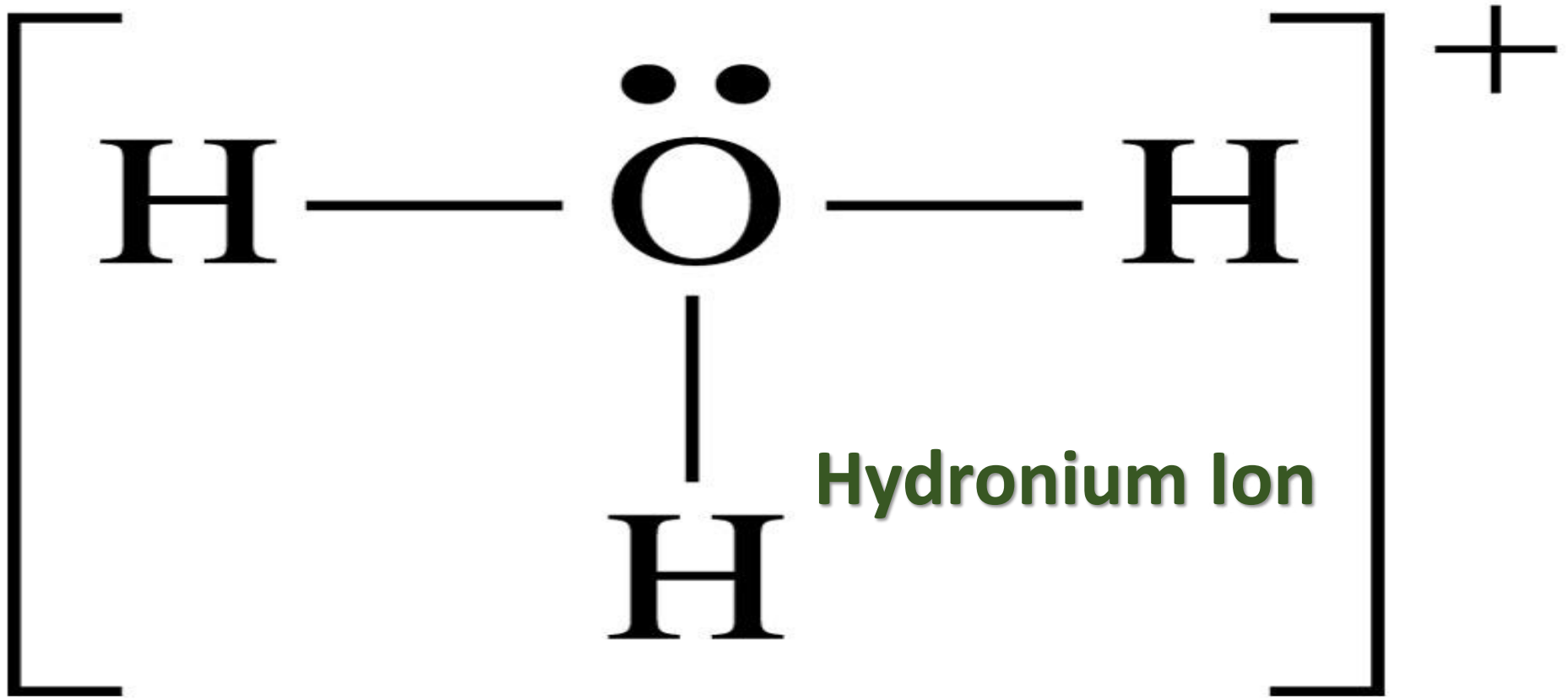
Ionization of Water

- Although many of the solvent properties of water can be explained in terms of the uncharged H_2O molecule, the small degree of ionization of water into hydrogen ions (H^+) and hydroxide ions (OH^-) must also be taken into account

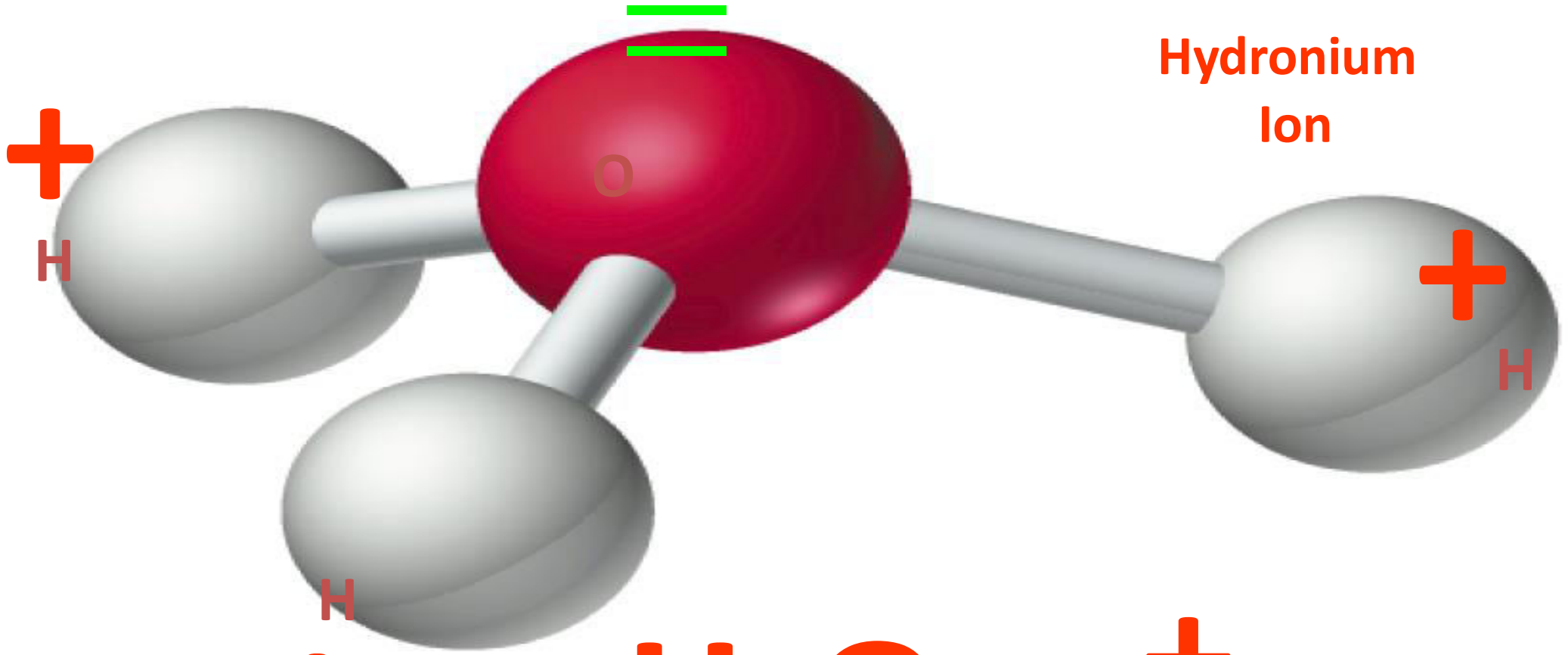
- Pure Water Is Slightly Ionized
- Water molecules have a slight tendency to undergo reversible ionization to yield a hydrogen ion (a proton) and a hydroxide ion, giving the equilibrium reaction

- $H_2O \leftrightarrow H^+ + OH^-$

- Hydrogen ions formed in water are immediately hydrated to hydronium ions (H_3O^+)
- The ionization of water can be measured by its electrical conductivity
- Pure water carries electrical current as H_3O^+ migrates toward the cathode and OH^- toward the anode



Hydronium Ion


Hydronium
Ion
O
H
H
H
+
+
+
H_3O

- The Ionization Of Water Is Expressed By an Equilibrium Constant

- The degree of ionization of water at equilibrium is small
- At 25^0C only about two out of every 10^9 molecules in pure water are ionized at any instant

- The equilibrium constant for the reversible ionization of water is

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]}$$

According to the **law of mass action** the rate of a chemical reaction is proportional to the product of the masses of the reactants. Necessarily, this implies that for a chemical reaction mixture that is in equilibrium, the ratio between the concentration of reactants and products is constant

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]}$$

- Since 1 mole (mol) of water weighs 18 g,
- 1 liter (L) (1000 g) of water contains
- 1000 ÷ 18 = 55.56 mol
- Pure water thus is 55.56 molar(M)
- Accordingly, we can substitute 55.5 M in the equilibrium constant expression to yield
- Keq = $[H^+][OH^-]/55.5$
- molecular wt. of water is 18.01528 g/mol

- $K_{eq} = [H^+][OH^-]/55.5$
- On rearranging, this becomes

$$(K_{eq})(55.5) = [H^+][OH^-] = K_w$$

- Where K_w designates the product (55.5M)(K_{eq}), the ion product of water at 25^0C
- The value for K_{eq}, determined by electrical conductivity measurements of pure water, is 1.8×10^{-16} M at 25^0C

- $K_w = (K_{eq})(55.5) = [H^+][OH^-]$
- $K_w = (1.8 \times 10^{-16} M)(55.5M) = [H^+][OH^-]$
- $K_w = 1.0 \times 10^{-14}\ M^2 = [H^+][OH^-]$
- $K_w = [H^+][OH^-] = [H^+]^2 = [OH^-]^2$
- Solving for $[H^+]$ gives:

  $[H^+] = \sqrt{K_w} = \sqrt{10^{-14}\ M^2}$

  $[H^+] = 10^{-7}\ M$

- Thus the product $[H^+][OH^-]$ in aqueous solutions at 25^0C always equals $1 \times 10^{-14}\ M^2$
- When there are exactly equal concentrations of H^+ and OH^- , as in pure water, the solution is said to be at neutral pH

- As the ion product of water is constant, whenever $[H^+]$ is greater than 1×10^{-7} M, $[OH^-]$ must be less than $1 X 10^{-7}$ M, and vice versa

END

WORKING EXAMPLES

- From the ion product of water we can calculate $[H^+]$
- if we know $[OH^-]$, and vice versa

- **What is the concentration of H^+ in a solution of 0.1 M NaOH?**

  Solution:

  Kw= $[H^+][OH^-]$
- With $[OH^-]$ = 0.1 M, solving for $[H^+]$ gives
- $[H^+] = K_w/[OH^-]$

  $= 1 \times 10^{-14}M^2/ 0.1M$

  $= 10^{-14}M^2/0.1M$

  $= 10^{-13}M$

- **What is the concentration of OH^- in a solution with an H^+ concentration of $1.3x\ 10^{-4}M$?**
- **Solution:**
- $Kw = [H^+][OH^-]$
- With $[H+] = 1.3x\ 10^{-4}M$, solving for $[OH^-]$ gives
- $[OH^-] = Kw/[H^+]$
- $= 1x10^{-14}M^2 / 1.3x\ 10^{-4}M$
- $= \mathbf{7.7 \times 10^{-11}}$

The pH Scale

- Designates the H^+ and OH^- Concentrations
- The **pH** of a solution is defined as the logarithm to the base 10 of the reciprocal of the $[H^+]$, i. e, the negative logarithm of the $[H^+]$
- $pH = \log 1/[H^+]$
- $= -\log[H^+]$

The symbol **p** denotes "negative logarithm of"

- For each pH unit less than 7.0, the $[H^+]$ is increased tenfold;
- for each pH unit above 7.0, it is decreased tenfold

- The pH of water at 25°C, in which H^+ and OH^- ions are present in equal numbers, is 7.0
- pH = $-\log[1 \times 10^{-7}] = 7$

- The symbol p denotes "negative logarithm of"

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
10^{-1}	1	10^{-13}	13
10^{-2}	2	10^{-12}	12
10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $pOH = -\log [OH^-]$, which is analogous to the expression for pH. Note that in all cases, $pH + pOH = 14$.

TABLE 2–6 The pH Scale

$[H^+]$ (M)	pH	$[OH^-]$ (M)	pOH*
10^0 (1)	0	10^{-14}	14
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10^{-3}	3	10^{-11}	11
10^{-4}	4	10^{-10}	10
10^{-5}	5	10^{-9}	9
10^{-6}	6	10^{-8}	8
10^{-7}	7	10^{-7}	7
10^{-8}	8	10^{-6}	6
10^{-9}	9	10^{-5}	5
10^{-10}	10	10^{-4}	4
10^{-11}	11	10^{-3}	3
10^{-12}	12	10^{-2}	2
10^{-13}	13	10^{-1}	1
10^{-14}	14	10^0 (1)	0

*The expression pOH is sometimes used to describe the basicity, or OH^- concentration, of a solution; pOH is defined by the expression $pOH = -\log [OH^-]$, which is analogous to the expression for pH. Note that in all cases, $pH + pOH = 14$.

- **What will be the pH of 0.1 M HCl?**

- Assuming that being a strong acid HCl is completely dissociated, it's 0.1 M solution will contain 0.1 or 10^{-1} grams H^+ per litre

- pH= - log $[H^+]$
- pH = - log $[10^{-1}]$
- = - [- 1]
- = **1**

Weak Acids and Bases

- Each acid has a characteristic tendency to ionize in an aqueous solution
- The stronger the acid, the greater its tendency ionize i.e. to lose its proton
- This tendency is measured by an acid dissociation constant

- Weak Acids and Bases have Characteristic Acid Dissociation Constants
- HCl, H_2SO_4, and HNO_3, commonly called strong acids, are fully ionized in aqueous solutions
- The strong bases NaOH and KOH are also completely ionized

- Of more interest is the behavior of weak acids and bases-those not completely ionized when dissolved in water
- These are ubiquitous in biological systems and play important roles in metabolism and its regulation

- Acids may be defined as proton donors and bases as proton acceptors
- A proton donor and its corresponding proton acceptor make up a conjugate acid-base pair

- Acetic acid (CH_3COOH), a proton donor, and the acetate anion (CH_3COO^-), the corresponding proton acceptor, constitute a conjugate acid-base pair, related by the reversible reaction:

- $CH_3COOH \leftrightarrow CH_3COO^- + H^+$

- The tendency of any acid (HA) to lose a proton and form its conjugate base (A-) is defined by the acid dissociation constant (Ka) for the reversible reaction
- $HA \leftrightarrow H^+ + A^-$;

- Ka = [H+][A-]/[HA]

- Stronger acids, have larger dissociation constants (Ka) i.e they ionize completely

- Weaker acids, have smaller dissociation constants (Ka) i.e. they ionize only partially.

pKa

- analogous to pH, pKa is defined by the equation
- **pKa = log 1/Ka**

  = -logKa

- The stronger the tendency to dissociate a proton, the stronger is the acid and the lower its pKa

END

Working with pKa

- Titration is used to determine the amount of an acid in a given solution
- A measured volume of the acid is titrated with a solution of a strong base, usually sodium hydroxide (NaOH), of known concentration

The NaOH is added in small increments until the acid is consumed (neutralized), as determined with an indicator dye or a pH meter

The concentration of the acid in the original solution can be calculated from the volume and concentration of NaOH added

- Consider the titration of a 0.1M solution of acetic acid with 0.1M NaOH at 25^0C
- Two reversible equilibria are involved in the process (here, for simplicity, acetic acid is denoted HAc)

$$H_2O \leftrightarrow H^+ + OH$$

$$HAc \leftrightarrow H^+ + Ac^-$$

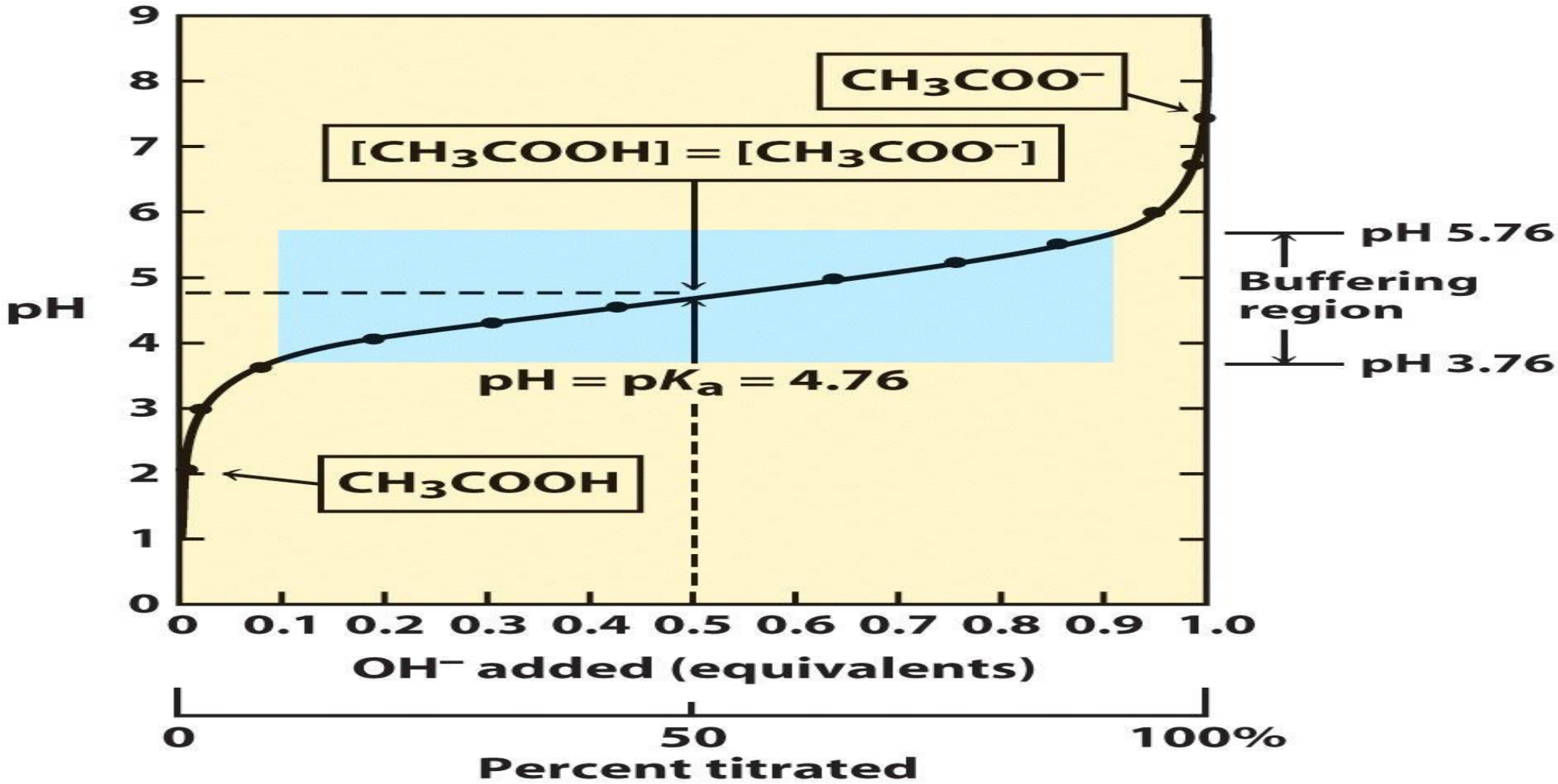
- The equilibria must simultaneously conform to their characteristic equilibrium constants, which are, respectively,
- $K_w = [H^+][OH^-]$

  $= 1 \times 10^{-14}\ M2$

- $K_a = [H^+][Ac^-]/[HAc]$

  $= 1.74 \times 10^{-5}\ M$

- At the beginning of the titration, the acetic acid is only slightly ionized
- As NaOH is gradually added, OH^- combines with the free H^+ in the solution to form H_2O,
- As free H+ is removed, HAc dissociates further to satisfy its equilibrium constant


9
8
7
6
5
4
3
2
1
0
pH
CH_3COO^-
$[CH_3COOH] = [CH_3COO^-]$
pH 5.76
Buffering region
pH 3.76
$pH = pK_a = 4.76$
CH_3COOH
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
OH^- added (equivalents)
0
50
100%
Percent titrated

- At the midpoint a very important relationship holds:
- The pH of the equimolar solution of acetic acid and acetate is exactly equal to the pKa of acetic acid (pKa = 4.76)

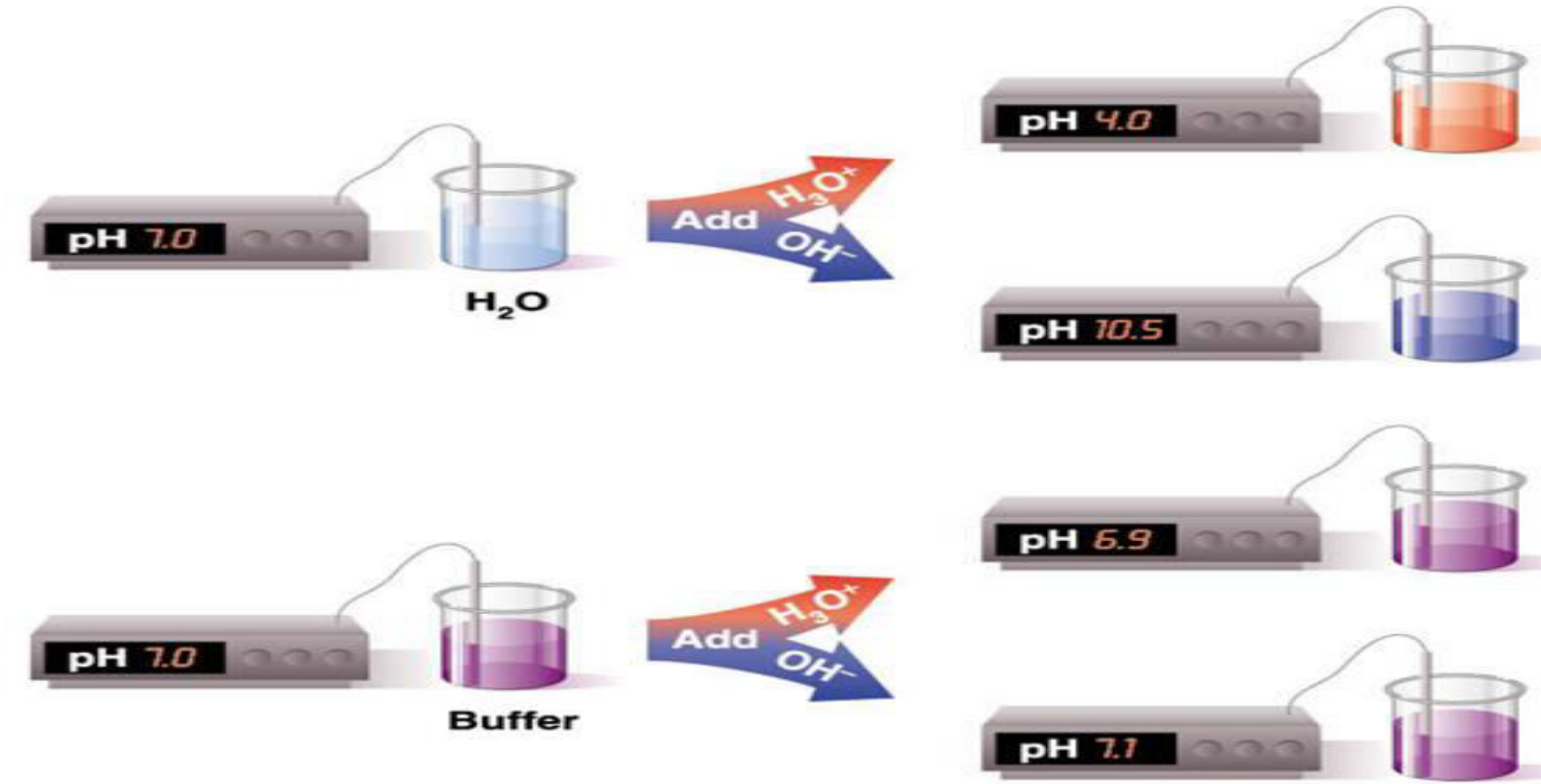
- At pKa of a weak acid, half of the acid is in dissociated form
- whereas other half is un-dissociated.

- Increasing the pH will result in an increased dissociation and vice versa.

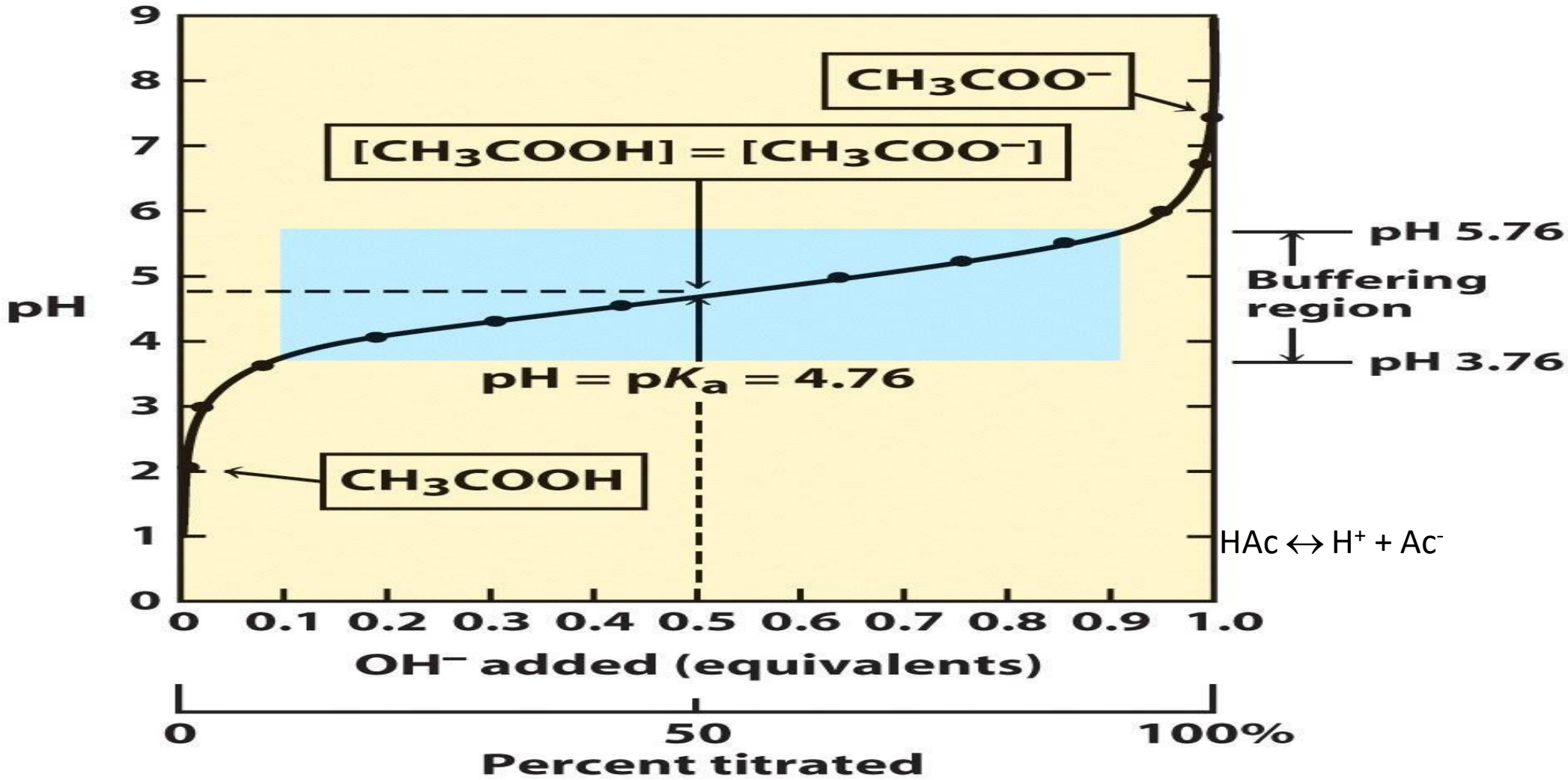
END

Buffer Solutions

- Buffers are aqueous systems that tend to resist changes in pH when small amounts of acid (H^+) or base (OH^-) are added
- A buffer system consists of a weak acid (the proton donor) and its conjugate base (the proton acceptor)


pH 7.0
H_2O
Add
H_3O^+
OH^-
pH 4.0
pH 10.5
pH 7.0
Buffer
Add
H_3O^+
OH^-
pH 6.9
pH 7.1

- As an example, a mixture of ;
- acetic acid and
- acetate ion,
- is a buffer system,



$HAc \leftrightarrow H^+ + Ac^-$

- At the midpoint of the buffering region, where the concentration of the proton donor (acetic acid) exactly equals that of the proton acceptor (acetate), the buffering power is maximal;
- The pH at this point in the titration curve of acetic acid is equal to its pKa

- The pH of the acetate buffer system does change slightly when a small amount of H^+ or OH^- is added, but this pH change is very small
- compared with the pH change that would result if the same amount of H+ or OH- were added to pure water.

- Each conjugate acid-base pair has a characteristic pH zone in which it is an effective buffer
- For example, the $H_2PO_4^-/HPO_4^{2-}$ pair has a pKa of 6.86 and thus can serve as an effective buffer system between approximately pH 5.9 and pH 7.9

END

Citric Acid Cycle- (CAC)- 1

Citric Acid Cycle

- Also called Tricarboxylic Acid Cycle (TCA) or Krebs Cycle (after its discoverer, Hans Krebs) .
- Three names for the same thing.
- A series of chemical reactions used by all aerobic organisms to generate energy through the **OXIDATION** of acetyl CoA … derived from carbohydrates, fatty acids and proteins into carbondioxide, water and chemical energy in the form of **ATP**
- Conversion of pyruvate to activated acetate
- Cellular respiration and intermediates for biosynthesis.
- Conversion of **acetate to carbohydrate precursors in the glyoxylate** cycle

Cellular Respiration

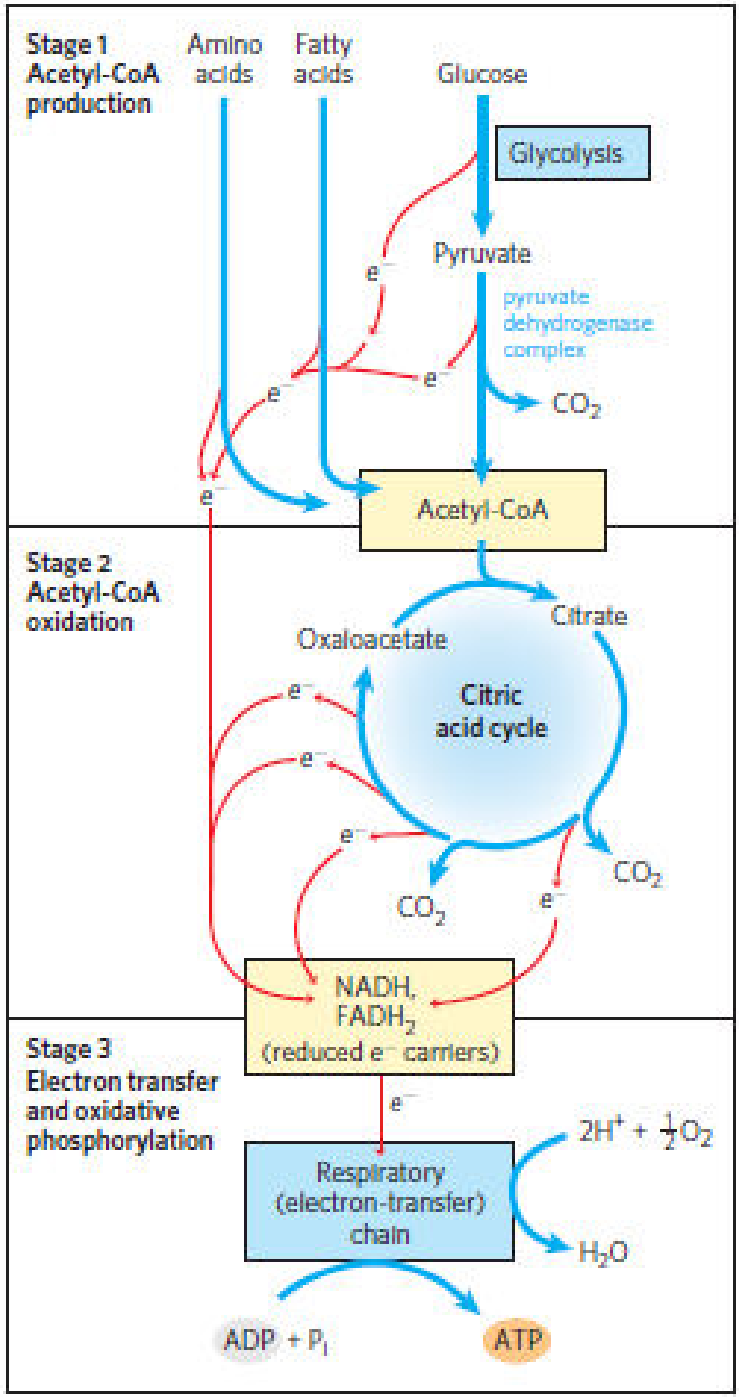
- For most eukaryotic cells and many bacteria, which live under aerobic conditions and oxidize their organic fuels to CO_2 and H2O, **glycolysis is the first stage in the complete oxidation of glucose.**
- Rather than being reduced to lactate, ethanol, or some other fermentation product, the pyruvate produced by glycolysis is further oxidized to H_2O and CO_2. This aerobic phase of catabolism is called **respiration**.
- Biochemists and cell biologists, however, use the term in a narrower sense to refer to the molecular processes by which *cells* consume O_2 and produce CO_2—processes more precisely termed **cellular respiration**.

Cellular Respiration…cont

- Cellular respiration occurs in three major stages.
- In the first stage, organic fuel **molecules—glucose, fatty acids, and some amino acids** are oxidized to **yield two-carbon fragments** in the form of the **acetyl group of acetyl-coenzyme A (acetyl-CoA).**
- In the second stage, the **acetyl groups are fed into the citric acid cycle**, which enzymatically oxidizes them to CO2; the energy released is conserved in the reduced electron carriers NADH and FADH2.
- In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up **protons (H^+) and electrons**. The electrons are transferred to **O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.**

FIGURE 16–1 Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration.

- **Stage 1:** oxidation of fatty acids, glucose, and some amino acids yields acetyl-CoA.
- **Stage 2:** oxidation of acetyl groups in the citric acid cycle includes four steps in which electrons are abstracted.
- **Stage 3:** electrons carried by NADH and $FADH_2$ are funneled into a chain of mitochondrial (or, in bacteria, plasma membrane–bound) electron carriers—the respiratory chain—ultimately reducing O_2 to H_2O. This electron flow drives the production of ATP.



In Cytosol

In Mitochondria

Generates more NADH, FADH2 and one GTP

Citric Acid Cycle

Production of Acetyl-CoA

Pyruvate Is Oxidized to Acetyl-CoA and CO_2

- The overall reaction catalyzed by the pyruvate dehydrogenase complex is an **oxidative decarboxylation**
- Irreversible oxidation process in which following things take place

1. the carboxyl group is removed from pyruvate as a molecule of CO2 and the two remaining carbons become the acetyl group of acetyl-CoA
2. The NADH formed in this reaction gives up a hydride ion (:H2) to the respiratory chain
3. NADH carries the two electrons to oxygen or, in anaerobic microorganisms, t an alternative electron acceptor such as nitrate or sulfate.

4. The **transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.**

- The irreversibility of the PDH complex reaction has been demonstrated by **isotopic labeling experiments**: the complex cannot reattach radioactively labeled CO2 to acetyl-CoA to yield carboxyl-labeled pyruvate.

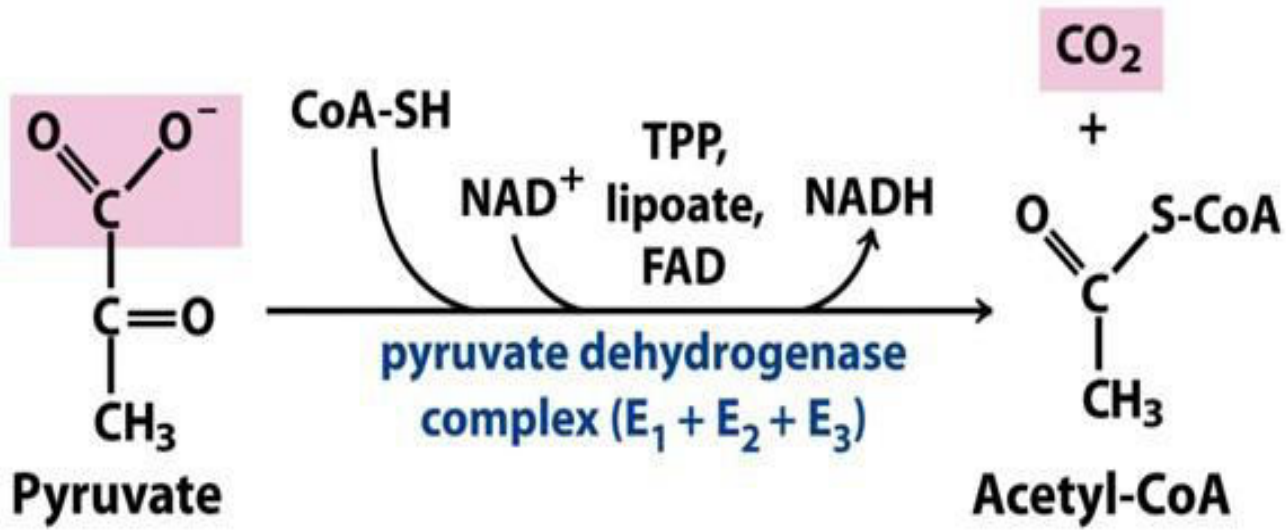


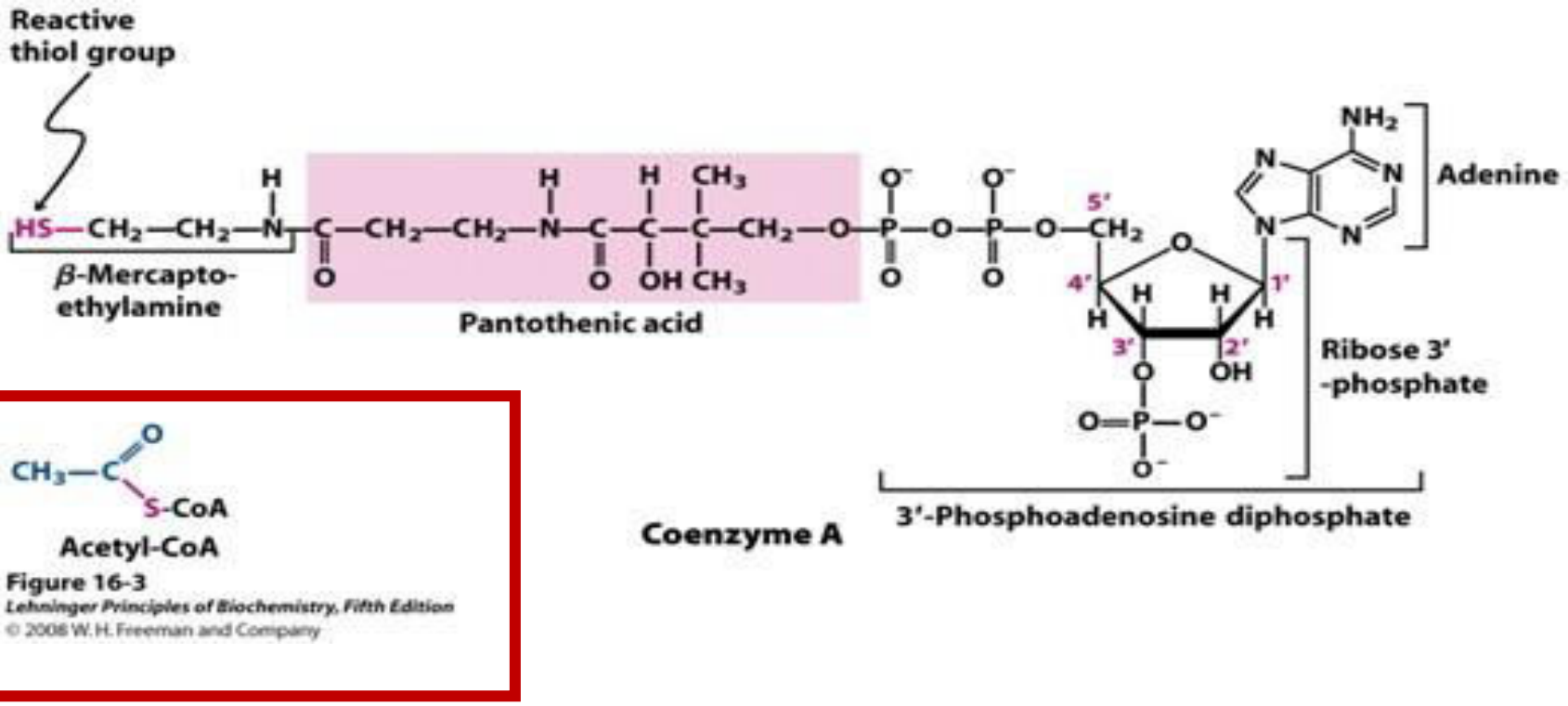
Figure 16-2
Lehninger Principles of Biochemistry, Fifth Edition

Citric Acid Cycle

Production of Acetyl-CoA-2

The Pyruvate Dehydrogenase Complex (PDH) Requires Five Coenzymes

- The process of conversion of Pyruvate to the acetyl group of acetyl-CoA requires the sequential action of ..
- **three different enzymes**
- **five different coenzymes or prosthetic groups**—
  - Thiamine pyrophosphate (TPP)
  - flavin adenine dinucleotide (FAD)
  - Coenzyme A (CoA, sometimes denoted CoA-SH, to emphasize the role of the OSH group)
  - Nicotinamide adenine dinucleotide (NAD)
  - Lipoate
- Four different vitamins required in human nutrition are vital components of this system: thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate (in CoA).
- FAD and NAD act as electron carriers ..TPP as the coenzyme of pyruvate decarboxylase
-



- Coenzyme A (CoA). A hydroxyl group of pantothenic acid
  is joined to a modified ADP moiety by a phosphate ester bond, and its carboxyl group is attached to b-mercaptoethylamine in amide linkage.
  The hydroxyl group at the 3’ position of the ADP moiety has a phosphoryl group not present in free ADP.
- The —SH group forms a thioester with acetate in acetyl-coenzyme A (acetyl-CoA)

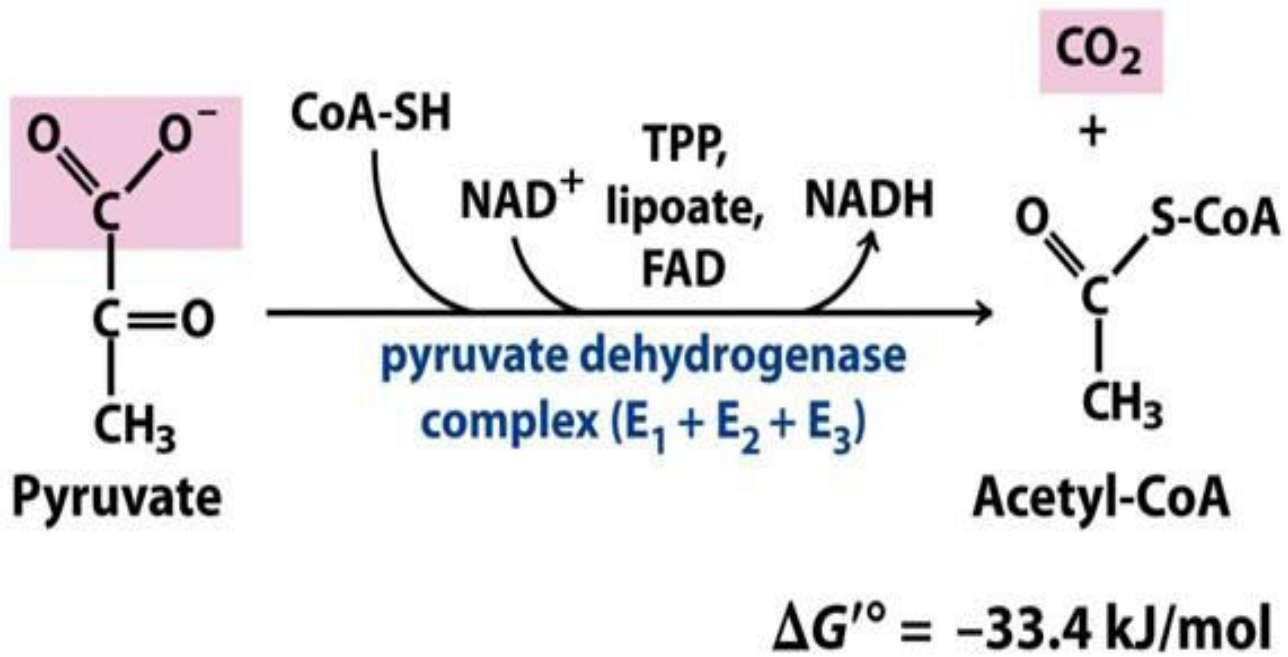


Figure 16-2
Lehninger Principles of Biochemistry, Fifth Edition

- Five **coenzymes** for the PDH complex
- TPP, FAD, NAD, CoA, lipoate

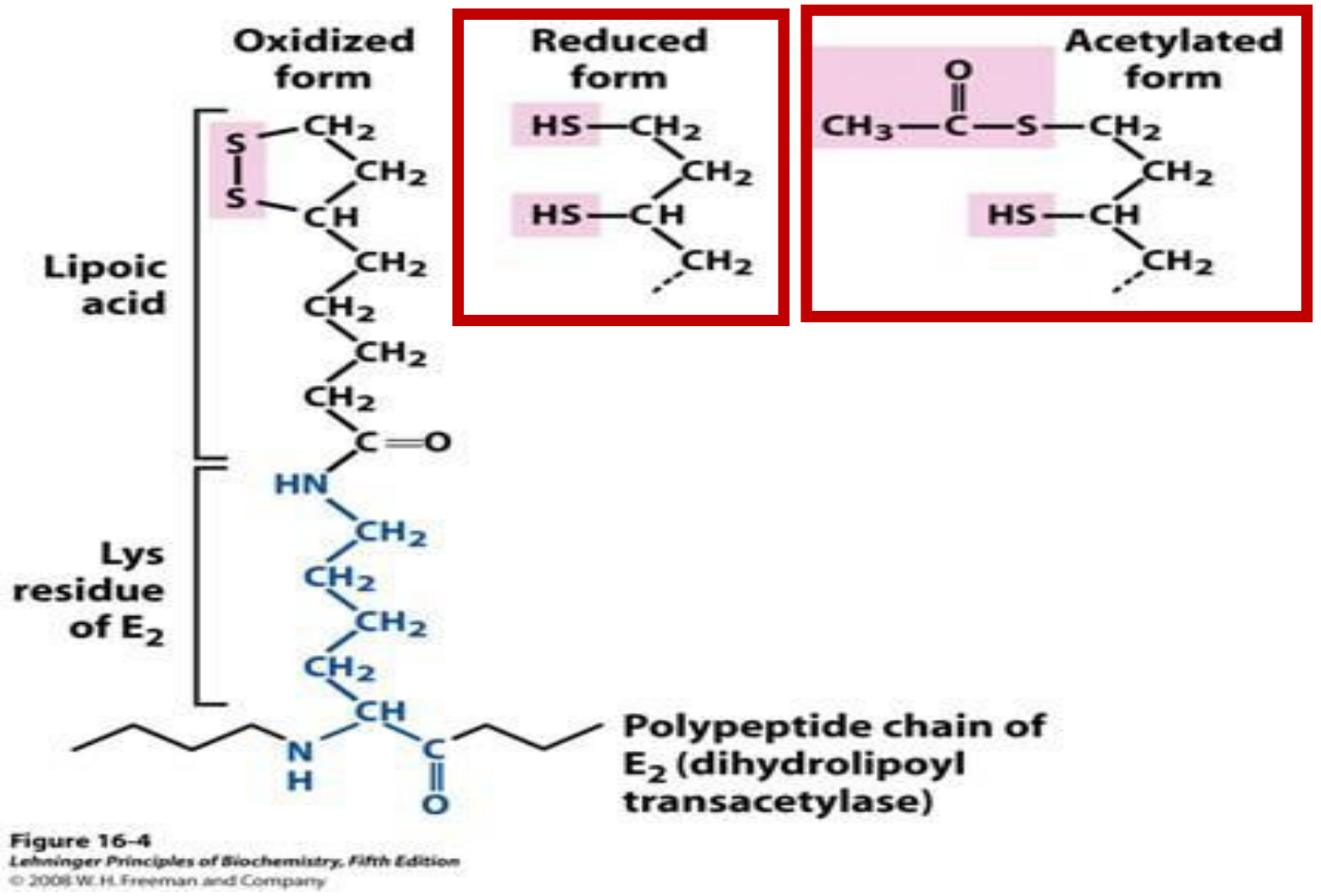


Figure 16-4
Lehninger Principles of Biochemistry, Fifth Edition

- **Lipoic acid (lipoate) in amide linkage with a Lys residue.**
- The lipoyllysyl moiety is the prosthetic group of **dihydrolipoyl transacetylase** (E2 of the PDH complex).
- The lipoyl group occurs in oxidized (disulfide) and reduced (dithiol) forms and acts as a carrier of both hydrogen and an acetyl (or other acyl) group.

Sulfhydryl (-SH group)

- A **sulfhydryl** is a functional **group** consisting of a sulfur bonded to a hydrogen atom. The **sulfhydryl group**, also called a **thiol**, is indicated in **chemistry** nomenclature by "-**thiol**" as a suffix and "mercapto-" or "sulfanyl" as a prefix.
- Thiols have great affinity for soft metals.

Acyl Group

- It contains a double bonded oxygen atom and an alkyl **group**. (R-C=O **group** is called **Acyl Group**)

Acetyl Group

- In organic chemistry, acetyl is a moiety, the acyl with chemical formula CH_3CO.
- (The **acetyl group** contains a methyl **group** single-bonded to a carbonyl).

Citric Acid Cycle

The Pyruvate Dehydrogenase (PDH) Complex

The PDH Complex Consists of 3 Enzymes

The PDH complex contains three enzymes—

1. **Pyruvate dehydrogenase** (E1)
2. **dihydrolipoyl transacetylase** (E2)
3. **dihydrolipoyl dehydrogenase** (E3)

The PDH complex isolated from mammals is about 50 nm in diameter—more than five times the size of an entire ribosome and big enough to be visualized with the electron microscope

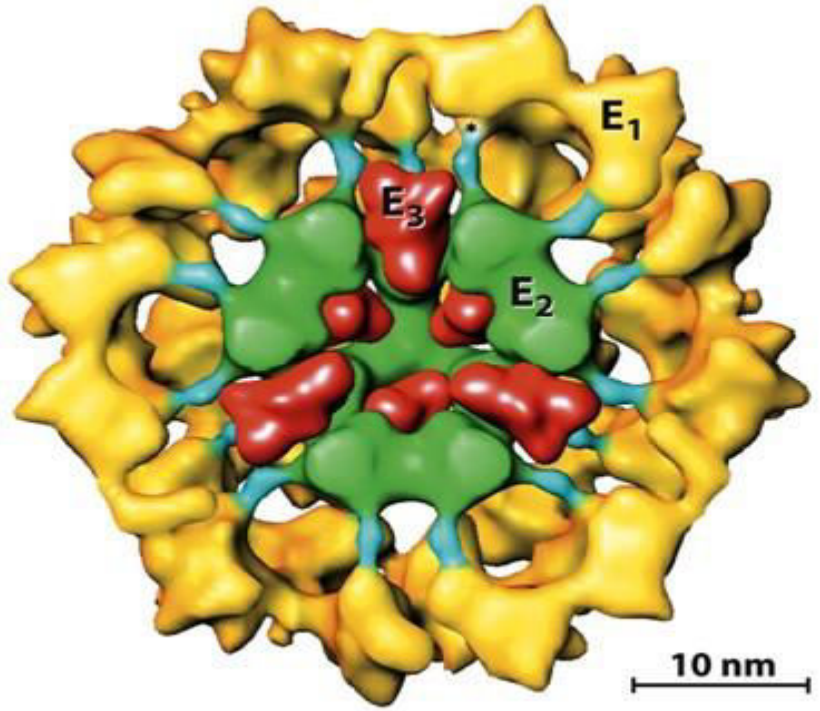


Figure 16-5b
Lehninger Principles of Biochemistry, Fifth Edition

- In the bovine enzyme, 60 identical copies of E2 form a pentagonal dodecahedron **(the core)** with a diameter of about 25 nm
- The core of the *Escherichia coli* enzyme contains 24 copies of E2.
- E2 is the point of connection for the prosthetic group lipoate **(blue),** attached through an amide bond to the amino group of a Lys residue …reaches outward to touch the active sites of E1 molecules **(yellow)** arranged on the E2 core.
- The domains of E2 are separated by linkers, sequences of 20 to 30 amino acid residues, rich in Ala and Pro and interspersed with charged residues; these linkers tend to assume their extended forms, holding the three domains apart.

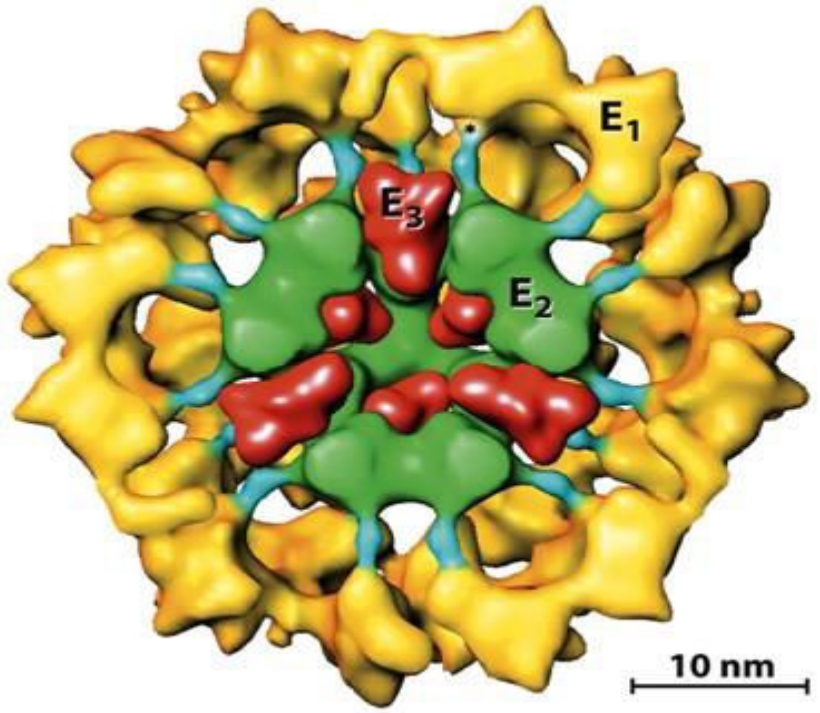


Figure 16-5b
Lehninger Principles of Biochemistry, Fifth Edition

- Several E3 subunits **(red)** are also bound to the core, where the E2 can reach their active sites.
- An **asterisk** marks the site where a lipoyl group is attached to the lipoyl domain of E2.
- To make the structure clearer, about half of the complex has been cut away from the front.
- This model was prepared by Z. H. Zhou and colleagues (2001)

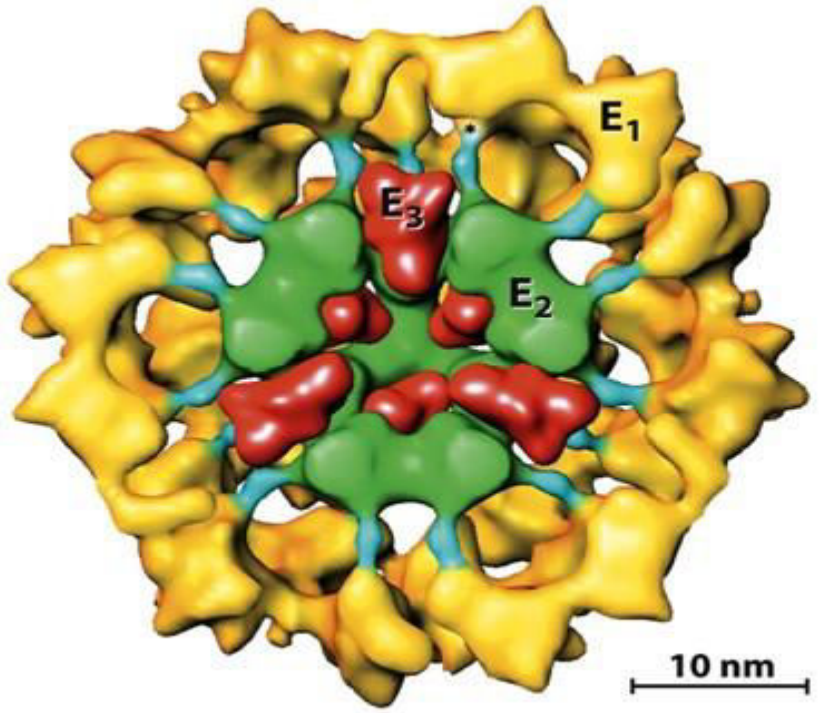


Figure 16-5b
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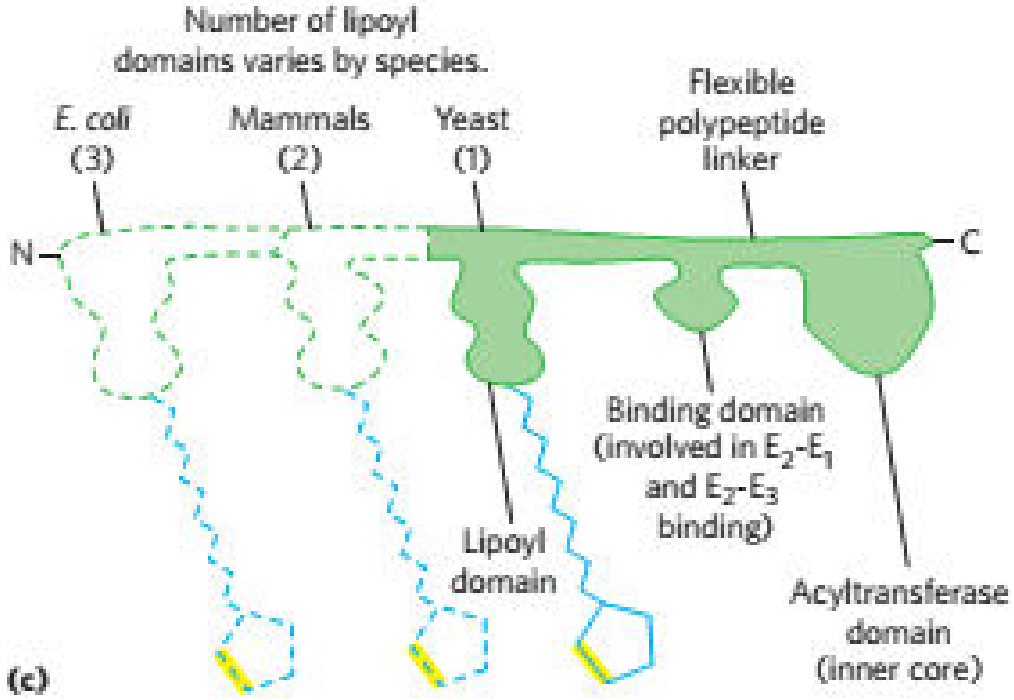
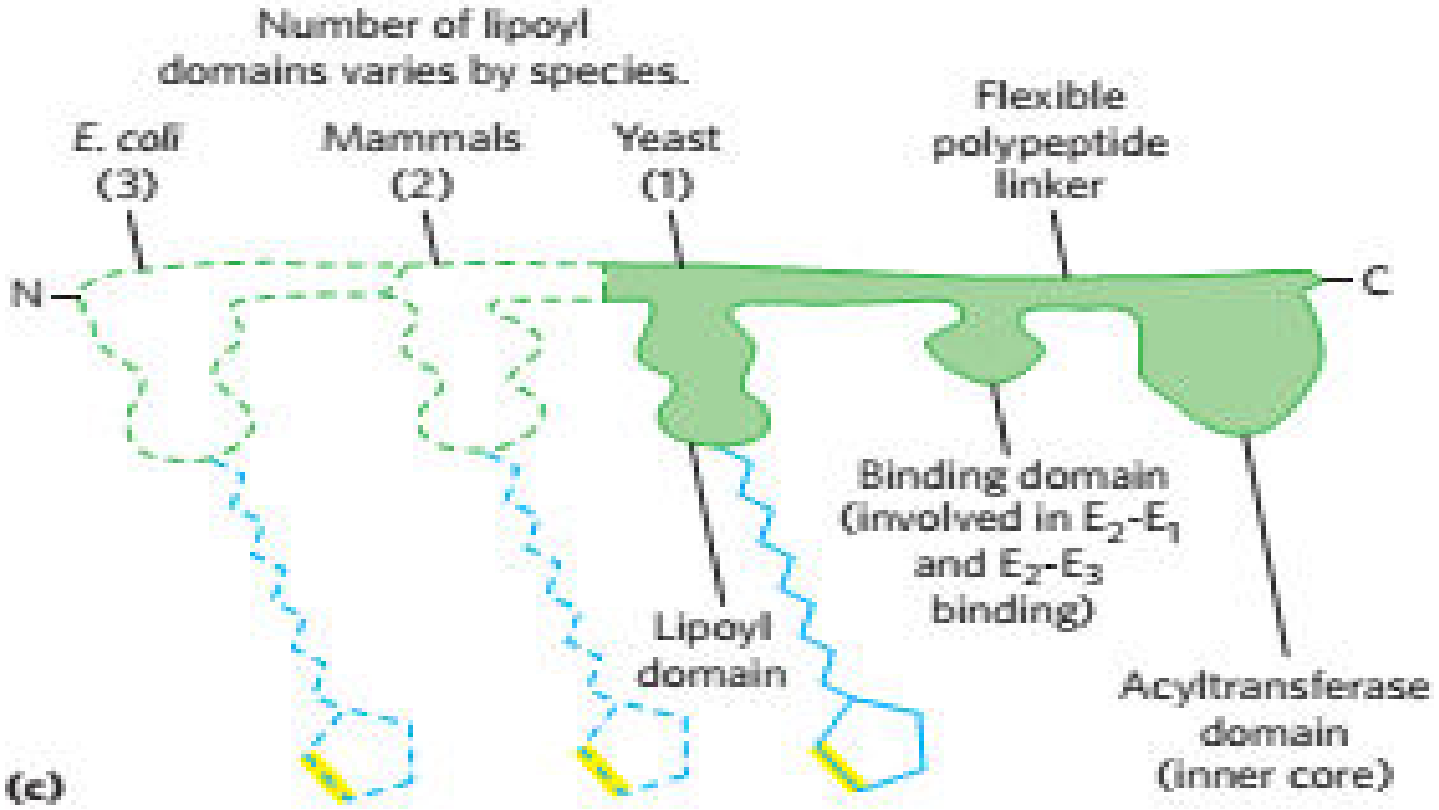
..cont

- **E2 has three functionally distinct domains**

1. the amino-terminal ***lipoyl domain***, containing the lipoyl-Lys residue(s)
2. the central E1- and E3-*binding domain*
3. the innercore ***acyltransferase*** *domain*, which contains the acyltransferase active site.





- The yeast PDH complex has a single lipoyl domain with a lipoate attached but the mammalian complex has two, and *E. coli* has three.

- The active site of E1 has bound TPP, and that of E3 has bound FAD.
- Also part of the complex are two regulatory proteins, a protein kinase and a phosphoprotein phosphatase,
- This basic E1–E2–E3 structure has been conserved during evolution and used in a number of similar metabolic reactions
- The attachment of lipoate to the end of a Lys side chain in E2 produces a long, flexible arm that can move from the active site of E1 to the active sites of E2 and E3, a distance of 5 nm

The Coenzymes and prosthetic groups of pyruvate dehydrogenase

Cofactor	Location	Function
Thiamine pyrophosphate	Bound to E1	Decarboxylates pyruvate
Lipoic acid	Covalently linked to a Lys on E2 (lipoamide)	Accepts hydroxyethyl carbanion from TPP
CoenzymeA	Substrate for E2	Accepts acetyl group from lipoamide
FAD (flavin)	Bound to E3	reduced by lipoamide
NADH	Substrate for E3	Reduced by FADH2

Citric Acid Cycle

The Pyruvate Dehydrogenase (PDH) Complex-2

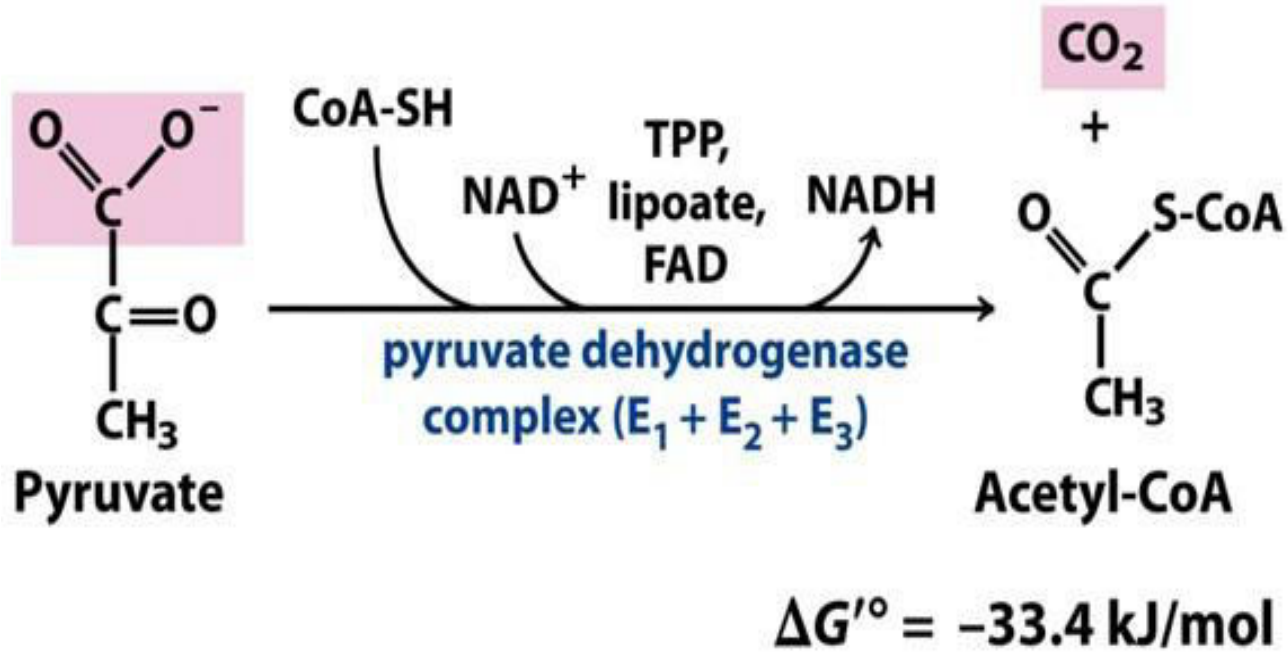


Figure 16-2
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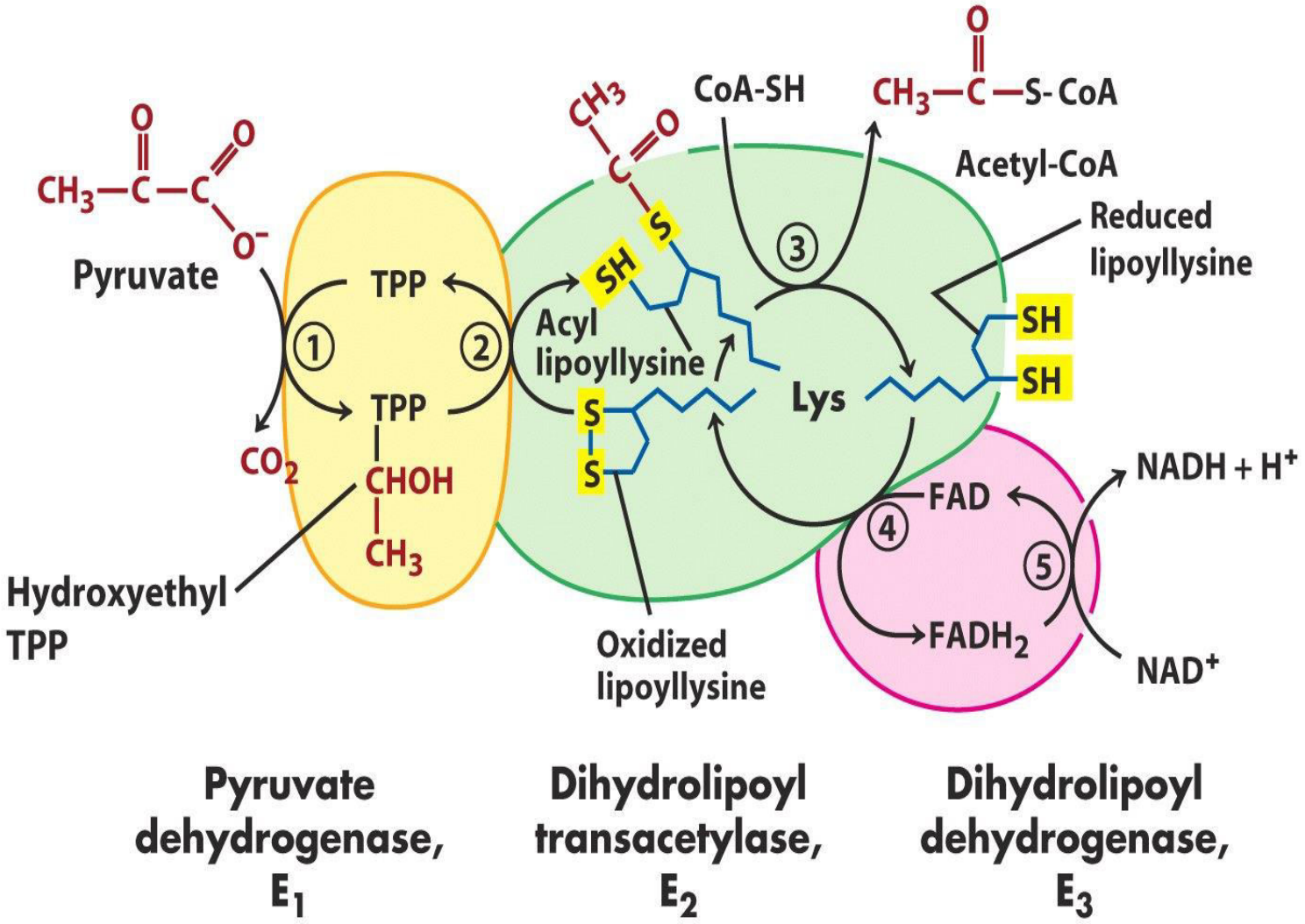
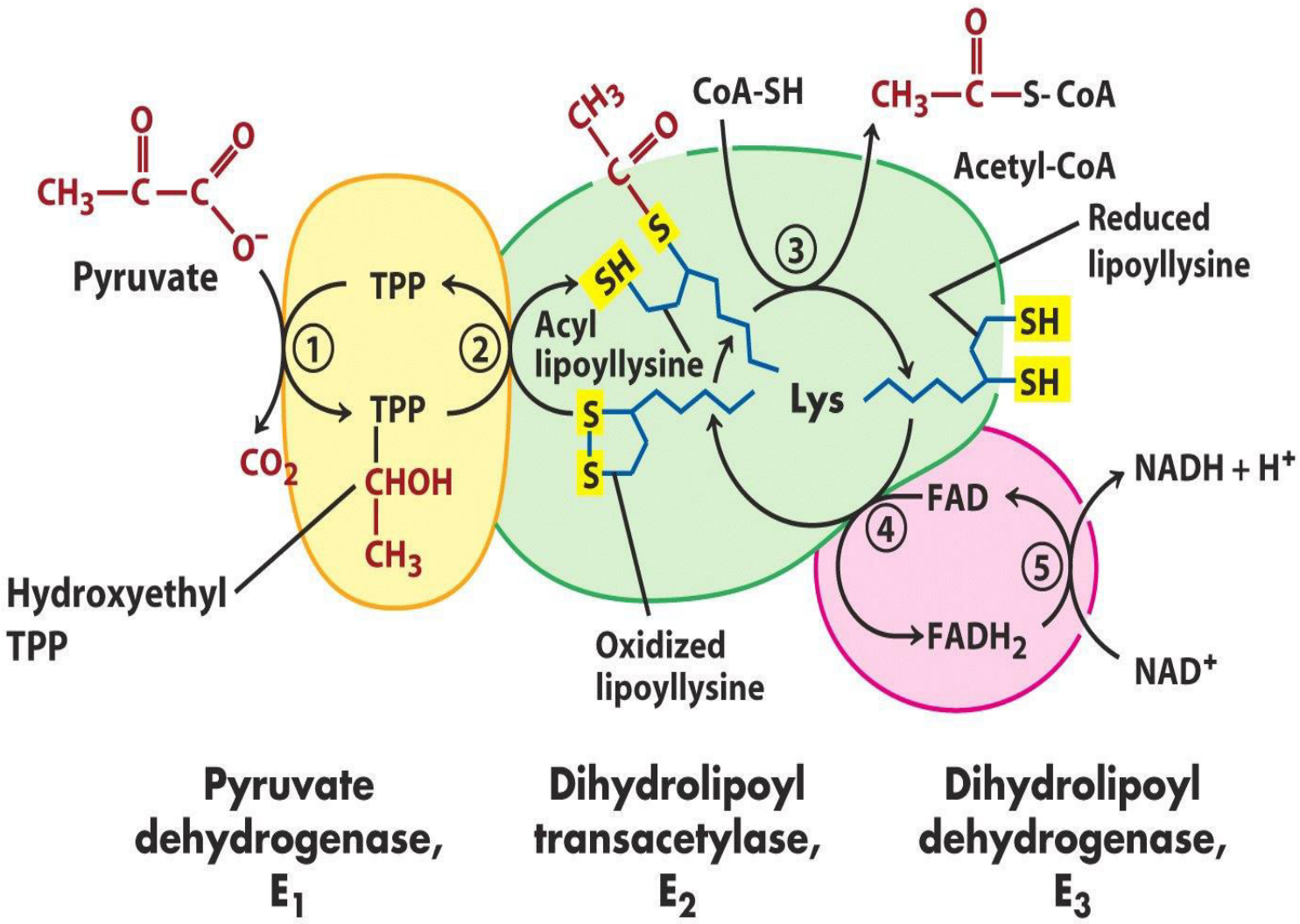
One Unit of PDH



The PDH Complex

- Pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate.
- **Step 1** is essentially identical to the reaction catalyzed by pyruvate decarboxylase.. C-1 of pyruvate is released as CO2, and C-2, which in pyruvate has the oxidation state of an aldehyde, is attached to TPP as a hydroxyethyl group.
- This first step is the slowest and therefore limits the rate of the overall reaction.
- It is also the point at which the PDH complex checks its substrate specificity.
- **Step 2** the hydroxyethyl group is oxidized to the level of a carboxylic acid (acetate).

- The two electrons removed in this reaction reduce the —S—S— of a lipoyl group on E2 to two thiol (—SH) groups.
- **Step 3** The acetyl group produced in this oxidation-reduction reaction is first transferred into thioester linkage (—SH group), then transesterified to CoA to form acetyl-CoA
- Thus the energy of oxidation drives the formation of a high-energy thioester of acetate.
- **Steps 4 and 5** The remaining reactions catalyzed by the PDH complex (by E3) are electron transfers necessary to regenerate the oxidized (disulfide) form of the lipoyl group of E2 to prepare the enzyme complex for another round of oxidation.
- The electrons removed from the hydroxyethyl group derived from pyruvate pass through FAD to NAD+.



Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex.

1. C-1 is released as CO2, C-2 is attached to TPP as a hydroxyethyl group
2. the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group to form the acetyl thioester of the reduced lipoyl group.

3. a transesterification to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group.
4. transfer of two hydrogen to the FAD (lipoyllysyl group gets oxidized)
5. the reduced FADH2 transfers a hydride ion to NAD+ ,forming NADH.

- **Substrate channeling**

Substrate channeling is the passing of the intermediary metabolic product of one enzyme directly to another enzyme or active site without its release into solution.

- The five-reaction sequence is thus an example of **substrate channeling**.

- **The most important component of PDH complex** are the swinging lipoyllysyl arms of E2, which accept from E1 the two electrons and the acetyl group derived from pyruvate, passing them to E3.
- All these enzymes and coenzymes are clustered, allowing the intermediates to react quickly without diffusing away from the surface of the enzyme complex.
- The intermediates of the multistep sequence never leave the complex, and the local concentration of the substrate of E2 is kept very high.
- Channeling also prevents theft of the activated acetyl group by other enzymes that use this group as substrate.
- A similar tethering mechanism for the channeling of substrate between active sites is used in some other enzymes, with lipoate, biotin, or a CoA-like moiety serving as cofactors.

Citric Acid Cycle

Acetyl-CoA enters Citric Acid Cycle

Citric Acid Cycle-8 enzymes

- **Acetyl CoA** enters TCA and undergoes oxidation.
- acetyl-CoA donates its acetyl group to the four-carbon compound **oxaloacetate** to form the six-carbon citrate.
- Citrate is then transformed into **isocitrate,** also a six-carbon molecule
- Isocitrate is dehydrogenated with loss of CO2 to yield the five-carbon compound **a-ketoglutarate** (also called oxoglutarate).
- *α*-Ketoglutarate undergoes loss of a second molecule of CO2 and yields the **four-carbon compound succinate.**
- Succinate is then enzymatically converted in three steps into the **four-carbon oxaloacetate**

- oxaloacetate is ready to react with another molecule of acetyl-CoA.
- In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO_2 leave
- one molecule of oxaloacetate (OAA) is used to form citrate and one molecule of oxaloacetate is regenerated. No net removal of oxaloacetate occurs
- **Four of the eight steps in this process are oxidations,** in which the energy of oxidation is very efficiently conserved in the form of the **reduced coenzymes NADH and $FADH_2$**.
- In eukaryotes, cycle takes place in mitochondria - the site of most energy- yielding oxidative reactions and of the coupled synthesis of ATP
- In prokaryotes cycle takes place in the cytosol, plasma membrane plays a role analogues to that of inner mitrochondrial membrane in ATP synthesis

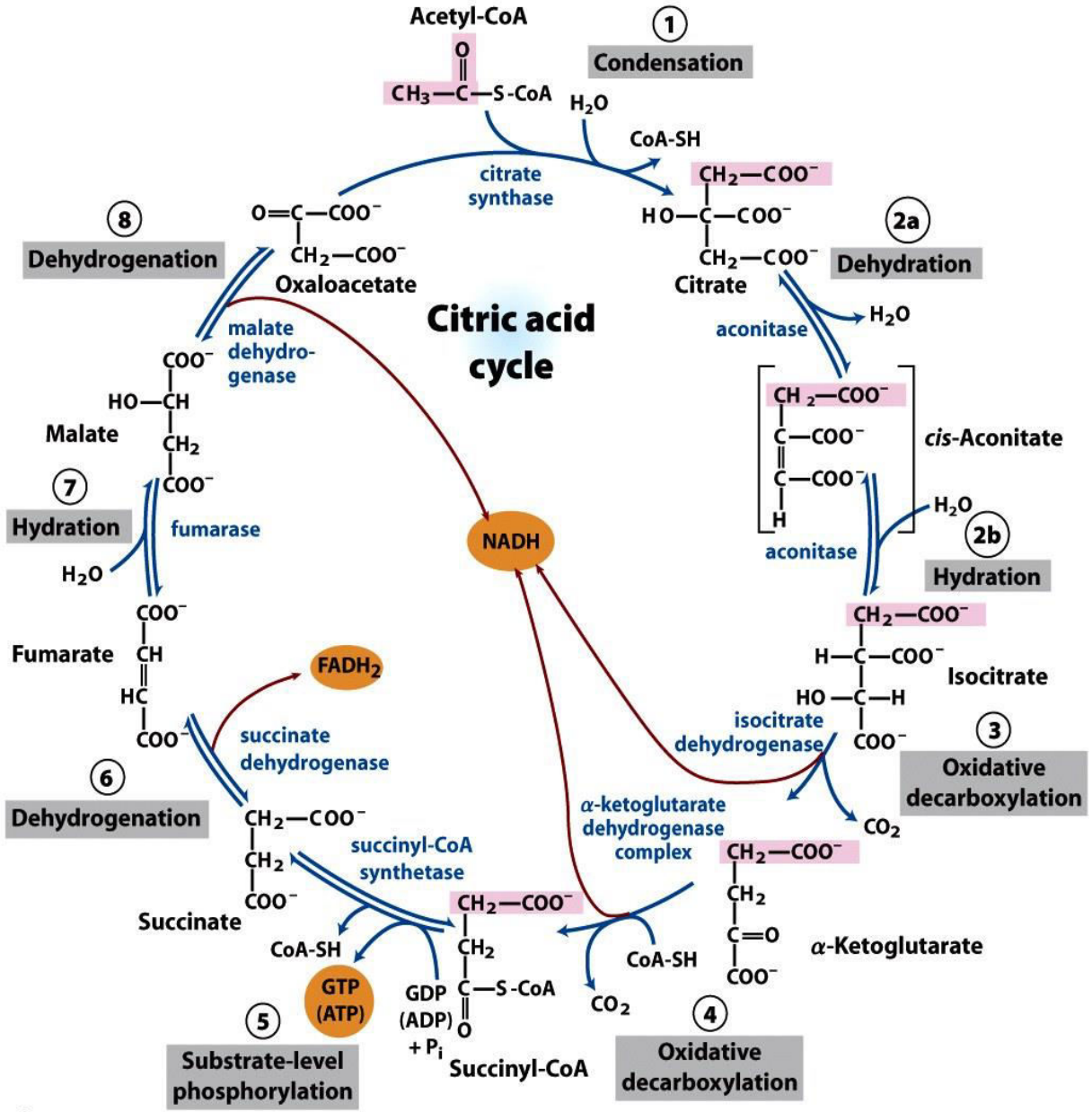


Figure 16-7
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FIGURE 16-7 Reactions of the citric acid cycle. The carbon atoms shaded in pink are those derived from the acetate of acetyl-CoA in the first turn of the cycle; these are *not* the carbons released as CO_2 in the first turn. Note that in **succinate and fumarate**, the two-carbon group derived from acetate can no longer be specifically denoted; because succinate and fumarate are symmetric molecules, C-1 and C-2 are indistinguishable from C-4 and C-3. The red arrows show where energy is conserved by electron transfer to FAD or NAD^+, forming $FADH_2$ or NADH + H^+. Steps 1, 3, and 4 are essentially irreversible in the cell; all other steps are reversible. The product of step 5 may be either ATP or GTP, depending on which succinyl-CoA synthetase isozyme is the catalyst.

Products

- Products of the first turn of the cycle are one GTP (or ATP), three NADH, 1$FADH_2$ and two CO_2.
- Because two acetyl-CoA are produced from each GLUCOSE molecule, two cycles are required per glucose molecule.
- Therefore, at the end of two cycles, the products are: two GTP, six NADH, two $FADH_2$, and four CO_2.

	Substrates	Products	Enzyme	Reaction type	Comment
0 / 10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	irreversible, extends the 4C oxaloacetate to a 6C molecule
1	Citrate	*cis*-Aconitate + H_2O	Aconitase	Dehydration	reversible isomerisation
2	*cis*-Aconitate + H_2O	Isocitrate		Hydration	
3	Isocitrate + NAD^+	Oxalosuccinate + $NADH + H^+$	Isocitrate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)
4	Oxalosuccinate	α-Ketoglutarate + CO_2		Decarboxylation	rate-limiting, irreversible stage, generates a 5C molecule
5	α-Ketoglutarate + NAD^+ + CoA-SH	Succinyl-CoA + $NADH + H^+$ + CO_2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)

6	Succinyl-CoA + GDP + P_i	Succinate + CoA-SH + GTP	Succinyl-CoA synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP,[12] generates 1 ATP or equivalent Condensation reaction of GDP + P_i and hydrolysis of Succinyl-CoA involve the H_2O needed for balanced equation.
7	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH_2)	Succinate dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→$FADH_2$ in the first step of the reaction) in the enzyme,[12] generates the equivalent of 1.5 ATP
8	Fumarate + H_2O	*L*-Malate	Fumarase	Hydration	Hydration of C-C double bond
9	*L*-Malate + NAD^+	Oxaloacetate + NADH + H^+	Malate dehydrogenase	Oxidation	reversible (in fact, equilibrium favors malate), generates NADH (equivalent of 2.5 ATP)
10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	This is the same as step 0 and restarts the cycle. The reaction is irreversible and extends the 4C oxaloacetate to a 6C molecule

The Citric acid Cycle.. cont

1.In each turn of the cycle, on acetyl group enters as acetyl-CoA and two Co2 leave; 1 OAA used and 1 OAA generated; NADH and FADH2, GTP or ATP

2.Four or five carbon intermediate serve as precursor of biomolecule

3.In eucaryotes, cycle takes place in mitochondria - the site of most energy-yielding oxidative reactions and of the coupled synthesis of ATP 3. In prokaryotes. cycle are in the cytosol, plasma memb. plays a role analogues to that of inner mitrochondrial membrane in ATP synthesis

Citric Acid Cycle

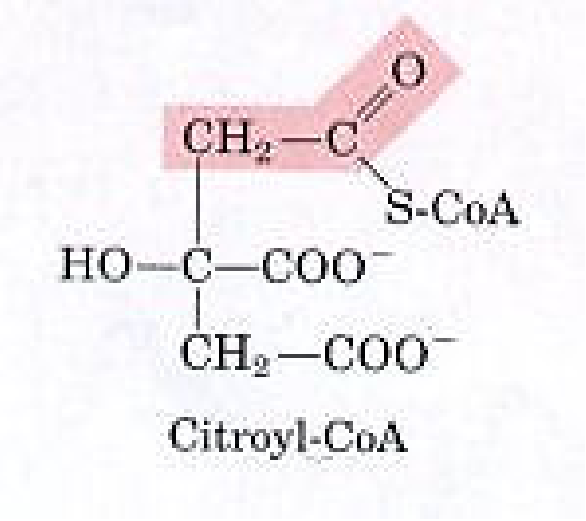
Steps of Citric Acid Cycle-1

1. Formation of Citrate (The Citrate Synthase Reaction)

- The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**
- The methyl carbon of the acetyl group is joined to the carbonyl group

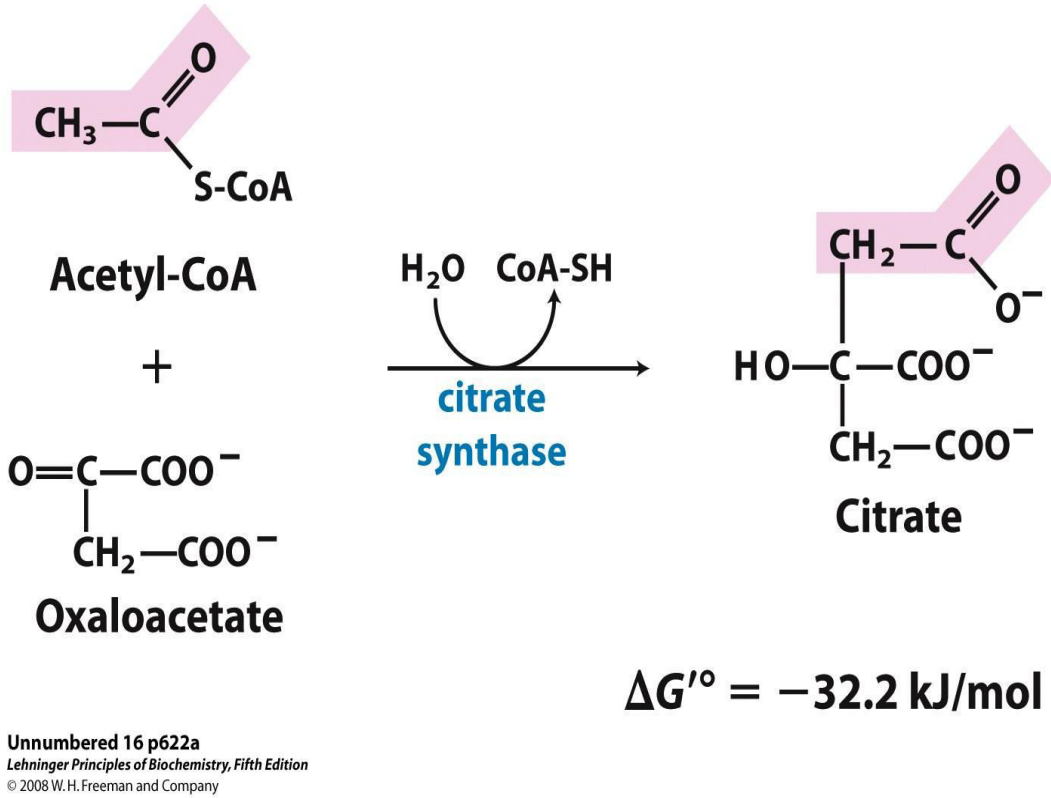
(C-2) of oxaloacetate.

- Citroyl-CoA is a transient intermediate formed on the active site of the enzyme …It rapidly undergoes hydrolysis to release CoA and citrate
- The hydrolysis of this high-energy thioester intermediate makes the forward reaction highly exergonic.
- The CoA **liberated** in this reaction is recycled to participate in the oxidative decarboxylation of another molecule of pyruvate by the PDH complex.



Citroyl-CoA

The Citrate Synthase Reaction



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- Formation of citrate: the condensation of acetyl-CoA with oxaloacetate. -Methyl carbon of acetyl group is joined to the **carbonyl group** (C-2) of OAA
- The hydrolysis of high-energy thioester makes the forward reaction
- Citrate sythase: homodimeric enzyme; induce conformational change by OAA binding, creating binding site for acetyl-CoA; when citroyl- CoA has formed, another conformational change brings about thioester hydorlysis; ordered bisubstrate mechanism

- **The only cycle reaction with C-C bond formation**
- **Essentially irreversible process**

The Citrate Synthase

- Citrate synthase : the homodimeric enzyme

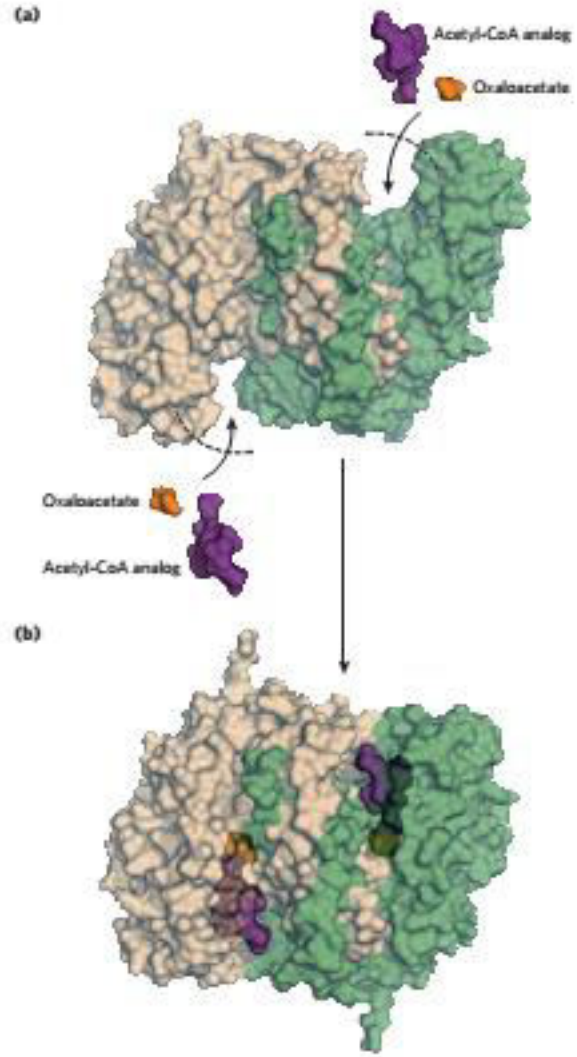
In these representations one subunit is colored tan and one green.

- Each subunit is a single polypeptide **with two domains**, **with the active site between them.**

1. **large and rigid,**
2. **smaller and more flexible**

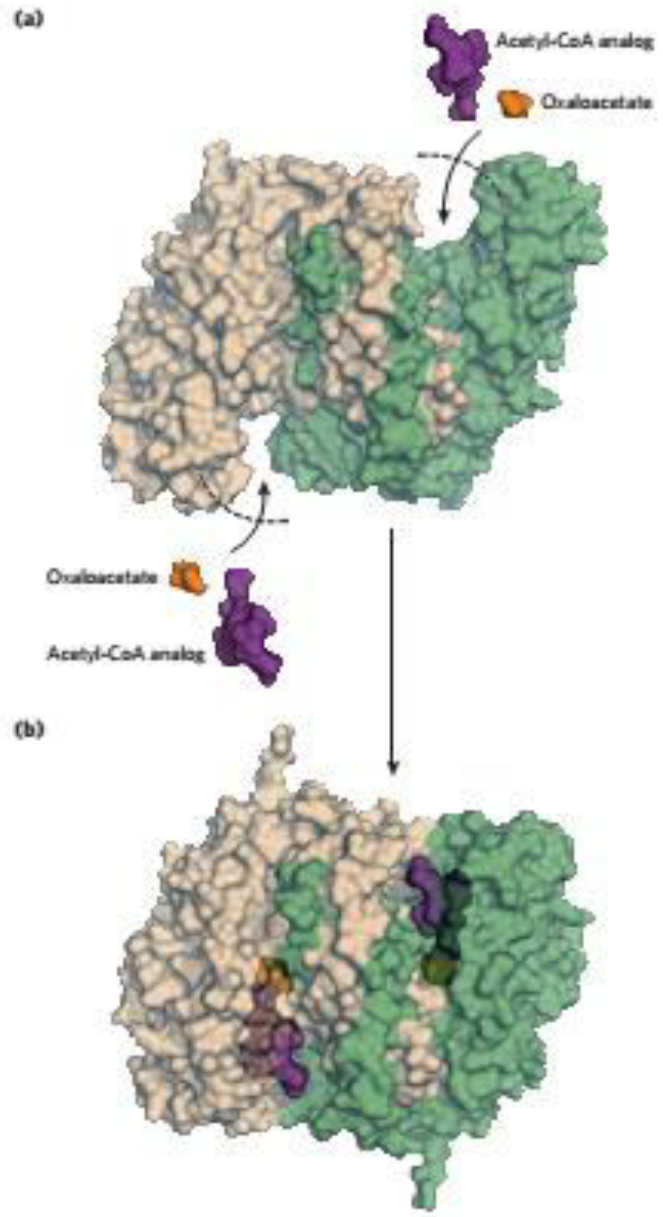
a) Open form of the enzyme alone
b) closed form with bound oxaloacetate and a stable analog of acetyl-CoA

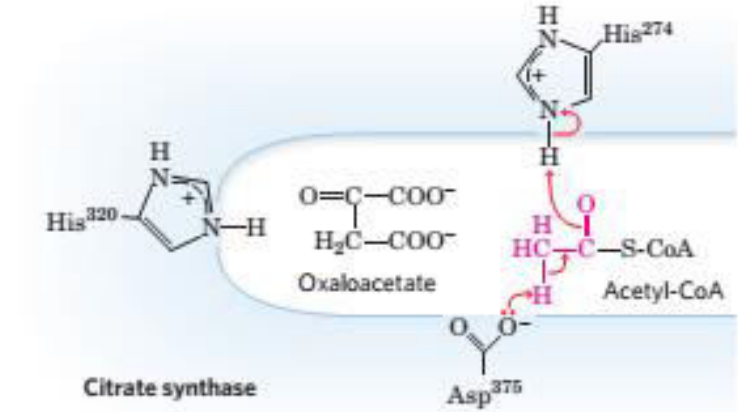
- The flexible domain of each subunit undergoes a large conformational change on binding oxaloacetate, creating a binding site for acetyl-CoA.



Structure of Citrate Synthase

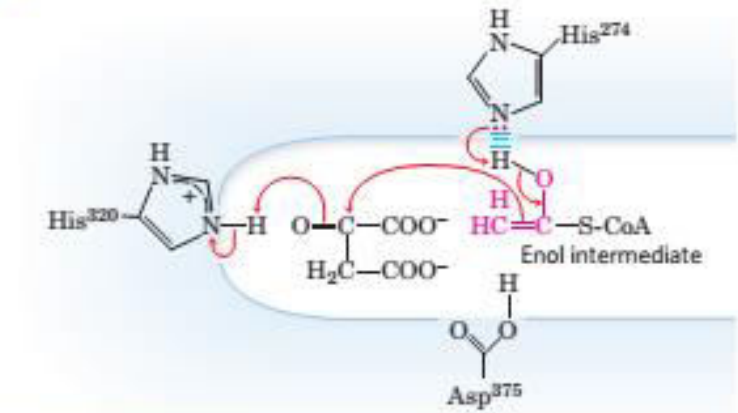
- **Ordered bisubstrate mechanism of Citrate synthase.**
- In the mammalian citrate synthase reaction, oxaloacetate binds first, in a strictly **ordered reaction sequence**.
- This binding triggers a conformation change that opens up the binding site for acetyl-CoA.
- Oxaloacetetate is specifically oriented in the active site of citrate synthase by interaction of its two carboxylates with two positively charged Arg residues





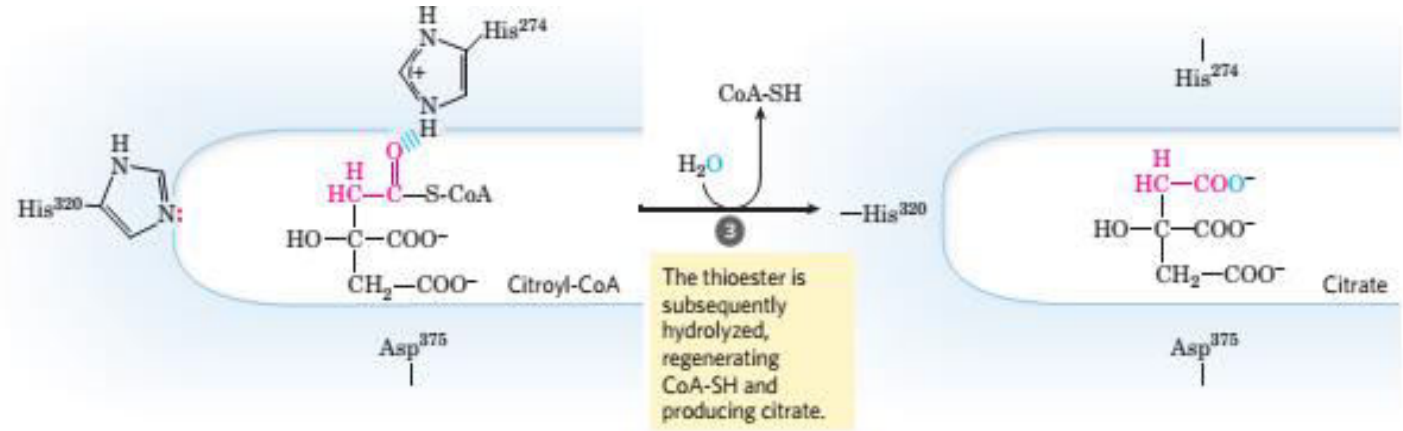
The thioester linkage in acetyl-CoA activates the methyl hydrogens. Asp^{375} abstracts a proton from the methyl group, forming an enolate intermediate. The intermediate is stabilized by hydrogen bonding to and/or protonation by His^{274} (full protonation is shown).

1



The enol(ate) rearranges to attack the carbonyl carbon of oxaloacetate, with His^{274} positioned to abstract the proton it had previously donated. His^{320} acts as a general acid. The resulting condensation generates citroyl-CoA.

2

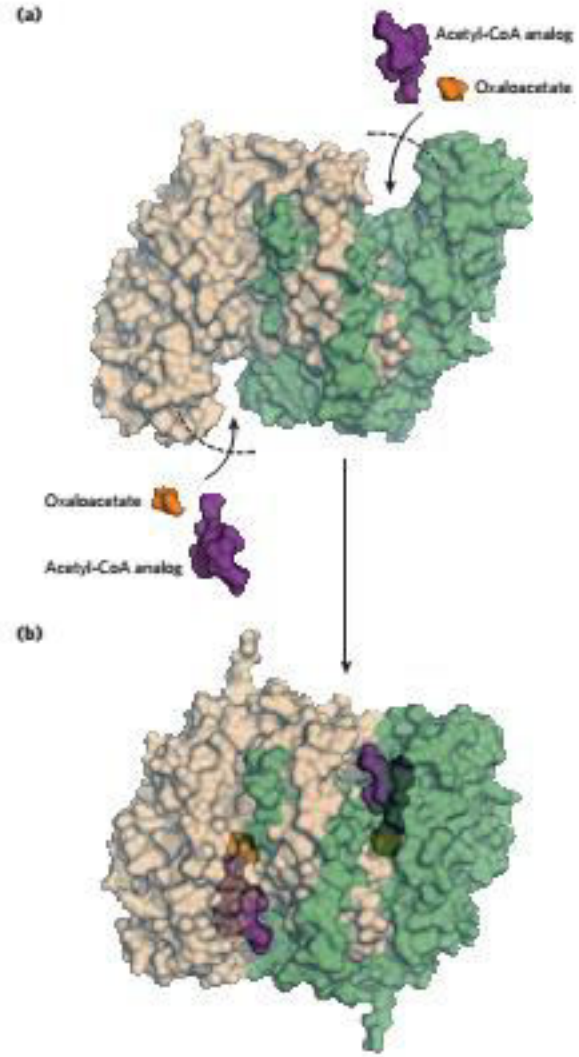


The thioester is subsequently hydrolyzed, regenerating CoA-SH and producing citrate.

- **Ordered bisubstrate mechanism of Citrate synthase.**
- **Oxaloacetate, the first substrate** to bind to the enzyme, induces a large conformational change in the flexible domain, creating a binding site for the **second substrate, acetyl-CoA**.
- When citroyl-CoA has formed in the enzyme active site..Another conformational change brings about thioester hydrolysis, releasing CoA-SH.
  This induced fit of the enzyme first to its substrate and then to its reaction intermediate decreases the likelihood of premature and unproductive cleavage of the thioester bond of acetyl-CoA.

The Citrate Synthase mechanism

- **Oxaloacetate, the first substrate** to bind to the enzyme, induces a large conformational change in the flexible domain, creating a binding site for the **second substrate, acetyl-CoA**.
- When citroyl-CoA has formed in the enzyme active site..Another conformational change brings about thioester hydrolysis, releasing CoA-SH. This induced fit of the enzyme first to its substrate and then to its reaction intermediate decreases the likelihood of premature and unproductive cleavage of the thioester bond of acetyl-CoA.
- Kinetic studies of the enzyme are consistent with this ordered bisubstrate mechanism The reaction catalyzed by citrate synthase is essentially a Claisen condensation involving a thioester (acetyl-CoA) and a ketone (oxaloacetate)

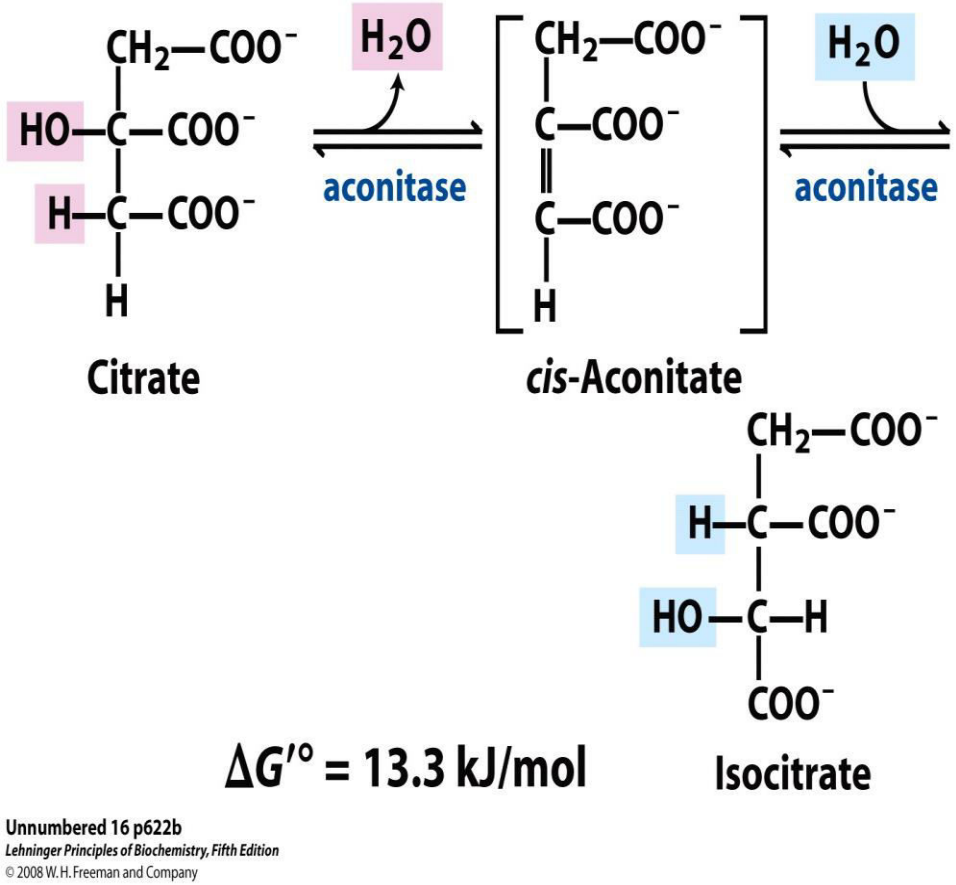


Citric Acid Cycle

Steps of Citric Acid Cycle-2

2. Isomerization of Citrate by Aconitase

- The enzyme **aconitase** (**aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**
- Reaction Intermediate of the tricarboxylic acid is formed..known as ***cis*-aconitate**
- ***cis*-aconitate does not dissociate from the active site**
- **Aconitase can promote the reversible addition of H2O to the double bond** of enzyme-bound *cis*-aconitate in two different ways,
  one leading to citrate and the other to isocitrate
- Under physiological conditions in the cell.. Conc. Of isocitrate is very little , the reaction is pulled to the right because isocitrate is rapidly consumed in the next step of the cycle



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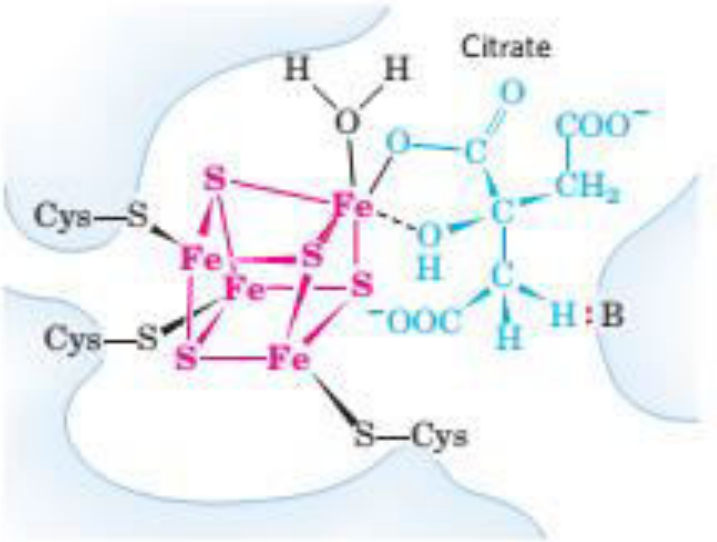
..cont

- Aconitase contains an **iron sulfur center** which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H2O.
- In iron-depleted cells, aconitase loses its iron-sulfur center and acquires a new role in the regulation of iron homeostasis.
- **Aconitase is one of many enzymes known to “moonlight” in a second role (protein with 2 jobs)**



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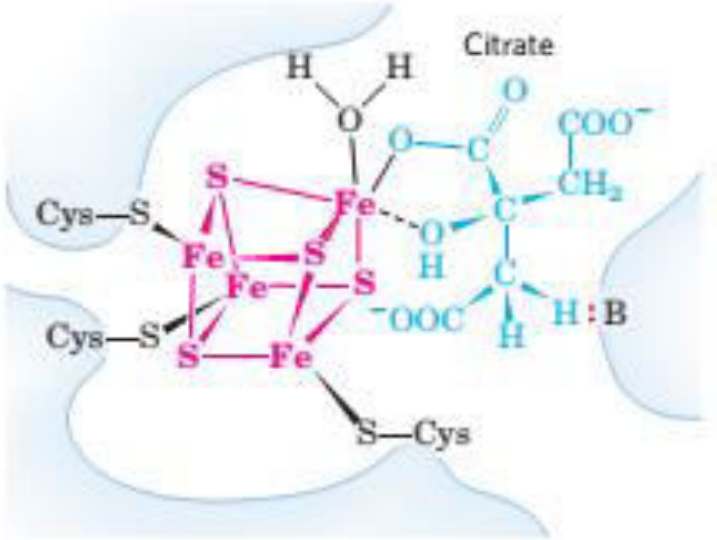
- **Iron-sulfur center in aconitase.**
- The iron-sulfur center is in red, the citrate molecule in blue. **Three Cys residues of the enzyme** bind three iron atoms
- the fourth iron is bound to one of the carboxyl groups of citrate and also interacts noncovalently with a hydroxyl group of citrate (dashed bond).
- A basic residue (**:**B) in the enzyme helps to position the citrate in the active site.
- The iron-sulfur center acts in both substrate binding and catalytic site.



3. The Isocitrate Dehydrogenase Reaction

- Oxidation of Isocitrate to *α*-Ketoglutarate and CO2 In the next step
- **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form ***α*-ketoglutarate**
- **Mn2+ in the active site interacts with the carbonyl group of the intermediate** oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to *α*-ketoglutarate.
- Mn2+ also stabilizes the enol formed transiently by decarboxylation.

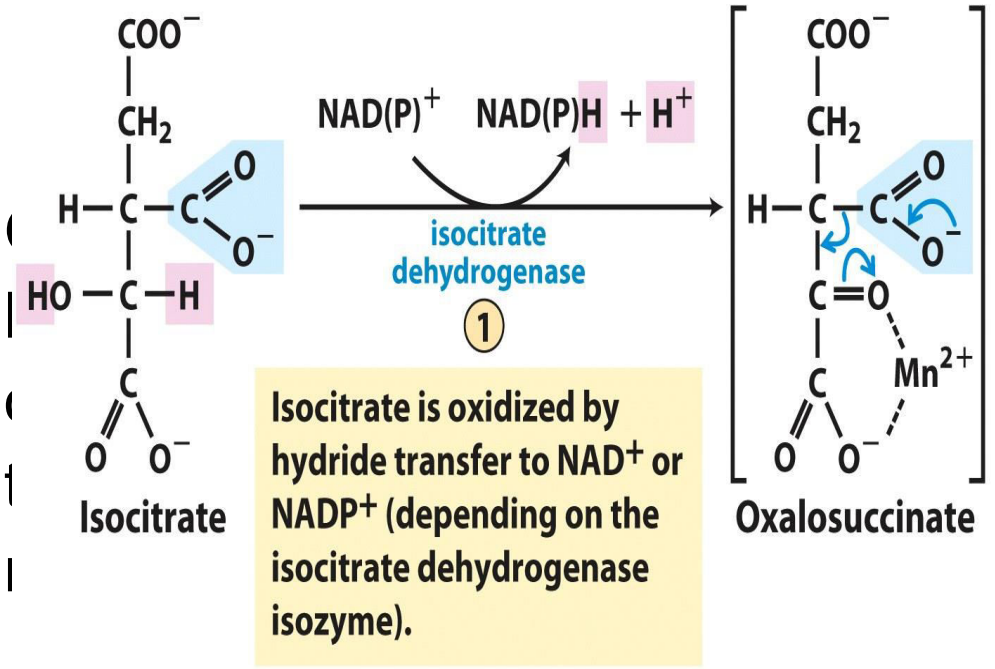


Figure 16-11 part 1
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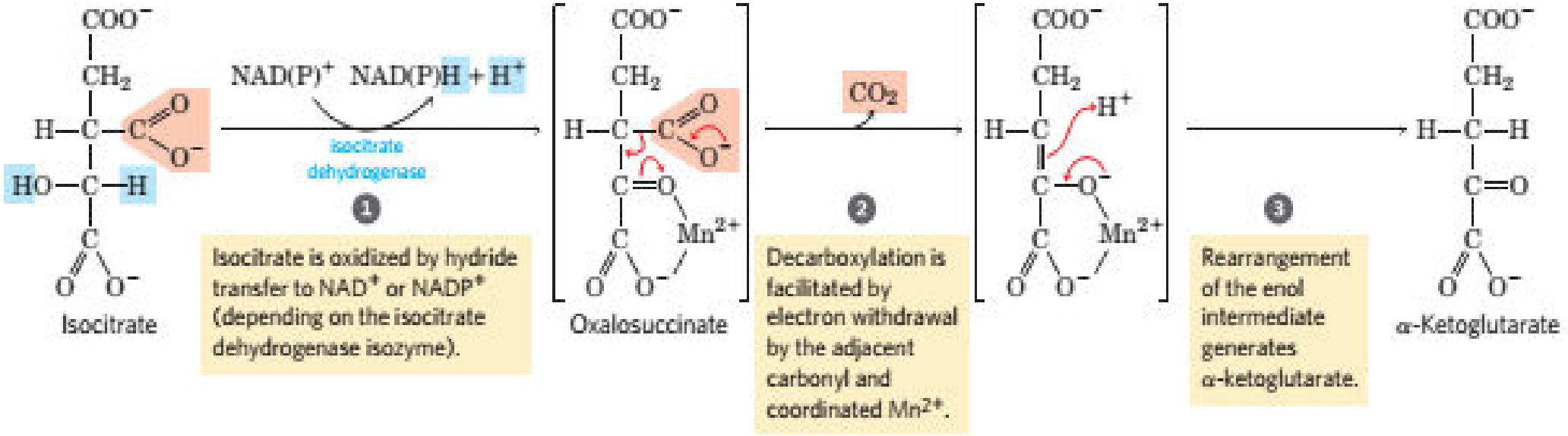


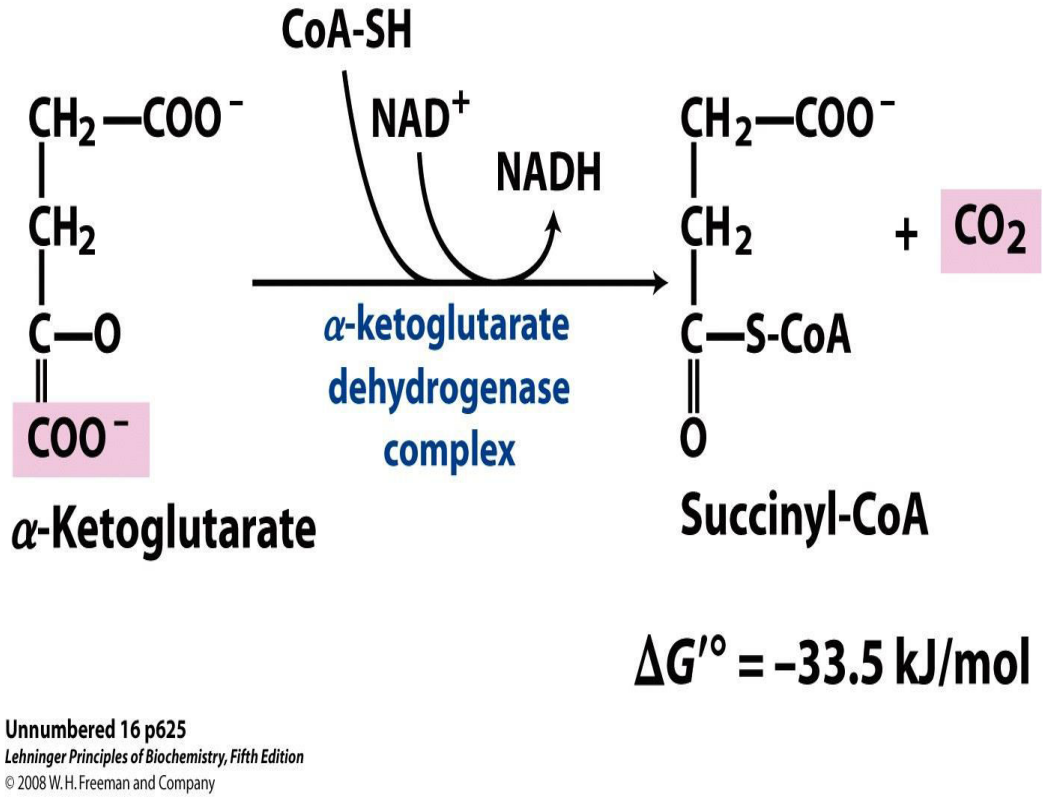
- **Isocitrate dehydrogenase reaction.**
- **In this reaction, the substrate, isocitrate, loses one carbon by oxidative decarboxylation**

Two different forms of isocitrate dehydrogenase (Isozymes)

- There are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD+ as electron acceptor and the other requiring NADP+.
- The overall reactions are otherwise identical
- In eukaryotic cells, the NAD-dependent enzyme occurs in the mitochondrial matrix and serves in the citric acid cycle.
- The main function of the NADP-dependent enzyme, found in both the mitochondrial matrix and the cytosol, may be the generation of NADPH, which is essential for reductive anabolic reactions

4. Oxidation of α-ketoglutarate

- **The next step is another oxidative decarboxylation**
- Oxidation of *α*-Ketoglutarate to Succinyl-CoA and CO2
- a-ketoglutarate is converted to **succinyl-CoA** and CO2 by the action of the **a-ketoglutarate dehydrogenase complex**
- NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.
- The energy of oxidation of *α*-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA
- This reaction is somehow identical to the pyruvate dehydrogenase reaction and to the reaction sequence responsible for the breakdown of branched chain amino acids



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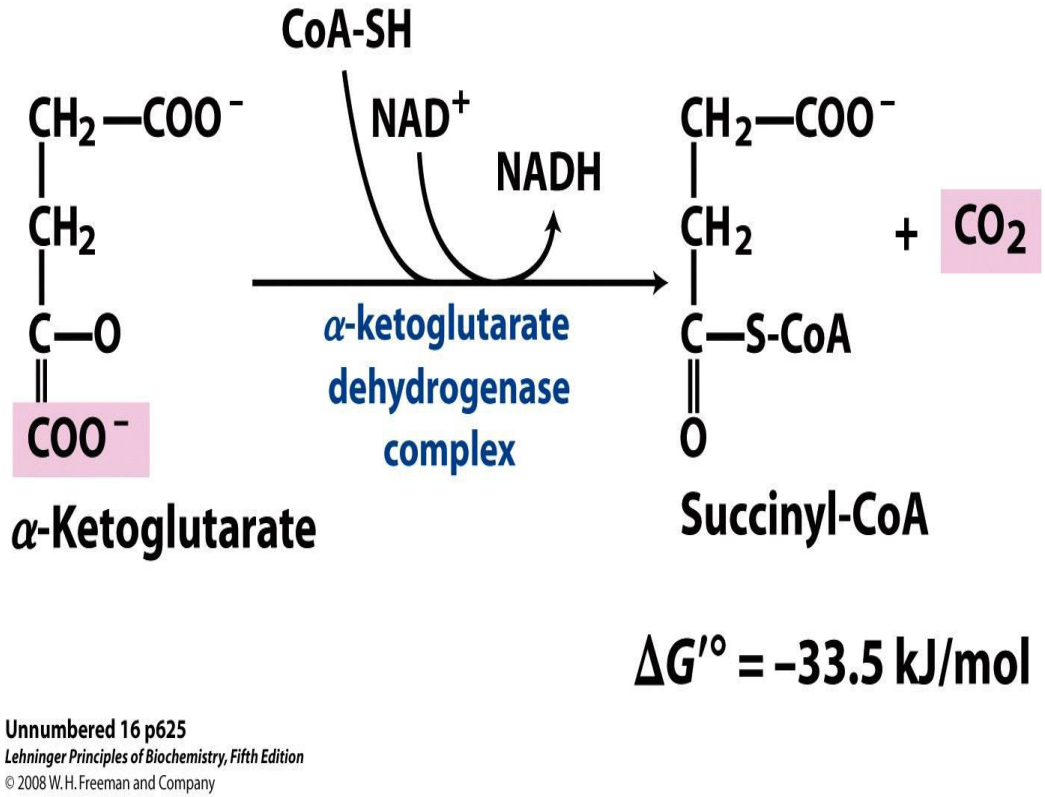
Citric Acid Cycle

Steps of Citric Acid Cycle-3

4. Oxidation of α-ketoglutarate

4. Oxidation of α-ketoglutarate

- **The next step is another oxidative decarboxylation**
- Oxidation of *α*-Ketoglutarate to Succinyl-CoA and CO2
- a-ketoglutarate is converted to **succinyl-CoA** and CO2 by the action of the **a-ketoglutarate dehydrogenase complex**
- NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.
- The energy of oxidation of *α*-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA
- This reaction is somehow identical to the pyruvate dehydrogenase reaction and to the reaction sequence responsible for the breakdown of branched chain amino acids



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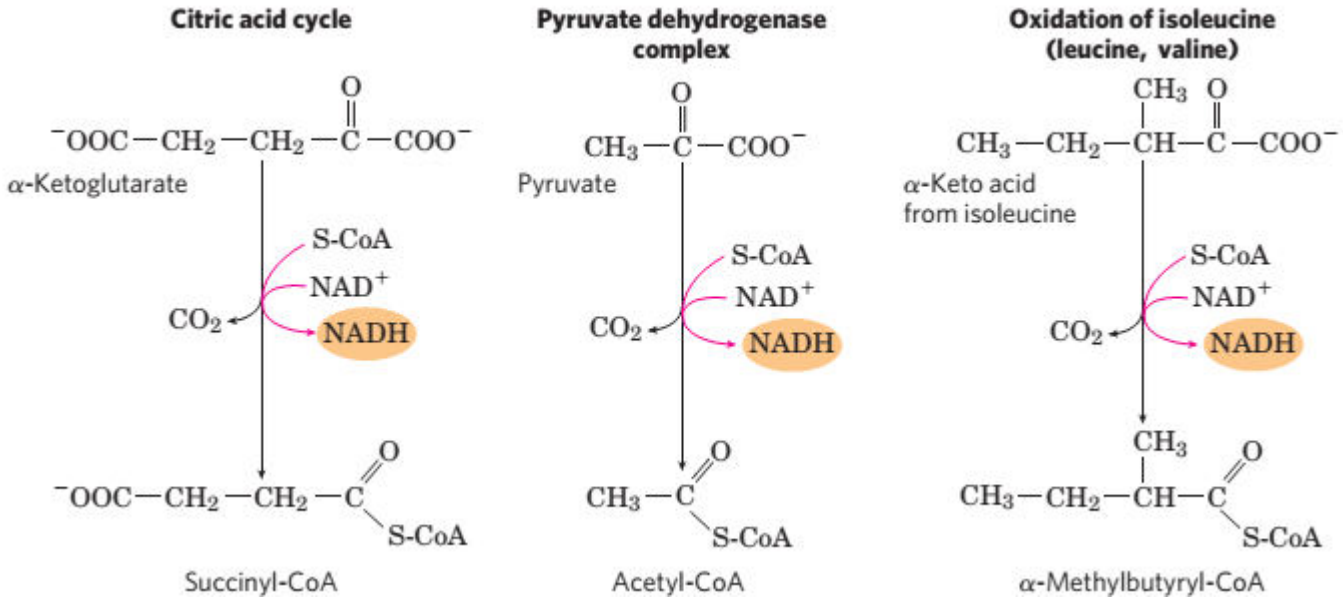
a-ketoglutarate dehydrogenase complex resembles PDH complex

- The a-ketoglutarate dehydrogenase complex closely resembles the PDH complex in both structure and function.
- It includes three enzymes, homologous to E1, E2, and E3 of the PDH complex, as well as enzyme-bound TPP, bound lipoate, FAD, NAD, and coenzyme A.
- Both complexes are certainly derived from a common evolutionary ancestor.
- **Although the E1 components of the two complexes are structurally similar, their amino acid sequences differ and, they have different binding specificities**
- E1 of the PDH complex binds pyruvate, and E1 of the *α*-ketoglutarate dehydrogenase complex binds *α*-ketoglutarate.
- The E2 components of the two complexes are also very similar, both having covalently bound lipoyl moieties.
- The subunits of E3 are identical in the two enzyme complexes.
- The enzymatic complex that degrades branched-chain *α*-keto acids catalyzes the same reaction sequence using the **same five cofactors**.
- This is a clear case of **divergent evolution**, in which the genes for an enzyme with one substrate specificity give rise, during evolution, to closely related enzymes with different substrate specificities but the same enzymatic mechanism.

A conserved mechanism for oxidative decarboxylation

- The pathways shown use the same five cofactors (thiamine pyrophosphate, coenzyme A, lipoate, FAD, and NAD+)
- closely similar multienzyme complexes, and the same enzymatic mechanism to carry out oxidative decarboxylations of pyruvate (by the pyruvate dehydrogenase complex) a-ketoglutarate (in the citric acid cycle) and the carbon skeletons of the three branched-chain amino acids, isoleucine leucine, and valine



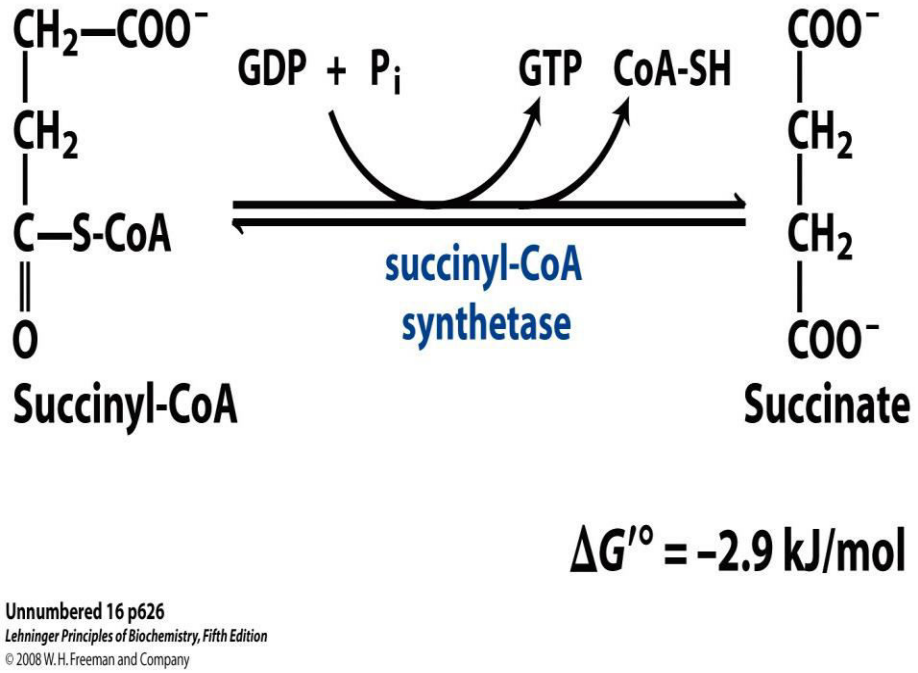
Citric Acid Cycle

Steps of Citric Acid Cycle-4

5. Conversion of Succinyl-CoA to Succinate

5. Conversion of Succinyl-CoA to Succinate

- **Succinyl-CoA** like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis (*G*= -36 kJ/mol).
- Energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net *G*= - 2.9 kJ/mol.
- The enzyme that catalyzes this reversible reaction is called **succinyl-CoA synthetase** or **succinic thiokinase**, names indicate the participation of a nucleoside triphosphate in the reaction



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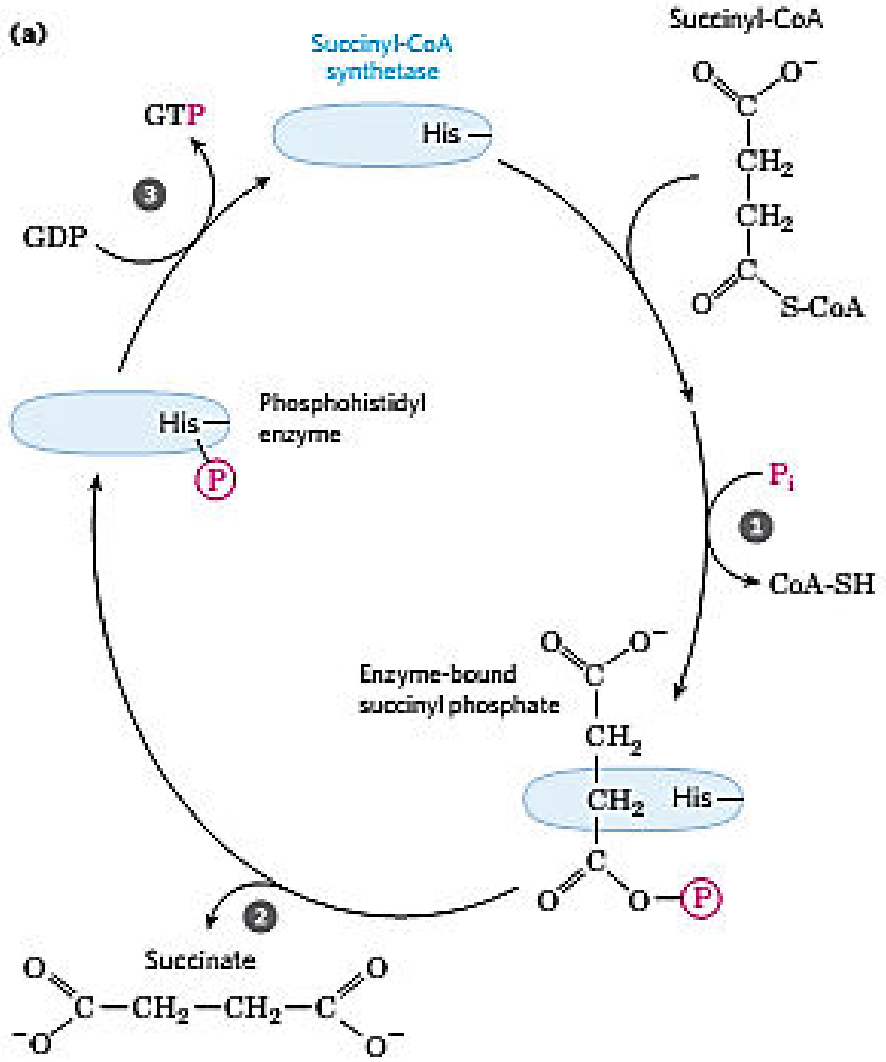
The succinyl-CoA synthetase reaction.

(a) In step 1 a phosphoryl group replaces the CoA of succinyl-CoA bound to the enzyme, forming a high-energy acyl phosphate.

(b) In step 2 the succinyl phosphate donates its phosphoryl group to a His residue of the enzyme, forming a high-energy phosphohistidyl enzyme.

(c) In step 3 the phosphoryl group is transferred from the His residue to the terminal phosphate of GDP (or ADP), forming GTP (or ATP).

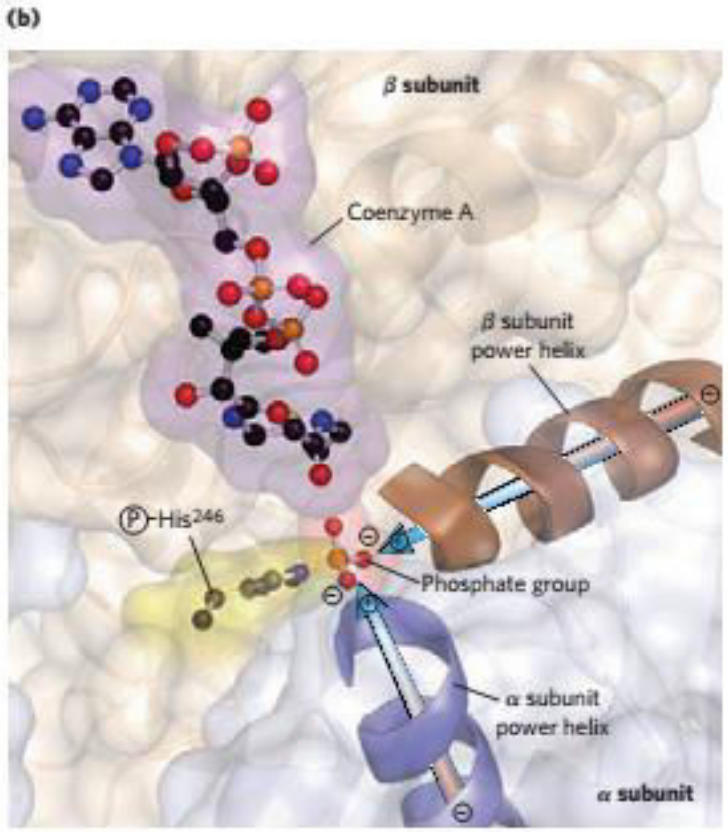


Nucleoside diphosphate kinase reaction:

The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, reversible reaction catalyzed by **Nucleoside diphosphate kinase reaction**

..cont

- The enzyme has two subunits,
  a subunit: **which has the P –His residue (His246) and the binding site for CoA**
- ***B subunit:*** **confers specificity for either ADP or GDP.**
- The active site is at the interface between subunits. The crystal structure of succinyl-CoA synthetase reveals two "power helices" (one from each subunit), oriented so that their electric dipoles situate partial positive charges close to the negatively charged P –His (Fig. 16–13b), stabilizing the phosphoenzyme intermediate.



Citric Acid Cycle

Steps of Citric Acid Cycle-5

6. Oxidation of Succinate to Fumarate

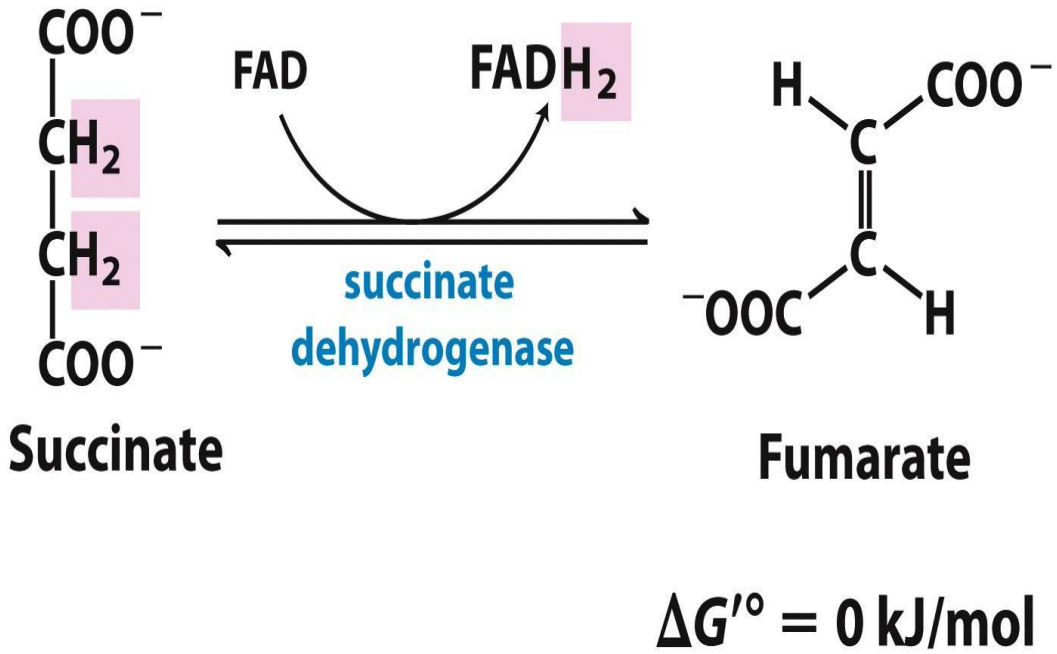
Citric Acid Cycle

Steps of Citric Acid Cycle-5

6. Oxidation of Succinate to Fumarate

6. Oxidation of Succinate to Fumarate

- **Succinate Dehydrogenase reaction**
- The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**
- In eukaryotes, succinate dehydrogenase is tightly bound to the mitochondrial inner membrane; in bacteria, to the plasma membrane.
- **The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD**
- Electron flow from succinate through these carriers to the final electron acceptor, O2, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).



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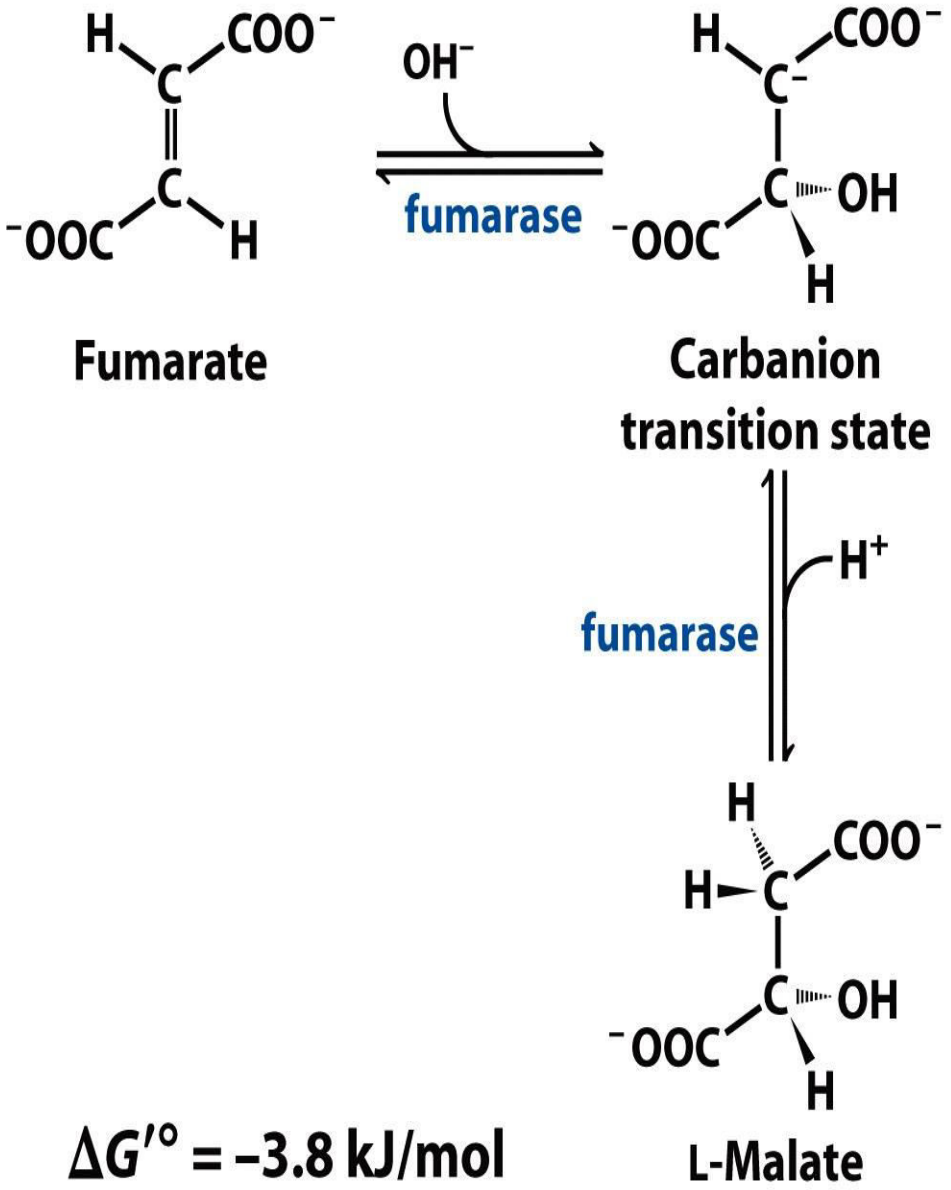
7. Hydration of Fumarate to Malate

- The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase** (**fumarate hydratase**).
- The transition state in this reaction is a carbanion
- This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate).
- In the reverse direction (from L-malate to fumarate),
  fumarase is equally stereospecific: D-malate is not a substrate.



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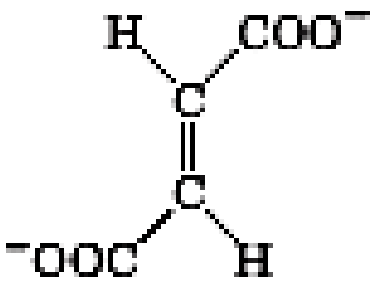
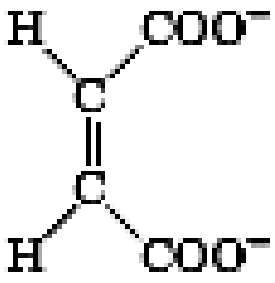
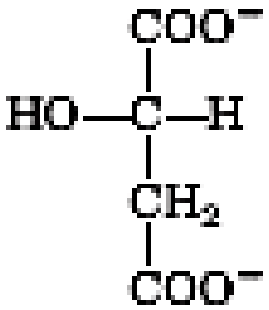


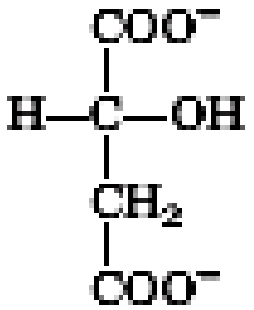
Fumarate



Maleate



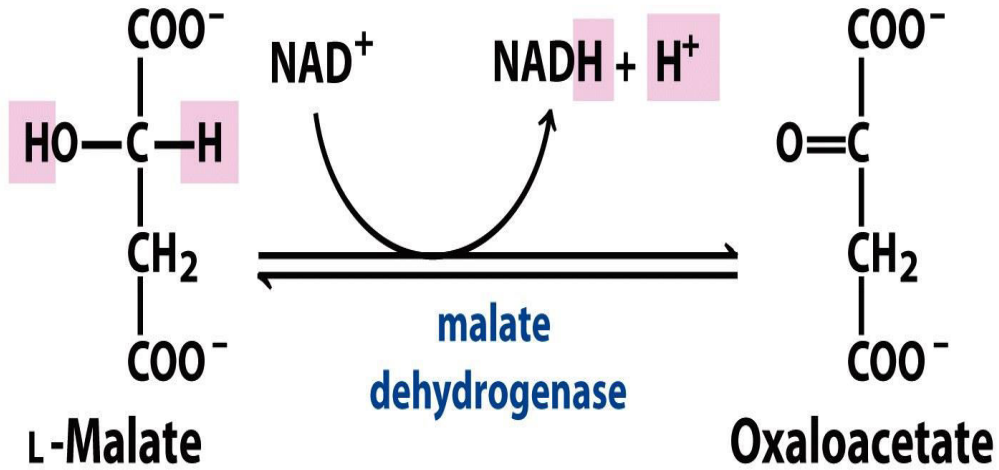
L-Malate



D-Malate

8. Oxidation of Malate to Oxaloacetate

- Oxidation of Malate to Oxaloacetate Is the last reaction of the citric acid cycle
- NAD-linked **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate
- In intact cells oxaloacetate is continually removed by the **highly exergonic citrate synthase** reaction
- This keeps the concentration of oxaloacetate in the cell remains extremely low pulling **the malate dehydrogenase** reaction toward the formation of oxaloacetate.



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Interesting facts

- The individual reactions of the citric acid cycle were initially worked out in vitro, using minced muscle tissue
- Later, the pathway and its regulation were studied in vivo.
- By using radioactively labeled precursors such as [14C] pyruvate and [14C]acetate
- Carbon-14, $^{14}\mathbf{C}$, or radiocarbon, is a radioactive **isotope** of carbon with an atomic nucleus containing 6 protons and 8 neutrons.
- Researchers have traced the fate of individual carbon atoms through the citric acid cycle.

Direct and Indirect ATP Yield

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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Products from One Turn of the Cycle

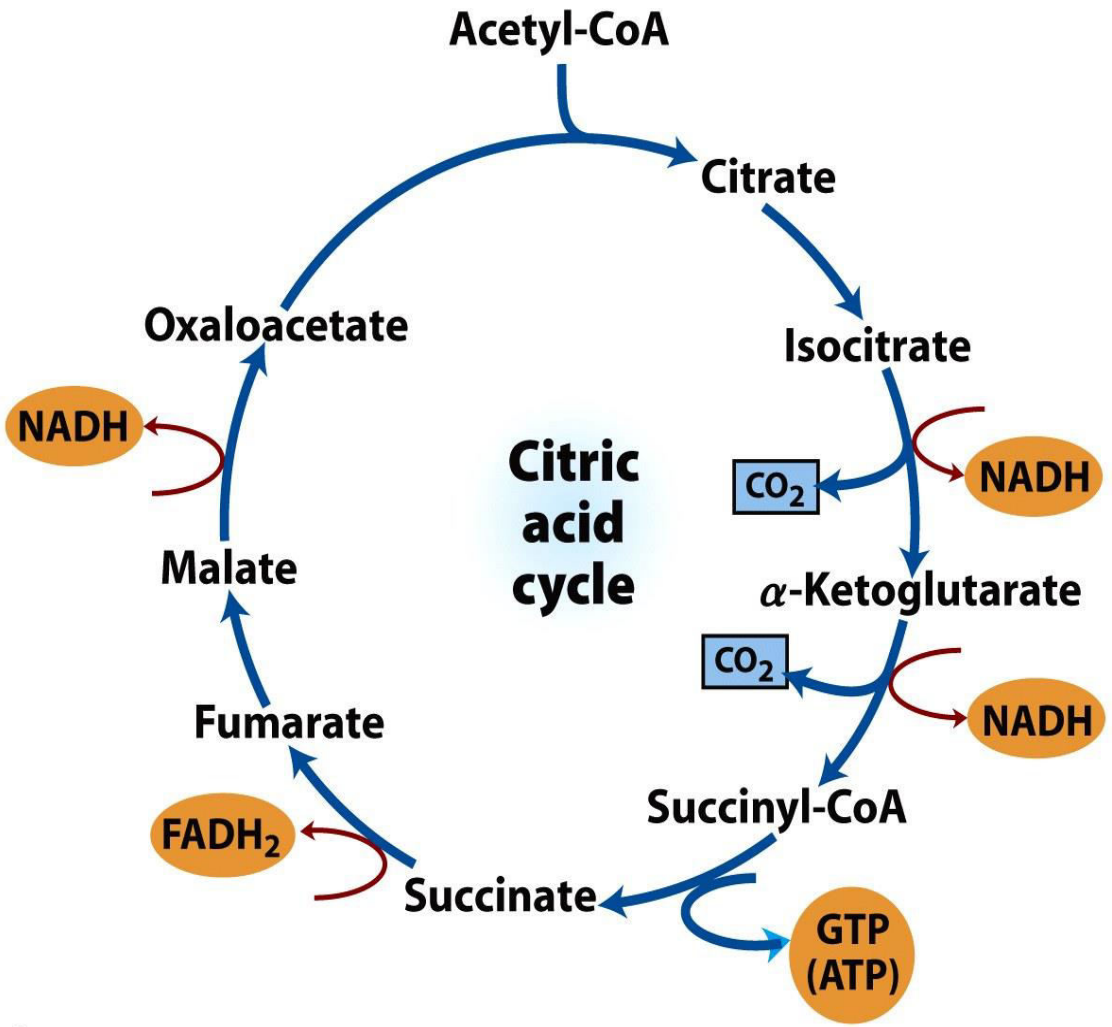

Figure 16-13
Lehninger Principles of Biochemistry, Fifth Edition

Substrates	Products	Enzyme	Reaction type	Comment	
0 / 10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	irreversible, extends the 4C oxaloacetate to a 6C molecule
1	Citrate	*cis*-Aconitate + H_2O	Aconitase	Dehydration	reversible isomerisation
2	*cis*-Aconitate + H_2O	Isocitrate		Hydration	
3	Isocitrate + NAD^+	Oxalosuccinate + NADH + H^+	Isocitrate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)
4	Oxalosuccinate	α-Ketoglutarate + CO_2		Decarboxylation	rate-limiting, irreversible stage, generates a 5C molecule
5	α-Ketoglutarate + NAD^+ + CoA-SH	Succinyl-CoA + NADH + H^+ + CO_2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)

6	Succinyl-CoA + GDP + P_i	Succinate + CoA-SH + GTP	Succinyl-CoA synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP,[12] generates 1 ATP or equivalent Condensation reaction of GDP + P_i and hydrolysis of Succinyl-CoA involve the H_2O needed for balanced equation.
7	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH_2)	Succinate dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→$FADH_2$ in the first step of the reaction) in the enzyme,[12] generates the equivalent of 1.5 ATP
8	Fumarate + H_2O	*L*-Malate	Fumarase	Hydration	Hydration of C-C double bond
9	*L*-Malate + NAD^+	Oxaloacetate + NADH + H^+	Malate dehydrogenase	Oxidation	reversible (in fact, equilibrium favors malate), generates NADH (equivalent of 2.5 ATP)
10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	This is the same as step 0 and restarts the cycle. The reaction is irreversible and extends the 4C oxaloacetate to a 6C molecule

Lecture No. 99

Citric Acid Cycle

Cycle

$$\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + 2\,H_2O$$

$$2CO_2 + 3\text{NADH} + \text{FADH}_2 + \text{GTP} + \text{CoA} + 3H^+$$

- Carbons of acetyl groups in acetyl-CoA are oxidized to CO_2
- Electrons from this process reduce NAD^+ and FAD
- One GTP is formed per cycle, this can be converted to ATP
- Intermediates in the cycle are not depleted

Direct and Indirect ATP Yield

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*
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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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Role of the Citric Acid Cycle in Anabolism

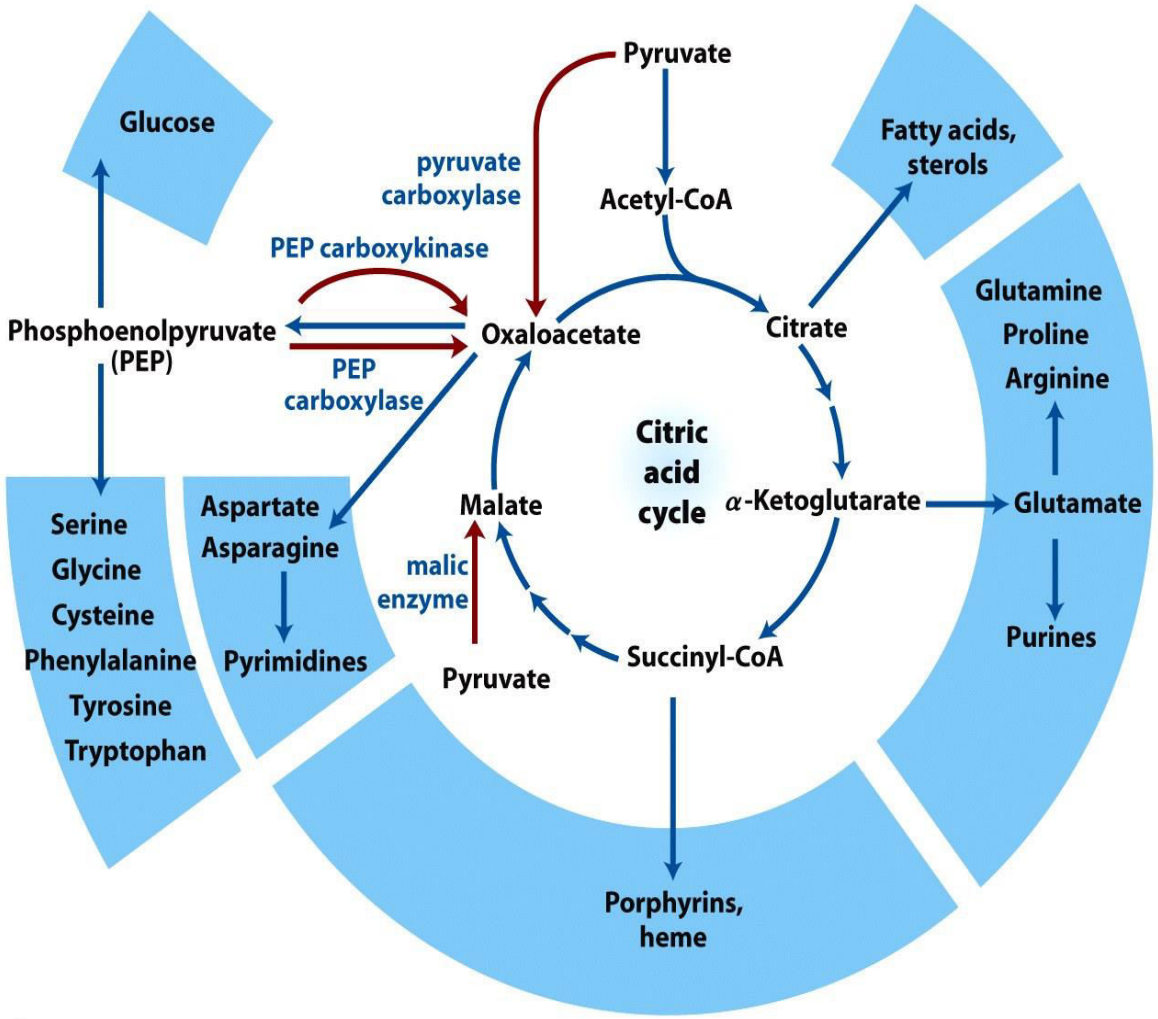

Figure 16-15
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- **The amphibolic nature of Citric acid cycle:** **This pathway is utilized for the both catabolic reactions to generate energy as well as for anabolic reactions to generate metabolic intermediates for biosynthesis.**

- **If the CAC intermediate are used for synthetic reactions, they are replenished by anaplerotic reactions in the cells (indicated by red colours).**

Anaplerotic Reactions

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

Table 16-2
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Summary

In this chapter, we learned that:

- Citric acid cycle is an important catabolic process: it makes GTP, and reduced cofactors that could yield ATP
- Citric acid cycle plays important anabolic roles in the cell
- A large multi-subunit enzyme, pyruvate dehydrogenase complex, converts pyruvate into acetyl-CoA
- Several cofactors are involved in reactions that harness the energy from pyruvate
- The rules of organic chemistry help to rationalize reactions in the citric acid cycle

Role of Citric Acid Cycle

Citric Acid Cycle

Citric Acid Cycle-8 enzymes

Citric Acid Cycle-8 enzymes

- **Acetyl CoA** enters TCA and undergoes oxidation.
- acetyl-CoA donates its acetyl group to the four-carbon compound **oxaloacetate** to form the six-carbon citrate.
- Citrate is then transformed into **isocitrate,** also a six-carbon molecule
- Isocitrate is dehydrogenated with loss of CO2 to yield the five-carbon compound **a-ketoglutarate** (also called oxoglutarate).
- *α*-Ketoglutarate undergoes loss of a second molecule of CO2 and yields the **four-carbon compound succinate.**
- Succinate is then enzymatically converted in three steps into the **four-carbon oxaloacetate**

- oxaloacetate is ready to react with another molecule of acetyl-CoA.
- In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO_2 leave
- one molecule of oxaloacetate (OAA) is used to form citrate and one molecule of oxaloacetate is regenerated. No net removal of oxaloacetate occurs
- **Four of the eight steps in this process are oxidations,** in which the energy of oxidation is very efficiently conserved in the form of the **reduced coenzymes NADH and $FADH_2$**.
- In eukaryotes, cycle takes place in mitochondria - the site of most energy- yielding oxidative reactions and of the coupled synthesis of ATP
- In prokaryotes cycle takes place in the cytosol, plasma membrane plays a role analogues to that of inner mitrochondrial membrane in ATP synthesis

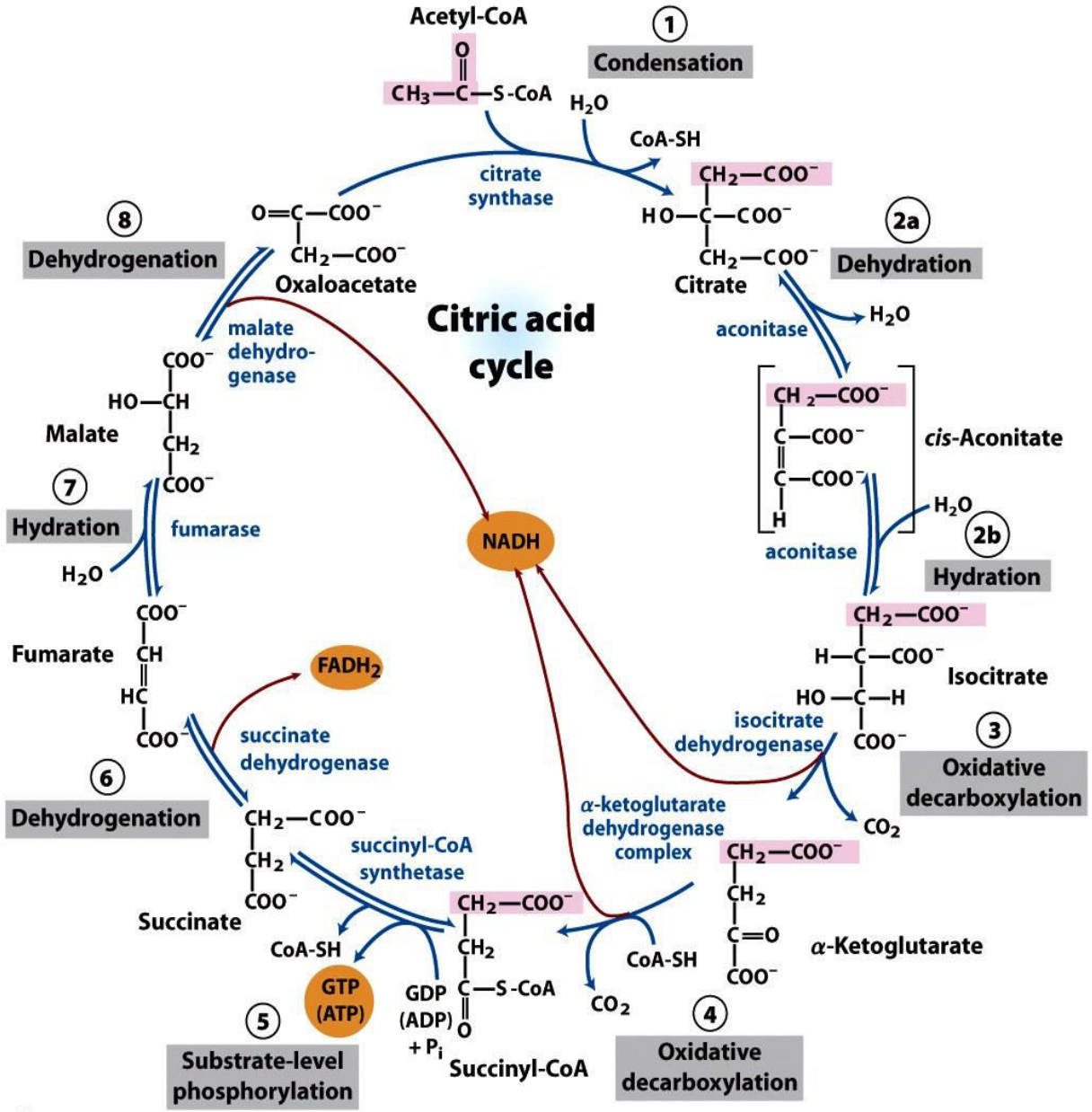

Figure 16-7
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FIGURE 16-7 Reactions of the citric acid cycle. The carbon atoms shaded in pink are those derived from the acetate of acetyl-CoA in the first turn of the cycle; these are *not* the carbons released as CO_2 in the first turn. Note that in **succinate and fumarate**, the two-carbon group derived from acetate can no longer be specifically denoted; because succinate and fumarate are symmetric molecules, C-1 and C-2 are indistinguishable from C-4 and C-3. The red arrows show where energy is conserved by electron transfer to FAD or NAD^+, forming $FADH_2$ or NADH + H^+. Steps 1, 3, and 4 are essentially irreversible in the cell; all other steps are reversible. The product of step 5 may be either ATP or GTP, depending on which succinyl-CoA synthetase isozyme is the catalyst.

Products

- Products of the first turn of the cycle are one GTP (or ATP), three NADH, 1$FADH_2$ and two CO_2.
- Because two acetyl-CoA are produced from each GLUCOSE molecule, two cycles are required per glucose molecule.
- Therefore, at the end of two cycles, the products are: two GTP, six NADH, two $FADH_2$, and four CO_2.

	Substrates	Products	Enzyme	Reaction type	Comment
0 / 10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	irreversible, extends the 4C oxaloacetate to a 6C molecule
1	Citrate	*cis*-Aconitate + H_2O	Aconitase	Dehydration	reversible isomerisation
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3	Isocitrate + NAD^+	Oxalosuccinate + $NADH + H^+$	Isocitrate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)
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5	α-Ketoglutarate + NAD^+ + CoA-SH	Succinyl-CoA + $NADH + H^+$ + CO_2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)

6	Succinyl-CoA + GDP + P_i	Succinate + CoA-SH + GTP	Succinyl-CoA synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP,[12] generates 1 ATP or equivalent Condensation reaction of GDP + P_i and hydrolysis of Succinyl-CoA involve the H_2O needed for balanced equation.
7	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH_2)	Succinate dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→$FADH_2$ in the first step of the reaction) in the enzyme,[12] generates the equivalent of 1.5 ATP
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9	*L*-Malate + NAD^+	Oxaloacetate + NADH + H^+	Malate dehydrogenase	Oxidation	reversible (in fact, equilibrium favors malate), generates NADH (equivalent of 2.5 ATP)
10	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	This is the same as step 0 and restarts the cycle. The reaction is irreversible and extends the 4C oxaloacetate to a 6C molecule

Citric Acid Cycle

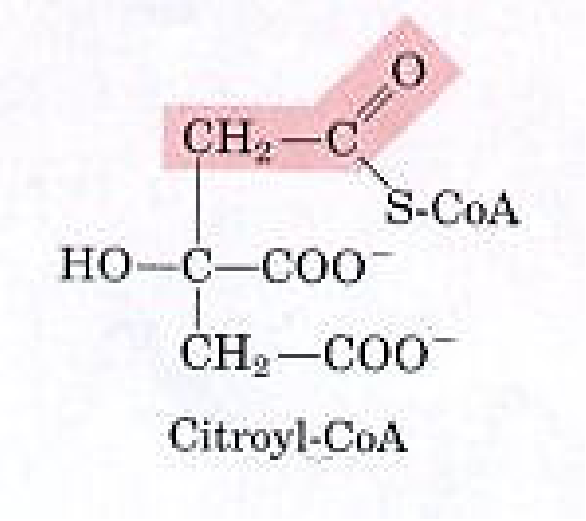
Steps of Citric Acid Cycle-1

1. Formation of Citrate (The Citrate Synthase Reaction)

- The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**
- The methyl carbon of the acetyl group is joined to the carbonyl group

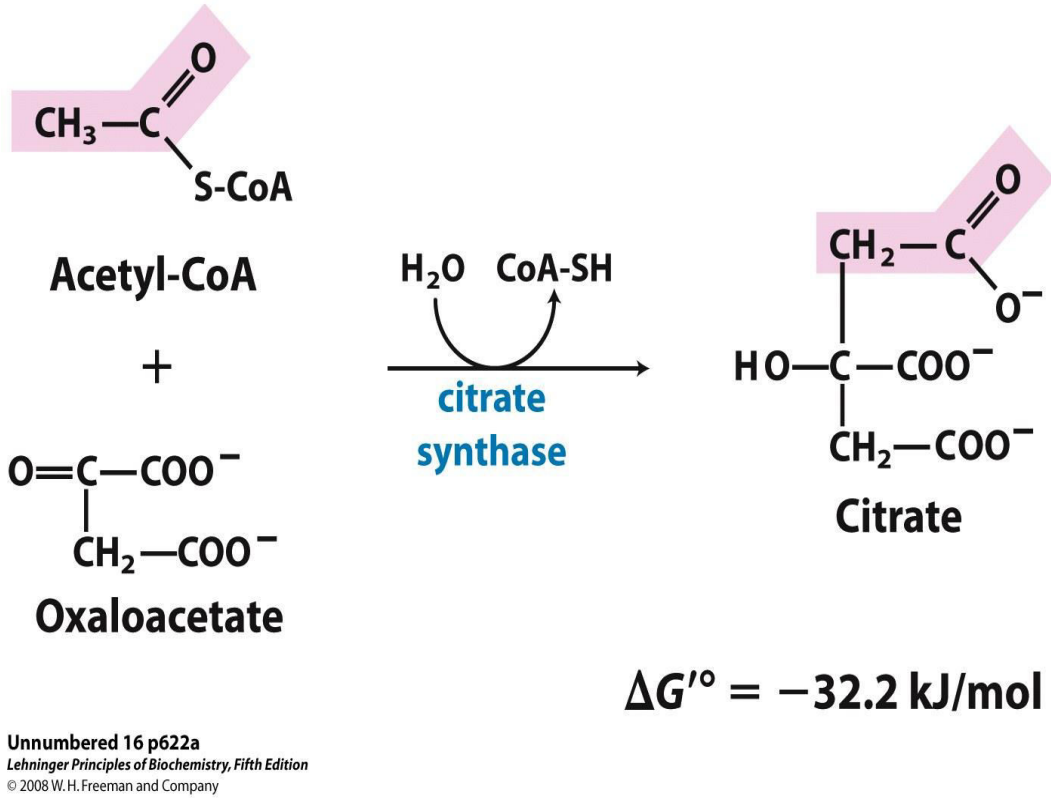
(C-2) of oxaloacetate.

- Citroyl-CoA is a transient intermediate formed on the active site of the enzyme …It rapidly undergoes hydrolysis to release CoA and citrate
- The hydrolysis of this high-energy thioester intermediate makes the forward reaction highly exergonic.
- The CoA **liberated** in this reaction is recycled to participate in the oxidative decarboxylation of another molecule of pyruvate by the PDH complex.



Citroyl-CoA

The Citrate Synthase Reaction



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- Formation of citrate: the condensation of acetyl-CoA with oxaloacetate. -Methyl carbon of acetyl group is joined to the **carbonyl group** (C-2) of OAA
- The hydrolysis of high-energy thioester makes the forward reaction
- Citrate sythase: homodimeric enzyme; induce conformational change by OAA binding, creating binding site for acetyl-CoA; when citroyl- CoA has formed, another conformational change brings about thioester hydorlysis; ordered bisubstrate mechanism

- **The only cycle reaction with C-C bond formation**
- **Essentially irreversible process**

The Citrate Synthase

- Citrate synthase : the homodimeric enzyme

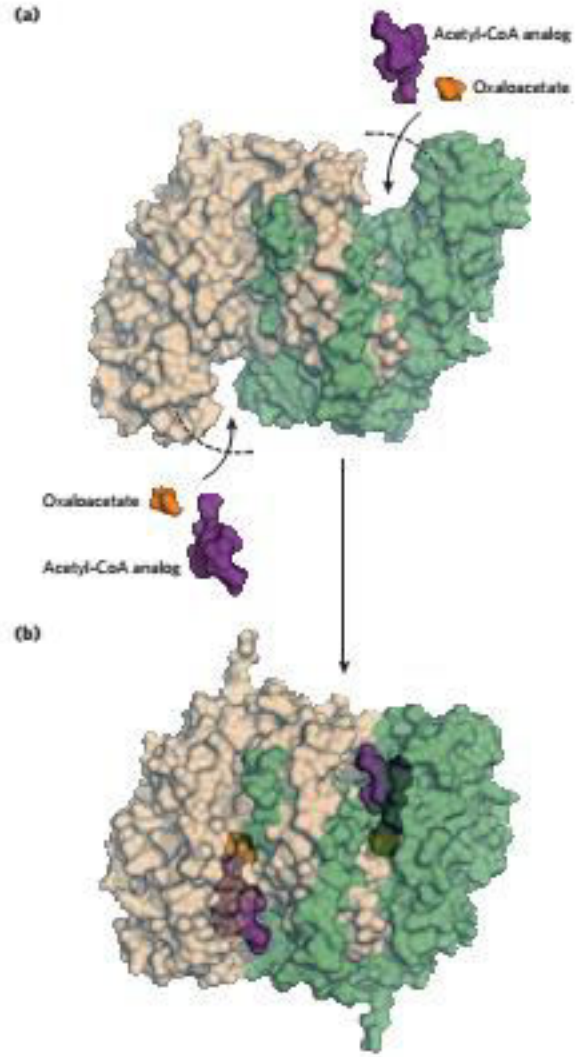
In these representations one subunit is colored tan and one green.

- Each subunit is a single polypeptide **with two domains**, **with the active site between them.**

1. **large and rigid,**
2. **smaller and more flexible**

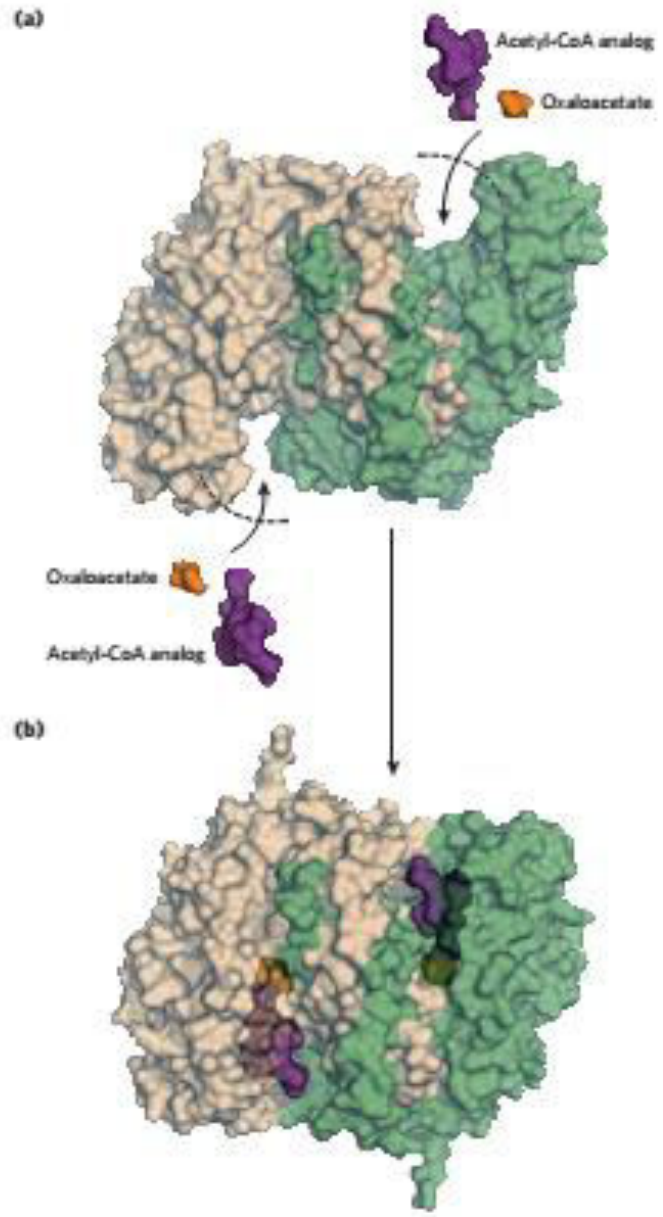
a) Open form of the enzyme alone
b) closed form with bound oxaloacetate and a stable analog of acetyl-CoA

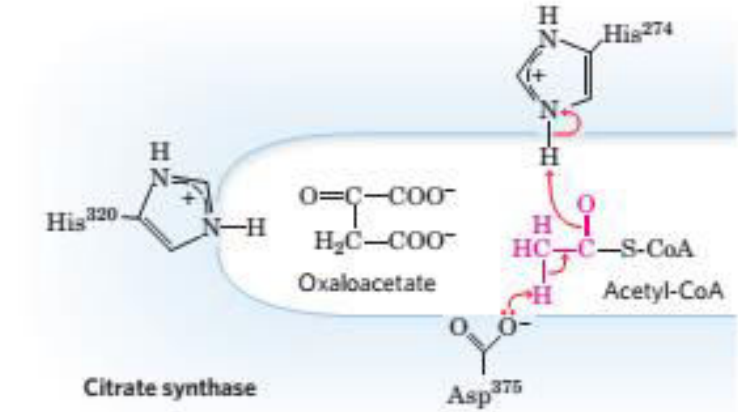
- The flexible domain of each subunit undergoes a large conformational change on binding oxaloacetate, creating a binding site for acetyl-CoA.



Structure of Citrate Synthase

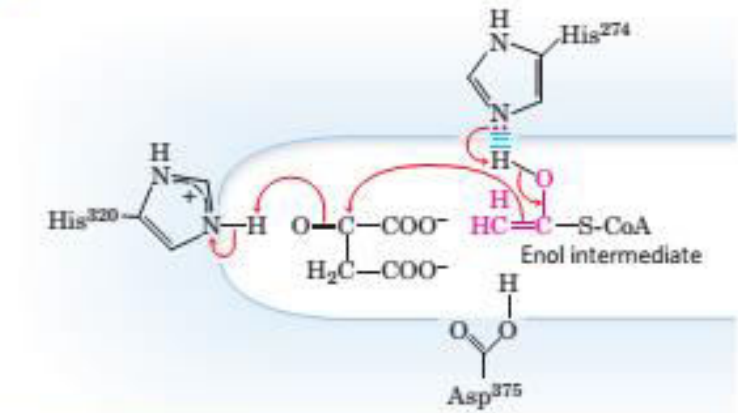
- **Ordered bisubstrate mechanism of Citrate synthase.**
- In the mammalian citrate synthase reaction, oxaloacetate binds first, in a strictly **ordered reaction sequence**.
- This binding triggers a conformation change that opens up the binding site for acetyl-CoA.
- Oxaloacetetate is specifically oriented in the active site of citrate synthase by interaction of its two carboxylates with two positively charged Arg residues





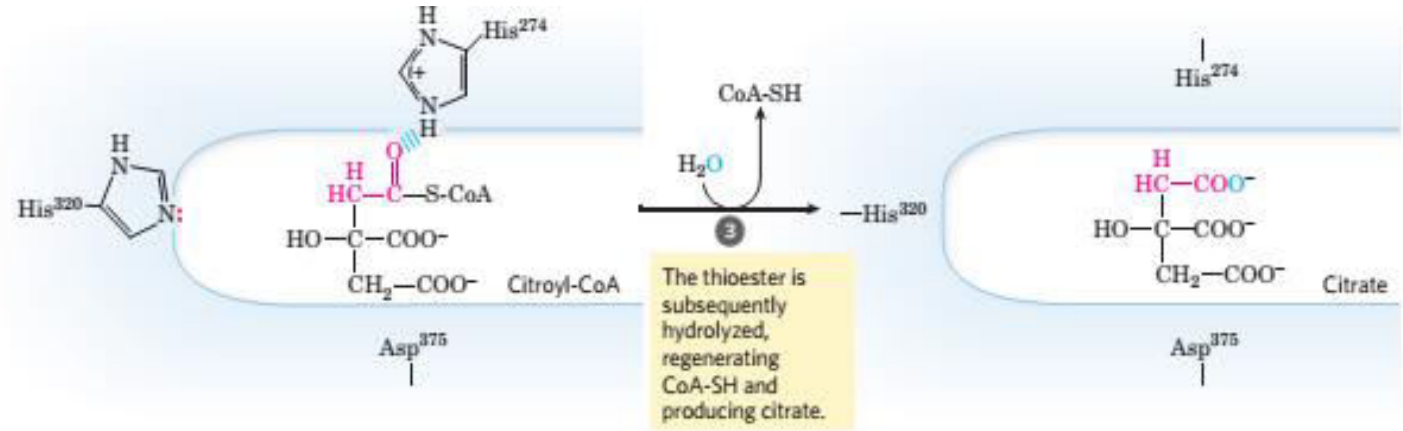
The thioester linkage in acetyl-CoA activates the methyl hydrogens. Asp^{375} abstracts a proton from the methyl group, forming an enolate intermediate. The intermediate is stabilized by hydrogen bonding to and/or protonation by His^{274} (full protonation is shown).

1



The enol(ate) rearranges to attack the carbonyl carbon of oxaloacetate, with His^{274} positioned to abstract the proton it had previously donated. His^{320} acts as a general acid. The resulting condensation generates citroyl-CoA.

2



The thioester is subsequently hydrolyzed, regenerating CoA-SH and producing citrate.

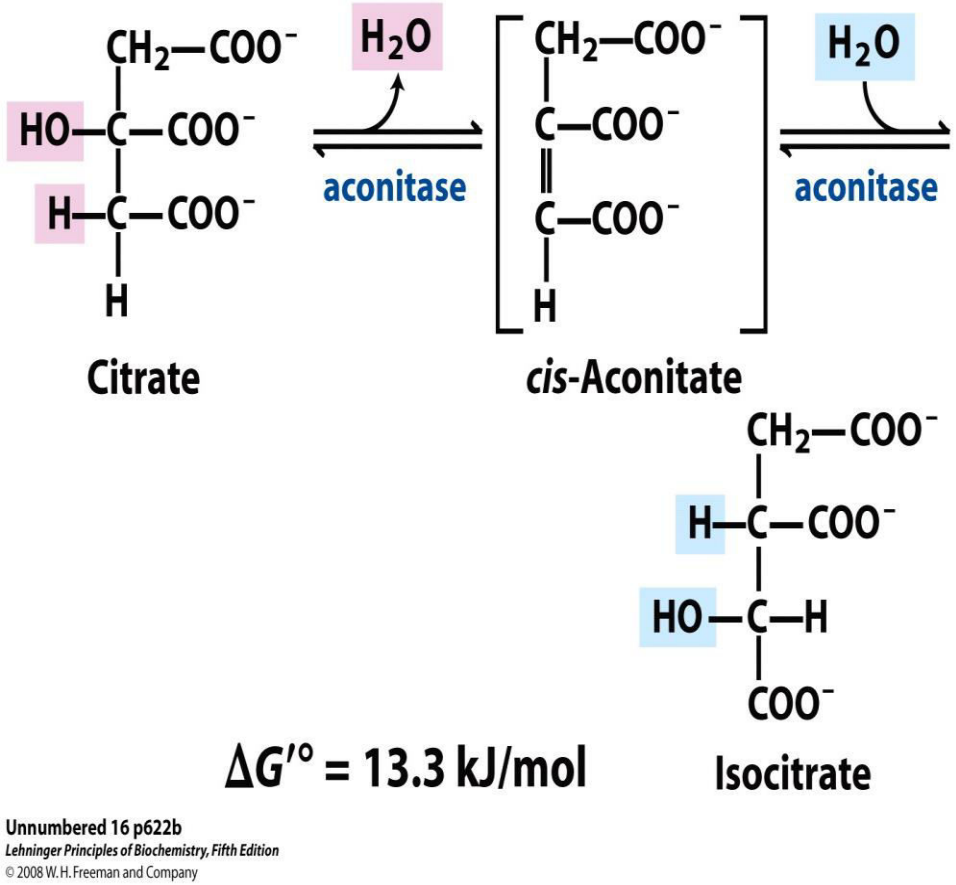
- **Ordered bisubstrate mechanism of Citrate synthase.**
- **Oxaloacetate, the first substrate** to bind to the enzyme, induces a large conformational change in the flexible domain, creating a binding site for the **second substrate, acetyl-CoA**.
- When citroyl-CoA has formed in the enzyme active site..Another conformational change brings about thioester hydrolysis, releasing CoA-SH.
  This induced fit of the enzyme first to its substrate and then to its reaction intermediate decreases the likelihood of premature and unproductive cleavage of the thioester bond of acetyl-CoA.

Citric Acid Cycle

Steps of Citric Acid Cycle-2

2. Isomerization of Citrate by Aconitase

- The enzyme **aconitase** (**aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**
- Reaction Intermediate of the tricarboxylic acid is formed..known as ***cis*-aconitate**
- ***cis*-aconitate does not dissociate from the active site**
- **Aconitase can promote the reversible addition of H2O to the double bond** of enzyme-bound *cis*-aconitate in two different ways,
  one leading to citrate and the other to isocitrate
- Under physiological conditions in the cell.. Conc. Of isocitrate is very little , the reaction is pulled to the right because isocitrate is rapidly consumed in the next step of the cycle



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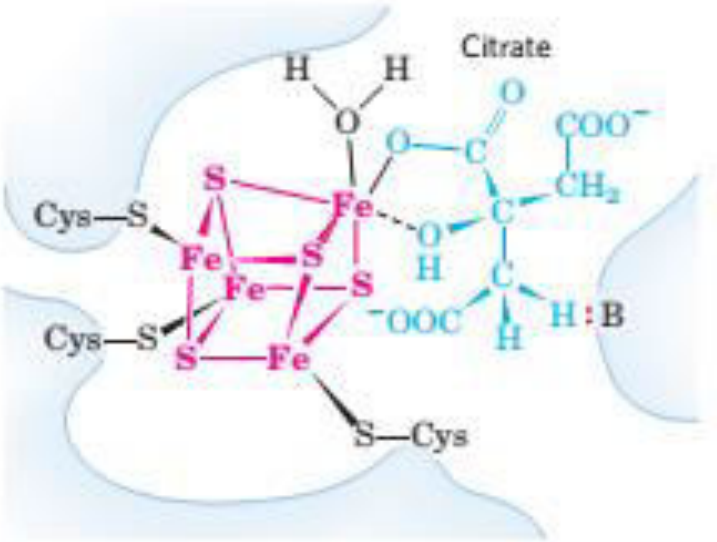
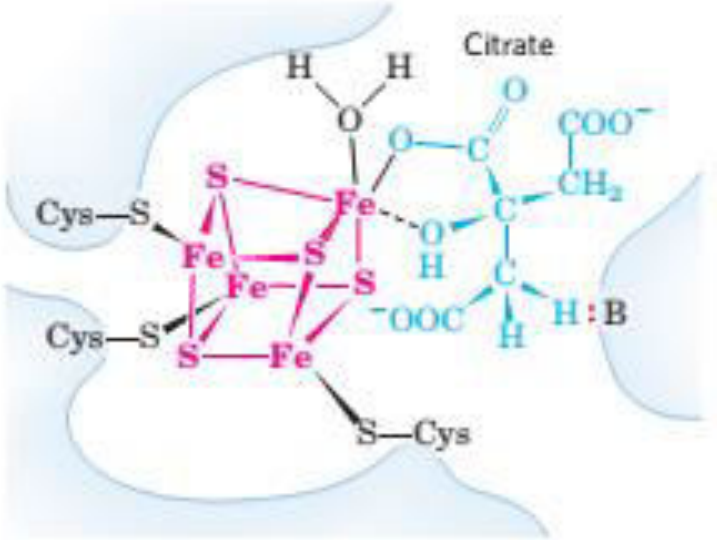
..cont

- Aconitase contains an **iron sulfur center** which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H2O.
- In iron-depleted cells, aconitase loses its iron-sulfur center and acquires a new role in the regulation of iron homeostasis.
- **Aconitase is one of many enzymes known to “moonlight” in a second role (protein with 2 jobs)**



..cont

- **Iron-sulfur center in aconitase.**
- The iron-sulfur center is in red, the citrate molecule in blue. **Three Cys residues of the enzyme** bind three iron atoms
- the fourth iron is bound to one of the carboxyl groups of citrate and also interacts noncovalently with a hydroxyl group of citrate (dashed bond).
- A basic residue (**:**B) in the enzyme helps to position the citrate in the active site.
- The iron-sulfur center acts in both substrate binding and catalytic site.



3. The Isocitrate Dehydrogenase Reaction

- Oxidation of Isocitrate to *α*-Ketoglutarate and CO2 In the next step
- **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form ***α*-ketoglutarate**
- **Mn2+ in the active site interacts with the carbonyl group of the intermediate** oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to *α*-ketoglutarate.
- Mn2+ also stabilizes the enol formed transiently by decarboxylation.

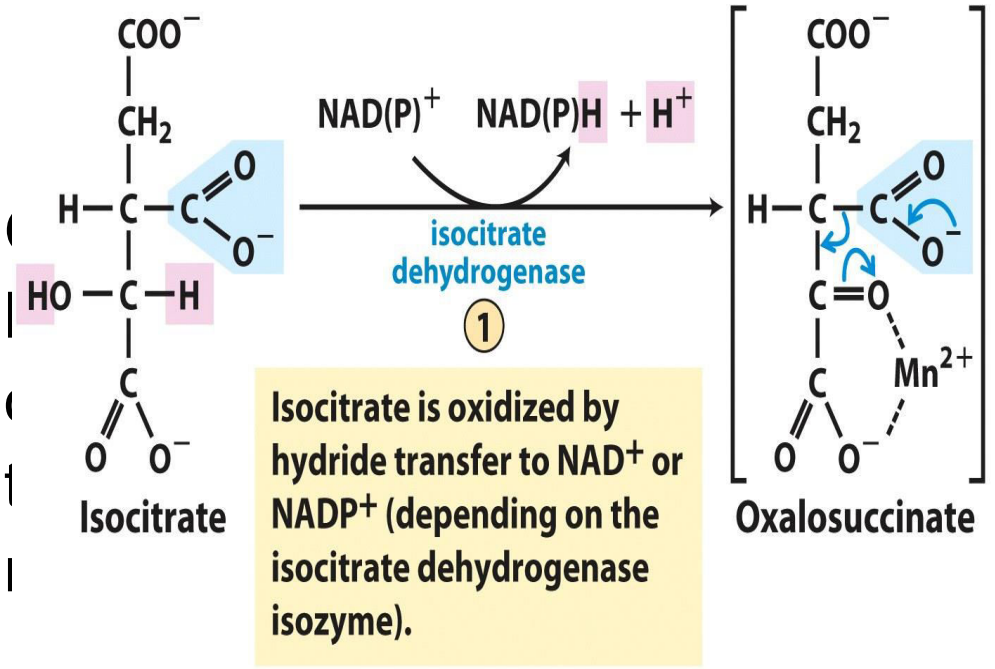


Figure 16-11 part 1
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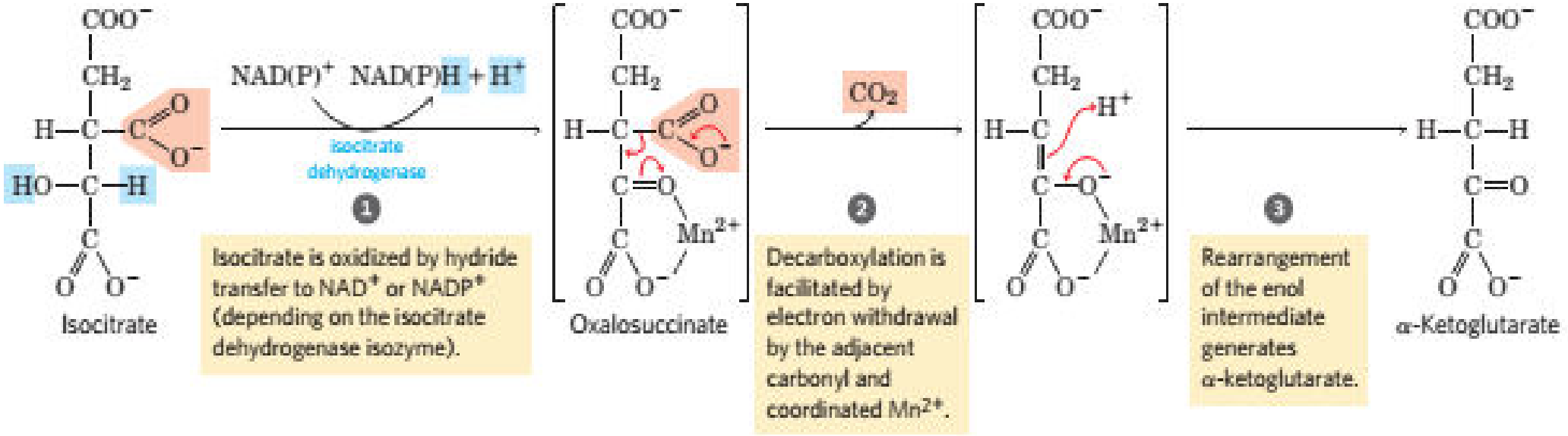


- **Isocitrate dehydrogenase reaction.**
- **In this reaction, the substrate, isocitrate, loses one carbon by oxidative decarboxylation**

Two different forms of isocitrate dehydrogenase (Isozymes)

- There are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD+ as electron acceptor and the other requiring NADP+.
- The overall reactions are otherwise identical
- In eukaryotic cells, the NAD-dependent enzyme occurs in the mitochondrial matrix and serves in the citric acid cycle.
- The main function of the NADP-dependent enzyme, found in both the mitochondrial matrix and the cytosol, may be the generation of NADPH, which is essential for reductive anabolic reactions

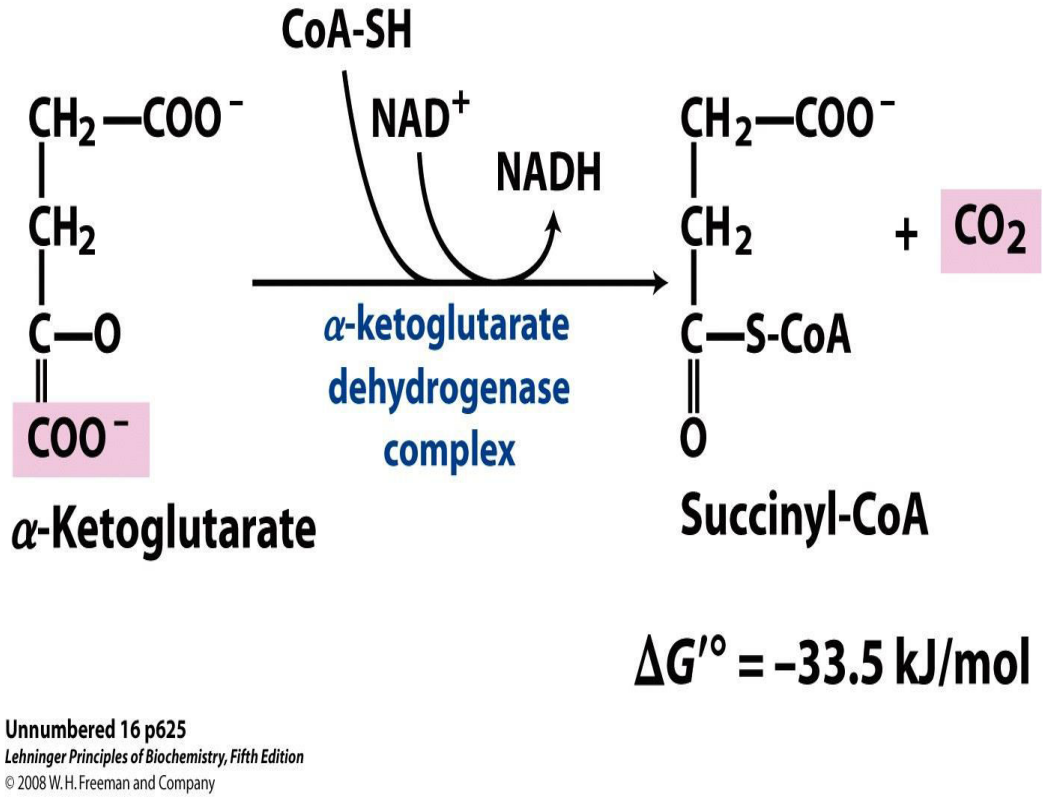
Citric Acid Cycle

Steps of Citric Acid Cycle-3

4. Oxidation of α-ketoglutarate

4. Oxidation of α-ketoglutarate

- **The next step is another oxidative decarboxylation**
- Oxidation of *α*-Ketoglutarate to Succinyl-CoA and CO2
- a-ketoglutarate is converted to **succinyl-CoA** and CO2 by the action of the **a-ketoglutarate dehydrogenase complex**
- NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.
- The energy of oxidation of *α*-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA
- This reaction is somehow identical to the pyruvate dehydrogenase reaction and to the reaction sequence responsible for the breakdown of branched chain amino acids



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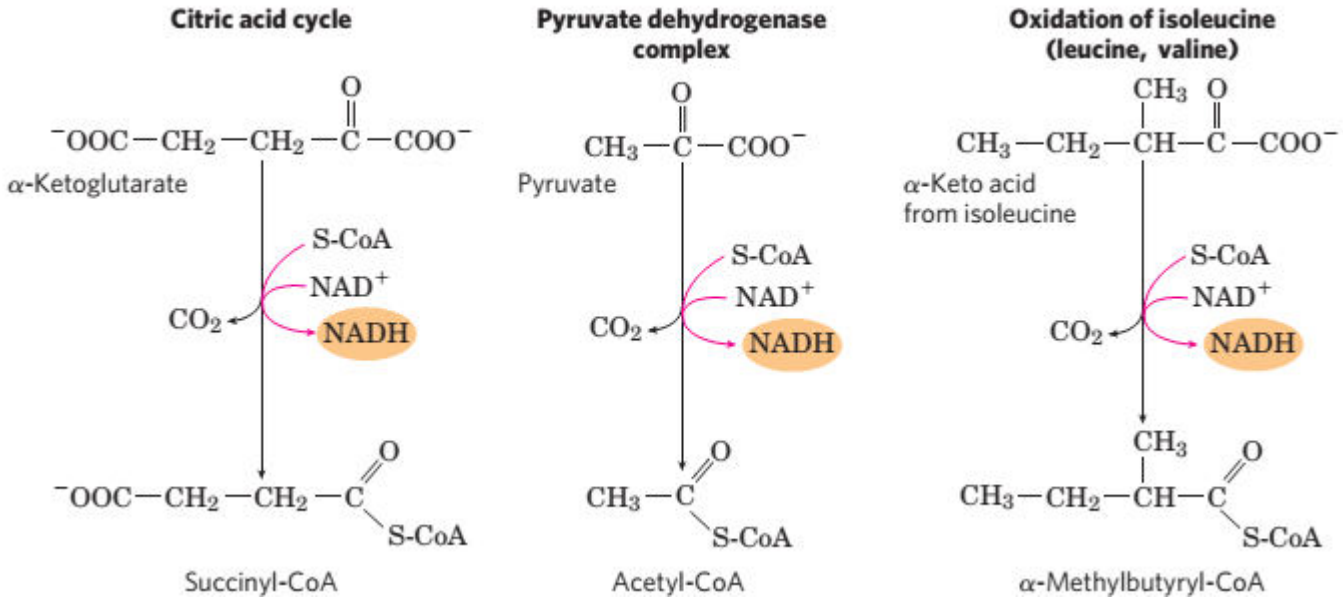
a-ketoglutarate dehydrogenase complex resembles PDH complex

- The a-ketoglutarate dehydrogenase complex closely resembles the PDH complex in both structure and function.
- It includes three enzymes, homologous to E1, E2, and E3 of the PDH complex, as well as enzyme-bound TPP, bound lipoate, FAD, NAD, and coenzyme A.
- Both complexes are certainly derived from a common evolutionary ancestor.
- **Although the E1 components of the two complexes are structurally similar, their amino acid sequences differ and, they have different binding specificities**
- E1 of the PDH complex binds pyruvate, and E1 of the *α*-ketoglutarate dehydrogenase complex binds *α*-ketoglutarate.
- The E2 components of the two complexes are also very similar, both having covalently bound lipoyl moieties.
- The subunits of E3 are identical in the two enzyme complexes.
- The enzymatic complex that degrades branched-chain *α*-keto acids catalyzes the same reaction sequence using the **same five cofactors**.
- This is a clear case of **divergent evolution**, in which the genes for an enzyme with one substrate specificity give rise, during evolution, to closely related enzymes with different substrate specificities but the same enzymatic mechanism.

A conserved mechanism for oxidative decarboxylation

- The pathways shown use the same five cofactors (thiamine pyrophosphate, coenzyme A, lipoate, FAD, and NAD+)
- closely similar multienzyme complexes, and the same enzymatic mechanism to carry out oxidative decarboxylations of pyruvate (by the pyruvate dehydrogenase complex) a-ketoglutarate (in the citric acid cycle) and the carbon skeletons of the three branched-chain amino acids, isoleucine leucine, and valine



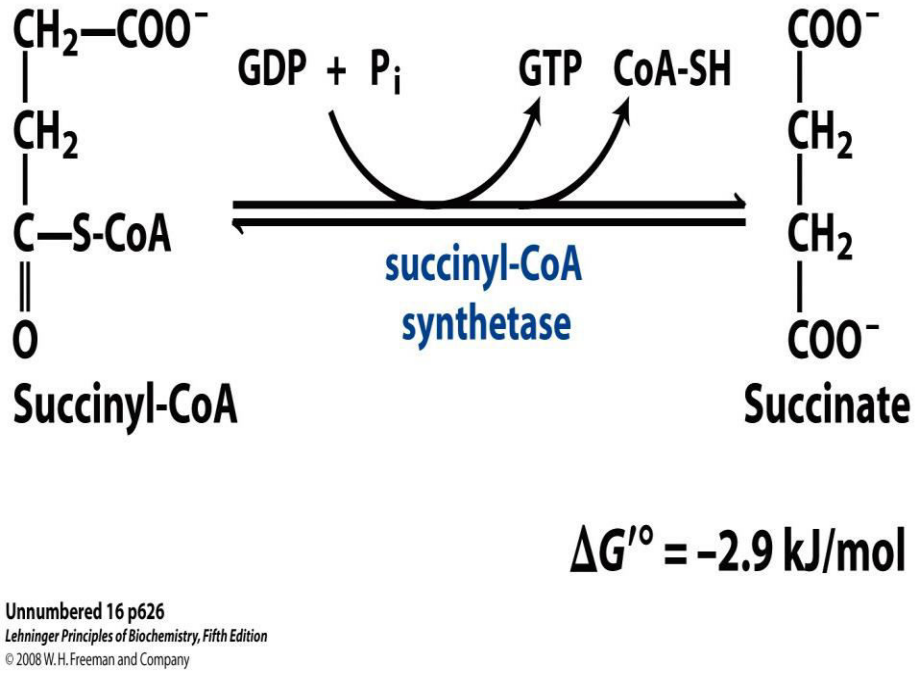
Citric Acid Cycle

Steps of Citric Acid Cycle-4

5. Conversion of Succinyl-CoA to Succinate

5. Conversion of Succinyl-CoA to Succinate

- **Succinyl-CoA** like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis (*G*= -36 kJ/mol).
- Energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net *G*= - 2.9 kJ/mol.
- The enzyme that catalyzes this reversible reaction is called **succinyl-CoA synthetase** or **succinic thiokinase**, names indicate the participation of a nucleoside triphosphate in the reaction



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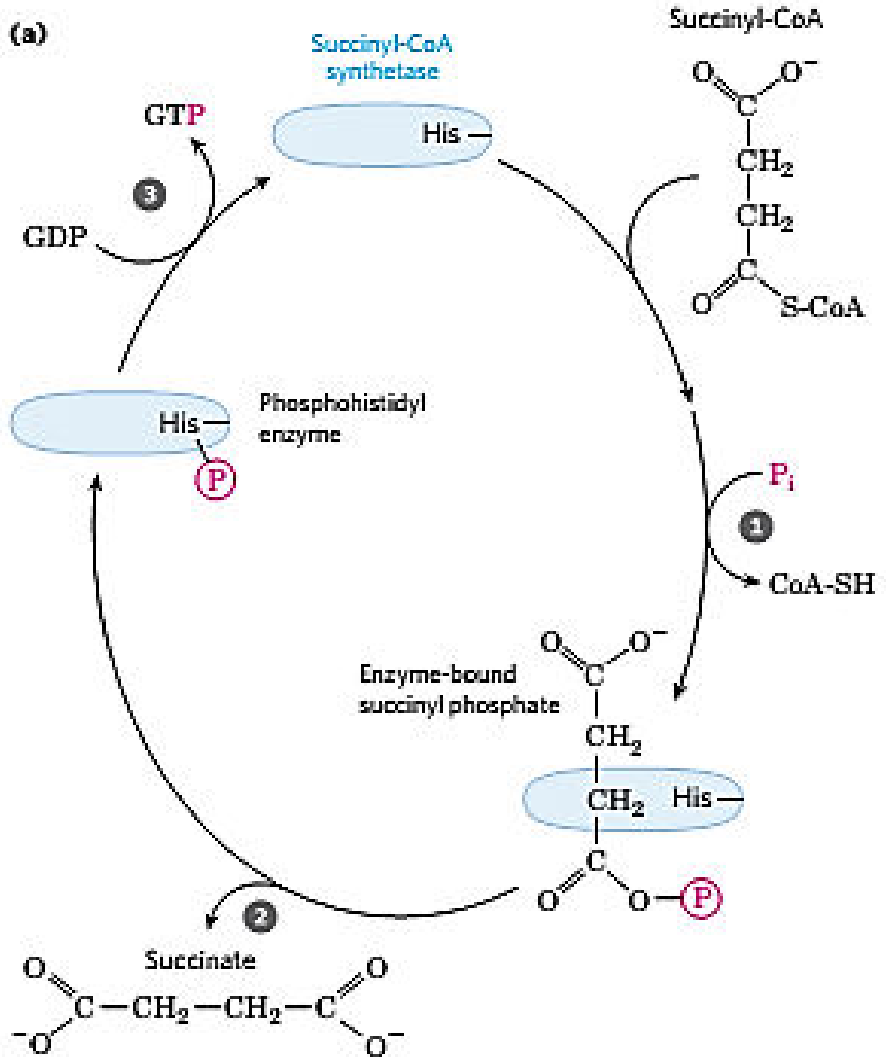
The succinyl-CoA synthetase reaction.

(a) In step 1 a phosphoryl group replaces the CoA of succinyl-CoA bound to the enzyme, forming a high-energy acyl phosphate.

(b) In step 2 the succinyl phosphate donates its phosphoryl group to a His residue of the enzyme, forming a high-energy phosphohistidyl enzyme.

(c) In step 3 the phosphoryl group is transferred from the His residue to the terminal phosphate of GDP (or ADP), forming GTP (or ATP).

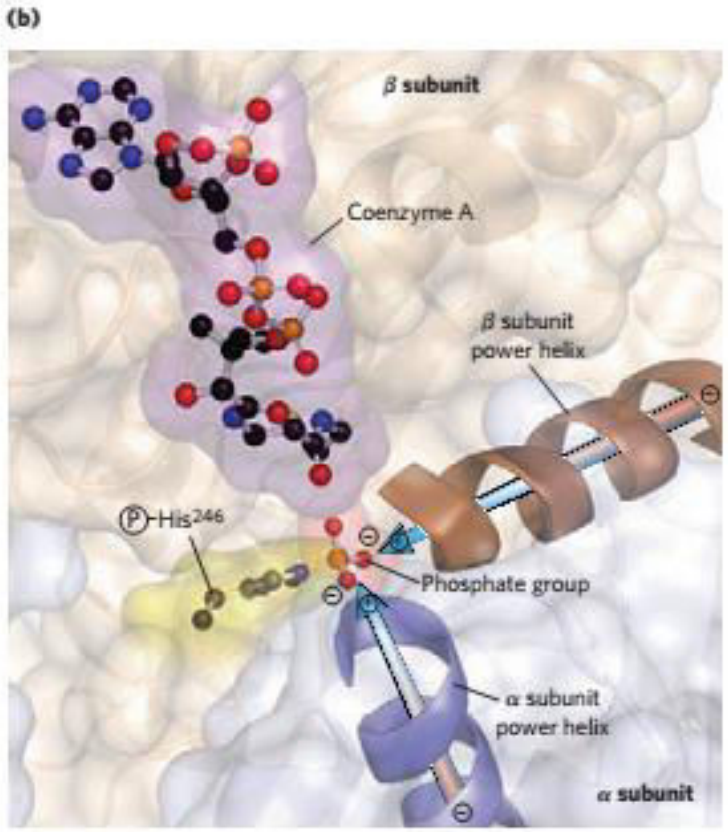


Nucleoside diphosphate kinase reaction:

The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, reversible reaction catalyzed by **Nucleoside diphosphate kinase reaction**

..cont

- The enzyme has two subunits,
  a subunit: **which has the P –His residue (His246) and the binding site for CoA**
- ***B subunit:*** **confers specificity for either ADP or GDP.**
- The active site is at the interface between subunits. The crystal structure of succinyl-CoA synthetase reveals two "power helices" (one from each subunit), oriented so that their electric dipoles situate partial positive charges close to the negatively charged P –His (Fig. 16–13b), stabilizing the phosphoenzyme intermediate.



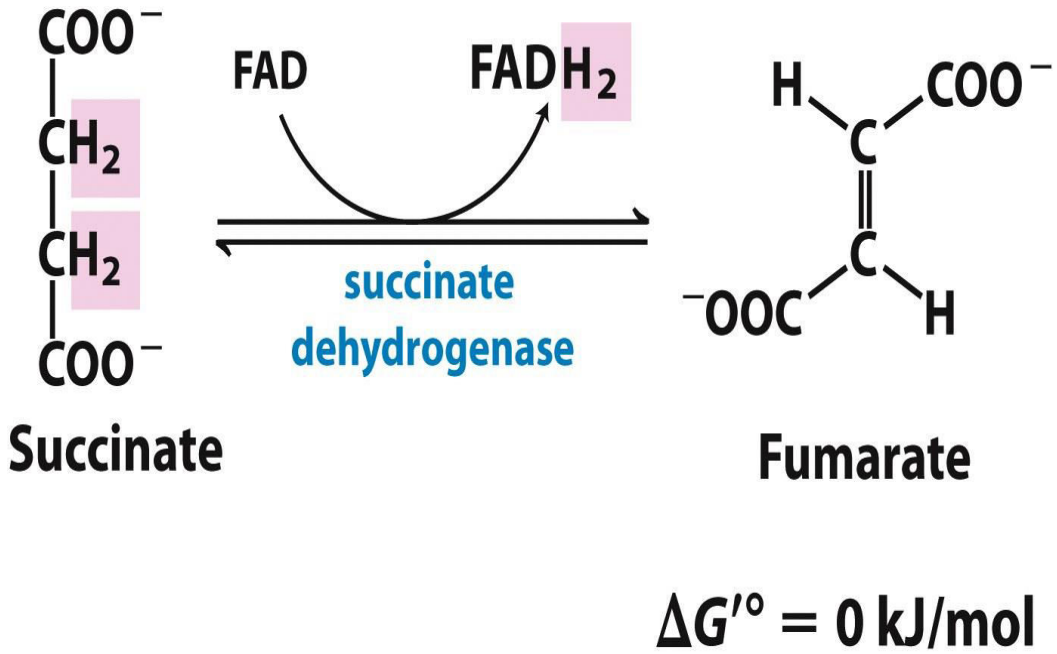
Citric Acid Cycle

Steps of Citric Acid Cycle-5

6. Oxidation of Succinate to Fumarate

6. Oxidation of Succinate to Fumarate

- **Succinate Dehydrogenase reaction**
- The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**
- In eukaryotes, succinate dehydrogenase is tightly bound to the mitochondrial inner membrane; in bacteria, to the plasma membrane.
- **The enzyme contains three different iron-sulfur clusters and one molecule of covalently bound FAD**
- Electron flow from succinate through these carriers to the final electron acceptor, O2, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).



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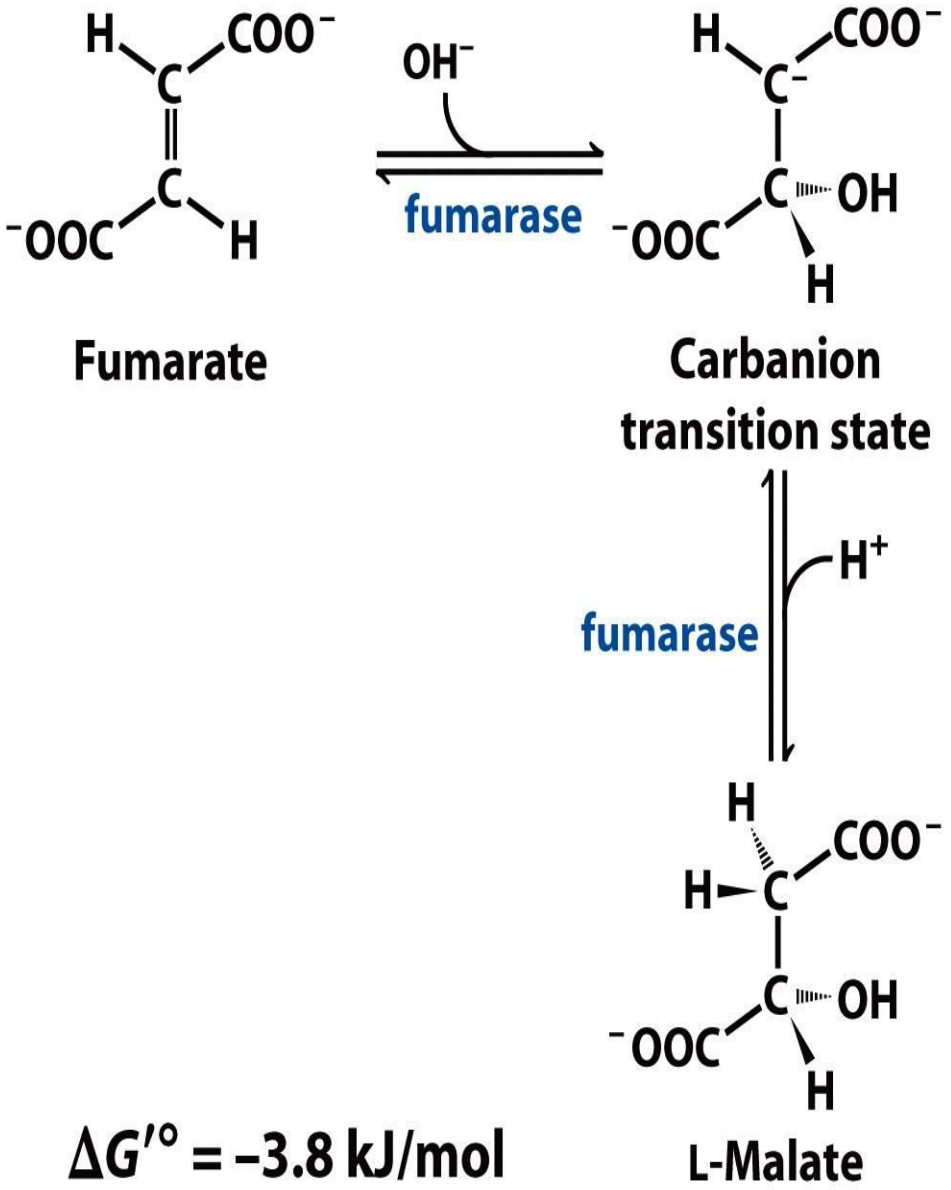
7. Hydration of Fumarate to Malate

- The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase** (**fumarate hydratase**).
- The transition state in this reaction is a carbanion
- This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate).
- In the reverse direction (from L-malate to fumarate),
  fumarase is equally stereospecific: D-malate is not a substrate.



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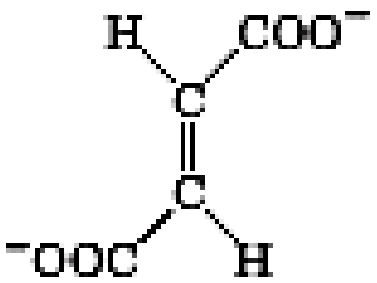
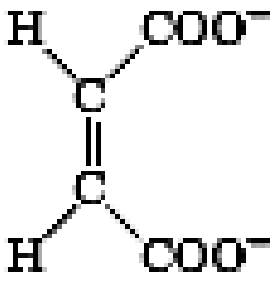
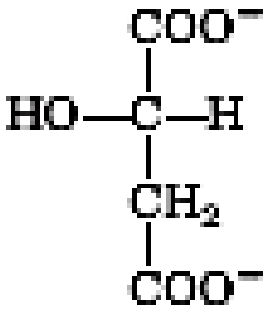


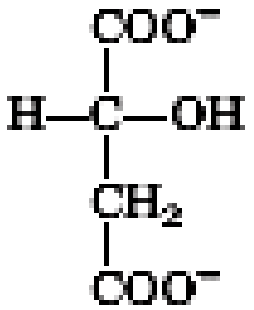
Fumarate



Maleate



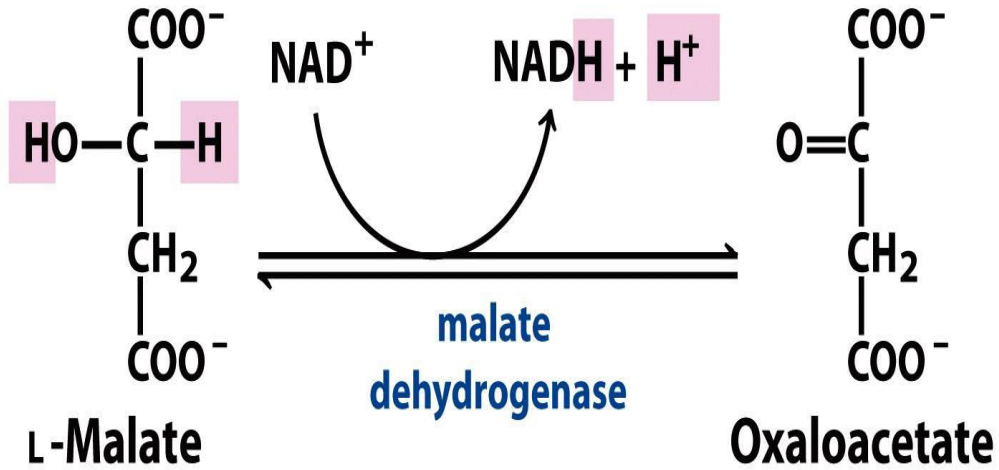
L-Malate



D-Malate

8. Oxidation of Malate to Oxaloacetate

- Oxidation of Malate to Oxaloacetate Is the last reaction of the citric acid cycle
- NAD-linked **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate
- In intact cells oxaloacetate is continually removed by the **highly exergonic citrate synthase** reaction
- This keeps the concentration of oxaloacetate in the cell remains extremely low pulling **the malate dehydrogenase** reaction toward the formation of oxaloacetate.



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Interesting facts

- The individual reactions of the citric acid cycle were initially worked out in vitro, using minced muscle tissue
- Later, the pathway and its regulation were studied in vivo.
- By using radioactively labeled precursors such as [14C] pyruvate and [14C]acetate
- Carbon-14, $^{14}\mathbf{C}$, or radiocarbon, is a radioactive **isotope** of carbon with an atomic nucleus containing 6 protons and 8 neutrons.
- Researchers have traced the fate of individual carbon atoms through the citric acid cycle.

Direct and Indirect ATP Yield

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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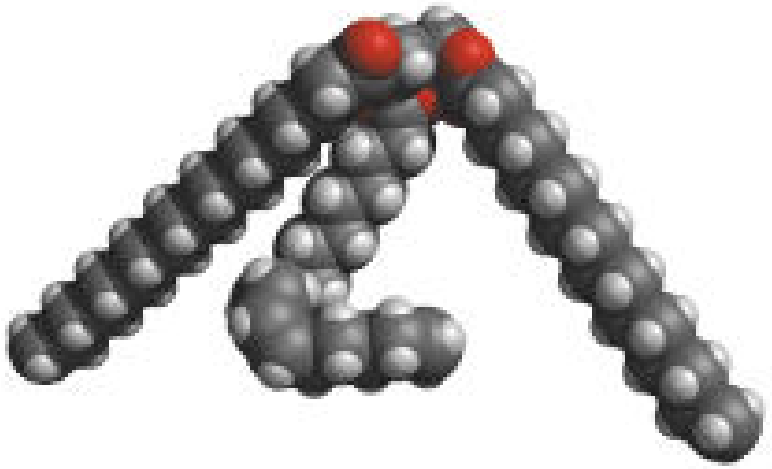
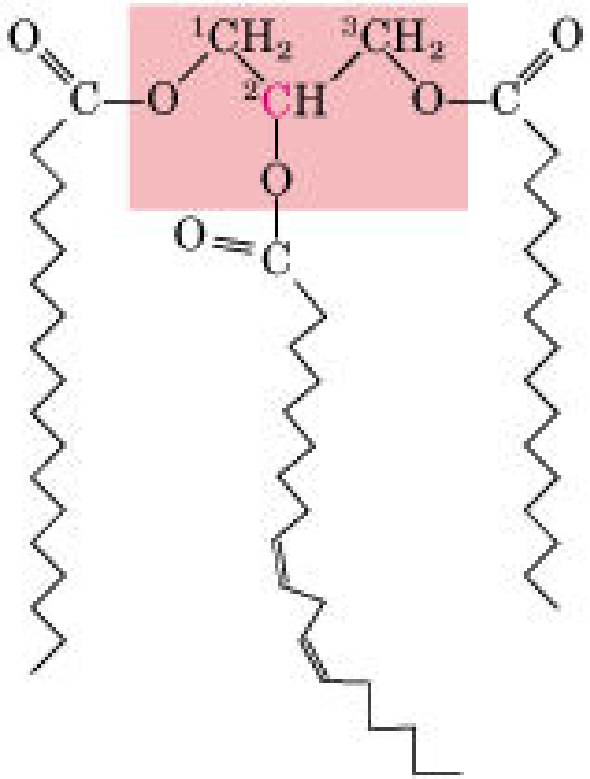
Lecture No. 104

Fatty Acid Catabolism





1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol,
a mixed triacylglycerol

Triacylglycerols
(fats or triglycerides)

Three fatty acids
ester-linked to a
glycerol molecule

Used as energy storage
molecules in eukaryotes

Stored in adipocytes

Advantages of using triacylglycerols for energy storage

1. Fats are highly reduced hydrocarbons with a large energy of oxidation.
2. Fats are insoluble molecules that aggregate into droplets. They are unsolvated and no storage mass is water.
3. Fats are chemically inert. They can be stored without fear of unfavorable reactions.

Disadvantages of triacylglycerols as energy storage

1. Fats must be emulsified before enzymes can digest them.
2. Fats are insoluble in the blood and must be carried in the blood as protein complexes.

Extraction of energy from fatty acids

Step 1. Oxidation of fatty acids to acetyl-CoA. This generates NADH and $FADH_2$.

Step 2. Oxidation of acetyl-CoA to CO_2 in the citric acid cycle. This generates NADH, $FADH_2$ and GTP (ATP).

Step 3. Transfer of electrons from NADH and $FADH_2$ to O_2. This results in the synthesis of ATP.

Citric Acid Cycle

Anaplerotic reactions

Net Effect of the Citric Acid Cycle

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2\,H_2O \longrightarrow$$

$$\longrightarrow 2CO_2 + 3NADH + FADH_2 + GTP + CoA + 3H^+$$

- Electrons from this process reduce NAD^+ and FAD
- One GTP is formed per cycle, this can be converted to ATP
- Intermediates in the cycle are not depleted

Anaplerotic reactions

- **This pathway is utilized for the both catabolic reactions to generate energy as well as for anabolic reactions to generate metabolic intermediates for biosynthesis.**
- As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by **anaplerotic reactions**

- **Anaplerotic reactions :**
  These are chemical **reactions** that form intermediates of a metabolic pathway.

Anaplerotic reactions

- Most common anaplerotic reactions convert either **pyruvate or phosphoenolpyruvate to oxaloacetate or malate.**
- The most important anaplerotic reaction in mammalian liver and kidney is the reversible **carboxylation of pyruvate by CO2 to form oxaloacetate**, catalyzed by **pyruvate carboxylase**.
- When the citric acid cycle is deficient in oxaloacetate or any other intermediates, pyruvate is carboxylated to produce more oxaloacetate.
- The enzymatic addition of a carboxyl group to pyruvate requires energy, supplied by ATP—required to attach a carboxyl group to pyruvate is about equal to the free energy available from ATP.

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

Table 16-2

Lehninger Principles of Biochemistry, Fifth Edition

.. cont

- **Pyruvate carboxylase** is inactive in the absence of acetyl-CoA.
- Whenever acetyl-CoA, the fuel for the citric acid cycle, is present in excess, it stimulates the pyruvate carboxylase reaction to produce more oxaloacetate, enabling the cycle to use more acetylCoA in the citrate synthase reaction
- **Phosphoenolpyruvate (PEP) carboxylase** activated by the glycolytic intermediate fructose 1,6-bisphosphate, which accumulates when the citric acid cycle operates too slowly to process the pyruvate generated by glycolysis.

Role of the Citric Acid Cycle in Anabolism

- **Role of the citric acid cycle in anabolism.**
- Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways.
- Pathways that replenish depleted cycle intermediates are shown in red (four anaplerotic reactions)

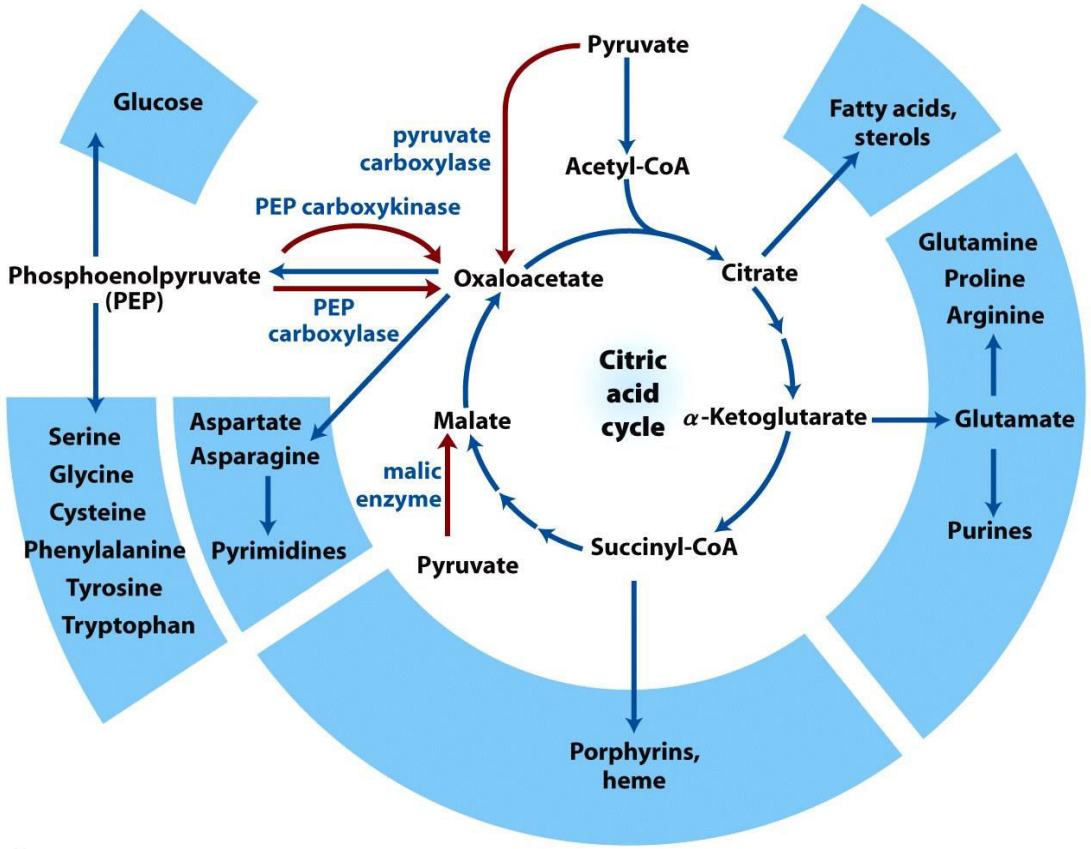


Figure 16-15
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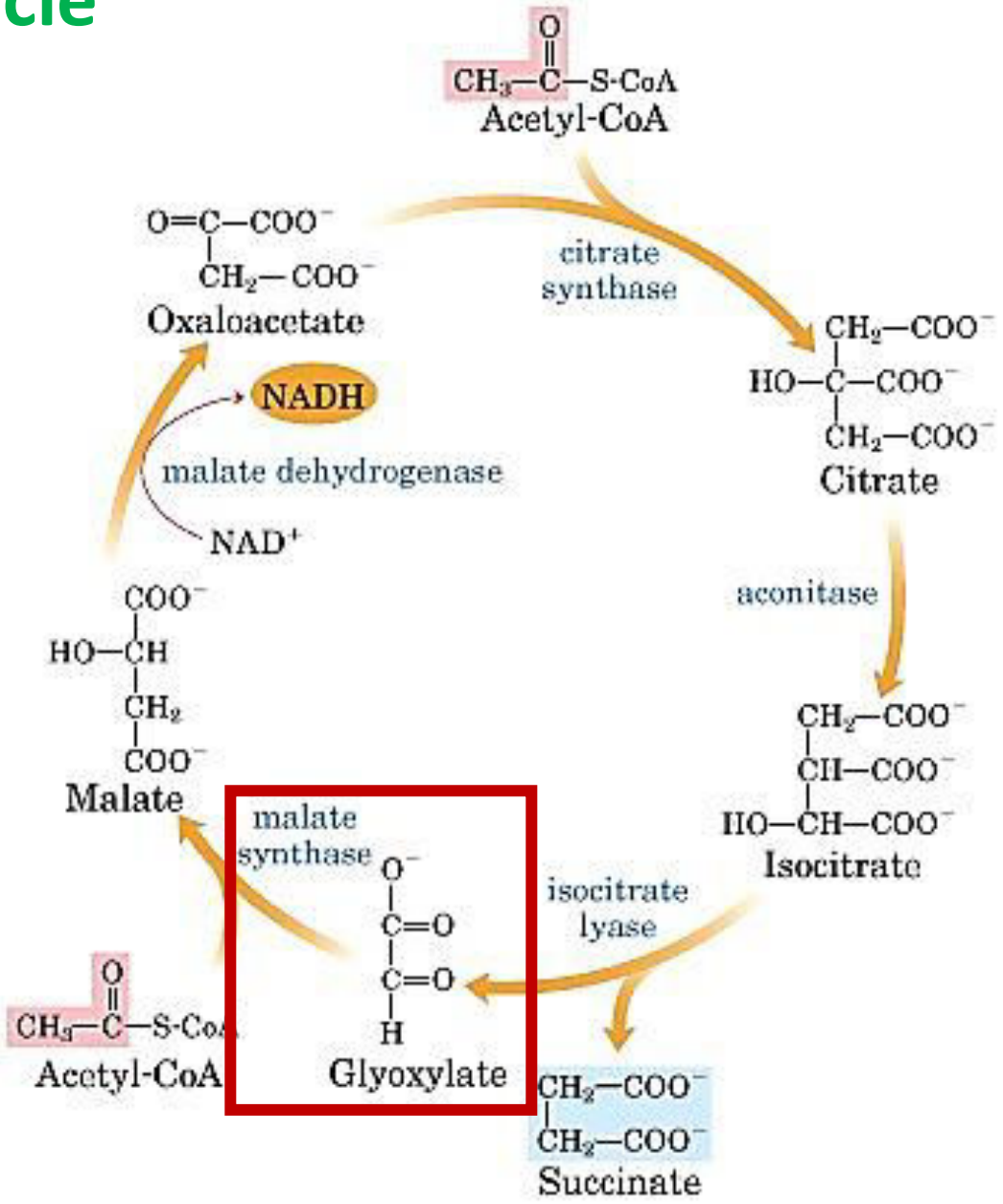
Citric Acid Cycle

Glyoxylate cycle

Glyoxylate cycle

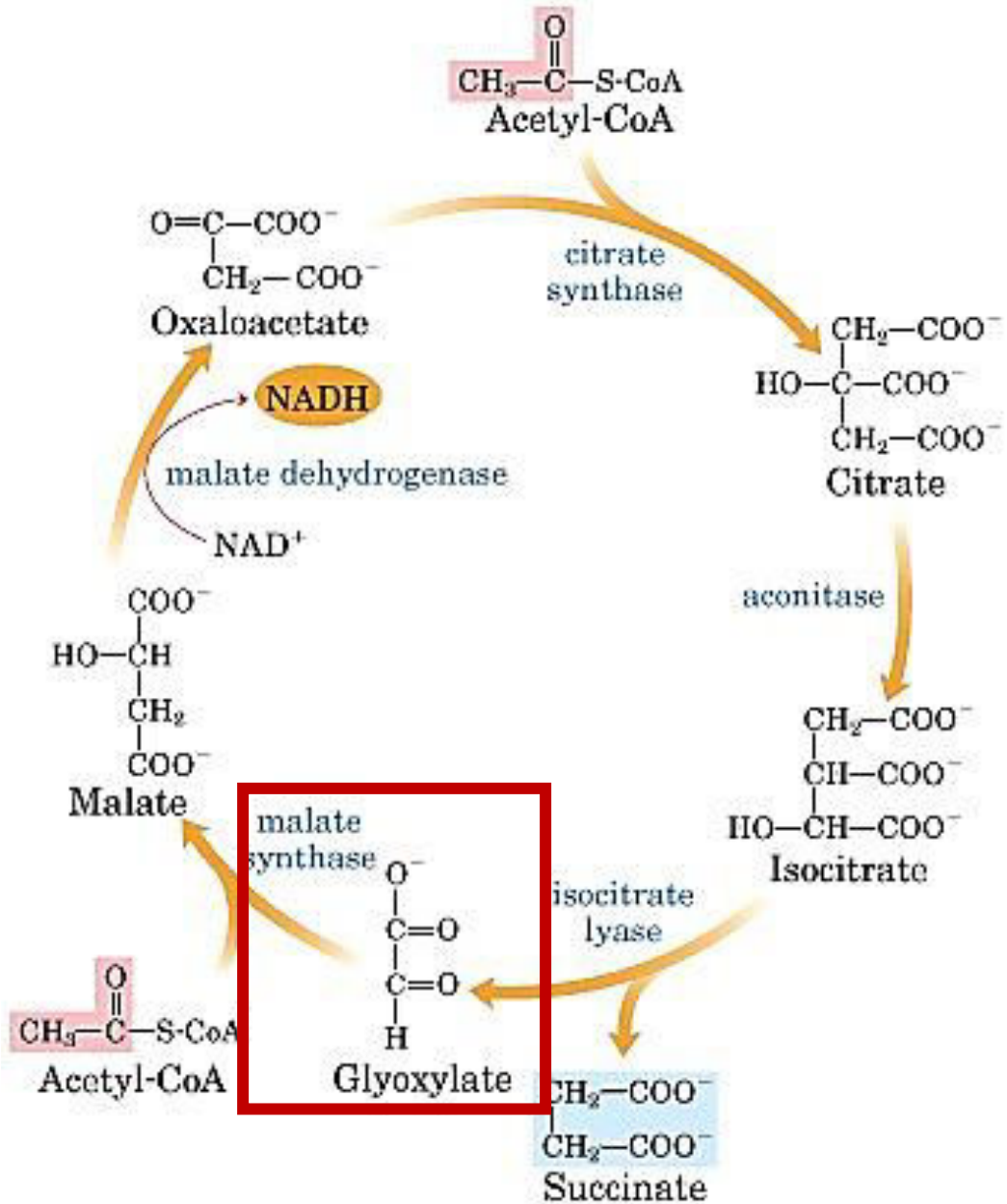
- The **glyoxylate cycle**, a variation of the tricarboxylic acid **cycle**, is an anabolic pathway occurring in plants, bacteria, protists, and fungi.
- The **glyoxylate cycle** relies on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates.
- In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available
- Discovered by H. Kornberg and Neil Madsen in the lab. Of Hans Krebs.
- Gets name from product “glyoxylate”

Glyoxylate cycle



Glyoxylate cycle

The two cycles differ in that **in the glyoxylate cycle, isocitrate is converted into glyoxylate and succinate by ICL** instead of into α-ketoglutarate.



Similarities with TCA cycle

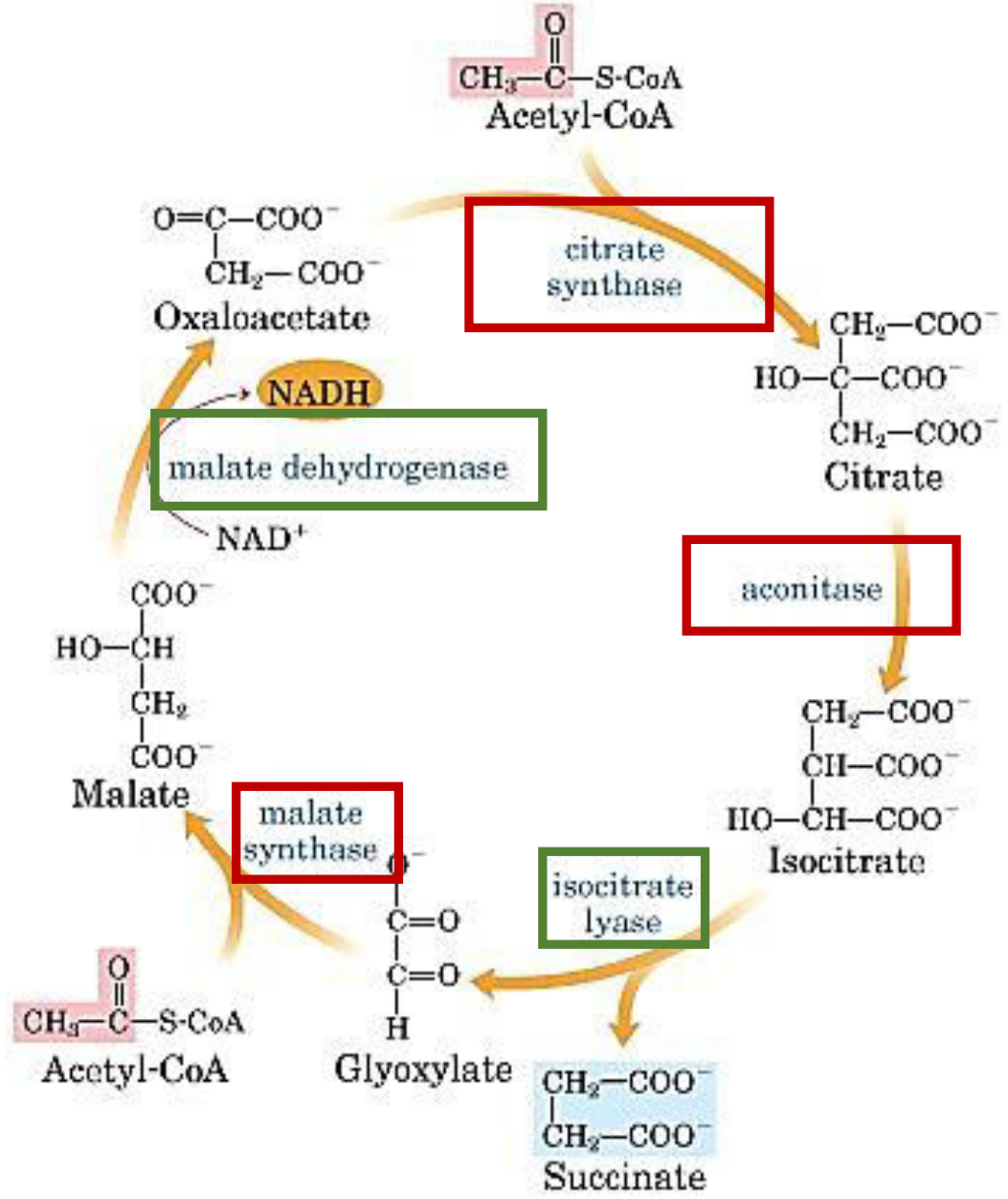
- The glyoxylate cycle utilizes five of the eight enzymes associated with **the citric acid cycle:**

1. citrate synthase
2. Aconitase
3. Succinate dehydrogenase
4. Fumarase
5. malate dehydrogenase.

Glyoxylate cycle

1. The citrate synthase, aconitase, and malate dehydrogenase of the glyoxylate cycle are isozymes of the citric acid cycle enzymes

2. **Isocitrate lyase and malate synthase are unique to the glyoxylate cycle.**
3. Notice that two acetyl groups (light red) enter the cycle and four carbons leave as succinate (blue).



1. The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis.
2. In recent years, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals and suggests that animals encode alternative enzymes of the cycle that differ in function from known MS and ICL in non-metazoan species.

- This bypasses **the decarboxylation steps** that take place in the TCA cycle, allowing simple carbon compounds to be used in the later synthesis of macromolecules, including glucose.
- Glyoxylate is subsequently combined **with acetyl-CoA to produce malate**, catalyzed by **malate synthase**
- **Malate is also formed in parallel from succinate by the action of succinate dehydrogenase and fumarase.**

Citric Acid Cycle

Glyoxylate cycle-2

Steps of Glyoxylate cycle

1. Acetyl-CoA condenses with oxaloacetate to form citrate
2. citrate is converted to isocitrate, exactly as in the citric acid cycle.
3. Then the cleavage of isocitrate by **isocitrate lyase**, forming succinate and **glyoxylate** (not the breakdown of isocitrate by isocitrate dehydrogenase)
4. The glyoxylate then condenses with a second molecule of acetyl-CoA to yield malate, in a reaction catalyzed by **malate synthase**
5. The malate is subsequently oxidized to oxaloacetate, which can condense with another molecule of acetyl-CoA to start another turn of the cycle

. cont

- Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate, which is then available for biosynthetic purposes.
- **The net result of the glyoxylate cycle is therefore the production of glucose from fatty acids.**
- Succinate generated in the first step can enter into the citric acid cycle to eventually form oxaloacetate.

Role in gluconeogenesis

- Fatty acids from lipids are commonly used as an energy source by vertebrates as fatty acids are degraded through beta oxidation into acetate molecules (used in acetyl coA).
- **This acetate**, bound to the active thiol group of coenzyme A, enters the Citric acid cycle (TCA or CCA cycle) where it is fully oxidized to carbon dioxide
- To utilize acetate from fat for biosynthesis of carbohydrates, the glyoxylate cycle, whose initial reactions are identical to the TCA cycle, is used.
- This pathway thus allows cells to obtain energy from fat.

- The oxaloacetate can be converted into **Phosphoenolpyruvate**, which is the product of phosphoenol pyruvate carboxykinase, the first enzyme in gluconeogenesis…

Function in Plants

- In plants the glyoxylate cycle occurs in specialized peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination.

In plants, certain invertebrates, and some microorganisms (*E. coli* and yeast) acetate can serve 2 purposes:

1. **as an energy-rich fuel**
2. **as a source of phosphoenolpyruvate** for carbohydrate synthesis

In these organisms, enzymes of the **glyoxylate cycle** catalyze conversion of acetate to succinate or other four-carbon intermediates of the citric acid cycle

..cont

- Those enzymes common to the citric acid and glyoxylate cycles have two isozymes, one specific to mitochondria, the other to **glyoxysomes**.
- Glyoxysomes are not present in all plant tissues at all times.
- They develop in lipid-rich seeds during germination, before the developing plant acquires the ability to make glucose by photosynthesis.
- In addition to glyoxylate cycle enzymes, glyoxysomes contain all the enzymes needed for the degradation of the fatty acids stored in seed oils

Germinating seeds can therefore convert the carbon of stored lipids into glucose

- The glyoxylate cycle can also provide plants with another aspect of metabolic diversity.
- Acetyl-CoA formed from lipid breakdown is converted to succinate via the glyoxylate cycle, and the succinate is exported to mitochondria, where citric acid cycle enzymes transform it to malate.
- A cytosolic isozyme of malate dehydrogenase oxidizes malate to oxaloacetate, a precursor for gluconeogenesis.
- Germinating seeds can therefore convert the carbon of stored lipids into glucose.
- The four carbon succinate molecule can be transformed into a variety of carbohydrates through combinations of other metabolic processes; the plant can synthesize molecules using acetate as a source for carbon.
- The Acetyl CoA can also react with glyoxylate to produce some NADPH from NADP+, which is used to drive energy synthesis in the form of ATP later in the Electron Transport Chain

Citric Acid Cycle

Glyoxylate cycle-3

Relationship between the glyoxylate and citric acid cycles

Glyoxylate cycle-3

- The two initial steps of the glyoxylate cycle are identical to those in the citric acid cycle: *acetate* → *citrate* → *isocitrate*.
- The glyoxylate cycle bypasses the steps in the citric acid cycle where carbon is lost in the form of CO_2.
- In the glyoxylate cycle, the bypassing of the two decarboxylation steps of the citric acid cycle makes possible the *net* formation of succinate, oxaloacetate, and other cycle intermediates from acetyl-CoA.
- Oxaloacetate thus formed can be used to synthesize glucose via gluconeogenesis.

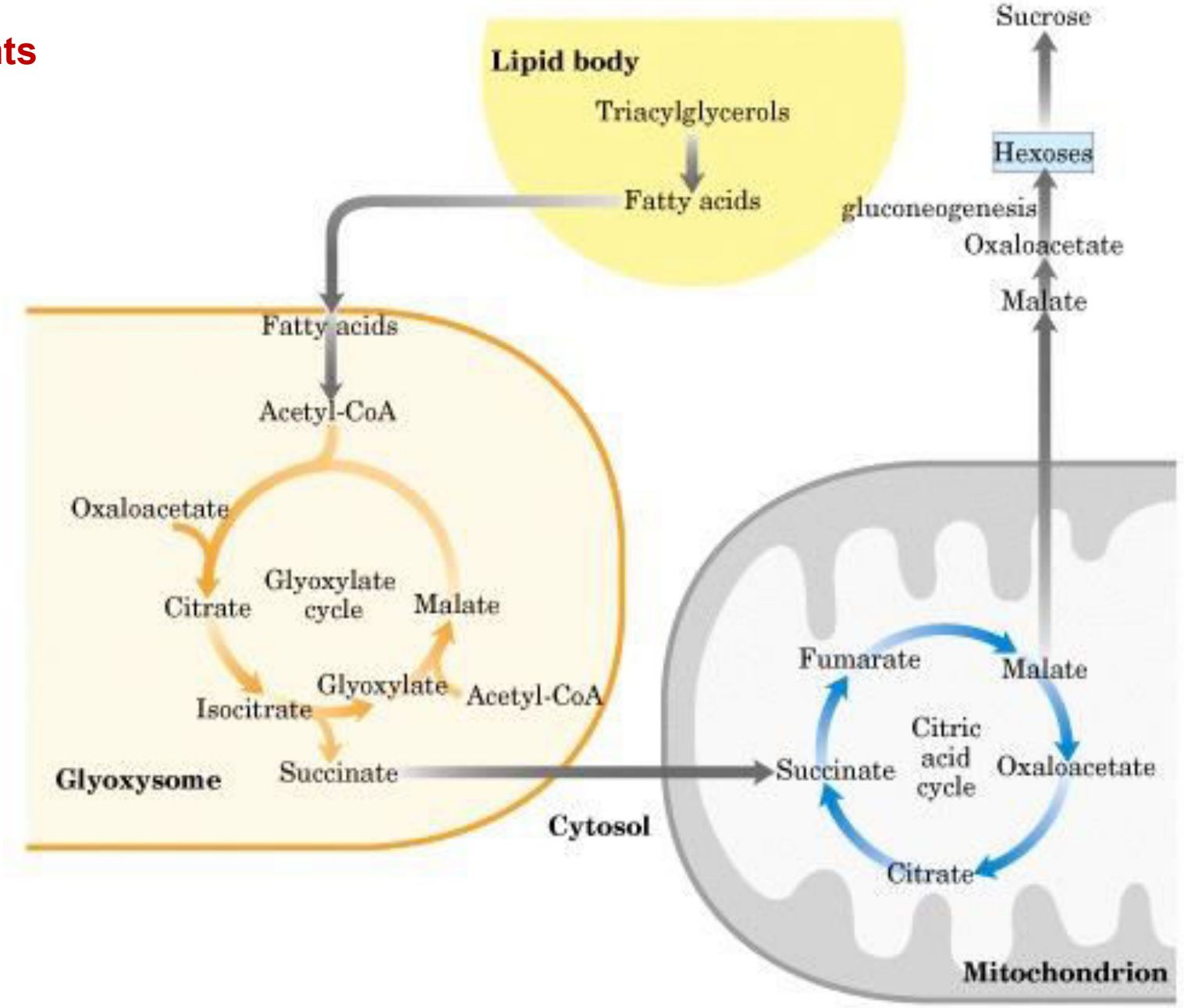
In germinating seeds, the enzymatic transformations of dicarboxylic and tricarboxylic acids occur in three intracellular compartments

and there is a continuous interchange of metabolites among these compartments

1. Mitochondria
2. glyoxysomes
3. cytosol

Linkage to Gluconeogenesis in Plants

- **Relationship between the glyoxylate and citric acid cycles.**
- The reactions of the glyoxylate cycle (in glyoxysomes) proceed simultaneously with, those of the citric acid cycle (in mitochondria) as intermediates pass between these compartments.
- The conversion of succinate to oxaloacetate is catalyzed by citric acid cycle enzymes.



The carbon skeleton of oxaloacetate from the citric acid cycle (in the mitochondrion) is carried to the glyoxysome in the form of aspartate.

Aspartate is converted to oxaloacetate, which condenses with acetyl-CoA derived from fatty acid breakdown.

The citrate thus formed is converted to isocitrate by aconitase, then split into glyoxylate and succinate by isocitrate lyase.

The succinate returns to the mitochondrion, where it reenters the citric acid cycle and is transformed into malate, which enters the cytosol and is oxidized (by cytosolic malate dehydrogenase) to oxaloacetate.

Oxaloacetate is converted via gluconeogenesis into hexoses and sucrose, which can be transported to the growing roots and shoot.

Four distinct pathways participate in these conversions:

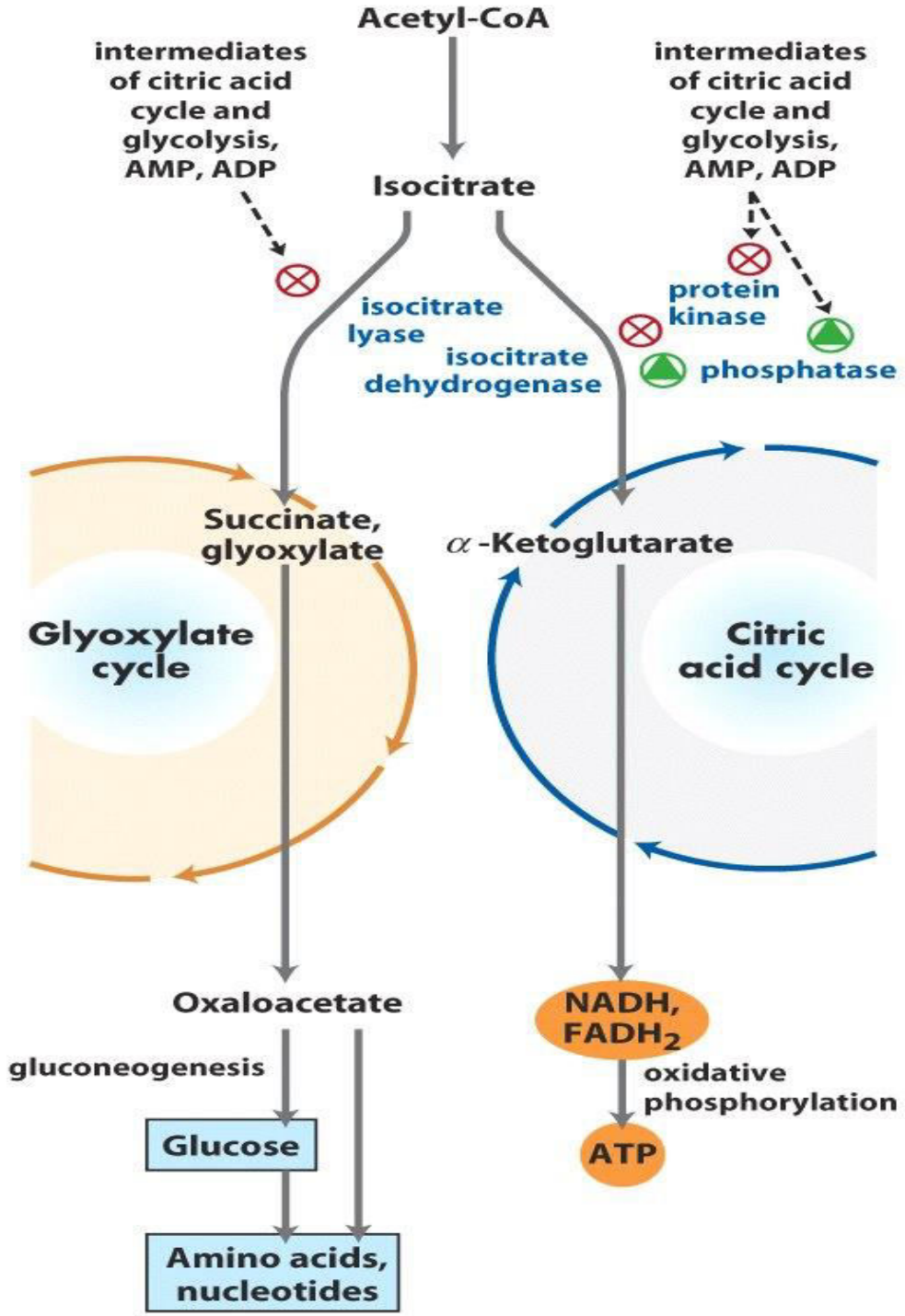
1. fatty acid breakdown to acetyl-CoA (in glyoxysomes)
2. the glyoxylate cycle (in glyoxysomes)
3. the citric acid cycle (in mitochondria)
4. gluconeogenesis (in the cytosol)

Coordinated regulation of glyoxylate and citric acid cycles

- The central molecules between 2 cycles is isocitrate
- The **translocation of isocitrate** between the citric acid cycle and the glyoxylate cycle is controlled by the activity of **isocitrate dehydrogenase**, which is regulated by reversible phosphorylation.
- **A specific protein kinase phosphorylates and inactivates the dehydrogenase.** This inactivation shunts isocitrate to the glyoxylate cycle, where it begins the synthetic route toward glucose.
- A phosphoprotein phosphatase removes the phosphoryl group from isocitrate dehydrogenase, **reactivating the enzyme**
- Reactivation of enzyme sends more isocitrate through the energy-yielding citric acid cycle
- The regulatory protein kinase and phosphoprotein phosphatase are separate enzymatic activities of a single polypeptide.

Regulation Linkage

- **Coordinated regulation of glyoxylate and citric acid cycles**.
- Regulation of isocitrate dehydrogenase activity determines the partitioning of isocitrate between the glyoxylate and citric acid cycles.
- When the enzyme is inactivated by phosphorylation (by a specific protein kinase), isocitrate is directed into biosynthetic reactions via the glyoxylate cycle.
- When the enzyme is activated by dephosphorylation (by a specific phosphatase), isocitrate enters the citric acid cycle and ATP is produced.



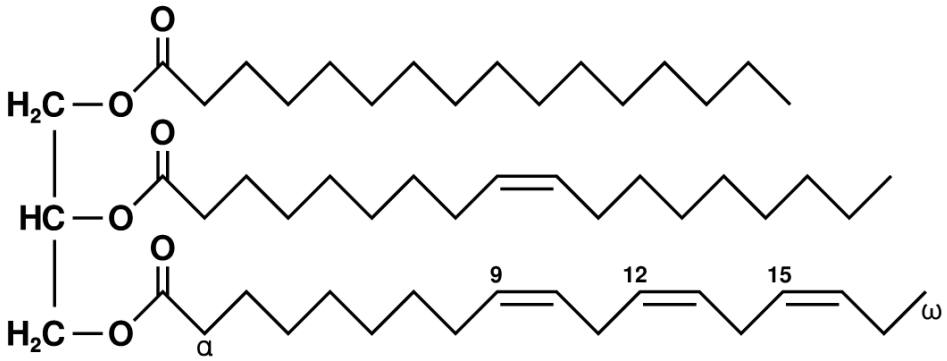
Fatty Acid Catabolism

Extraction of energy from fatty acids

- Fatty acids yield the maximum amount of ATP on an energy per gram basis, when they are completely oxidized to CO_2 and water by β-oxidation and the citric acid cycle.
- Fatty acids (mainly in the form of triglycerides) are the foremost storage form of fuel in most animals, and to a lesser extent in plants.
- ***Beta* oxidation**
- The repetitive four-step process, **by which fatty acids are converted into acetyl-CoA** called ***Beta* oxidation.**

- To overcome the relative stability of the C—C bonds in a fatty acid, the carboxyl group at C-1 is activated by attachment to coenzyme A, which allows stepwise oxidation of the fatty acyl group at the C-3, or *beta*, position-hence the name *beta* oxidation.

- Triacylglycerols
- triesters of fatty acids and glycerol with 1 molecule of glycerol (3 –OH).
- Three fatty acids ester-linked to a glycerol molecule
- Each –OH bonded to –COO group of 1 fatty acid (forming ester)



Example of an unsaturated fat triglyceride ($C_{55}H_{98}O_6$).
Left part: glycerol
Right part, from top to bottom: palmitic acid, oleic acid, alpha-linolenic acid.

- Triglycerides are the main constituents of body fat in humans and other animals, as well as vegetable fat
- Used as energy storage molecules in eukaryotes
- Stored in adipocytes

Advantages of using triacylglycerols for energy storage

1. Fats are highly reduced hydrocarbons with a large energy of complete oxidation (38 kJ/g) more than twice that for the same weight of carbohydrate or protein.
2. Fats are insoluble molecules that aggregate into droplets.
3. They are not dissolved due to which do not raise the osmolarity of the cytosol. (In storage polysaccharides, by contrast, water of solvation makes two-thirds of the overall weight of the stored molecules.)
4. Fats are chemically inert (triglycerides or neutral fats) that make them especially suitable as storage fuels so they can be stored without fear of unfavorable reactions.

Disadvantages of triacylglycerols as energy storage

1. The properties that make triacylglycerols good storage compounds present problems in their role as fuels.
2. Fats must be emulsified before enzymes can digest them.
3. Fats are insoluble in the blood and must be carried in the blood as protein complexes.

- **Emulsification**: The breakdown of fat globules in the duodenum into tiny droplets, which provides a larger surface area on which the enzyme pancreatic lipase can act to digest the fats into fatty acids and glycerol. **Emulsification** is assisted by the action of the bile salts.

Extraction of energy from fatty acids

- Complete oxidation of fatty acids to CO2 and H2O takes place in three stages:

- Step 1. Oxidation of fatty acids to acetyl-CoA. This generates NADH and $FADH_2$.

- Step 2. Oxidation of acetyl-CoA to CO_2 in the citric acid cycle. This generates NADH, $FADH_2$ and GTP (ATP).

- Step 3. Transfer of electrons from NADH and $FADH_2$ to O_2. This results in the synthesis of ATP.

Fatty Acid Catabolism-2

Digestion, Mobilization, and Transport of Fats

Digestion, Mobilization, and Transport of Fats

Cells can obtain fatty acid fuels from three sources:

1. Fats consumed in the diet
2. fats stored in cells as lipid droplets
3. fats synthesized in one organ for export to another

   Some species use all three sources under various circumstances, others use one or two.

Vertebrates, obtain fats in the diet, mobilize fats stored in specialized tissue (adipose tissue, consisting of cells called adipocytes), and, in the liver, convert excess dietary carbohydrates to fats for export to other tissues.

1. 40% or more of the daily energy requirement of humans in highly industrialized countries is supplied by **dietary triacylglycerols**
2. Triacylglycerols provide more than half the energy requirements of some organs, particularly the liver, heart, and resting skeletal muscle.
3. **In hibernating animals and migrating birds**, stored triacylglycerols are sole source of energy
4. Protists obtain fats by consuming organisms lower in the food chain, and some also store fats as cytosolic lipid droplets.
5. Vascular plants mobilize fats stored in **seeds during germination**, but do not otherwise depend on fats for energy.

Dietary Fats Are Absorbed in the Small Intestine

- **step 1** In vertebrates, before ingested triacylglycerols can be absorbed through the intestinal wall they must be converted from insoluble macroscopic fat particles to microscopic micelles (soluble) by bile salts, such as taurocholic acid
  step 2 Lipase action converts triacylglycerols to monoacylglycerols (monoglycerides) and diacylglycerols (diglycerides), free fatty acids, and glycerol .
- **step 3** These products of lipase action diffuse into the epithelial cells lining the intestinal surface (the intestinal mucosa)
- **step 4** In the intestinal mucosa they are reconverted to triacylglycerols and packed with dietary cholesterol and specific proteins into lipoprotein aggregates called **chylomicrons**

Molecular Structure of a Chylomicron

- The surface is a layer of phospholipids, with head groups facing the aqueous phase. Triacylglycerols sequestered in the interior (yellow) make up more than 80% of the mass. Several apolipoproteins that protrude from the surface (B-48, C-III, C-II) act as signals in the uptake and metabolism of chylomicron contents.
- The diameter of chylomicrons ranges from about 100 to 500 nm.

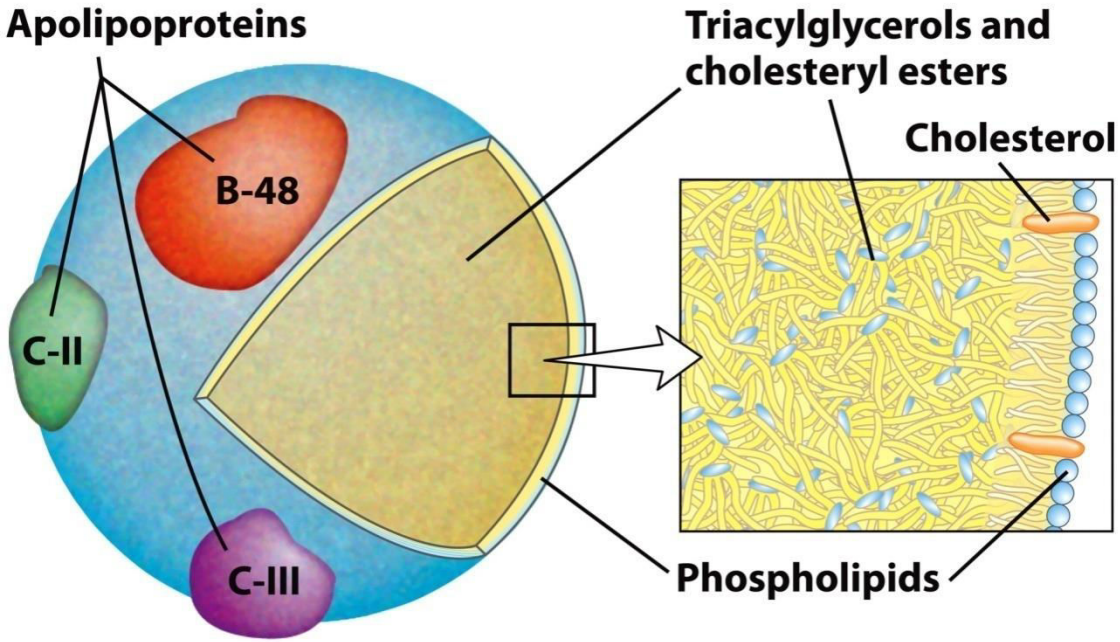


Figure 17-2
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- **step 5** The protein moieties of lipoproteins are recognized by receptors on cell surfaces. In lipid uptake from the intestine, chylomicrons, which contain apolipoprotein C-II (apoC-II), move from the intestinal mucosa into the lymphatic system, and then enter the blood, which carries them to muscle and adipose tissue
- **step 6** In the capillaries of these tissues, the extracellular enzyme **lipoprotein lipase**, activated by apoC-II, hydrolyzes triacylglycerols to fatty acids and glycerol
- **step 7** fatty acids and glycerol are taken up by cells in the target tissues .
- **step 8** In muscle, the fatty acids are oxidized for energy; in adipose tissue, they are reesterified for storage as triacylglycerols
- Bile salts are synthesized from cholesterol in the liver, stored in the gallbladder, and released into the small intestine after ingestion of a fatty meal.
- Bile salts act as biological detergents, converting dietary fats into mixed micelles of bile salts and triacylglycerols
- Micelle formation makes lipid molecules accessible to the action of water-soluble lipases in the intestine

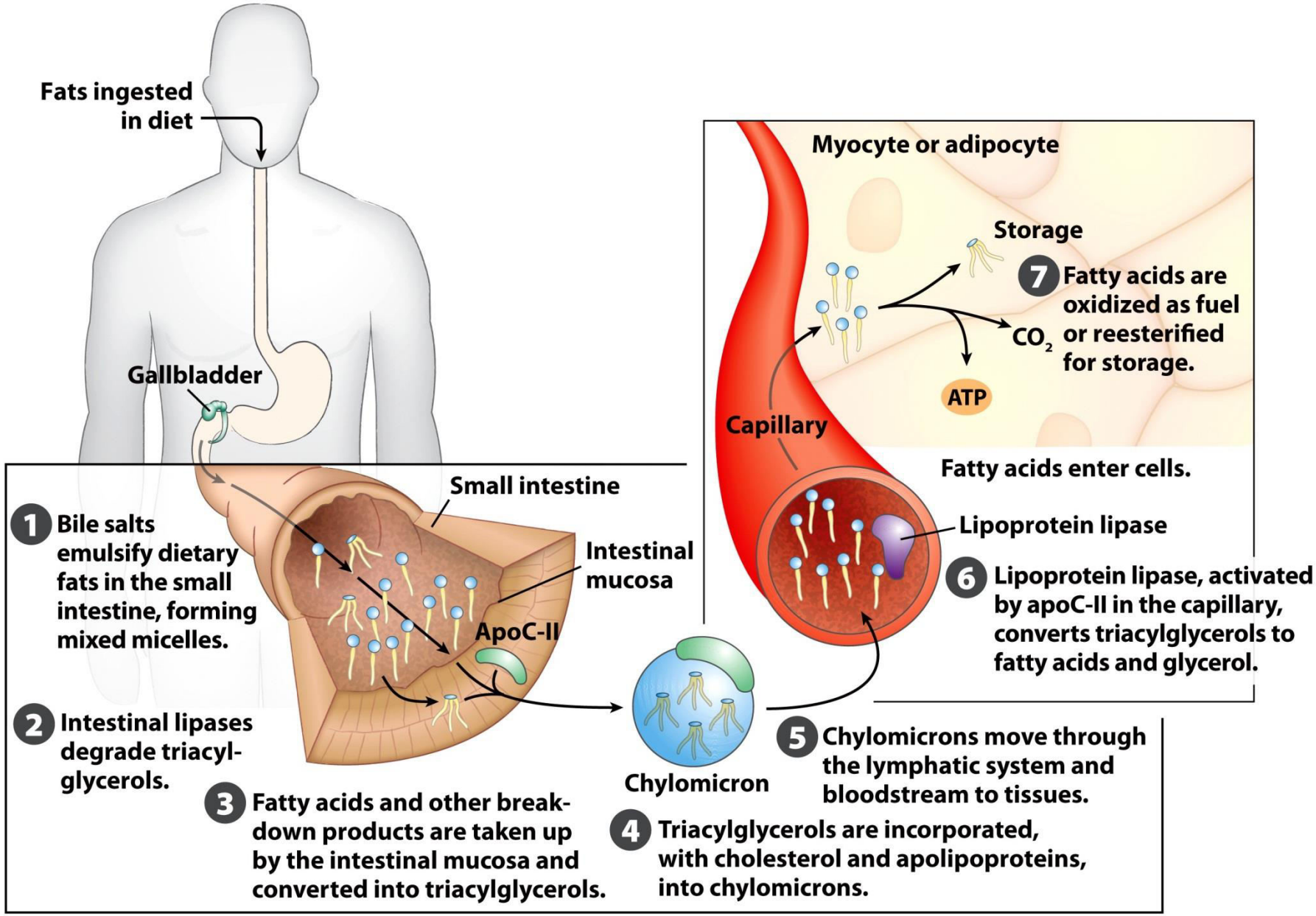

Figure 17-1
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Types of Dietary Fats

Apolipoproteins are lipid-binding proteins in the blood, responsible for the transport of triacylglycerols, phospholipids, cholesterol, and cholesteryl esters between organs. Apolipoproteins ("apo" means "detached" or "separate," designating the protein in its lipid-free form) combine with lipids to form several classes of **lipoprotein** particles, spherical aggregates with hydrophobic lipids at the core and hydrophilic protein side chains and lipid head groups at the surface.

Various combinations of lipid and protein produce particles of different densities, ranging from chylomicrons

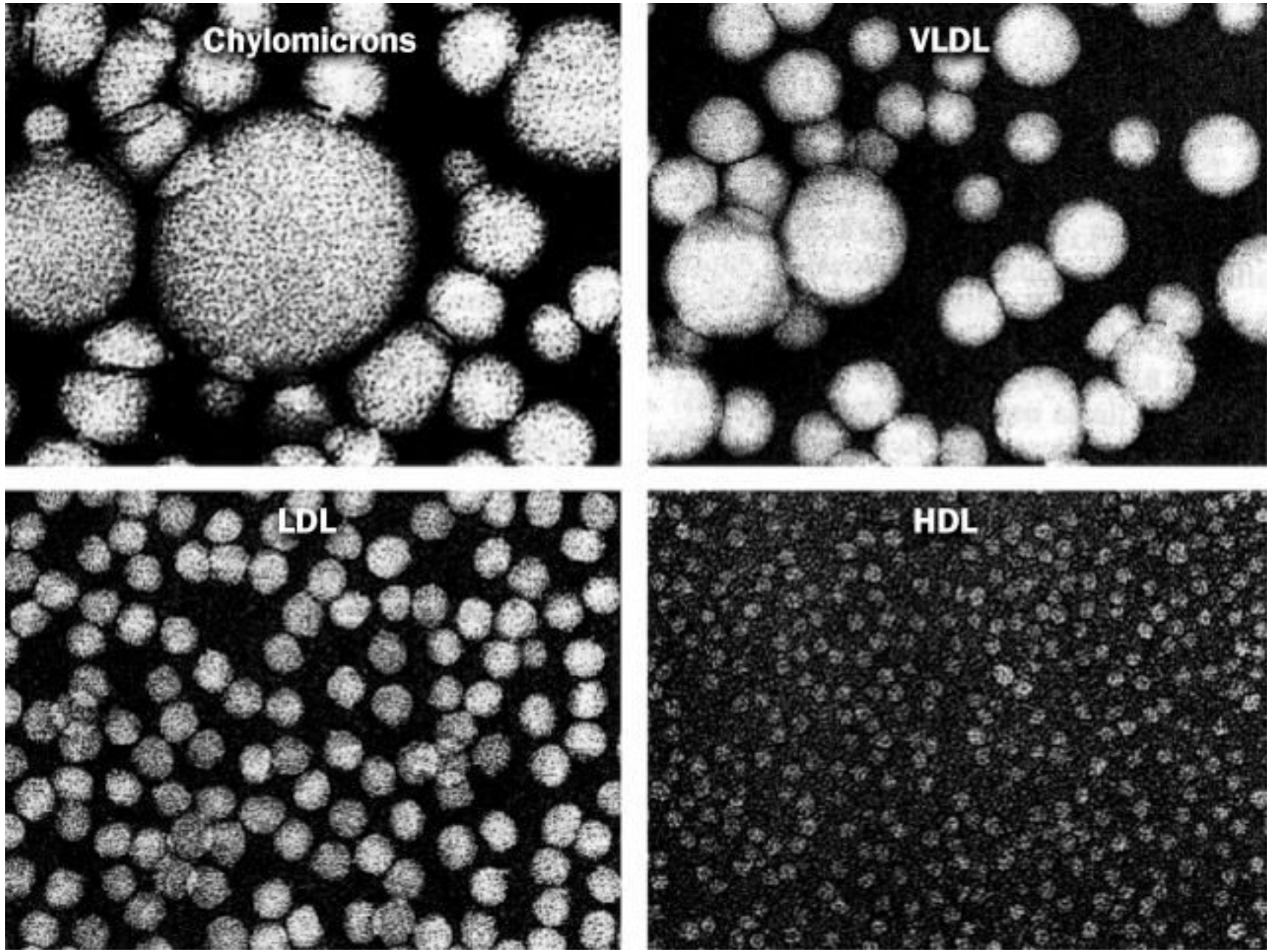
Lipoproteins are large complexes of lipids and proteins designed to transport lipids in the blood. The lipoproteins are classified by particle density.

Very-low-density lipoproteins (VLDL)

Very-high-density lipoproteins (VHDL)

The protein moieties of lipoproteins are recognized by receptors on cell surfaces.

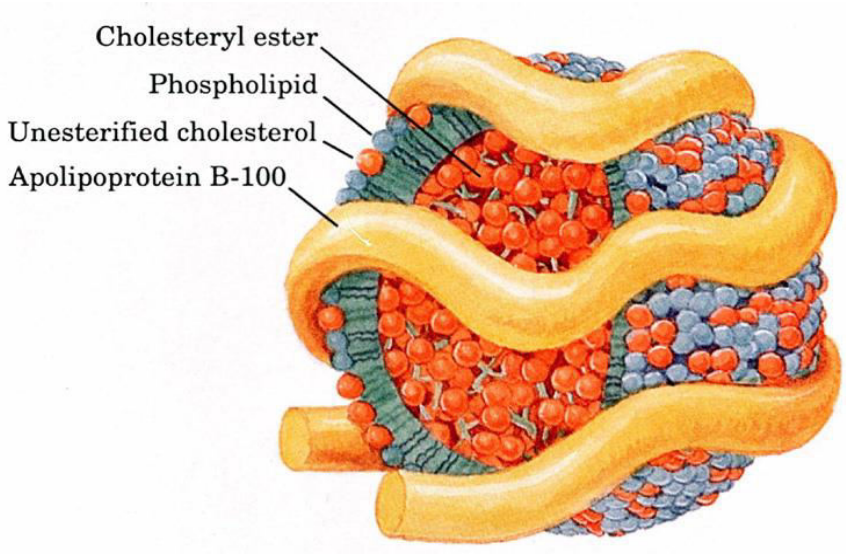
In lipid uptake from the intestine, chylomicrons, which contain apolipoprotein C-II (apoC-II)


Chylomicrons
VLDL
LDL
HDL

Dietry fats are absorbed in small intestine

1. Chylomicrons (transport dietary cholesterol to tissues)
2. Very low density lipoprotein (VLDL) transport cholesterol
3. Intermediate density lipoprotein (IDL) produced by the liver
4. Low density lipoprotein (LDL)
- 1st four transport cholesterol produced by the liver to tissues

5. High density lipoprotein (HDL) (transport cholesterol from tissue to liver)



Fatty Acid Catabolism-3

Hormones trigger metabolization of Triacylglycerols

Hormones trigger metabolization of Triacylglycerols

1. Neutral lipids are stored in adipocytes (and in steroid synthesizing cells of the adrenal cortex, ovary, and testis) in the form of lipid droplets, with a core of sterol esters and triacylglycerols surrounded by a monolayer of phospholipids.
2. The surface of these droplets is coated with **perilipins**
3. **Perilipins** are a family of proteins that restrict access to lipid droplets, preventing untimely lipid mobilization.
4. When hormones signal the need for metabolic energy, triacylglycerols stored in adipose tissue are mobilized (brought out of storage) and transported to tissues (skeletal muscle, heart, and renal cortex) in which fatty acids can be oxidized for energy production.

..cont

- Two hormones are secreted in response to low blood glucose levels
- **Epinephrine and glucagon**
- **Epinephrine and glucagon** stimulate the enzyme **adenylyl cyclase** in the adipocyte plasma membrane (which produces the intracellular second messenger cyclic AMP)
- Cyclic AMP–dependent protein kinase (PKA) triggers changes that open the lipid droplet up to the action of three lipases, which act on tri-, di-, and monoacylglycerols, releasing fatty acids and glycerol.
- These **free fatty acids,** (**FFA**) pass from the adipocyte into the blood, bind to the blood protein **serum albumin** noncovalently, carried to tissues skeletal muscle, heart, and renal cortex
- **serum albumin** makes up about half of the total serum protein, binds as many as 10 fatty acids per protein monomer
- 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

- The glycerol released by lipase action is phosphorylated by **glycerol kinase** and glycerol 3-phosphate is formed
- Glycerol 3-phosphate is oxidized to dihydroxyacetone phosphate.
- The glycolytic enzyme triose phosphate isomerase converts this compound to glyceraldehyde 3-phosphate, which is **oxidized via glycolysis.**

Mobilization of triacylglycerols stored in adipose tissue

1. When low levels of glucose in the blood trigger the release of glucagon, the hormone binds its receptor in the adipocyte membrane and thus stimulates adenylyl cyclase, via a G protein, to produce cAMP.
2. This activates PKA, which phosphorylates the hormone-sensitive lipase (HSL) And **perilipin molecules on the surface of the lipid droplet**.
3. Phosphorylation of perilipin causes dissociation of the protein CGI from perilipin.
4. CGI then associates with the enzyme adipose triacylglycerol lipase (ATGL), activating it.
5. Active ATGL converts triacylglycerols to diacylglycerols.
6. The phosphorylated perilipin associates with phosphorylated hormone-sensitive lipase **HSL**, allowing it access to the surface of the lipid droplet, where it converts diacylglycerols to monoacylglycerols.
7. A third lipase, monoacylglycerol lipase (MGL) hydrolyzes monoacylglycerols.

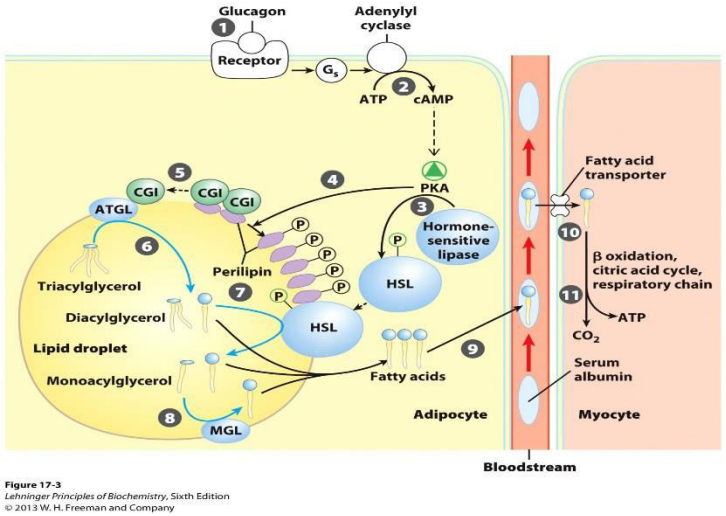


Figure 17-3
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Utilization of stored triacylglycerols

8. Fatty acids leave the adipocyte, bind serum albumin in the blood, and are carried in the blood; they are released from the albumin and enter a myocyte via a specific fatty acid transporter.

9. In the myocyte, fatty acids are oxidized to CO2

10. the energy of oxidation is conserved in ATP, which fuels muscle contraction and other

11. energy-requiring metabolism in the myocyte.

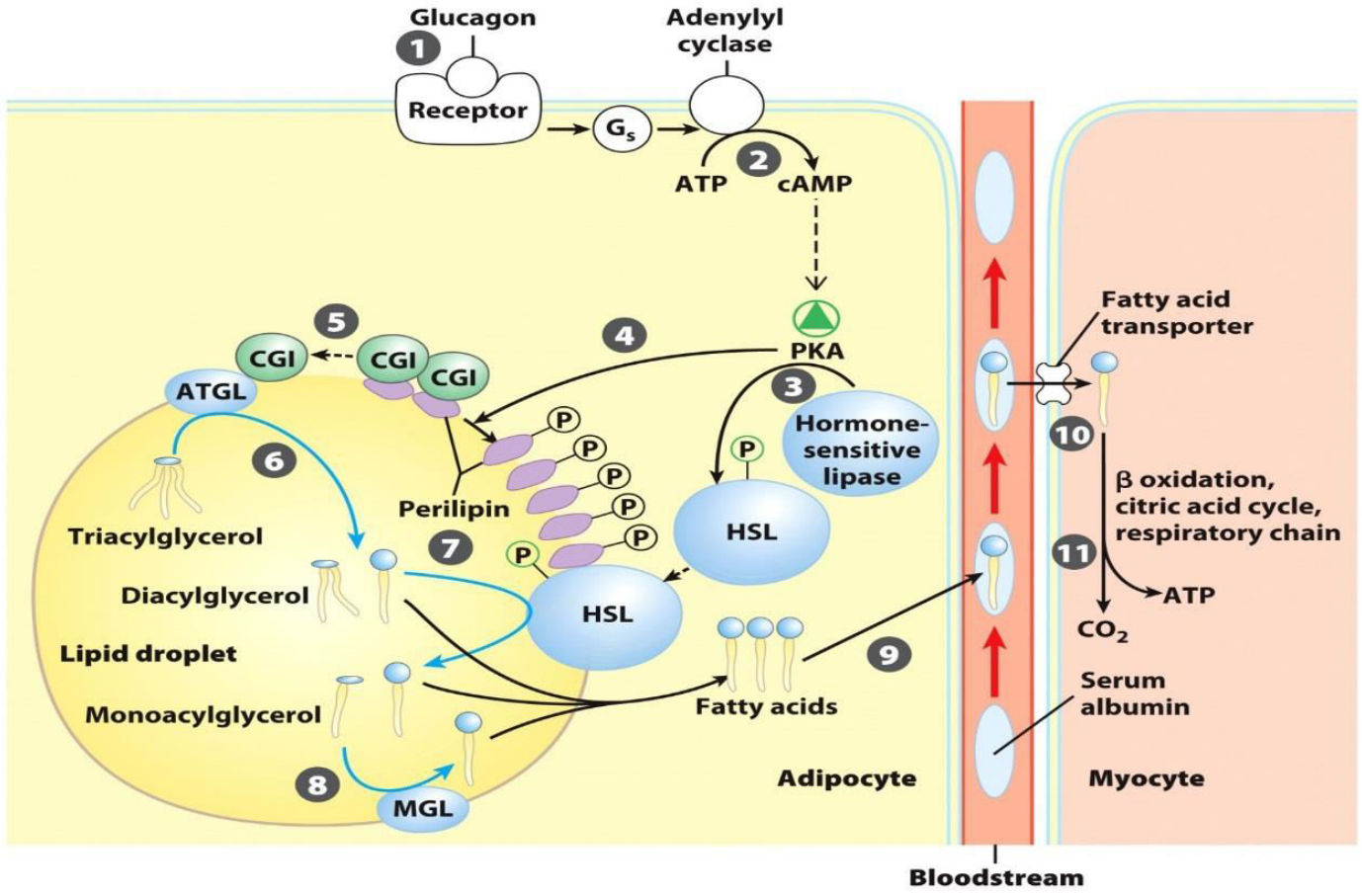

Figure 17-3
Lehninger Principles of Biochemistry, Sixth Edition

Fatty Acids Are Activated and Transported into Mitochondria

- The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix, as demonstrated in 1948 by Eugene P. Kennedy and Albert Lehninger.
- The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of **membrane transporters.**
- The fatty acids with **chain lengths of 14 or more carbons**, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes
- The fatty acids with chain lengths of 14 or more carbons must first undergo the three enzymatic reactions of the **carnitine shuttle**.

1. Esterification to CoA
2. Transesterification to carnitine followed by transport
3. Transesterification back to CoA

Three enzymatic reactions of the carnitine shuttle

- **The first reaction**
- This is catalyzed by a family of isozymes (different isozymes specific for fatty acids having short, intermediate, or long carbon chains) present in the outer mitochondrial membrane, the **acyl- CoA synthetases**
- **Acyl–CoA synthetases** catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a **fatty acyl– CoA**, coupled to the cleavage of ATP to AMP and PPi.
  (free energy released by **cleavage of phosphoanhydride bonds in ATP can be coupled to the formation of a high-energy compound.)**
- The reaction occurs in two steps and involves **a fatty acyl-adenylate intermediate (Fatty acyl–CoAs)**
- **Fatty acyl–CoAs** like acetyl-CoA, are high-energy compounds; their hydrolysis to FFA and CoA has a large, negative standard free-energy change (G= -31 kJ/mol).
- **The formation of a fatty acyl–CoA is made more favorable by the hydrolysis of *two* high-energy bonds in ATP**; the pyrophosphate formed in the activation reaction is immediately hydrolyzed by inorganic pyrophosphatase which pulls the reaction in the direction of fatty acyl–CoA formation. The overall reaction is

Fatty Acyl–CoA Synthetase Mechanism

Conversion of a fatty acid to a fatty acyl–CoA.
The conversion is catalyzed by fatty **acyl–CoA synthetase** and inorganic pyrophosphatase.
Fatty acid activation by formation of the fatty acyl–CoA derivative occurs in two steps.
The overall reaction is highly exergonic.

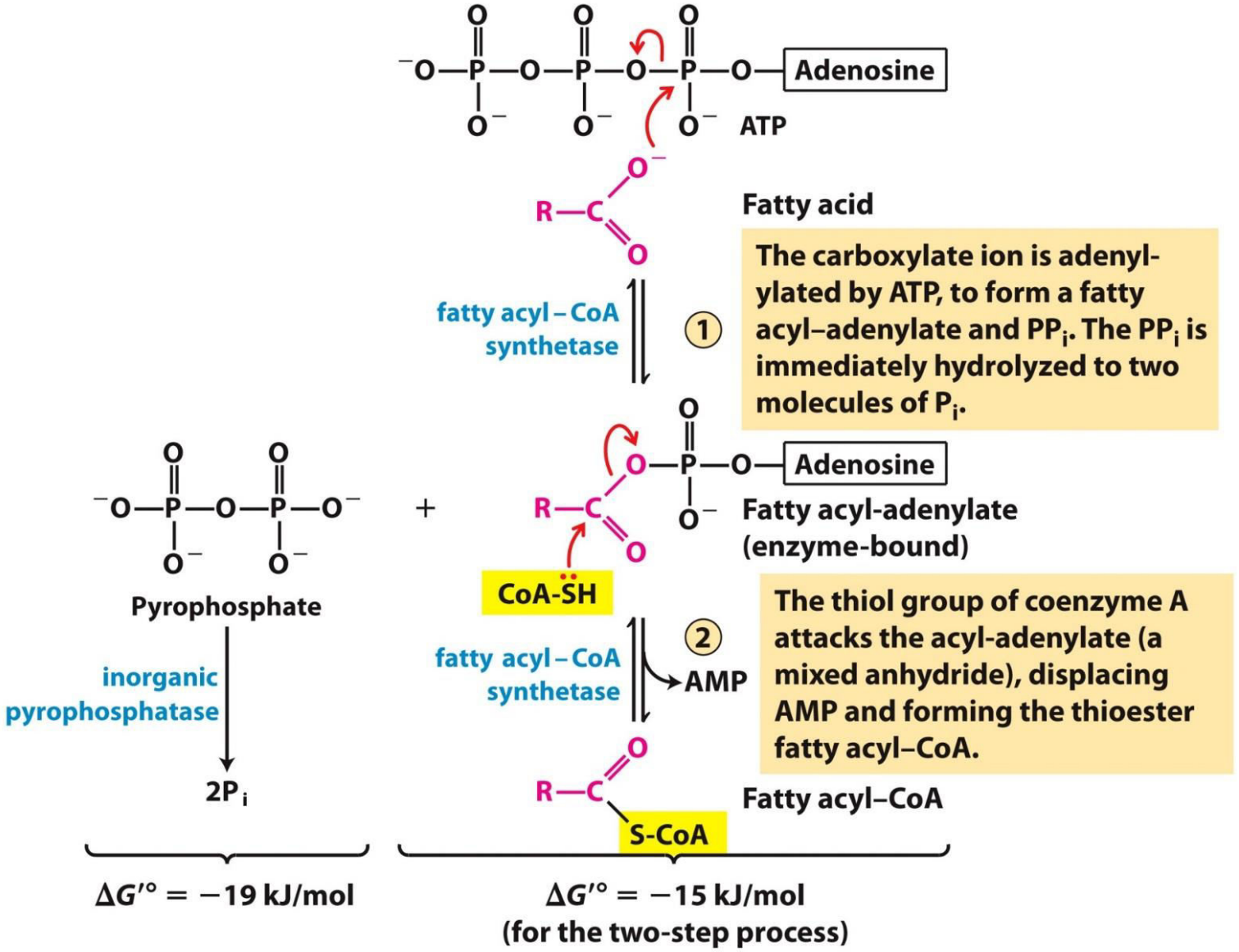

Figure 17-5
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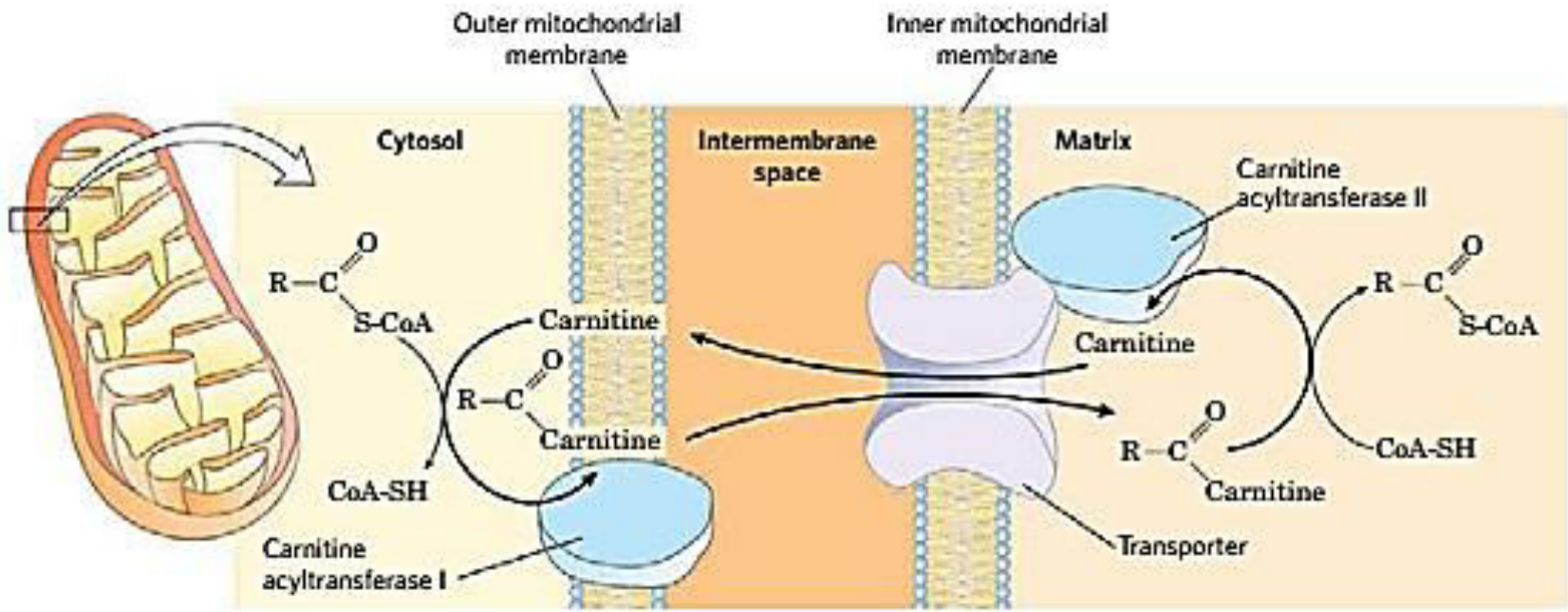
.. cont

- **The second reaction**
- Fatty acyl–CoA esters formed at the cytosolic side of the outer mitochondrial membrane can be oxidized to produce ATP in the mitochondrion and or they can be used in the cytosol to synthesize membrane lipids.
- Fatty acids destined for mitochondrial oxidation are transiently attached to the hydroxyl group of **carnitine** to form fatty acyl–carnitine— the second reaction of the shuttle.
- This transesterification is catalyzed by **carnitine acyltransferase I,** in the outer membrane.
- So the carnitine ester is formed then moved across the outer membrane to the intermembrane space
- This passage into the intermembrane space (the space between the outer and inner membranes) occurs through large pores (formed by the protein porin) in the outer membrane.
- The fatty acyl–carnitine ester then enters the matrix by facilitated diffusion through the **acyl-carnitine/carnitine transporter** of the inner mitochondrial membrane

- **Third (final step) of the carnitine shuttle**
- The fatty acyl group is enzymatically transferred from carnitine to intramitochondrial coenzyme A by **carnitine acyltransferase II**.
- This isozyme, located on the inner face of the inner mitochondrial membrane, regenerates fatty acyl–CoA and releases it, along with free carnitine, into the matrix .
- Carnitine reenters the intermembrane space via the acylcarnitine/carnitine transporter.



Fatty acid entry into mitochondria via the acyl-carnitine/ carnitine transporter.

After fatty acyl–carnitine is formed at the outer membrane or in the intermembrane space, it moves into the matrix by facilitated diffusion through the transporter in the inner membrane.

In the matrix, the acyl group is transferred to mitochondrial coenzyme A,

freeing carnitine to return to the intermembrane space through the same transporter.

This three-step process for transferring fatty acids into the mitochondrion—links two separate pools of coenzyme A and of fatty acyl–CoA, one in the cytosol, the other in mitochondria.

- These pools have different functions.
- **Coenzyme A in the mitochondrial matrix** is used in oxidative degradation of pyruvate, fatty acids, and some amino acids
- **Coenzyme A in cytosol..** Is conversely, is used in the biosynthesis of fatty acids

- Fatty acyl–CoA in the cytosolic pool can be used for membrane lipid synthesis or can be moved into the mitochondrial matrix for oxidation and ATP production.
- Conversion to the carnitine ester commits the fatty acyl moiety to the oxidative fate.
- **Rate limiting step for oxidation of fatty acids in mitochondria**
- The carnitine-mediated entry process is the rate limiting step for oxidation of fatty acids in mitochondria
- And is a regulation point.
- Once inside the mitochondrion, the fatty acyl–CoA is acted upon by a set of enzymes in the matrix.

Fatty Acid Catabolism-5

Stages for Oxidation of fatty acids

Oxidation of fatty acids

- The mitochondrial oxidation of fatty acids takes place in three stages
- **In First Sage** fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain.
- The **16-carbon palmitic acid** undergoes seven passes through the oxidative sequence
- In each pass two carbons are lost as acetyl-CoA.
- At the end of seven cycles the last two carbons of palmitate (originally C-15 and C-16) remain as acetyl-CoA.
- The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules.
- Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H+) from the fatty acyl moiety by dehydrogenases.

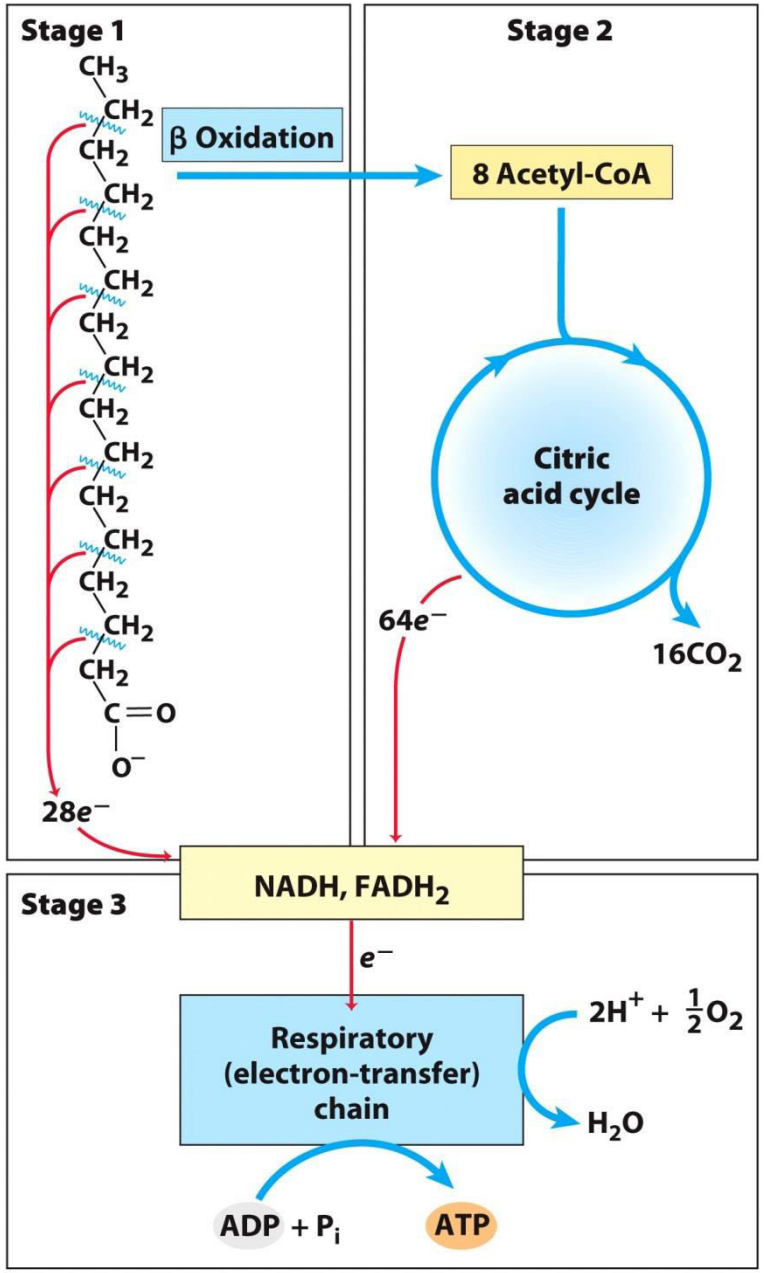


Figure 17-7
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Stages of fatty acid oxidation.
Stage 1:
A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA.
This process is called oxidation.
Stage 2:
The acetyl groups are oxidized to CO_2 via the citric acid cycle.
Stage 3:
Electrons derived from the oxidations of stages 1 and 2 pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.

- **In the second stage** of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO2 in the citric acid cycle, which also takes place in the mitochondrial matrix.
- Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation
- **Third stage** **Electrons derived from the oxidations of stages 1 and 2 pass to O2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.**
- This is due to production of reduced electron carriers NADH and FADH2 in first two stages of fatty acid oxidation

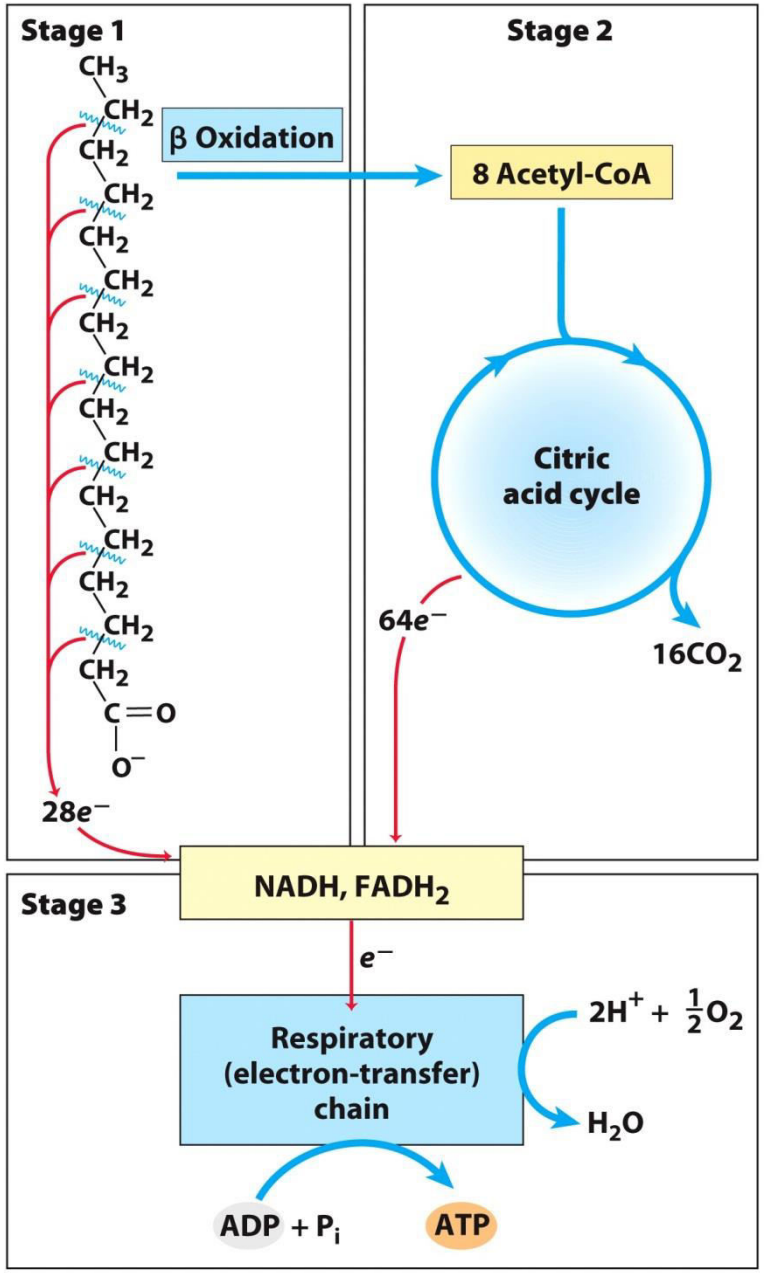

Figure 17-7
Lehninger Principles of Biochemistry, Sixth Edition

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Fatty Acid Catabolism-6

Steps of *Beta* Oxidation

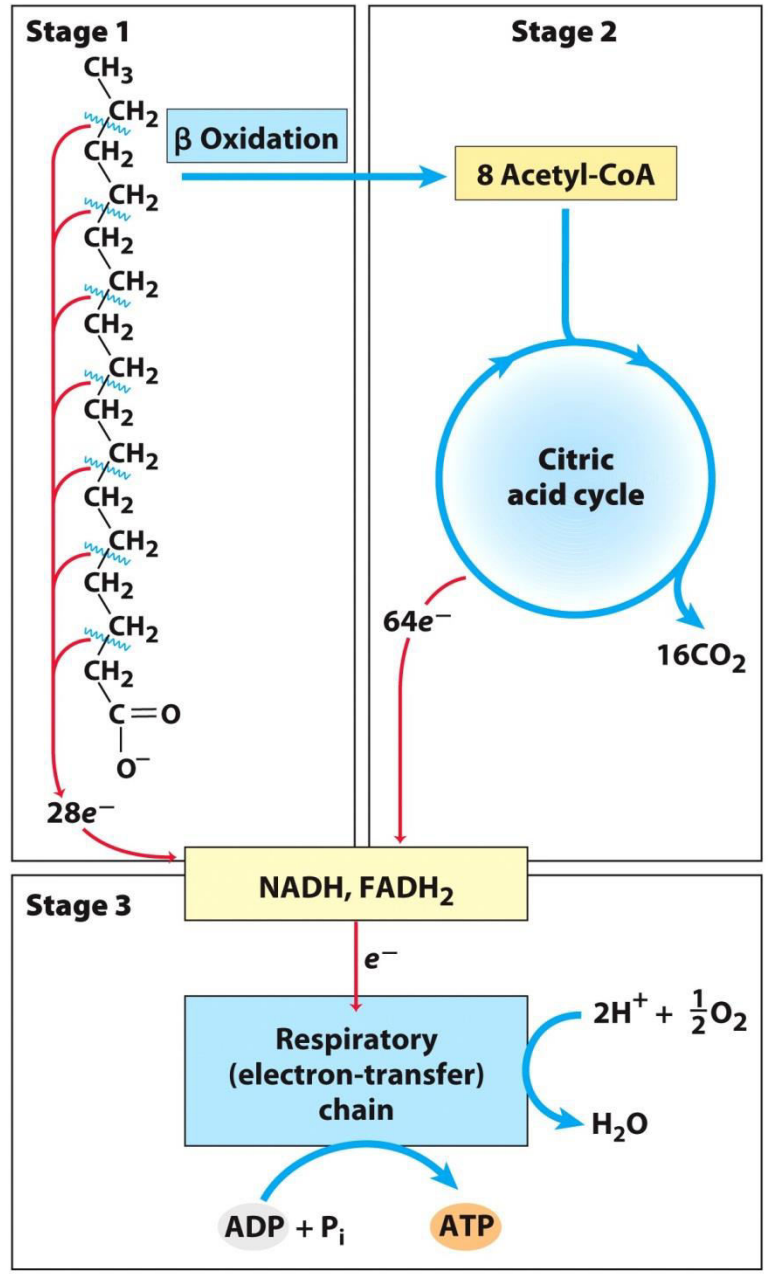

Figure 17-7

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Beta - Oxidation of saturated fatty acids

- First, dehydrogenation of fatty acyl–CoA produces a double bond between the ***alpha*** and ***beta*** carbon atoms (C-2 and C-3), yielding a ***trans*-Δ^2-enoyl-CoA** (the symbol Δ^2 designates the position of the double bond).
- This first step is catalyzed by **acyl- CoA dehydrogenase**
- **Acyl- CoA dehydrogenase:**
- Three isozymes of each specific for a range of fatty acyl chain lengths. All three isozymes are flavoproteins with FAD as a prosthetic group.

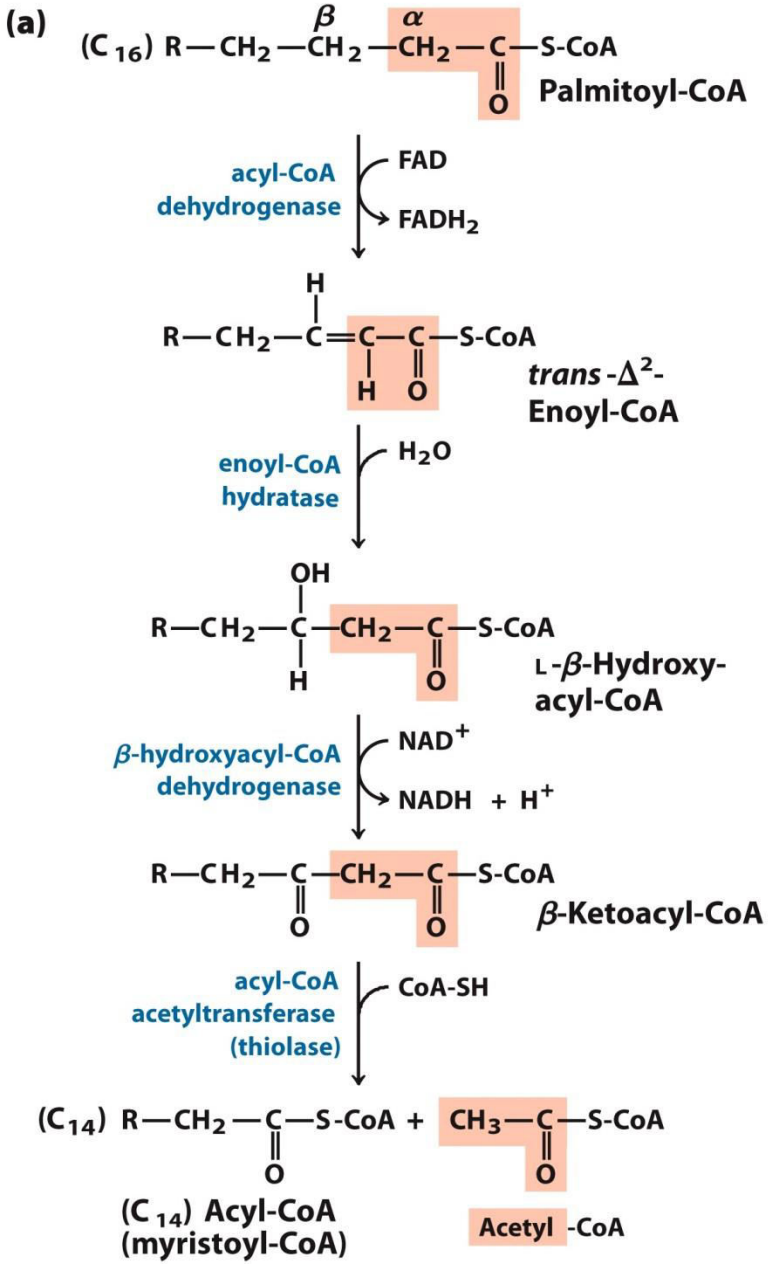
a) Very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons
b) Medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons
c) Short-chain (SCAD), acting on fatty acids of 4 to 8 carbons.

- The electrons removed from the fatty acyl–CoA are transferred to FAD, the reduced form of the **dehydrogenase** immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the **electron-transferring flavoprotein (ETF)**
- The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle in both reactions the enzyme is bound to the inner membrane
- A double bond is introduced into a carboxylic acid between ***alpha*** and ***beta*** **carbons**
- FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O2, with the synthesis of about 1.5 ATP molecules per electron pair.

2nd and 3rd Steps: β-Oxidation of saturated fatty acids

- **Second Step:** In the second step of the beta-oxidation cycle water is added to the double bond of the *trans*- D2-enoyl-CoA to form the L-stereoisomer of beta-**hydroxyacyl- CoA (3-hydroxyacyl-CoA)**.
- Enzyme: **Enoyl-CoA hydratase**, is analogous to the fumarase reaction in the citric acid cycle, in which H2O adds across an ***alpha*** and ***beta*** double bond

- **Third step:** In the third step, L-*beta*- hydroxyacyl-CoA is dehydrogenated to form beta-**ketoacyl-CoA**, by the action of beta-**hydroxyacyl-CoA dehydrogenase**
- NAD+ is the electron acceptor.
- The NADH formed in the reaction donates its electrons to **NADH dehydrogenase**, an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O2.
- **Enzyme:** The reaction catalyzed by **beta-hydroxyacyl- CoA dehydrogenase** is closely analogous to the malate dehydrogenase reaction of the citric acid cycle



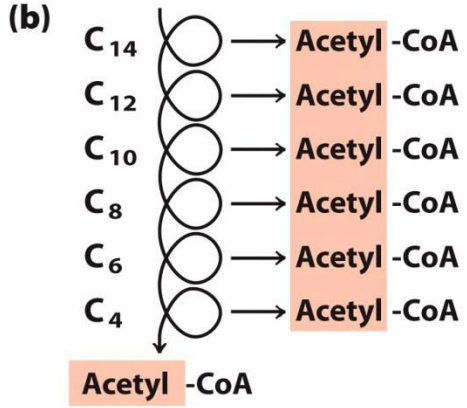


Figure 17-8
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The beta -oxidation pathway.

(a) In each pass through this four-step sequence, one acetyl residue (shaded in pink) is removed in the form of acetyl-CoA from the carboxyl end of the fatty acyl chain—in this example palmitate (C_{16}) is shown, which enters as palmitoyl-CoA.

(b) Six more passes through the pathway yield seven more molecules of acetyl-CoA, the seventh arising from the last two carbon atoms of the 16-carbon chain. Eight molecules of acetyl-CoA are formed in all.

4th Step: β-Oxidation of saturated fatty acids

- Last step is catalyzed by **acyl-CoA acetyltransferase**, called **thiolase**, which promotes reaction of *beta*-ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA.
- **Another product is the coenzyme A; thioester of the fatty acid, now shortened by two carbon atoms**
- **This reaction is called thiolysis,** by analogy with the process of hydrolysis, because the **β** -ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.
- The thiolase reaction is a reverse Claisen condensation

β Oxidation of saturated fatty acids

- **The last three steps of this four-step sequence are catalyzed by either of two sets of enzymes, with the enzymes employed depending on the length of the fatty acyl chain.**
- For fatty acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the **trifunctional protein (TFP)**. TFP is a heterooctamer of **α4β4 subunits.**
- Each subunit contains two activities, **the enoyl-CoA hydratase and the -hydroxyacyl-CoA dehydrogenase; the β subunits contain the thiolase activity.**
- When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxidations are catalyzed by a set of four soluble enzymes in the matrix.

… breakage of bonds in saturated fatty acids

- The single bond between methylene (—CH2—) groups in fatty acids is relatively stable.
- During first three reactions of **β** -oxidation less stable C—C bonds are created, in which the carbon (C-2) is bonded to *two* carbonyl carbons (the **β** -ketoacyl-CoA intermediate).
- The ketone function on the carbon (C-3) makes it a good target for nucleophilic attack by the —SH of coenzyme A, catalyzed by thiolase.
- The acidity of **the α- hydrogen** and the resonance stabilization of the carbanion generated by the departure of this hydrogen make the terminal —CH2—CO—S-CoA a good leaving group, facilitating breakage of the **α-β** bond.

Fatty Acid Catabolism-

The four steps are repeated to yield acetyl CoA

The four steps are repeated to yield acetyl CoA and ATP

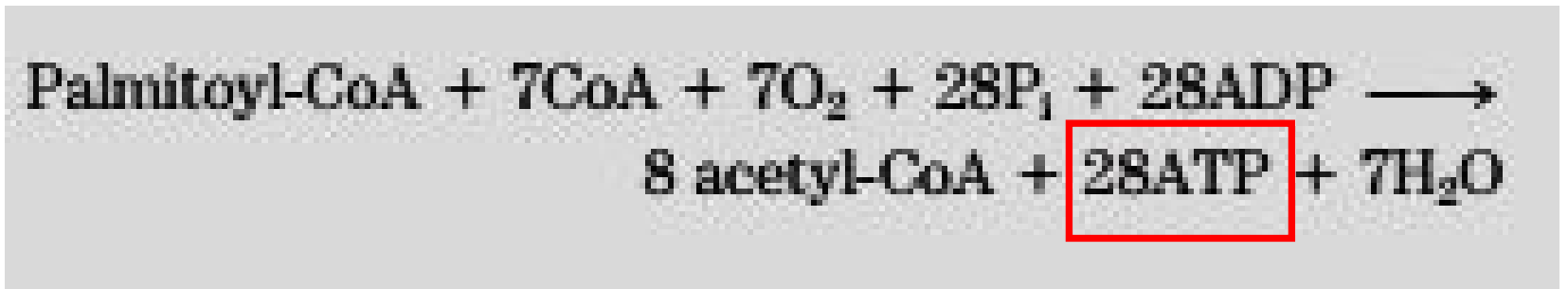
- In one pass through the *B*-oxidation sequence, one molecule of acetyl-CoA, two pairs of electrons, and four protons (H+) are removed from the long-chain fatty acyl–CoA, shortening it by two carbon atoms.
- The equation for one pass, beginning with the coenzyme A ester of our example, palmitate, is oxidized as following equation:

$$\text{Palmitoyl-CoA} + \text{CoA} + \text{FAD} + \text{NAD}^+ + \text{H}_2\text{O} \longrightarrow$$
$$\text{myristoyl-CoA} + \text{acetyl-CoA} + \text{FADH}_2 + \text{NADH} + \text{H}^+$$

…cont

- Following removal of one acetyl-CoA unit from palmitoyl- CoA, the coenzyme A thioester of the shortened fatty acid (now the 14-carbon myristate) remains
- **The myristoyl- CoA** can now go through the four steps **β**–oxidation, exactly analogous to the first, to yield a second molecule of acetyl-CoA and **lauroyl-CoA,** the coenzyme A thioester of the **12-carbon laurate.**
- Altogether, seven passes through **the β-oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight** molecules of acetyl-CoA
- The overall equation is:

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \longrightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$


Palmitoyl-CoA + 7CoA + 7O2 + 28Pi + 28ADP
8 acetyl-CoA + 28ATP + 7H2O

Beta oxidation in bears during hybernation

- Many animals depend on fat stores for energy during hibernation, during migratory periods, and in other situations involving metabolic adjustments.
- One of the most pronounced adjustments of fat metabolism occurs in hibernating grizzly bears.
- These animals remain in a continuous state of dormancy for periods as long as seven months.
- the bear maintains a body temperature of between 32 and 35C, close to the normal (nonhibernating) level
- Although expending about 25,000 kJ/day (6,000 kcal/day), the bear does not eat, drink, urinate, or defecate for months at a time.
- Fat oxidation yields sufficient energy for maintenance of body temperature, active synthesis of amino acids and proteins, and other energy-requiring activities, such as membrane transport.
- Fat oxidation also releases large amounts of water, which replenishes water lost in breathing.

- The glycerol released by degradation of triacylglycerols is converted into blood glucose by gluconeogenesis.
- Urea formed during breakdown of amino acids is reabsorbed in the kidneys and recycled**, the amino groups reused to make new amino acids for maintaining body proteins**.
- Bears store an enormous amount of body fat in preparation for their long sleep.
- An adult grizzly consumes about 38,000 kJ/day during the late spring and summer, but as winter approaches it feeds 20 hours a day, consuming up to 84,000 kJ daily.
- Large amounts of triacylglycerols are formed from the huge intake of carbohydrates during the fattening-up period.

Acetyl CoA produced by β-oxidation can enter Citric acid cycle

- **Acetyl CoA produced by β-oxidation can be** oxidized to CO2 and H2O by the citric acid cycle

$$8\ \text{Acetyl-CoA} + 16O_2 + 80P_i + 80\text{ADP} \longrightarrow 8\text{CoA} + 80\text{ATP} + 16CO_2 + 16H_2O \qquad (17\text{–}5)$$

Acetyl CoA produced by β-oxidation can enter Citric acid cycle

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7O_2 + 28P_i + 28\text{ADP} \longrightarrow 8\text{ acetyl-CoA} + 28\text{ATP} + 7H_2O$$

$$8\text{ Acetyl-CoA} + 16O_2 + 80P_i + 80\text{ADP} \longrightarrow 8\text{CoA} + 80\text{ATP} + 16CO_2 + 16H_2O \qquad (17\text{–}5)$$

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 108\text{ATP} + 16CO_2 + 23H_2O$$

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

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. 526).

Table 17-1
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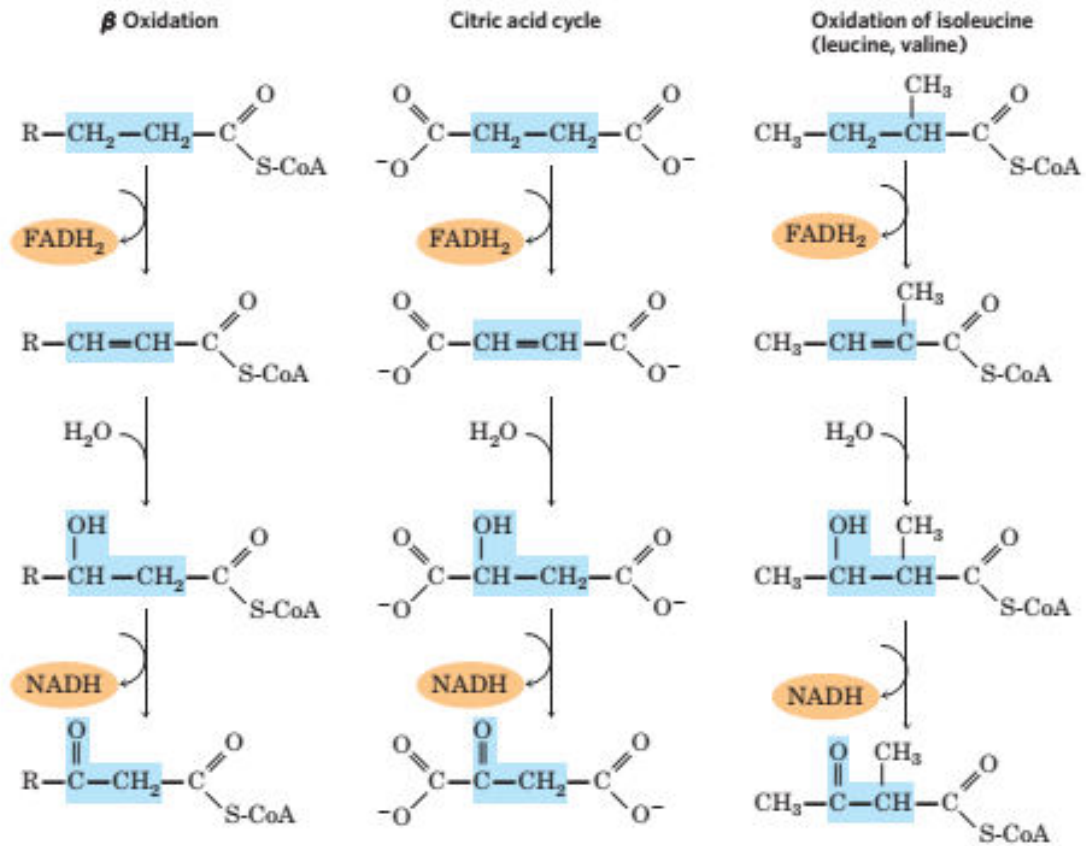


A conserved reaction sequence to introduce a carbonyl function on the carbon β- to a carboxyl group. The β-oxidation pathway for fatty acyl–CoAs, the pathway from succinate to oxaloacetate in the citric acid cycle, and the pathway by which the deaminated carbon skeletons from isoleucine, leucine, and valine are oxidized as fuels, use the same reaction sequence.

Fatty Acid Catabolism-4

Oxidation of unsaturated fatty acids requires 2 additional steps

Oxidation of unsaturated fatty acids requires 2 additional steps

- The fatty acid oxidation sequence just described is typical when the incoming fatty acid is saturated (that is, has only single bonds in its carbon chain).
- These bonds are in the **cis configuration and cannot be acted upon by enoyl-CoA hydratase,** the enzyme catalyzing the addition of H2O to the trans double bond of the D2-enoyl-CoA generated during oxidation.
- Two auxiliary enzymes are needed for oxidation of the common unsaturated fatty acids:

1. an isomerase
2. a reductase

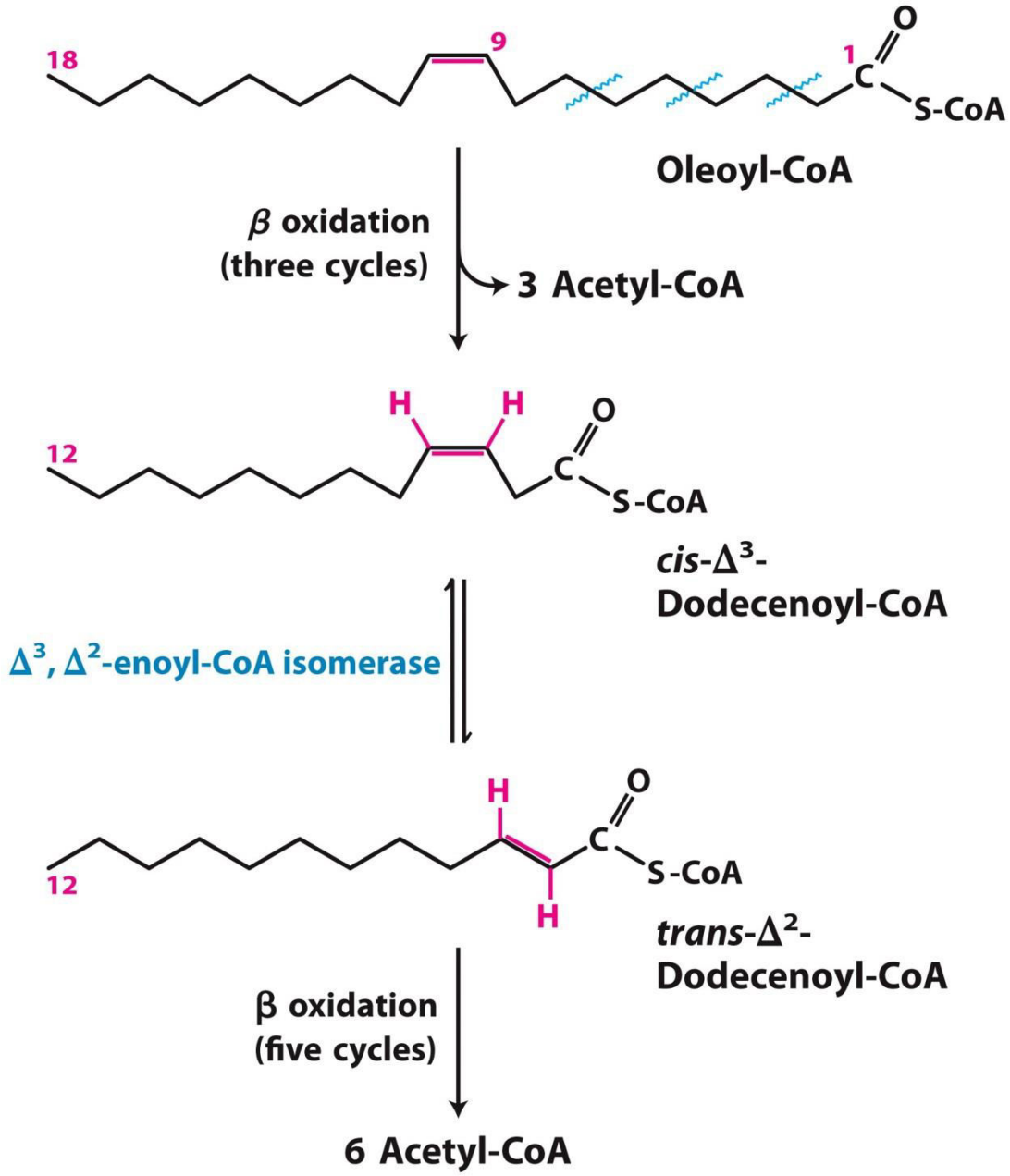


Figure 17-10
Lehninger Principles of Biochemistry, Sixth Edition

Beta oxidation of oleate 18:1Δ^9

Oxidation of Oleate (example of unsaturated fatty acids)

- Oleate is an abundant 18-carbon monounsaturated fatty acid **with a cis double bond between C-9 and C-10 (denoted Δ9).**
- In the first step of oxidation, oleate is converted to oleoyl-CoA and, like the saturated fatty acids, enters the mitochondrial matrix via the carnitine shuttle
- Oleoyl-CoA then undergoes three passes through the fatty acid oxidation cycle to yield three molecules of acetyl-CoA and the coenzyme A ester of a D3, 12-carbon unsaturated fatty acid, *cis*- Δ 3-dodecenoyl-CoA
- This product cannot serve as a substrate for enoyl-CoA hydratase, which acts only on trans double bonds.

- The auxiliary enzyme D3,D2**-enoyl-CoA isomerase** isomerizes the *cis*-D3-enoyl-CoA to the *trans*-D2-enoyl- CoA, which is converted **by enoyl-CoA hydratase** into the corresponding L-hydroxyacyl-CoA (*trans*-D2-dodecenoyl-CoA).
- This intermediate is now acted upon by the remaining enzymes of oxidation to yield acetyl-CoA and the coenzyme A ester of a 10-carbon saturated fatty acid, **decanoyl-CoA**.
- **decanoyl-CoA** undergoes four more passes through the -oxidation pathway to yield five more molecules of acetyl-CoA.
- Altogether, nine acetyl-CoAs are produced from one molecule of the 18-carbon oleate.

- The other auxiliary enzyme (a reductase) is required for oxidation of polyunsaturated fatty acids — for example, the 18-carbon linoleate, which has a ***cis*-D9,*cis*-D12 configuration** (Fig. 17–11).
- Linoleoyl- CoA undergoes three passes through the –oxidation sequence to yield three molecules of acetyl-CoA and the **coenzyme A ester of a 12-carbon** unsaturated fatty acid with a ***cis*-D3,*cis*-D6 configuration**.
- This intermediate cannot be used by the enzymes of the **β**–oxidation pathway; its double bonds are in the wrong position

- The other auxiliary enzyme (a reductase) is required for oxidation of polyunsaturated fatty acids — for example, the 18-carbon linoleate, which has a ***cis*-D9,*cis*-D12 configuration** (Fig. 17–11).
- Linoleoyl- CoA undergoes three passes through the –oxidation sequence to yield three molecules of acetyl-CoA and the **coenzyme A ester of a 12-carbon** unsaturated fatty acid with a ***cis*-D3,*cis*-D6 configuration**.
- This intermediate cannot be used by the enzymes of the **β**–oxidation pathway; its double bonds are in the wrong position

- And have the wrong configuration (cis, not trans).
- However, the combined action of enoyl-CoA isomerase and **2,4-dienoyl-CoA reductase**, as shown in **Figure 17–11**, allows reentry of this intermediate into the –oxidation pathway and its degradation to six acetyl-CoAs.
- The overall result is conversion of linoleate to nine molecules of acetyl-CoA.

Auxiliary enzymes

We investigated the enzyme activity of the blank in the spectrophotometric determination of the aminotransferase activities and aspartate aminotransferase activity. 6 lactate dehydrogenase and 3 malate dehydrogenase preparations from different manufactures and from different organs showed additional and contaminating activity. The additional activity depends upon the 2-oxoglutarate concentration. The contaminating activity is caused by alanine aminotransferase and aspartate aminotransferase in the auxiliary enzymes. We propose that exact definitions must be given for the auxiliary enzymes in the recommendations of standard determinations for enzyme activities.

Fatty Acid Catabolism

Complete Oxidation of Odd-numbered fatty acids requires 3 additional reactions

Oxidation of Odd-numbered fatty acids

- Fatty acids with an odd number of carbons are found in the lipids of many plants and marine organisms.
- Cattle and other ruminant animals form large amounts of the three-carbon **propionate** ($CH_3—CH_2—COO_2$) during fermentation of carbohydrates in the rumen.
- The propionate is absorbed into the blood and oxidized by the liver and other tissues
- Small quantities of propionate are added as a mold inhibitor to some breads and cereals, thus entering the human diet.
- Long-chain odd-number fatty acids are oxidized in the same pathway as the even-number acids, … at the carboxyl end of the chain.
- the substrate for the last pass through the β-oxidation sequence is a fatty acyl–CoA with a five-carbon fatty acid.

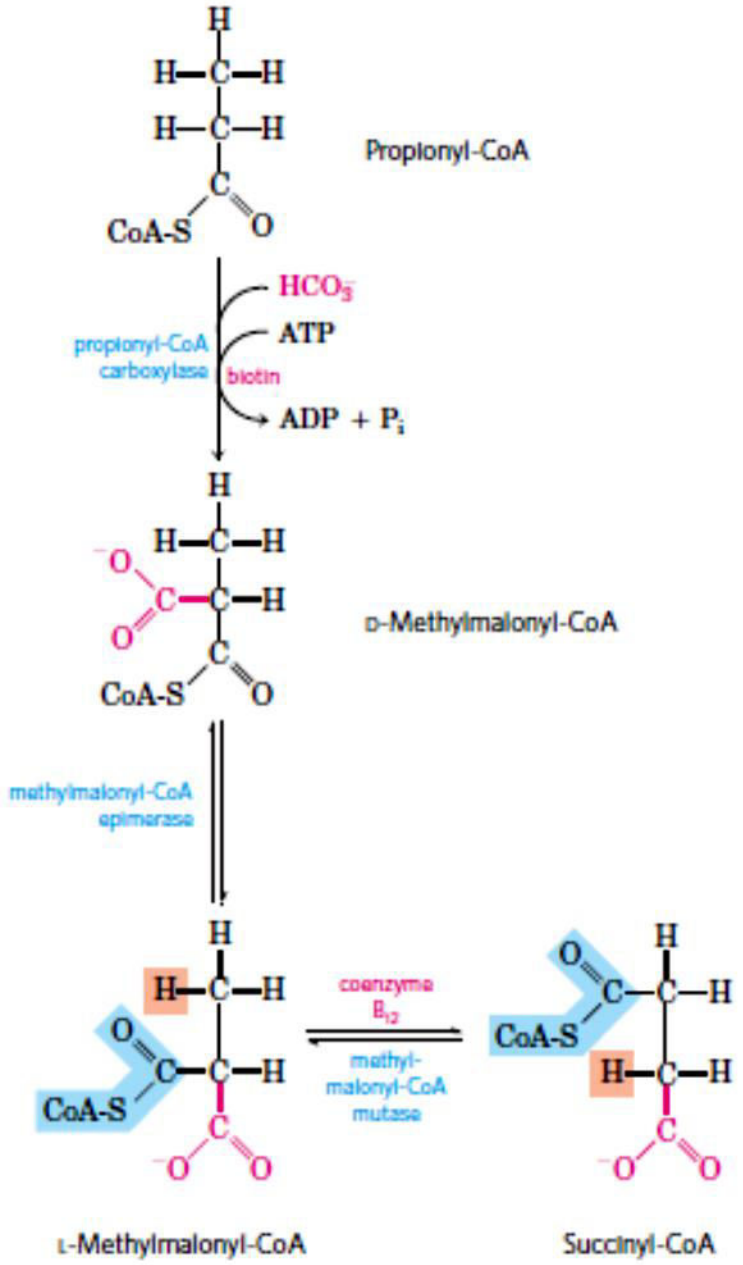
Oxidation of propionyl-CoA

- The oxidation and cleaving of this gives acetyl-CoA and **propionyl-CoA** as the products
- The acetyl-CoA can be oxidized in the citric acid cycle, but propionyl-CoA enters a different pathway having three enzymes.
- Propionyl-CoA is first carboxylated to form the D stereoisomer of **methylmalonyl-CoA** by **propionyl-CoA carboxylase**, which contains the cofactor biotin. In this enzymatic reaction, as in the pyruvate carboxylase reaction (see Fig. 16–17), CO2 (or its hydrated ion, HCO) is activated by attachment to biotin before its transfer to the substrate, in this case the propionate moiety.
- Formation of the carboxybiotin intermediate requires energy, which is provided by ATP.
- The D-methylmalonyl-CoA thus formed is enzymatically epimerized to its L stereoisomer by **methylmalonyl- CoA epimerase** (Fig. 17–12).
- The L-methylmalonyl-CoA then undergoes an intramolecular rearrangement to form succinyl-CoA, which can enter the citric acid cycle.
- This rearrangement is catalyzed by **methylmalonyl- CoA mutase**, which requires as its coenzyme **5'-deoxyadenosylcobalamin**, or **coenzyme B12**, which is derived from vitamin B12 (cobalamin).

Oxidation of propionyl-CoA produced by oxidation of odd-number fatty acids.

The sequence involves the carboxylation of propionyl-CoA to D-methylmalonyl-CoA and conversion of the latter to succinyl-CoA.

This conversion requires epimerization of D- to L-methylmalonyl- CoA, followed by a reaction in which substituents on adjacent carbon atoms exchange positions



Fatty Acid Catabolism-
72

Transcription Factors Turn on the Synthesis of Proteins for Lipid Catabolism

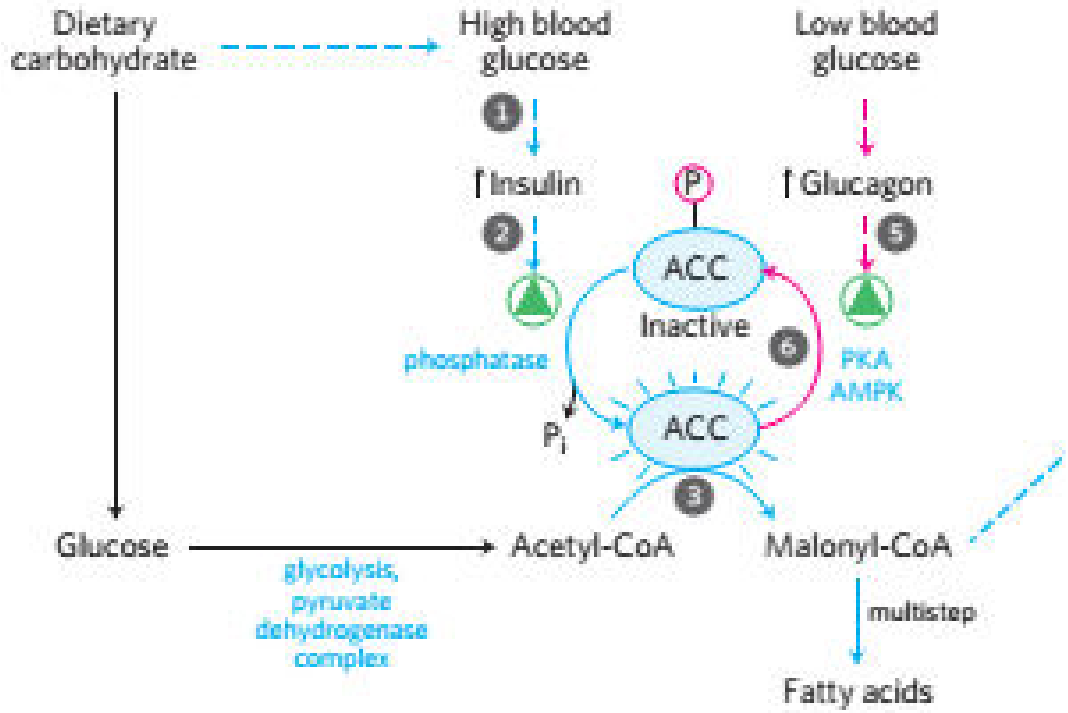
Transcription Factors Turn on the Synthesis of Proteins for Lipid Catabolism

Transcription Factors:

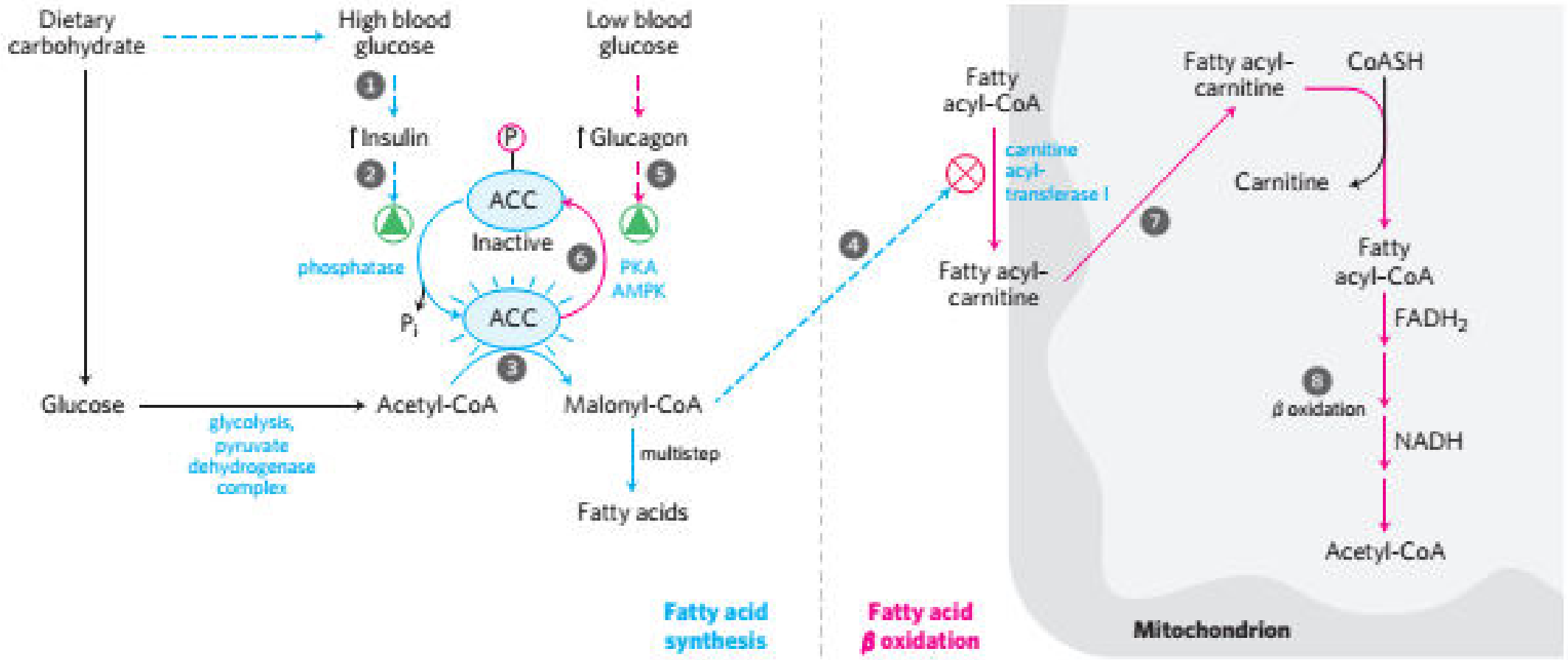
*a **transcription factor** (TF) (or sequence-specific DNA-binding **factor**) is a protein that controls the rate of **transcription** of particular genes; gene expression*

- Transcriptional regulation can change the number of molecules of the enzymes of fatty acid oxidation on a longer time scale
- The **PPAR** family of nuclear receptors are transcription factors that affect many metabolic processes in response to a variety of fatty acid–like ligands. (They were originally recognized as *p*eroxisome *p*roliferator-*a*ctivated *r*eceptors, then were found to function more broadly.)
- PPAR acts in muscle, adipose tissue, and liver to turn on a set of genes essential for fatty acid oxidation, including the fatty acid transporter, carnitine acyltransferases I and II, fatty acyl–CoA dehydrogenases for short, medium, long, and very long acyl chains, and related enzymes.

- This response is triggered when a cell or organism has an increased demand for energy from fat catabolism, **such as during a fast between meals or under conditions of longer-term starvation.**
- **Glucagon, released in response to low blood glucose**, can act through cAMP and the transcription factor CREB to turn on certain genes for lipid catabolism.
-



Fatty acid synthesis


Dietary carbohydrate
High blood glucose
Low blood glucose
1
↑ Insulin
↑ Glucagon
2
5
P
ACC
Inactive
6
phosphatase
PKA
AMPK
P_i
ACC
3
Glucose
glycolysis, pyruvate dehydrogenase complex
Acetyl-CoA
Malonyl-CoA
multistep
Fatty acids
4
Fatty acyl-CoA
carnitine acyl-transferase I
Fatty acyl-carnitine
7
Fatty acyl-carnitine
CoASH
Carnitine
Fatty acyl-CoA
$FADH_2$
8
β oxidation
NADH
Acetyl-CoA
Fatty acid synthesis
Fatty acid β oxidation
Mitochondrion

When the diet provides a ready source of carbohydrate as fuel, oxidation of fatty acids is unnecessary and is therefore downregulated. Two enzymes are key to the coordination of fatty acid metabolism: **acetyl-CoA carboxylase (ACC), the first enzyme in the synthesis of fatty acids, and carnitine acyltransferase I,** which limits the transport of fatty acids into the mitochondrial matrix for oxidation

Ingestion of a high-carbohydrate meal raises the blood glucose level and thus

1. triggers the release of insulin.
2. Insulin dependent protein phosphatase dephosphorylates ACC, activating it.
3. **ACC catalyzes the formation of malonyl-CoA (the first intermediate of fatty acid synthesis),** and
4. malonyl-CoA inhibits carnitine acyltransferase I, thereby preventing fatty acid entry into the mitochondrial matrix. When blood glucose levels drop between meals,
5. Glucagon release activates cAMP-dependent protein kinase **(PKA)**
6. **PKA phosphorylates and inactivates ACC.**
7. The concentration of malonylCoA falls, the inhibition of fatty acid entry into mitochondria is relieved, and fatty acids enter the mitochondrial matrix
8. become the major fuel. Because glucagon also triggers the mobilization of fatty acids in adipose tissue, a supply of fatty acids begins arriving in the blood.

- This response is triggered when a cell or organism has an increased demand for energy from fat catabolism, **such as during a fast between meals or under conditions of longer-term starvation.**
- **Glucagon, released in response to low blood glucose**, can act through cAMP and the transcription factor CREB to turn on certain genes for lipid catabolism.
- Another situation that is accompanied by major changes in the expression of the enzymes of fatty acid oxidation is the **transition from fetal to neonatal metabolism in the heart.**
- In the fetus the **principal fuels are glucose and lactate,** but **in the neonatal heart, fatty acids are the main fuel.**
- At the time of this transition, PPAR is activated and in turn activates the genes essential for fatty acid metabolism.
- The major site of fatty acid oxidation, at rest and during exercise, is skeletal muscle.
- Endurance training increases PPAR expression in muscle, leading to increased levels of fatty acid–oxidizing enzymes and increased oxidative capacity of the muscle.

Genetic Defects in Fatty Acyl–CoA Dehydrogenases Cause Serious Disease

- Stored triacylglycerols are typically the chief source of energy for muscle contraction, and an inability to oxidize fatty acids from triacylglycerols has serious consequences for health.
- The most common genetic defect in fatty acid catabolism in U.S. and northern European populations is due to a mutation in the gene encoding the **medium-chain acyl-CoA dehydrogenase (MCAD)**.
- Among northern Europeans, the frequency of carriers (individuals with this recessive mutation on one of the two homologous chromosomes) is about 1 in 40, and about 1 individual in 10,000 has the disease—that is, has two copies of the **mutant MCAD allele and is unable to oxidize fatty acids of 6 to 12 carbons.**
- **Symptoms:** The disease is characterized by recurring episodes of a syndrome that includes fat accumulation in the liver, **high blood levels of octanoic acid (8:0), low blood glucose (hypoglycemia), sleepiness, vomiting, and coma**.
- **Diagnosis :** The pattern of organic acids in the urine helps in the diagnosis of this disease: the urine commonly contains high levels of 6-carbon to 10-carbon dicarboxylic acids (produced by oxidation) and low levels of urinary ketone bodies (we discuss oxidation below and ketone bodies in Section 17.3).
- Although individuals may have no symptoms between episodes, the episodes are very serious; mortality from this disease is 25% to 60% in early childhood.

- If the genetic defect is detected shortly after birth, the infant can be started on a low-fat, high-carbohydrate diet.
- With early detection and careful management of the diet—including avoiding long intervals between meals, to prevent the body from turning to its fat reserves for energy—the prognosis for these individuals is good.

  More than 20 other human genetic defects in fatty acid transport or oxidation have been documented, most much less common than the defect in MCAD.
- One of the most severe disorders results from loss of the **long-chain *Beta*-hydroxyacyl-CoA** dehydrogenase activity of the **trifunctional protein, TFP.**
- Other disorders include defects in the ***alpha*** or ***Beta*** **subunits** that affect all three activities of TFP and cause serious heart disease and abnormal skeletal muscle.

Transcription Factors:

a **transcription factor** (TF) (or sequence-specific DNA-binding **factor**) is a protein that controls the rate of **transcription** of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence.

Fatty Acid Catabolism-
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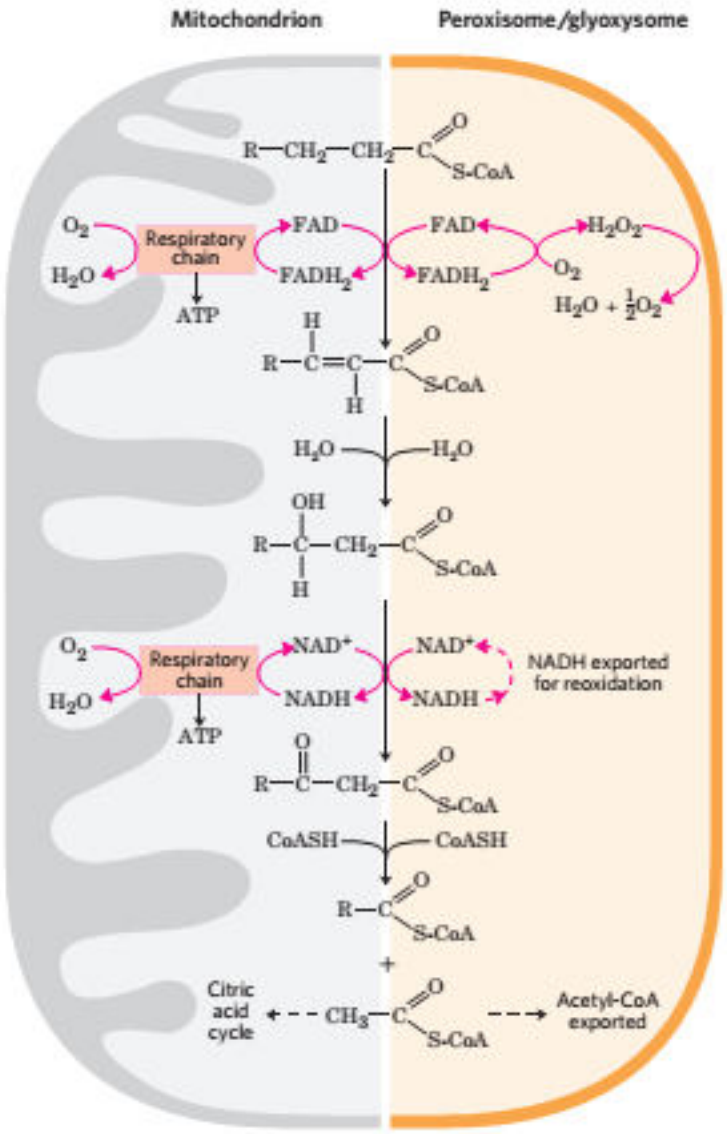
Peroxisomes Also Carry Out β-Oxidation

Peroxisomes Also Carry Out *beta* Oxidation

- The mitochondrial matrix is the major site of fatty acid oxidation in animal cells, but in certain cells other compartments also contain enzymes capable of oxidizing fatty acids to acetyl-CoA, by a pathway not identical to that in mitochondria.
- In plant cells, the major site of *beta* oxidation is not mitochondria but peroxisomes.
  In **peroxisomes**, membrane-enclosed organelles of animal and plant cells, the intermediates for oxidation of fatty acids are coenzyme A derivatives, and the process consists of four steps, as in mitochondrial *beta* oxidation

(1) Dehydrogenation
(2) addition of water to the resulting double bond,
(3) oxidation of the *beta*-hydroxyacyl-CoA to a ketone, and
(4) thiolytic cleavage by coenzyme A. (The identical reactions also occur in glyoxysomes)



- One difference between the peroxisomal and mitochondrial pathways is in the chemistry of the first step.
- In peroxisomes, the flavoprotein acyl-CoA oxidase that introduces the double bond passes electrons directly to O2, producing H2O2 (hydrogen peroxide). (Thus the name peroxisomes.)
- This strong and potentially damaging oxidant is immediately cleaved to H2O and O2 by **catalase**.
- Recall that in mitochondria, the electrons removed in the first oxidation step pass through the respiratory chain to O2 to produce H2O, and this process is accompanied by ATP synthesis.
- In peroxisomes, the energy released in the first oxidative step of fatty acid breakdown is not conserved as ATP, but is dissipated as heat

- A second important difference between mitochondrial and peroxisomal *Beta* oxidation in mammals is in the specificity for fatty acyl–CoAs; the peroxisomal system is much more active on very-long-chain fatty acids such as hexacosanoic acid (26:0) and on branched chain fatty acids such as phytanic acid and **pristanic acid** (see Fig. 17–18).
- These less-common fatty acids are obtained in the diet from dairy products, the fat of ruminant animals, meat, and fish. Their catabolism in the peroxisome involves several auxiliary enzymes unique to this organelle.

- The inability to oxidize these compounds is responsible for several serious human diseases.
- Individuals with **Zellweger syndrome** are unable to make peroxisomes and therefore lack all the metabolism unique to that organelle.
- In **X-linked adrenoleukodystrophy (XALD)**, peroxisomes fail to oxidize very-long-chain fatty acids, apparently for lack of a functional transporter for these fatty acids in the peroxisomal membrane.
- Both defects lead to accumulation in the blood of very-long-chain fatty acids, especially 26:0.
- XALD affects young boys before the age of 10 years, causing loss of vision, behavioral disturbances, and death within a few years.

- **In mammals, high concentrations of fats in the diet result in increased synthesis of the enzymes of peroxisomal *Beta* oxidation in the liver.**
- Liver peroxisomes do not contain the enzymes of the citric acid cycle and cannot catalyze the oxidation of acetyl-CoA to CO2. Instead, long-chain or branched fatty acids are catabolized to shorter-chain products, such as hexanoyl-CoA, which are exported to mitochondria and completely oxidized.

Fatty Acid Catabolism-
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Peroxisomes Also Carry Out β- Oxidation-cont..

The *beta*-Oxidation Enzymes of Different Organelles Have Diverged during Evolution

Glyoxysomes

- Plant peroxisomes and glyoxysomes are similar in structure and function
- **Glyoxysomes** are specialized peroxisomes found in plants (particularly in the fat storage tissues of germinating seeds) and also in filamentous fungi.
- Seeds that contain fats and oils include corn, soybean, sunflower, peanut and pumpkin.

Plant Peroxisomes and Glyoxysomes Use Acetyl-CoA as a Biosynthetic Precursor

- **In plants, fatty acid oxidation does not occur primarily in mitochondria but in the peroxisomes of leaf tissue and in the glyoxysomes of germinating seeds.**
-
- The biological role of oxidation in these organelles is to use stored lipids primarily to provide biosynthetic precursors, not energy.
  During seed germination, stored triacylglycerols are converted into glucose, sucrose, and a wide variety of essential metabolites **(Fig. 17–15)**.
- Fatty acids released from the triacylglycerols are first activated to their coenzyme A derivatives and oxidized in glyoxysomes by the same four-step process that takes place in peroxisomes
- The acetyl-CoA produced is converted via the glyoxylate cycle to four-carbon precursors for gluconeogenesis
- **Glyoxysomes, like peroxisomes, contain high concentrations of catalase,** which converts the H2O2 produced by oxidation to H2O and O2.

- Enzymes found in the **glyoxysome** catalyze the formation of acetyl CoA from fatty acids stored within germinating seeds

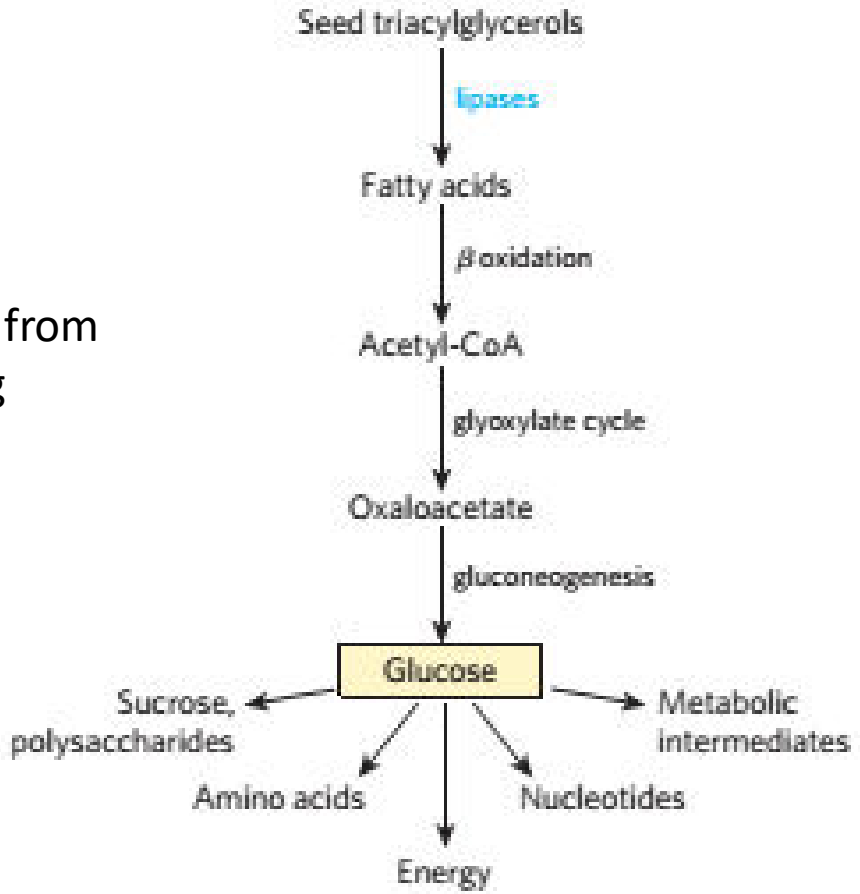


FIGURE 17–15 Triacylglycerols as glucose source in seeds. β Oxidation is one stage in a pathway that converts stored triacylglycerols to glucose in germinating seeds. For more detail, see Figure 16–24.

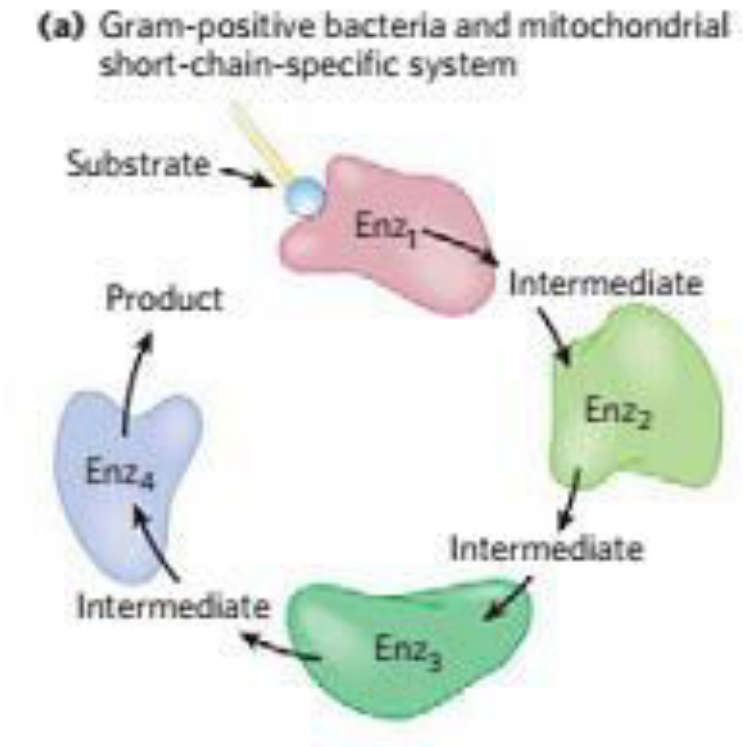
The *beta*-Oxidation Enzymes of Different Organelles Have Diverged during Evolution

- Although the beta-oxidation reactions in mitochondria are essentially the same as those in peroxisomes and glyoxysomes, the enzymes (isozymes) differ significantly between the two types of organelles.
- The differences reflect an evolutionary divergence that occurred very early, with the separation of gram-positive and gram-negative bacteria
- **In mitochondria, the four -oxidation enzymes that act on short-chain fatty acyl–CoAs are separate,** soluble proteins similar in structure to the analogous enzymes of gram-positive bacteria
- The gram-negative bacteria have four activities in three soluble subunits (Fig. 17–16b), and the eukaryotic enzyme system that acts on long-chain fatty acids—the trifunctional protein, **TFP—has three enzyme activities in two subunits that are membrane-associated**

- The *beta*-oxidation enzymes of plant peroxisomes and glyoxysomes, form a complex of proteins, one of which contains four enzymatic activities in a single polypeptide
- The first enzyme, acyl-CoA oxidase, is a single polypeptide chain; the **multifunctional protein (MFP)** contains the second and third enzyme activities (enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase) as well as two auxiliary activities needed for the oxidation of unsaturated fatty acids (D-3-hydroxyacyl-CoA epimerase and D3,D2-enoyl-CoA isomerase); the fourth enzyme, thiolase, is a separate, soluble polypeptide.

- Interesting thing is that the enzymes that catalyze the synthesis of fatty acids are also organized differently in bacteria and eukaryotes; in bacteria, the seven enzymes needed for fatty acid synthesis are separate polypeptides, but in mammals, all seven activities are part of a single, huge polypeptide chain.
-

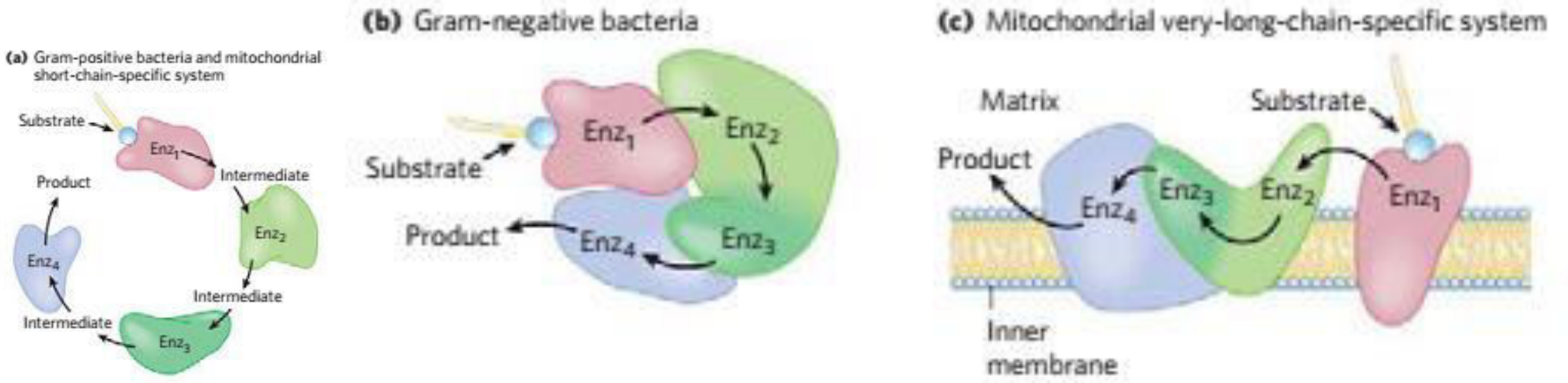
- One advantage to the cell in having several enzymes of the same pathway encoded in a single polypeptide chain is that this solves the problem of regulating the synthesis of enzymes that must interact functionally; regulation of the expression of *one* gene ensures production of the same number of active sites for all enzymes in the path.
- When each enzyme activity is on a separate polypeptide, some mechanism is required to coordinate the synthesis of all the gene products.
- The *disadvantage* of having several activities on the same polypeptide is that the longer the polypeptide chain, the greater is the probability of a mistake in its synthesis: a single incorrect amino acid in the chain may make all the enzyme activities in that chain useless.



The enzymes of oxidation.

In the picture Shown on next slides, are the different subunit structures of the enzymes of *Beta* oxidation in gram-positive and gram-negative bacteria, mitochondria, and plant peroxisomes and glyoxysomes.

Enz1 is acyl-CoA dehydrogenase; Enz2, enoyl-CoA hydratase; Enz3, L- *Beta* -hydroxyacyl-CoA dehydrogenase; Enz4, thiolase; Enz5, D-3-hydroxyacyl-CoA epimerase, and Enz6, D3,D2-enoyl-CoA isomerase.

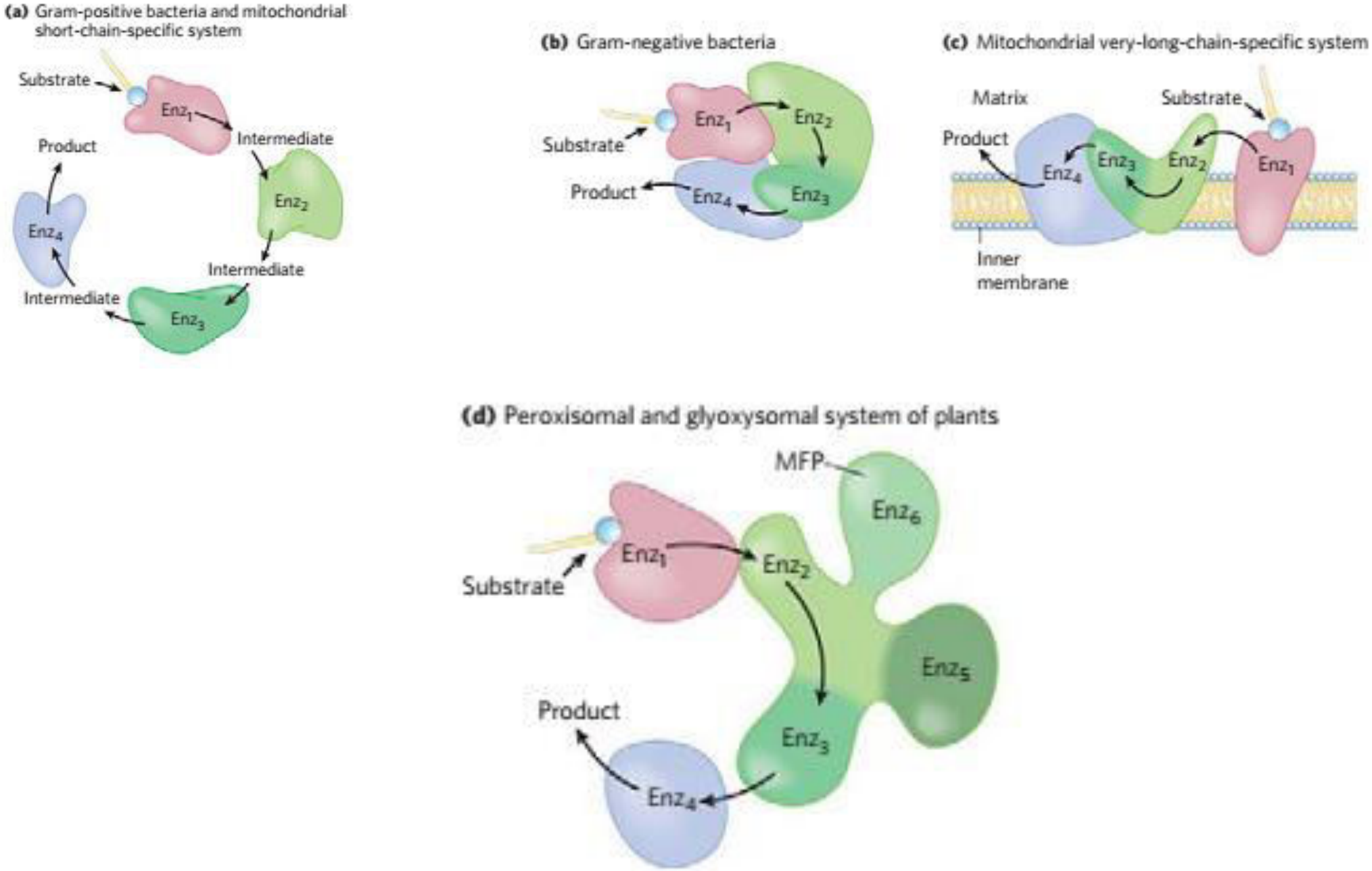


(a) The four enzymes of *beta* oxidation in gram-positive bacteria are separate, soluble entities, as are those of the short-chain-specific system of mitochondria

(b) In gram negative bacteria, the four enzyme activities reside in three polypeptides; Enz2 and Enz 3 are parts of a single polypeptide chain.

(c) The very-long-chain-specific system of mitochondria is also composed of three polypeptides, one of which includes the activities of Enz2 and Enz3; in this case, the system is bound to the inner mitochondrial membrane.

(d) In the peroxisomal and glyoxysomal *beta* oxidation systems of plants, Enz 1 and Enz4 are separate polypeptides, but Enz2 and Enz3, as well as two auxiliary enzymes (Enz5 and Enz6), are part of a single polypeptide chain: the multifunctional protein, MFP.


(a) Gram-positive bacteria and mitochondrial short-chain-specific system
Substrate
Enz1
Intermediate
Enz2
Intermediate
Enz3
Intermediate
Enz4
Product
(b) Gram-negative bacteria
Substrate
Enz1
Enz2
Enz3
Product
Enz4
(c) Mitochondrial very-long-chain-specific system
Matrix
Substrate
Product
Enz4
Enz3
Enz2
Enz1
Inner membrane
(d) Peroxisomal and glyoxysomal system of plants
MFP
Enz6
Substrate
Enz1
Enz2
Enz5
Product
Enz3
Enz4

- **Isozymes**
- In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants, of enzymes.
- Isozymes represent enzymes from different genes that process or catalyse the same reaction

Fatty Acid Catabolism-
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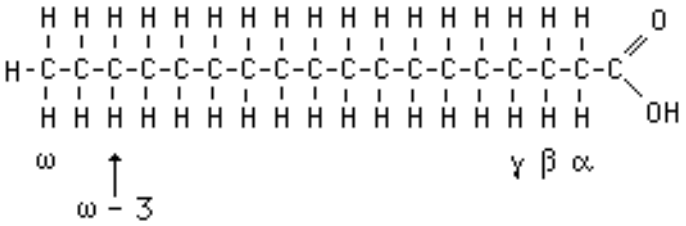
The *Omega*-Oxidation of Fatty Acids Occurs in the Endoplasmic Reticulum

The *Omega*-Oxidation of Fatty Acids

- **Omega oxidation** (ω-**oxidation**) is a process of fatty acid metabolism in some species of animals.
- **The carbon atom closest to the carboxyl group is the alpha carbon, the next carbon is the beta carbon and so on.**
- It is an alternative pathway to beta **oxidation** that, instead of involving the β carbon, involves the **oxidation** of the ω carbon (the carbon most distant from the carboxyl group of the fatty acid).
- In vertebrates, the enzymes for ω oxidation are located in the smooth ER of liver and kidney cells, instead of in the mitochondria where β oxidation occurs



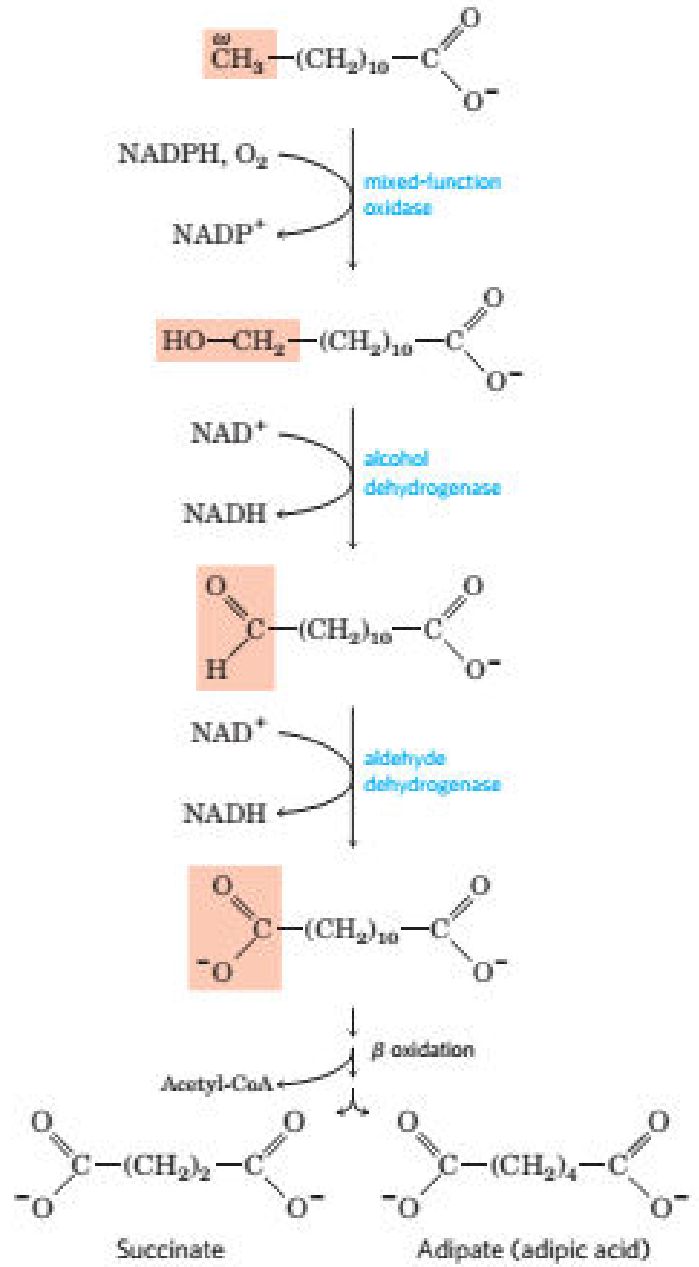
Use of Greek letters to designate carbons

The carbon next to the -COOH group is designated α; the next one is β, and so forth. The most distant carbon is designated ω. Sometimes carbon atoms close to the ω carbon are designated in relation to it. *E.g.*, the third from the end is ω - 3 (omega minus 3).

The *Omega*-Oxidation of Fatty Acids Occurs in the Endoplasmic Reticulum

- Although mitochondrial ***Beta*** oxidation, in which enzymes act at the carboxyl end of a fatty acid, is by far the most important catabolic fate for fatty acids in animal cells, there is another pathway in some species, including vertebrates, that involves oxidation of **the (omega) carbon—the carbon most distant from the carboxyl group.**
- The enzymes unique to ***Omega*** **oxidation are located (in vertebrates) in the endoplasmic reticulum of liver and** kidney, and the preferred substrates are fatty acids of 10 or 12 carbon atoms.
- In mammals ***Omega -*** oxidation is normally a minor pathway for fatty acid degradation, but when ***beta-***oxidation is defective (because of mutation or a carnitine deficiency) it becomes more important.

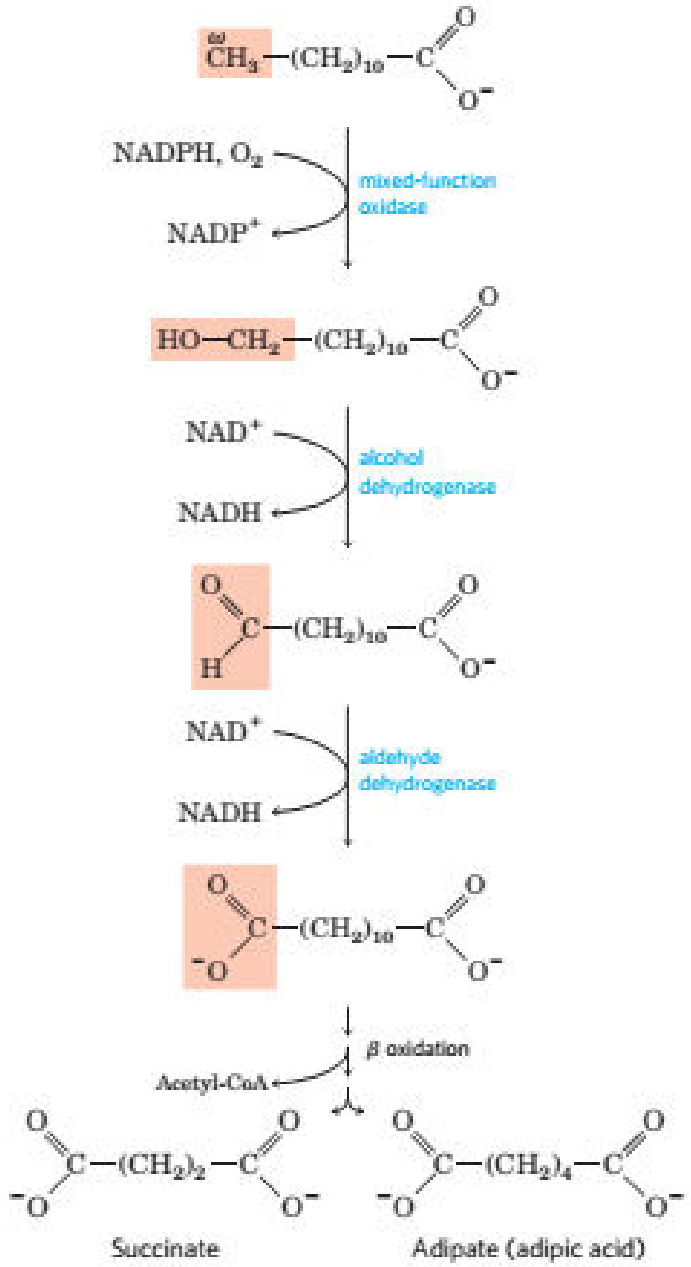
FIGURE 17-17 **The ω oxidation of fatty acids in the endoplasmic reticulum.** This alternative to β oxidation begins with oxidation of the carbon most distant from the β carbon—the ω (omega) carbon. The substrate is usually a medium-chain fatty acid; shown here is lauric acid (laurate). This pathway is generally not the major route for oxidative catabolism of fatty acids.



1. The first step introduces a hydroxyl group onto the ***Omega* carbon ..** Hydroxylation**.** The oxygen for this group comes from molecular oxygen (O2) in a complex reaction that involves **cytochrome P450** and the electron donor NADPH. Reactions of this type are catalyzed by **mixed-function oxidases**
2. Oxidation of the hydroxyl group to an aldehyde by **alcohol dehydrogenase**
3. The third step is the Oxidation of the aldehyde group to a carboxylic acid by NAD^+. The product of this step is a fatty acid with a carboxyl group at each end, by **aldehyde dehydrogenase**

- At this point, either end can be attached to coenzyme A, and the molecule can enter the mitochondrion and undergo oxidation by the normal route.

- In each pass through beta -oxidation pathway, **the "double-ended" fatty acid yields final products dicarboxylic acids such as succinic acid, which can enter the citric acid cycle, and adipic acid.**



- **Adipic acid**
- **Adipic acid** or hexanedioic **acid (6 carbon chain)** is the organic compound with the formula $(CH_2)_4(COOH)_2$. From an industrial perspective, it is the most important dicarboxylic **acid**
- About 2.5 billion kilograms of this white crystalline powder are produced annually, mainly as a precursor for the production of nylon.

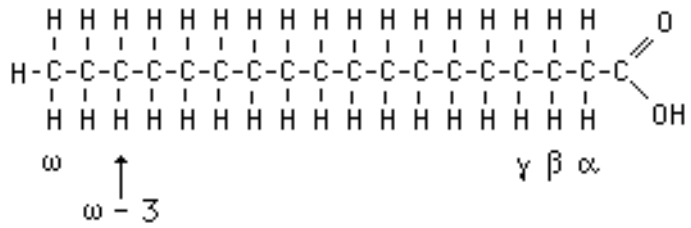
Fatty Acid Catabolism-76

Phytanic Acid Undergoes alpha-Oxidation in Peroxisomes

- ***Alpha*-Oxidation**

- **α-Oxidation** is important in the catabolism of branched-chain fatty acids.
- **Takes place at alpha carbon atom in peroxisomes**

- **Phytanic acid:**
- **Phytanic acid** (or **3,7,11,15-tetramethyl hexadecanoic acid-**$C_{20}H_{40}O_2$) is a branched chain fatty acid that humans can obtain through the consumption of dairy products, ruminant animal fats, and certain fish
- Unlike most fatty acids, phytanic acid cannot be metabolized by β-oxidation

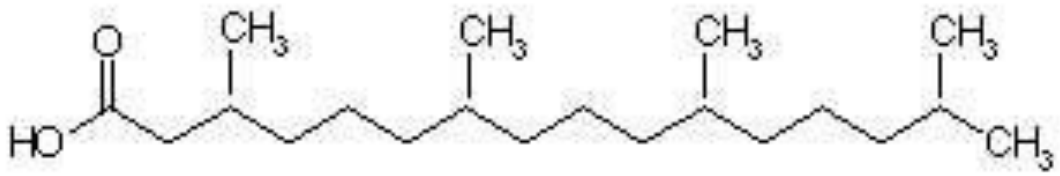


Use of Greek letters to designate carbons

The carbon next to the –COOH group is designated α; the next one is β, and so forth. The most distant carbon is designated ω. Sometimes carbon atoms close to the ω carbon are designated in relation to it. *E.g.*, the third from the end is ω – 3 (omega minus 3).

Phytanic Acid Undergoes *Alpha*-Oxidation in Peroxisomes

- Phytanic acid has a **methyl-substituted *beta*- carbon** and therefore cannot undergo ***beta*-** oxidation.
- These branched fatty acids are **catabolized in peroxisomes** of animal cells by ***Alpha*-oxidation.**

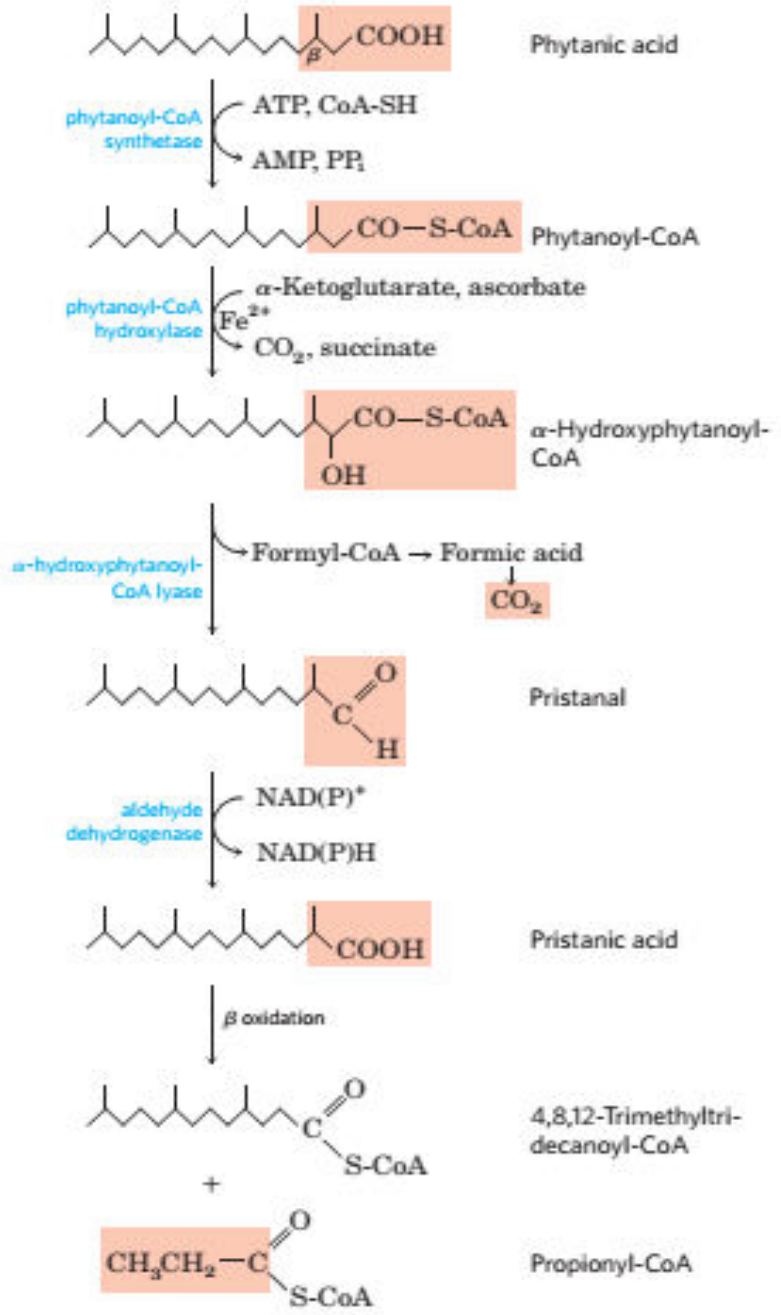


PHYTANIC ACID

Steps of *Alpha*-Oxidation

1. **Phytanic acid** is first attached to **CoA** to form **phytanoyl CoA**
2. **Phytanoyl CoA** is oxidized by **phytanoyl CoA hydroxylase** in a process using Fe^{2+} and O_2, to yield 2-hydroxyphytanoyl-CoA.
3. 2-hydroxyphytanoyl-CoA is cleaved to form pristanal and **formyl-CoA** (in turn later broken down into formate and eventually CO_2).
4. Pristanal is oxidized by **aldehyde dehydrogenase** to form **pristanic acid** (which can then undergo **beta-Oxidation**

- Notice that ***beta-*** oxidation of pristanic acid releases propionyl-CoA, not acetyl-CoA.



Steps of *Alpha*-Oxidation

- Propionyl-CoA is further catabolized as in case of odd numbered fatty acids
- **alpha carbon was decarboxylated to form an aldehyde, that is now having a carbon chain shorter of 1 carbon but has no functional group on beta carbon**
- **Now it's oxidized at beta carbon and process continues in same way**

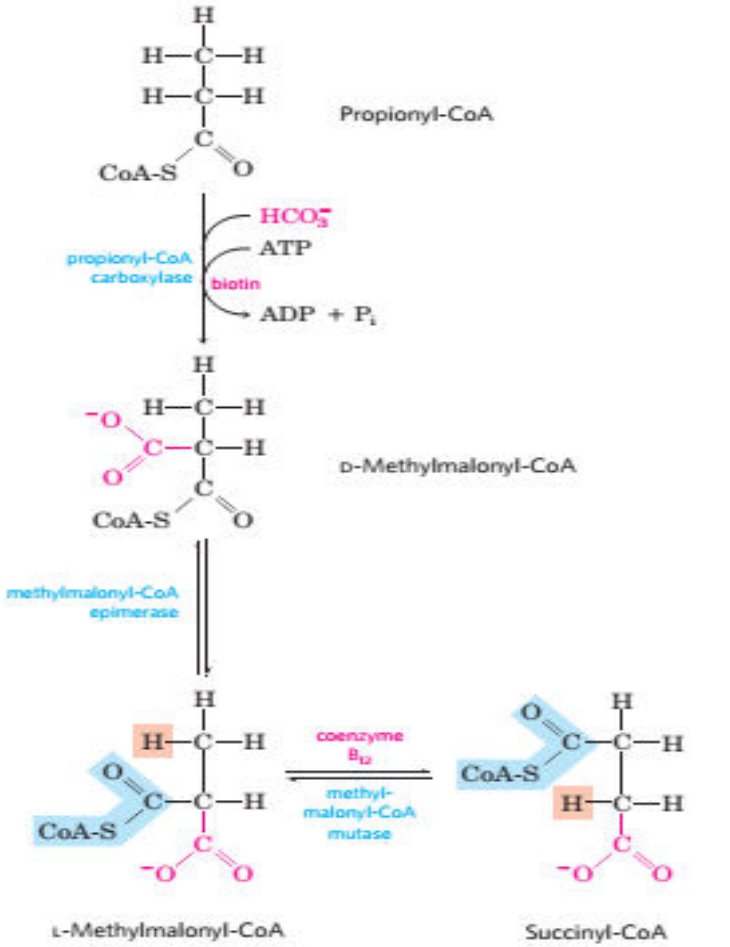


FIGURE 17–12 Oxidation of propionyl-CoA produced by β oxidation of odd-number fatty acids. The sequence involves the carboxylation of propionyl-CoA to D-methylmalonyl-CoA and conversion of the latter to succinyl-CoA. This conversion requires epimerization of D- to L-methylmalonyl-CoA, followed by a remarkable reaction in which substituents on adjacent carbon atoms exchange positions (see Box 17–2).

Refsum disease

- **Refsum disease:**
- A disorder resulting from a genetic defect in phytanoyl-CoA hydroxylase, leads to very high blood levels of phytanic acid and severe neurological problems, including blindness and deafness.
- Phytanic acid accumulates in patients suffering from a peroxisome biogenesis disorder, which includes Zellweger Syndrome, Neonatal Adrenoleukodystrophy, and Infantile Refsum Disease.
- Formic acid and not CO_2 is the primary reaction product of phytanic acid oxidation. The amount of formic acid was shown to be about 9-fold higher as compared to CO_2

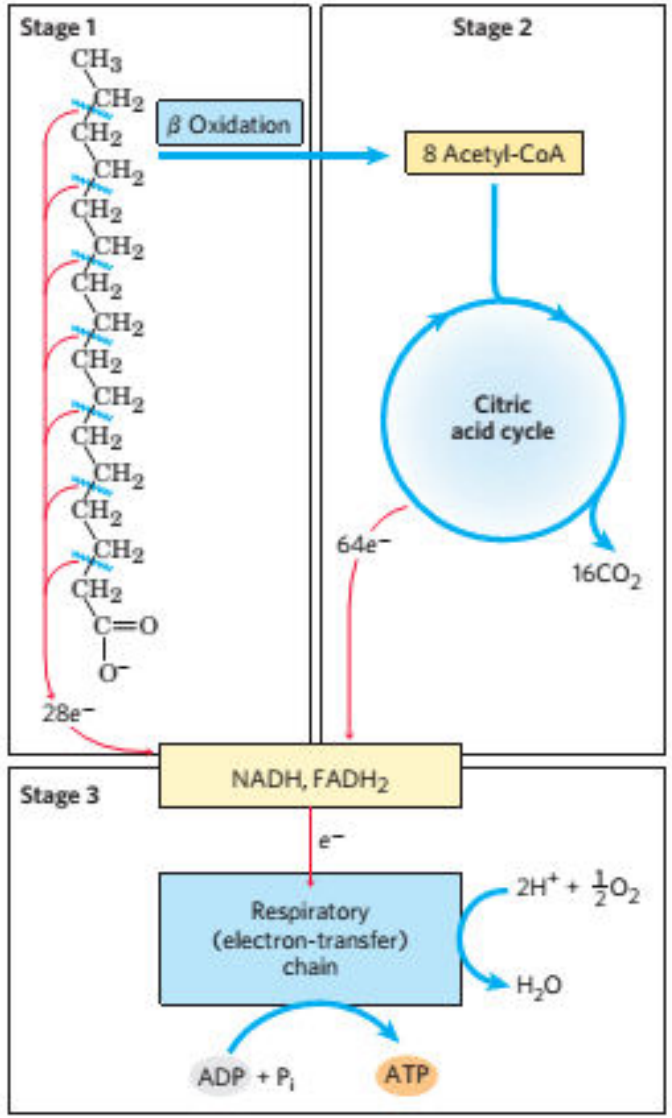
- **Origin of Phytanic Acid**
- Phytol is the alcohol moiety of the chlorophyll molecule and, as an integral part of chlorophyll, it is abundantly present in plants and even in the marine environment
- Phytol can be released from chlorophyll by the action of bacteria present in the rumen of ruminant animals.
- The resulting free **phytol** can be converted to phytanic acid in the rumen of animals

Fatty Acid Catabolism-77

Ketone Bodies

Ketone Bodies

- In humans and most other mammals, acetyl-CoA formed in the liver during oxidation of fatty acids can either enter the citric acid cycle or undergo conversion to the “ketone bodies,”
- **These include acetone**, **acetoacetate**, and **D-*beta*-hydroxybutyrate,** for export to other tissues**.**
- **The term “bodies” is a historical artifact; the term is occasionally applied to insoluble particles, but these compounds are soluble in blood and urine.**



Stages of fatty acid oxidation

- **Acetone**, produced in smaller quantities than the other ketone bodies, is exhaled.
- **Acetoacetate** and **D-*beta*- hydroxybutyrate** are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing much of the energy required by tissues such as skeletal and heart muscle and the renal cortex.
- The brain, which preferentially uses glucose as fuel, can adapt to the use of **acetoacetate** or **D-*beta*- hydroxybutyrate** under starvation conditions, when glucose is unavailable.
- The production and export of ketone bodies from the liver to extrahepatic tissues allows continued oxidation of fatty acids in the liver when acetyl CoA is not being oxidized in the citric acid cycle.

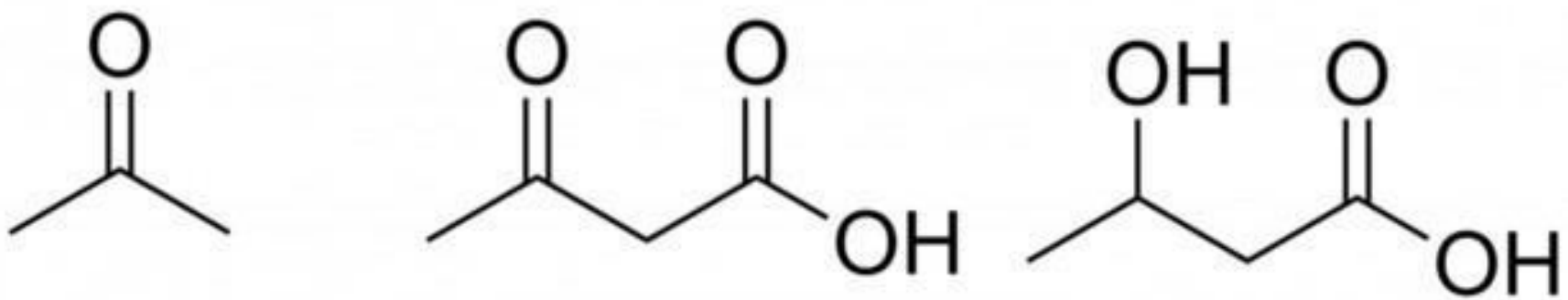
Ketone Bodies, Formed in the Liver, Are Exported to Other Organs as Fuel

- The first step in the formation of acetoacetate, occurring in the liver, is the enzymatic condensation of two molecules of acetyl-CoA, catalyzed by thiolase; this is simply the reversal of the last step of oxidation of fatty acids.
- The acetoacetyl-CoA then condenses with acetyl-CoA to form ***Beta*-hydroxy-*Beta*-methylglutaryl-CoA (HMG-CoA), which is cleaved to free acetoacetate and acetyl-CoA.**
- **The acetoacetate is reversibly reduced by D-*Beta*-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-*Beta*-hydroxybutyrate.**
- **This enzyme is specific for the D stereoisomer; it does not act on L *Beta*-hydroxyacyl-CoAs and is not to be confused with L-*Beta*-hydroxyacyl-CoA dehydrogenase of the *Beta*-oxidation pathway.**

Fatty Acid Catabolism-78

Ketone Bodies.. cont

Ketone Bodies



Acetone

Acetoacetic acid

Beta-hydroxybutyric acid
(Often referred to as
Beta-hydroxybutyrate)

Ketone Bodies in healthy condition

- In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of **acetoacetate decarboxylase** (Fig. 17–19).
- **Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic.**
- **Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes.**
- In extrahepatic tissues, D-***Beta***-hydroxybutyrate is oxidized to acetoacetate by D-***Beta-*** hydroxybutyrate dehydrogenase **(Fig. 17–20)**.
- **The acetoacetate is activated to its coenzyme A ester** by transfer of CoA from succinylCoA, an intermediate of the citric acid cycle (see Fig.16–7), in a reaction catalyzed by ***Beta-*****ketoacyl-CoA transferase, also called thiophorase.**
- **The acetoacetylCoA is then cleaved by thiolase to yield two acetylCoAs, which enter the citric acid cycle.**
- **Thus the ketone bodies are used as fuels in all tissues except liver, which lacks thiophorase.**

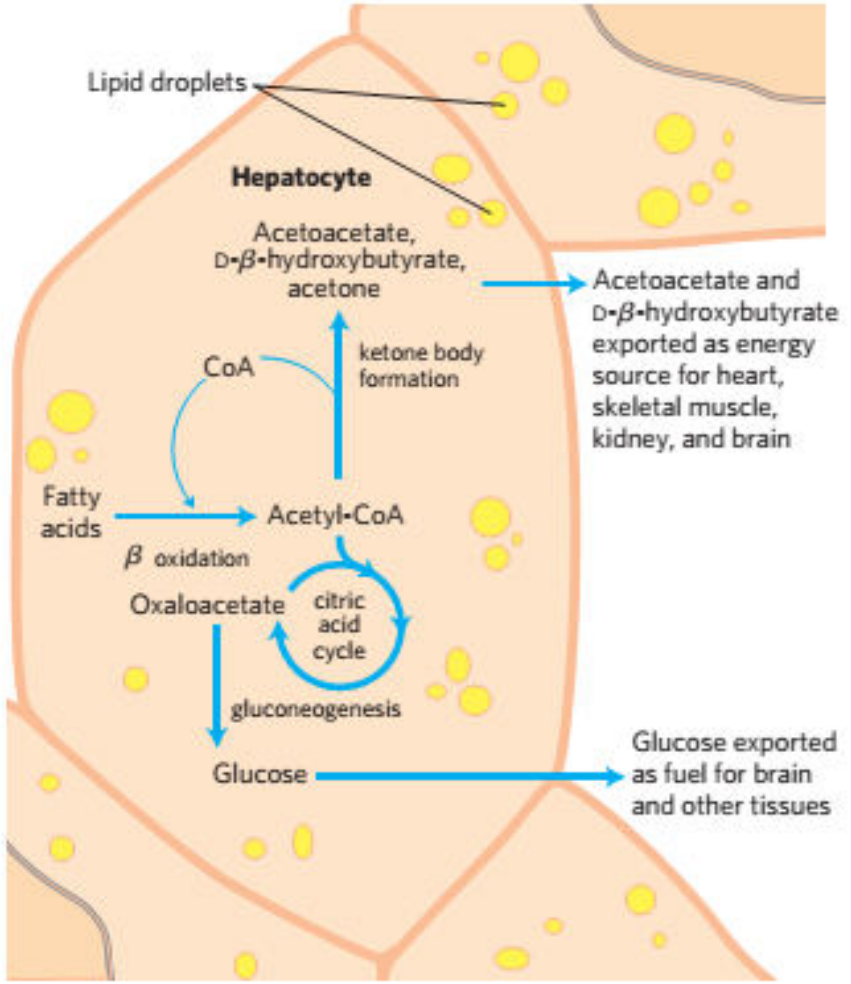
- **The liver is therefore a producer of ketone bodies for the other tissues, but not a consumer.**
  The production and export of ketone bodies by the liver allows continued oxidation of fatty acids with only minimal oxidation of acetyl-CoA.
- **When intermediates of the citric acid cycle are being siphoned off for glucose synthesis by gluconeogenesis, for example, oxidation of** cycle intermediates slows—and so does acetyl-CoA oxidation.
- Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is tied up in acetyl-CoA, ***Beta-***oxidation slows for want of the free coenzyme.
- The production and export of ketone bodies frees coenzyme A, allowing continued fatty acid oxidation.

Ketone Bodies Are Overproduced in Diabetes and during Starvation

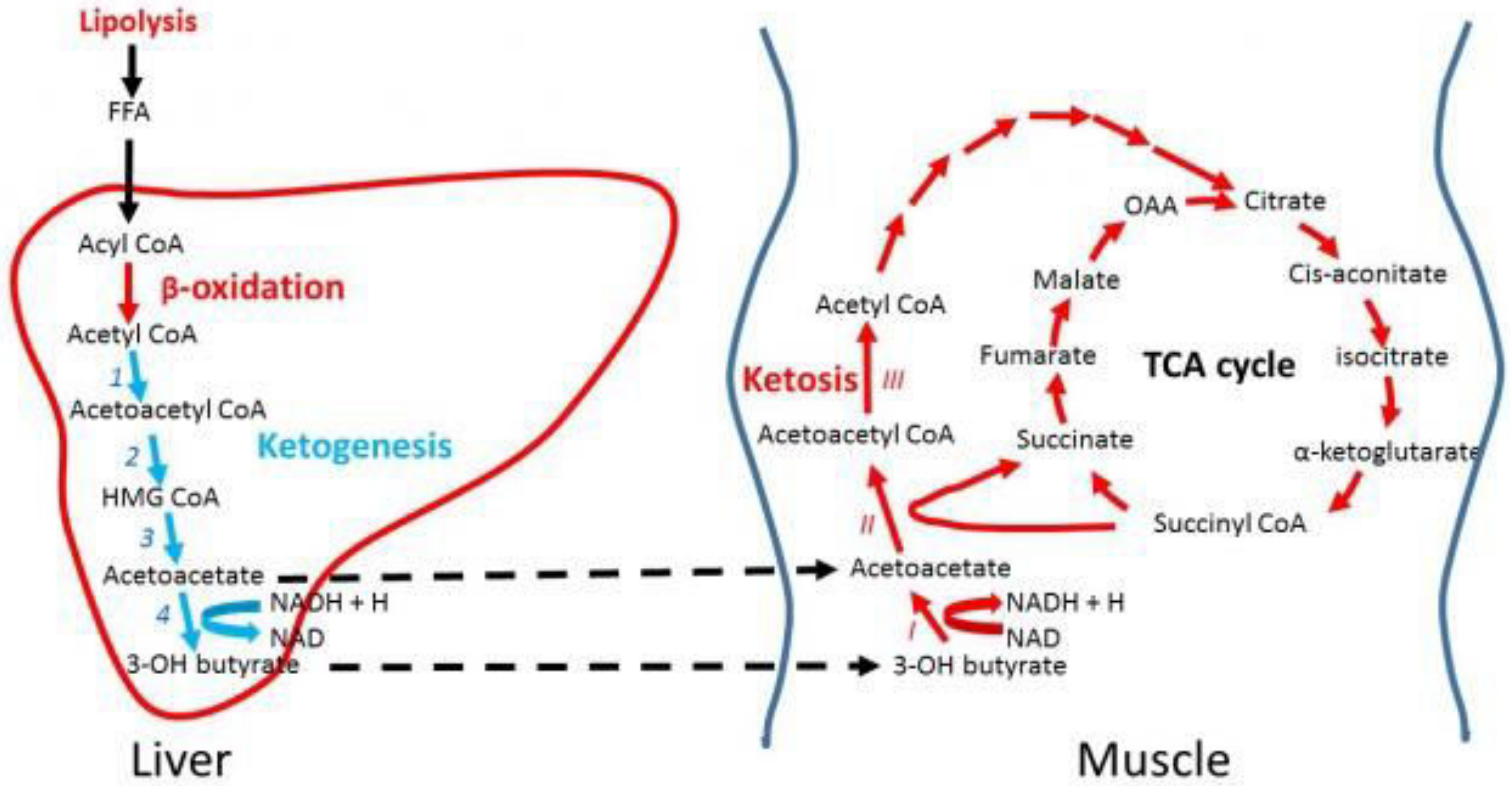
- Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies, with several associated medical problems. During starvation, gluconeogenesis depletes citric acid cycle intermediates, diverting acetyl-CoA to ketone body production **(Fig. 17–21)**.
- In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat.
- Under these conditions, levels of malonyl-CoA (the starting material for fatty acid synthesis) fall, inhibition of carnitine acyltransferase I is relieved, and fatty acids enter mitochondria to be degraded to **acetylCoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis**.
- The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies beyond the capacity of extrahepatic tissues to oxidize them.

…cont

- The increased blood levels of acetoacetate and **D-*betA*-hydroxybutyrate** lower the blood pH, causing the condition known as **acidosis**.
- Extreme acidosis can lead to coma and in some cases death. Ketone bodies in the blood and urine of individuals with untreated diabetes can reach extraordinary levels—a blood concentration of 90 mg/100 mL (compared with a normal level of ,3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of #125 mg/24 hr). This condition is called **ketosis**.
- Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine. These levels must be monitored to avoid the dangers of acidosis and ketosis (ketoacidosis).



Ketone body formation and export from the liver.
Conditions that promote gluconeogenesis (untreated diabetes, severely reduced food intake) slow the citric acid cycle (by drawing off oxaloacetate) and enhance the conversion of acetyl-CoA to acetoacetate.
The released coenzyme A allows continued *Beta* oxidation of fatty acids.


Lipolysis
FFA
Acyl CoA
β-oxidation
Acetyl CoA
1
Acetoacetyl CoA
Ketogenesis
2
HMG CoA
3
Acetoacetate
4
NADH + H
NAD
3-OH butyrate
Liver
Acetyl CoA
Ketosis
III
Acetoacetyl CoA
II
Acetoacetate
I
NADH + H
NAD
3-OH butyrate
OAA
Citrate
Malate
Cis-aconitate
Fumarate
TCA cycle
isocitrate
Succinate
α-ketoglutarate
Succinyl CoA
Muscle

ALLAH HAFIZ

WISH YOU BEST OF LUCK