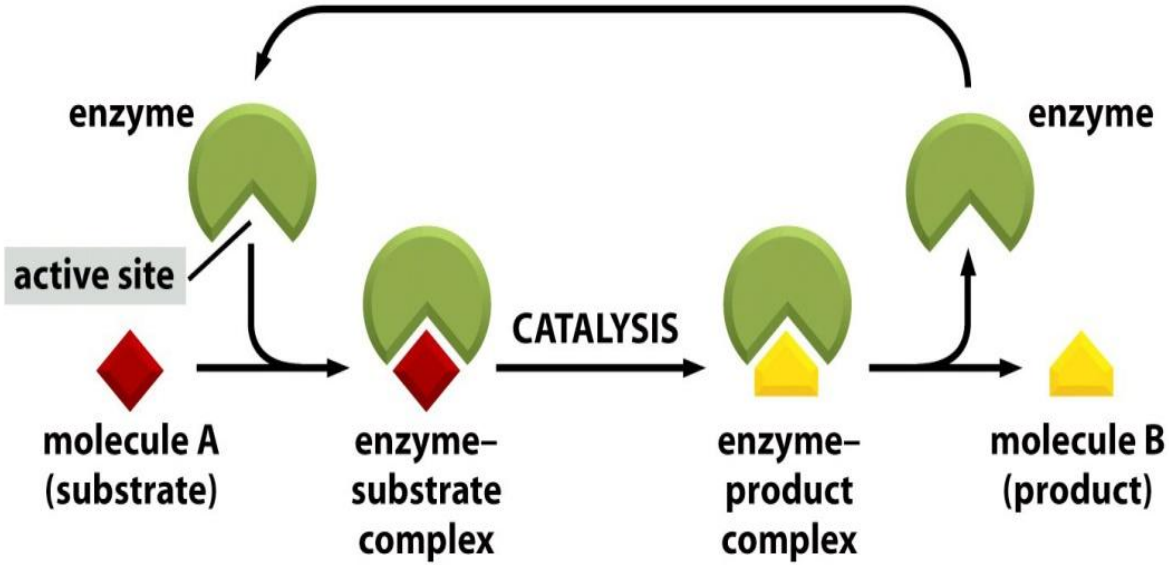
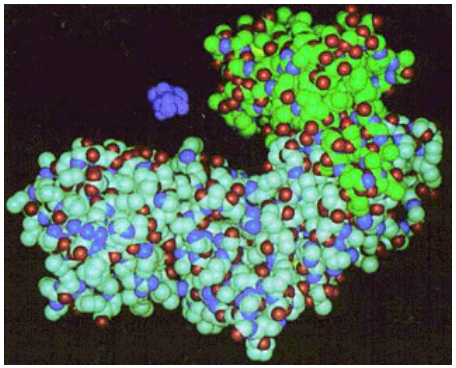
Enzymes II.

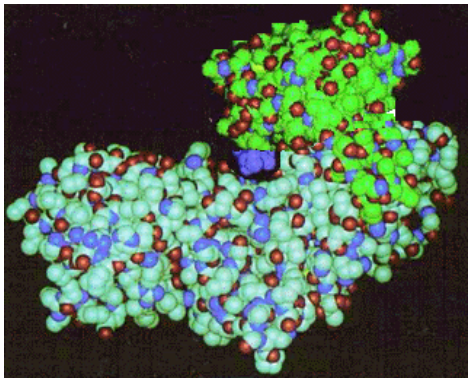


Active site: a pocket on the enzyme, where the enzyme-catalyzed reaction takes place

Substrate: The molecule that is bound in the active site and acted upon by the enzyme

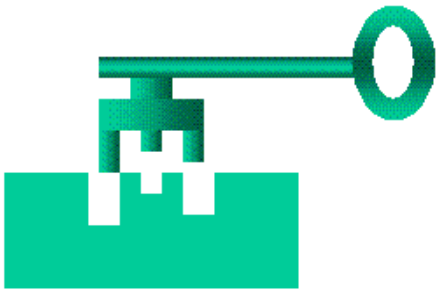
Protein structure and function

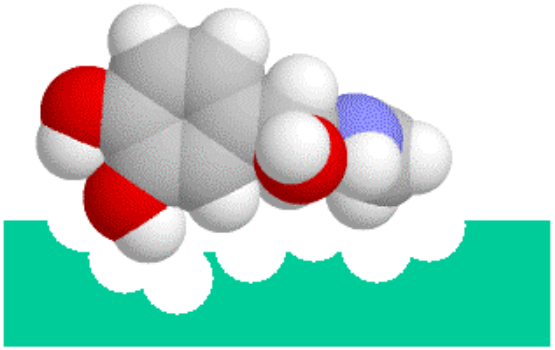




E + S → [ES]

1. **Key-lock theory**: the substrate is exactly fits into the pocket of the enzyme, just like the key into the lock. Emil Fischer 1890





active centrum

binding pocket

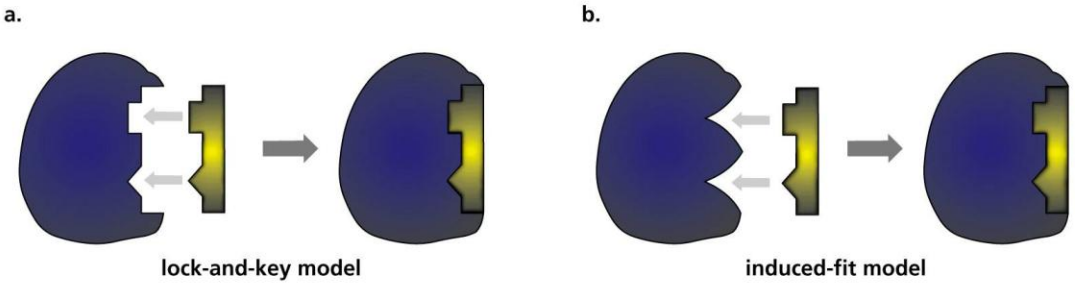
catalytic residues

coenzymes

E-S binding: weak secondary bonds: ionic bond, hidrogene bond, hydrofobic interactions

2. **Induced fit theory**: the binding of the substrate induce a change in the protein structure of the enzyme Koshland 1958.

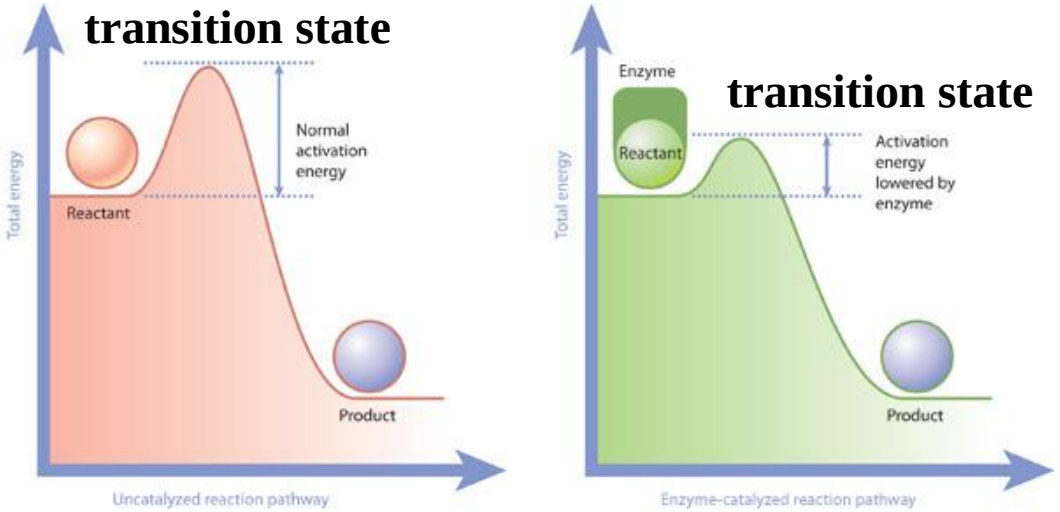
The new conformation of enzyme protein contributes to the catalytic activity of the enzyme.



$$E + S \longleftrightarrow ES \longleftrightarrow EP \longleftrightarrow E + P$$

ES and EP are transient complexes of the enzyme with the substrate and with the product

The function of a catalyst is to **increase the rate of a reaction**. **Catalysts do not affect reaction equilibria.**

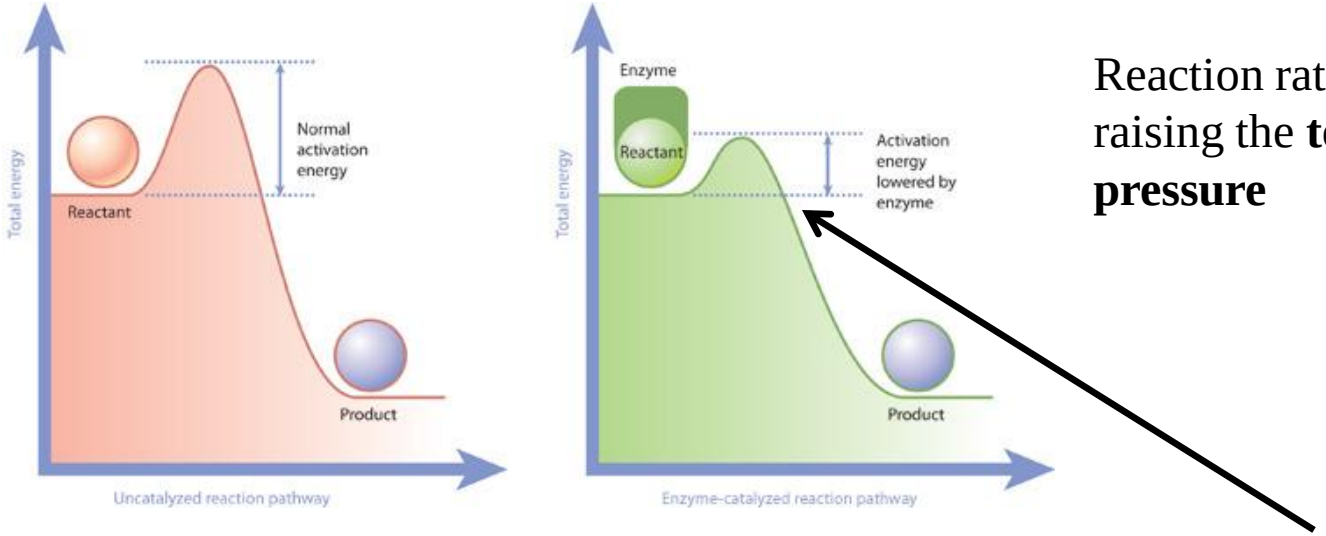


The free energy of the ground state of P is lower than that of S

ΔG is negative $\longrightarrow$ the equilibrium favours P.

The position and direction of equilibrium are not affected by any catalyst.

Higher activation energy corresponds to slower reaction



Reaction rates can be increased by raising the **temperature** and/or **pressure**

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

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

Rate-limiting step: the step (or steps) with the highest activation energy

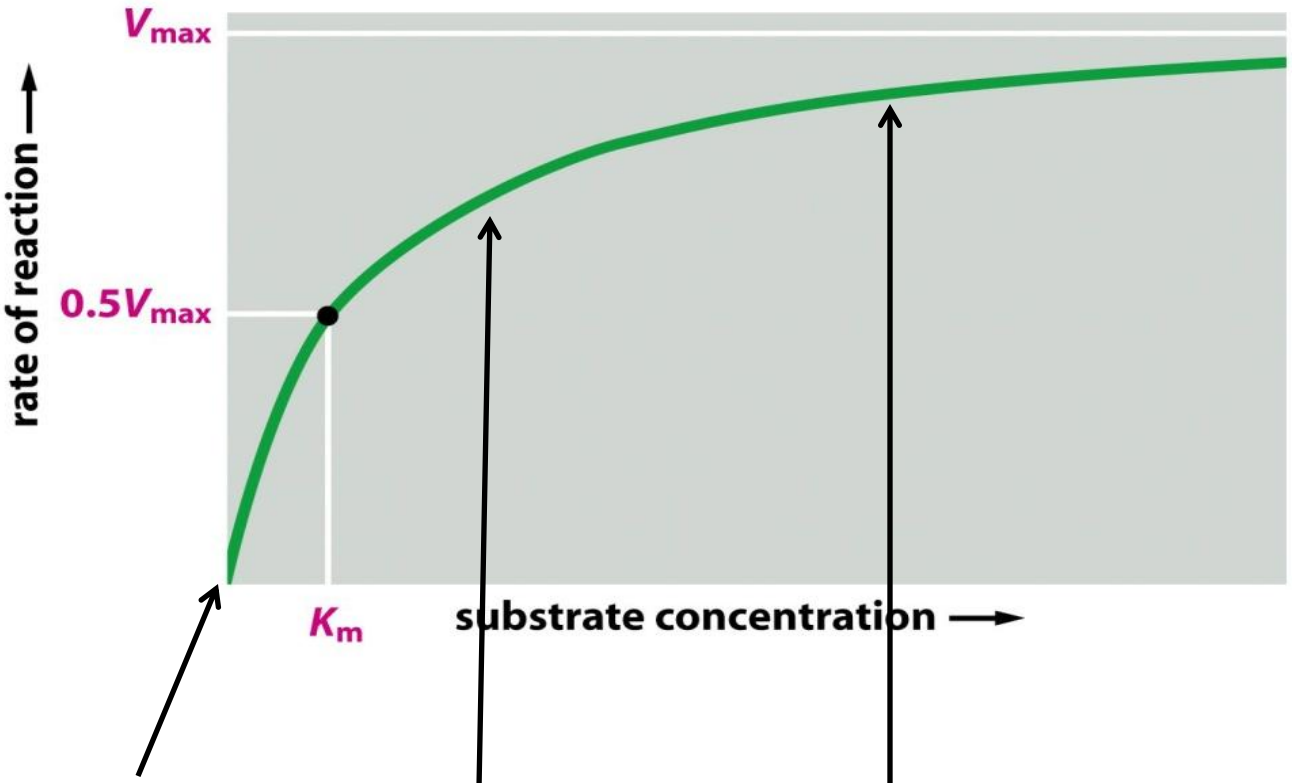
Enzyme Kinetics

The rate of an enzymatic reaction and how it changes in response to changes in experimental parameters

Key factor affecting the rate: **concentration of substrate, [S]**

Enzyme concentration: ~ nanomolar quantities
[S]: milimolar (five or six orders of magnitude higher).

If only the beginning of the reaction is monitored (often the first 60 seconds or less), changes in [S] can be limited to a few percent and [S] can be regarded as constant.
V_0 can then be explored as a function of [S], which is adjusted by the investigator.



At relatively low [S]: V_0 increases almost linearly with an increase of [S].

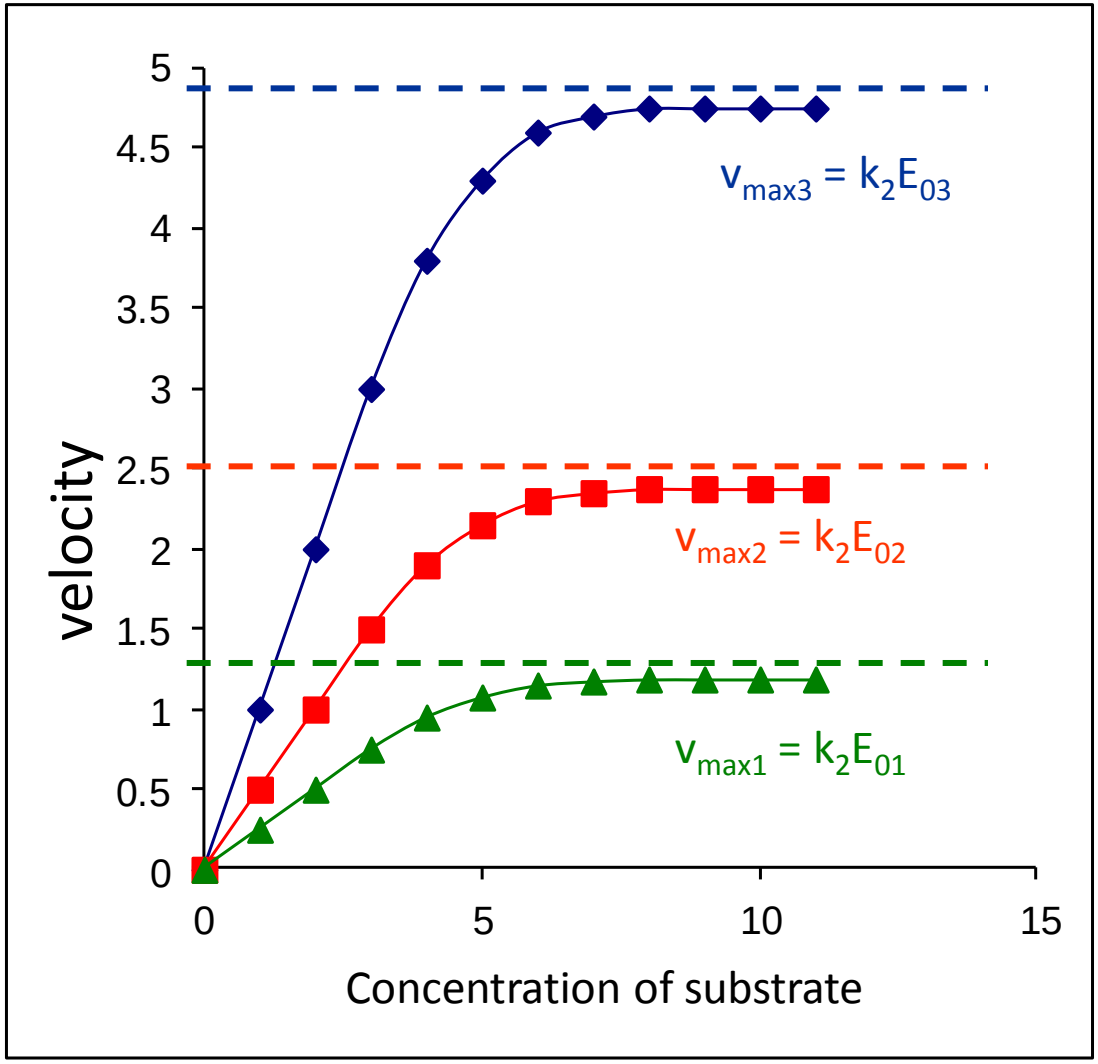
At higher [S]: V_0 increases by smaller and smaller amounts in response to increases of [S].

Finally, a point is reached beyond which increases in V_0 are vanishingly small as [S] increases. This **plateau-like** V_0 region is close to the **maximum velocity, V_{max}.**

Two postmen deliver 250 letters in an hour

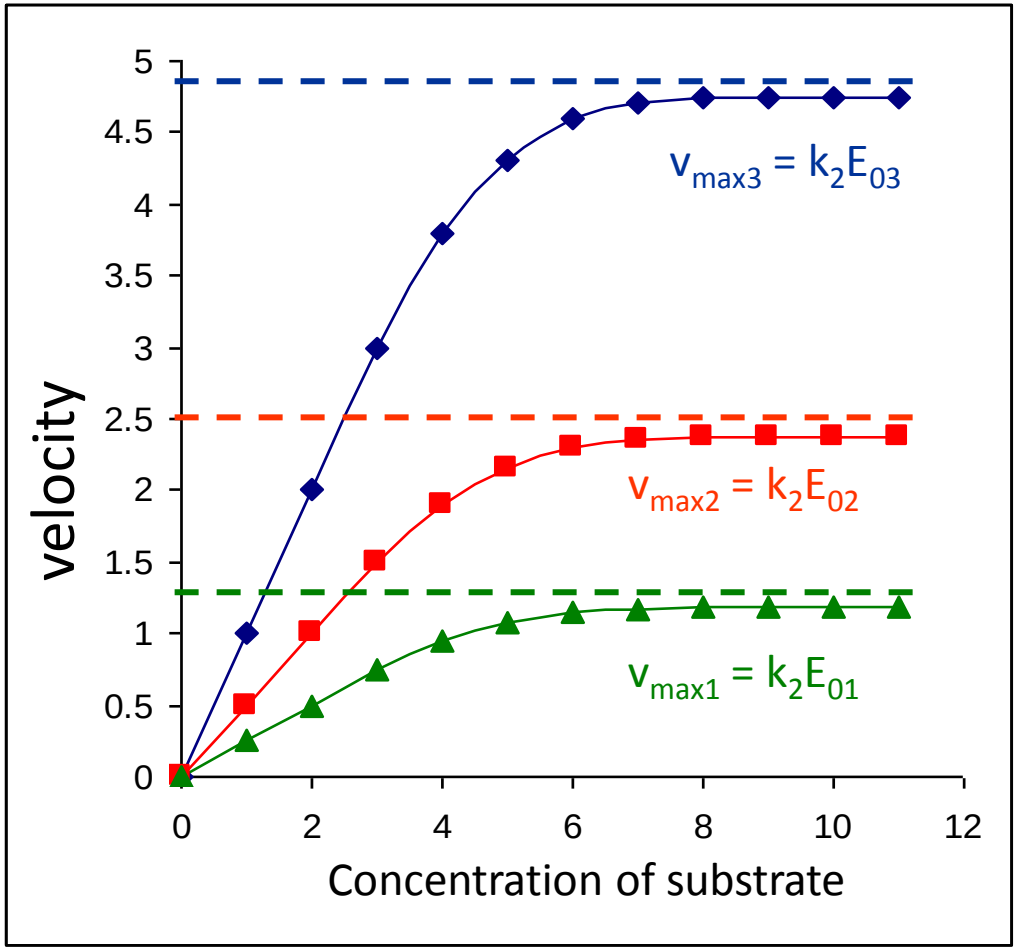
How many letters can be delivered by four postmen in an hour?

The conditions are same.


5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
velocity
0
5
10
15
Concentration of substrate
$v_{max3} = k_2E_{03}$
$v_{max2} = k_2E_{02}$
$v_{max1} = k_2E_{01}$

What do the kinetical parameters tell us?

v_{max}: It is proportional with the amount of enzyme. Essentially it gives the activity of the enzyme. It serves information on the amount of enzyme.



It depends on the amount of enzyme (protein), it is not an enzyme feature

The **k_2** speed constant is an enzyme feature

Turnover number: the quotient of v_{max} and the concentration of the enzyme

$k_{cat} = v_{max}/E_0$ (its value is between 1 and 10 000, generally ~ 1000)

The number of converted substrate molecules in a second by one enzyme molecule.

K_M:

1. It gives the concentration of substrate in the surrounding space of the enzyme.
2. It is constant for a certain enzyme. It can be applied for the recognition of the enzyme.
3. It can be influenced by different compound to regulate the activity of the enzyme.
4. It gives the affinity of substrate for the enzyme

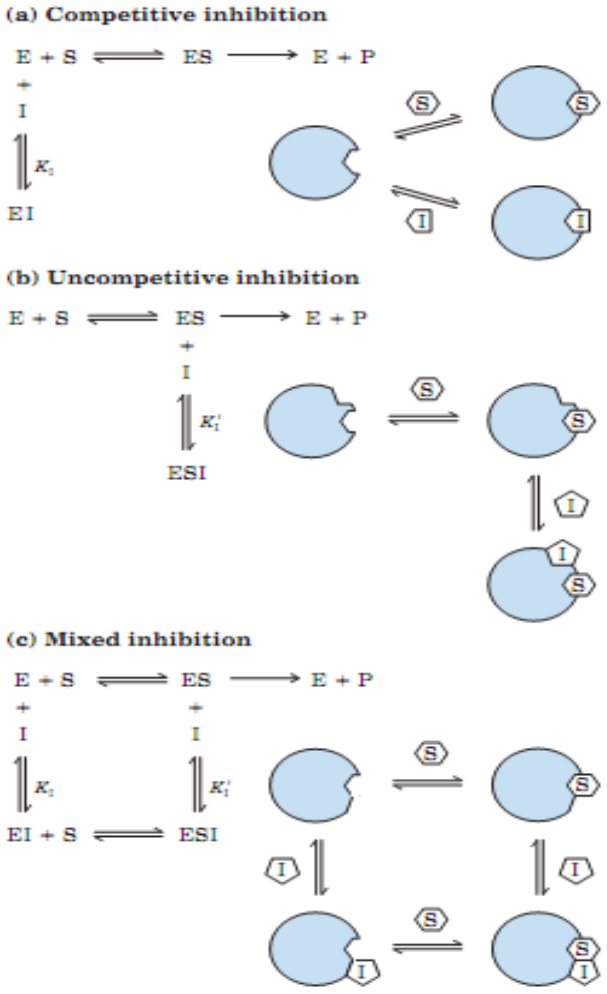
Enzyme inhibitors

Enzyme inhibitors are molecules that interfere with catalysis, slowing or halting enzymatic reactions.

Reversible and Irreversible

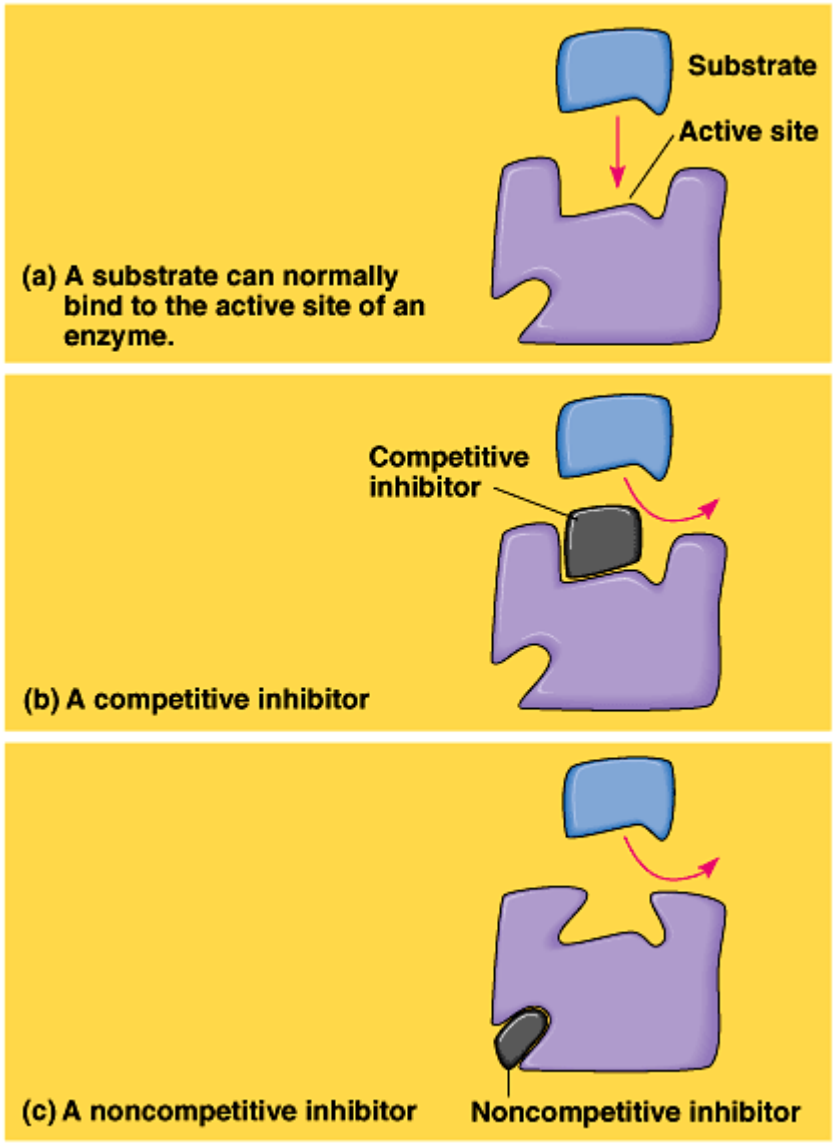
Irreversible inhibition: The irreversible inhibitors bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or form a particularly stable noncovalent association (e.g.: heavy metals). An irreversible inhibitor decrease the real amount of an active enzyme.

Reversible inhibition: The reversible inhibitors form dynamic complex with the enzyme.



Reversible inhibition: It has 3 different types

1. **Competitive inhibitor**: It competes with the substrate for the active site of an enzyme. The K_M increase
2. **Uncompetitive inhibitor**: An uncompetitive inhibitor binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex. v_{max}, K_M decrease
3. **Mixed inhibitor**: also binds at a site distinct from the substrate active site, but it binds to either E or ES. v_{max} decrease



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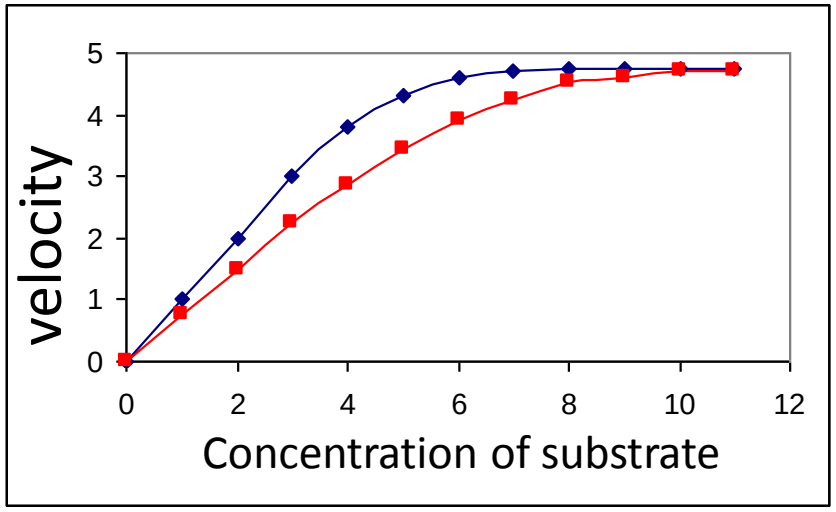
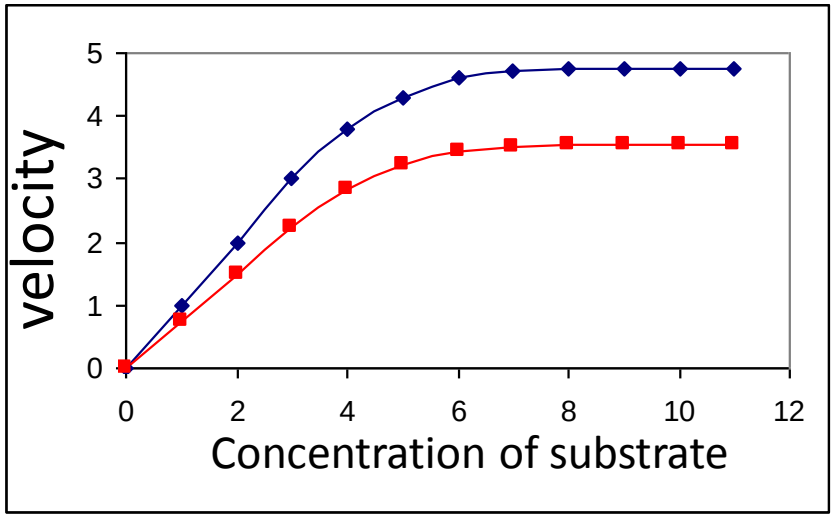


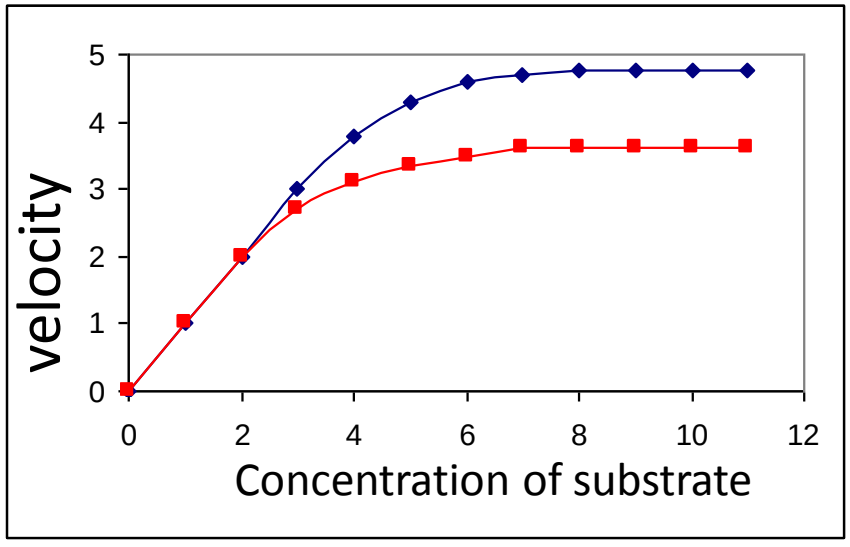
Competitive inhibition:

v_{max} same, K_M increase



Mixed inhibition:

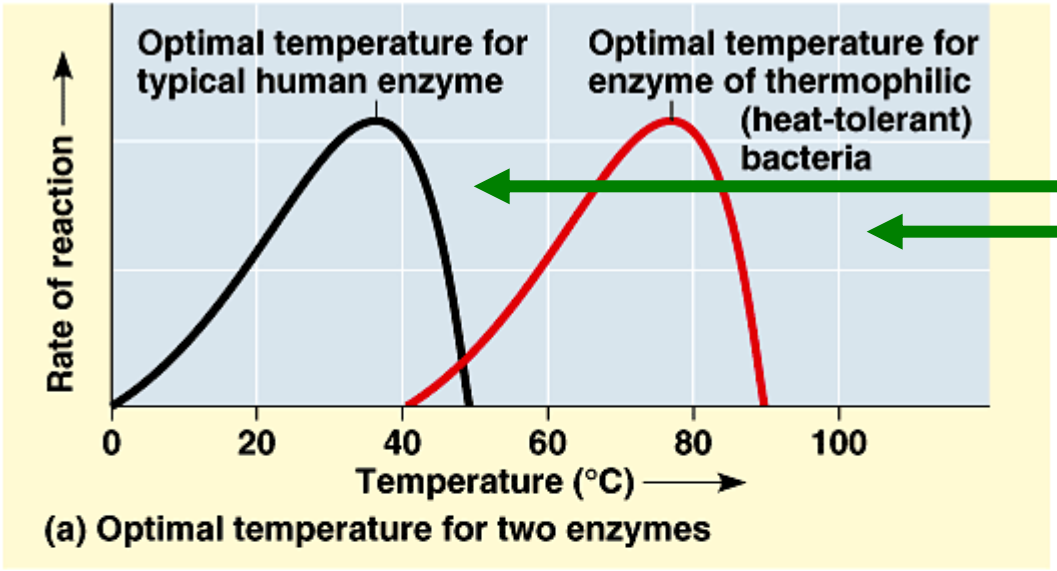
v_{max} decrease, K_M same



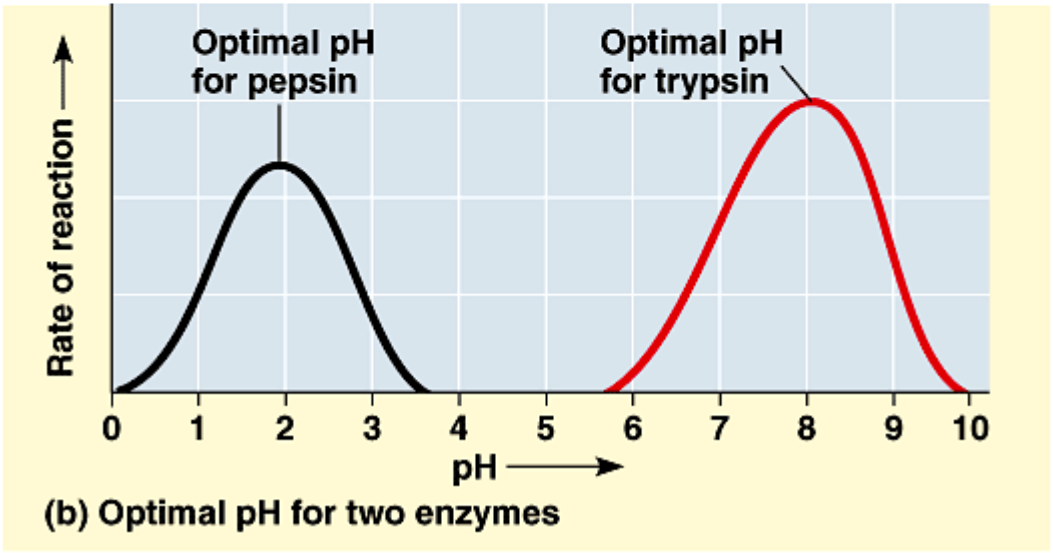
Uncompetitive inhibition:

v_{max} decrease K_M decrease

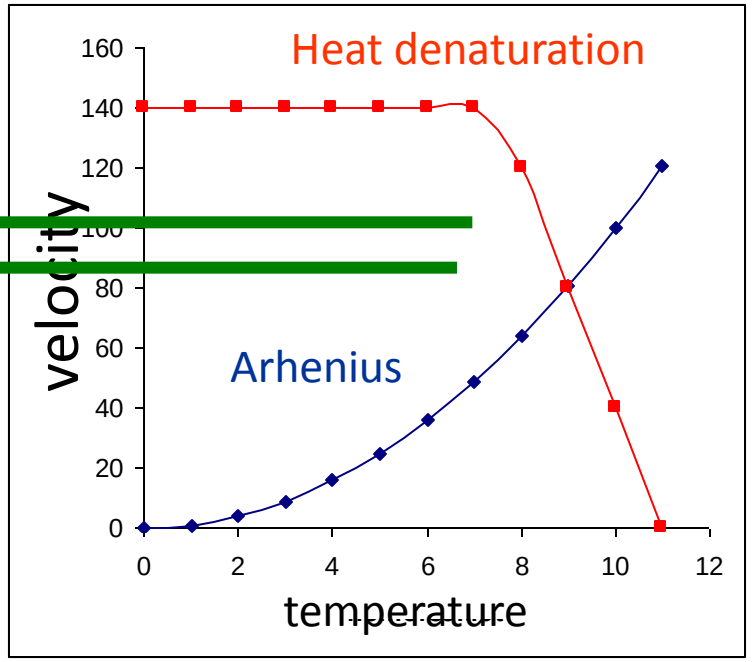
Enzyme activity depends on the pH and on the temperature



(a) Optimal temperature for two enzymes



(b) Optimal pH for two enzymes



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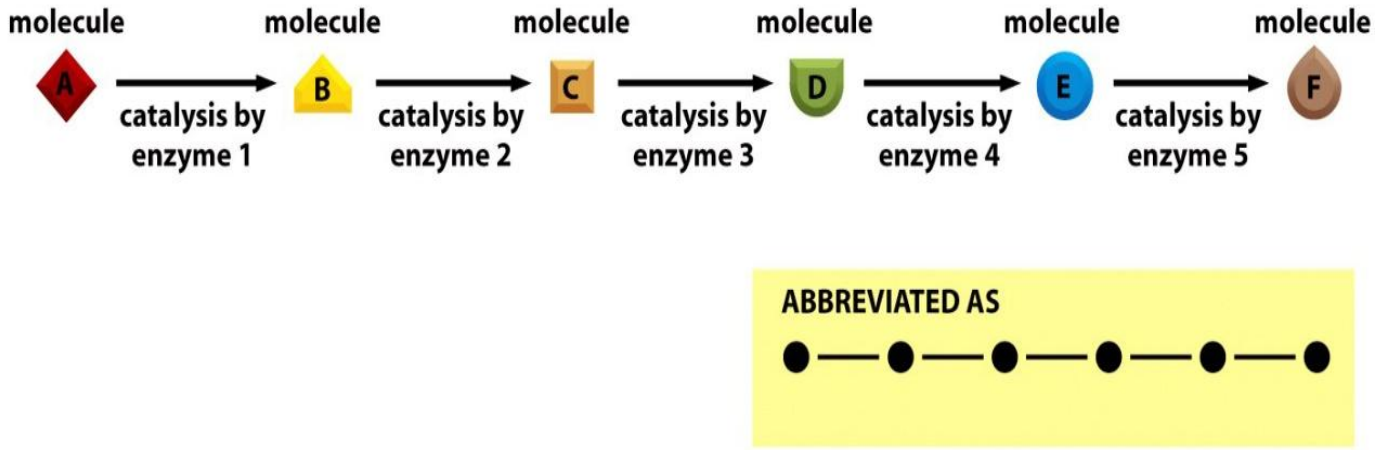
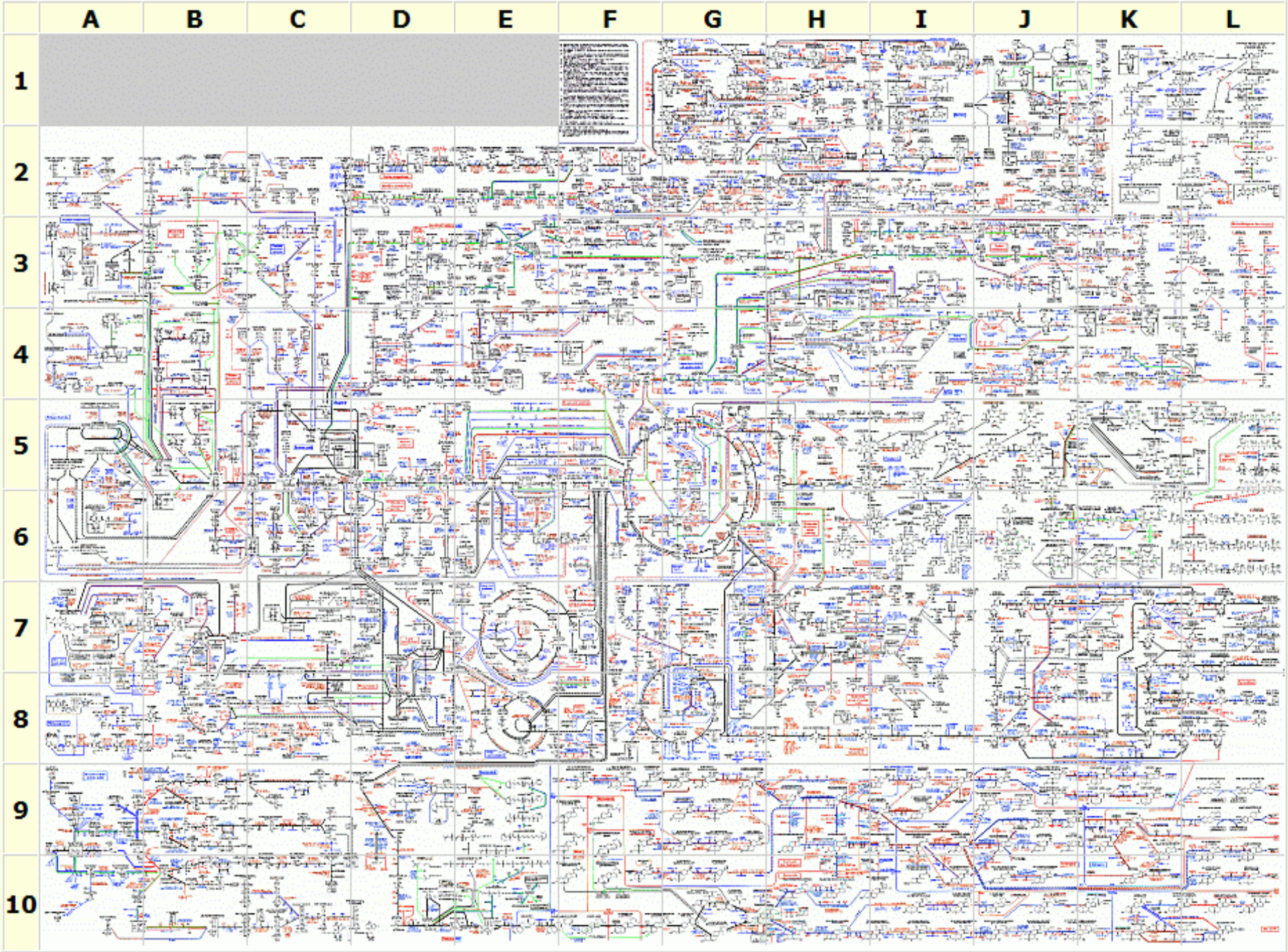
Enzyme-catalyzed reactions are usually connected in series



The product of one reaction becomes the starting material, or substrate, for the next.

Each pathway includes one or more enzymes that have a greater effect on the rate of the overall sequence.
These regulatory enzymes **exhibit increased or decreased catalytic activity in response to certain signals**.
Adjustments in the rate of reactions catalyzed by regulatory enzymes allow the cell to meet changing needs for energy and for biomolecules required in growth an maintenance.


A
B
C
D
E
F
G
H
I
J
K
L
1
2
3
4
5
6
7
8
9
10

Source of energy

Phototroph: an organism that obtains energy from sunlight for the synthesis of organic compounds (they convert the solar energy to chemical one)

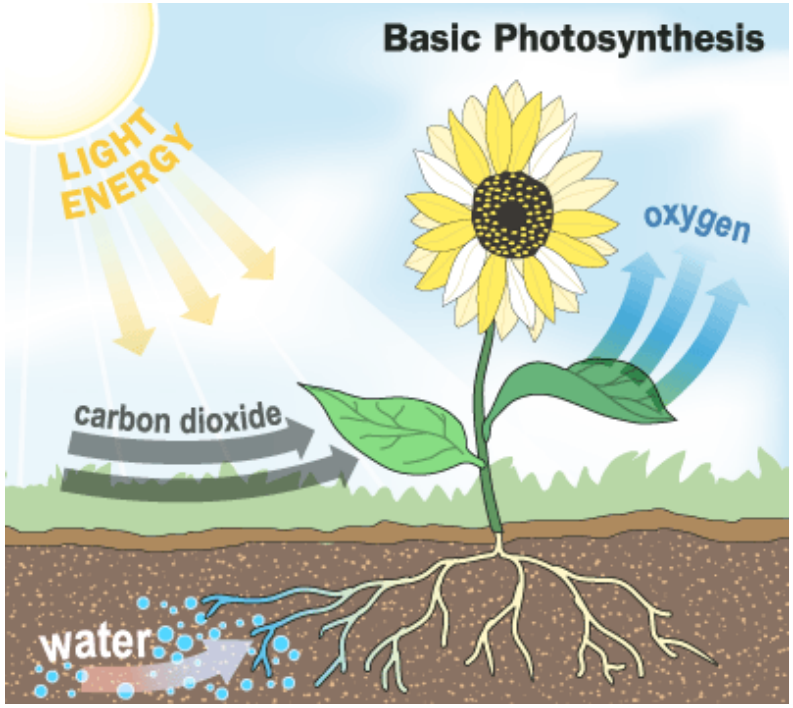
Chemotroph: an organism that cannot harvest and convert the solar energy, instead of it take up organic compounds and oxydize them to gain energy.

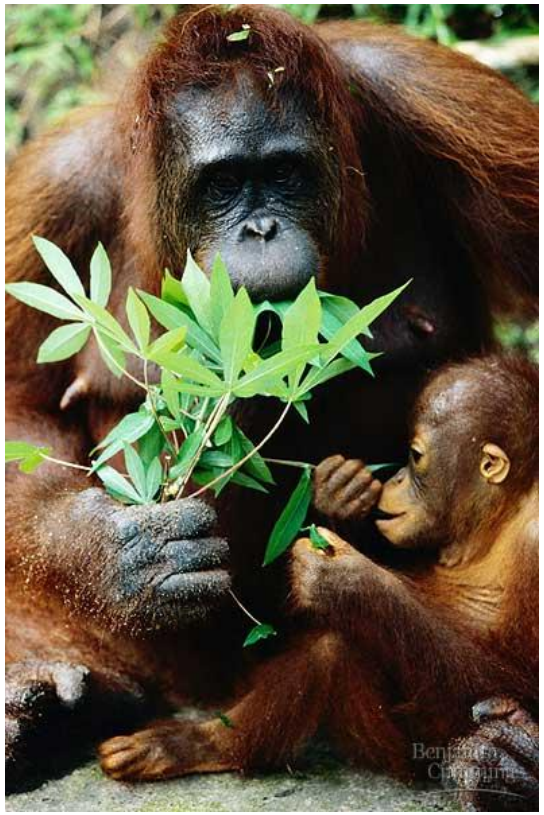
Source of carbon

Autotroph: An organism capable of synthesizing its own food from inorganic substances, using light or chemical energy. Green plants, algae, and certain bacteria are autotrophs.

Heterotroph: An organism that cannot synthesize its own food and is dependent on complex organic substances for nutrition.

Solar energy → Photosynthesis → Chemical energy → Contraction / Transport / Biosynthesis





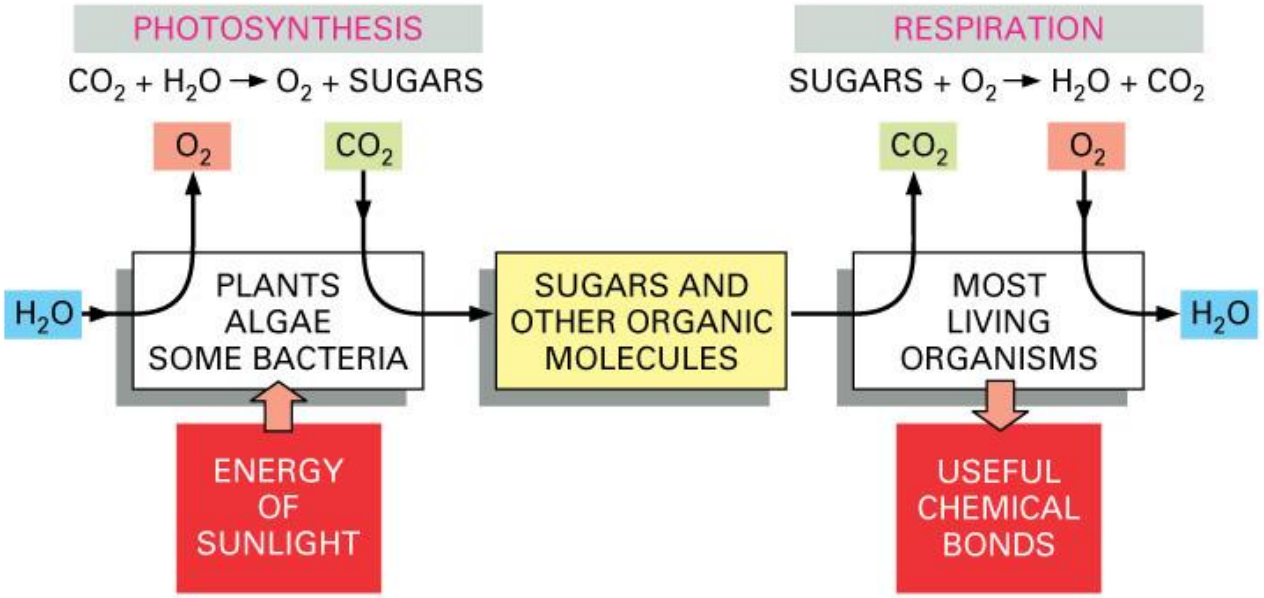
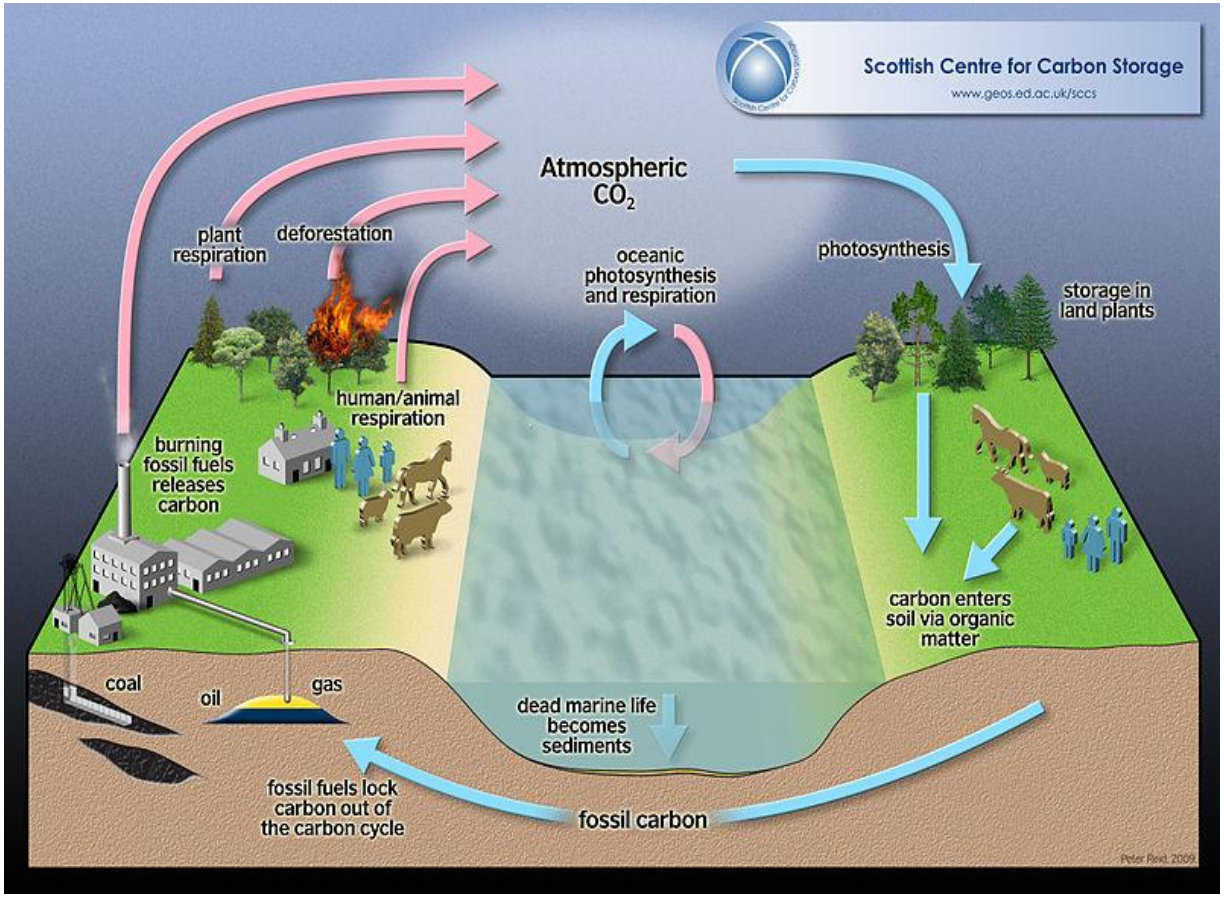


Figure 3-10 Essential Cell Biology, 2/e. (© 2004 Garland Science)

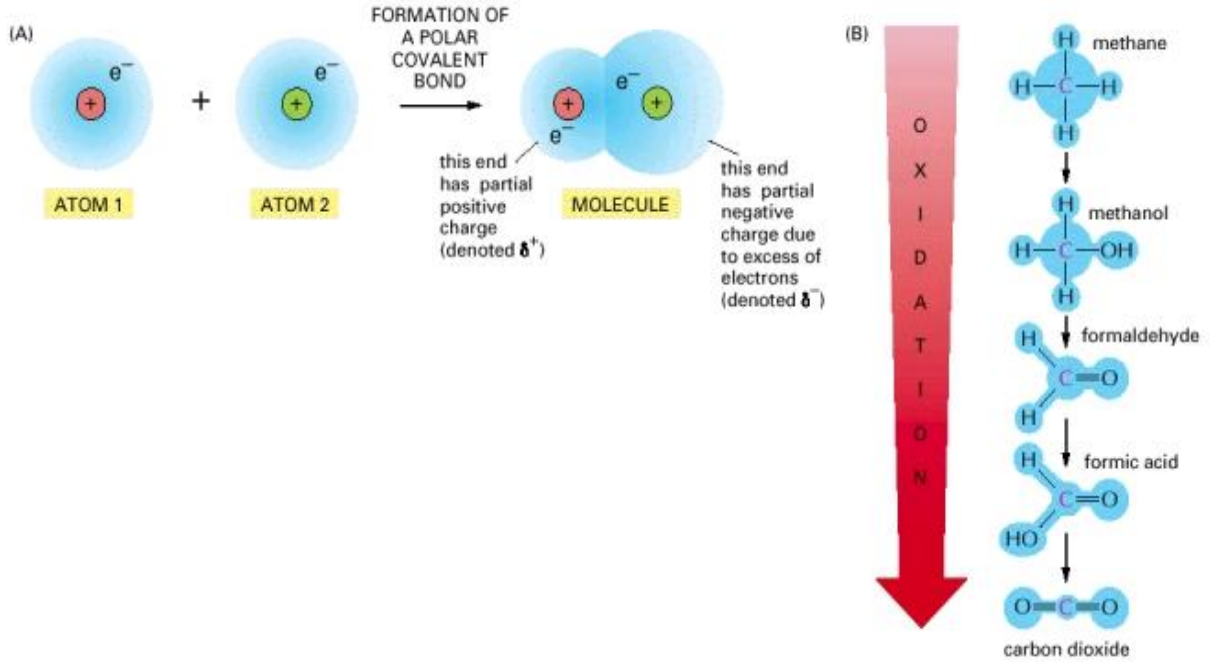
The plants, in turn, trap energy directly from sunlight.

All animals use the energy stored in the chemical bonds of organic molecules made by other organisms, which they take in as food.

All of the energy used by animal cells is derived ultimately from the sun



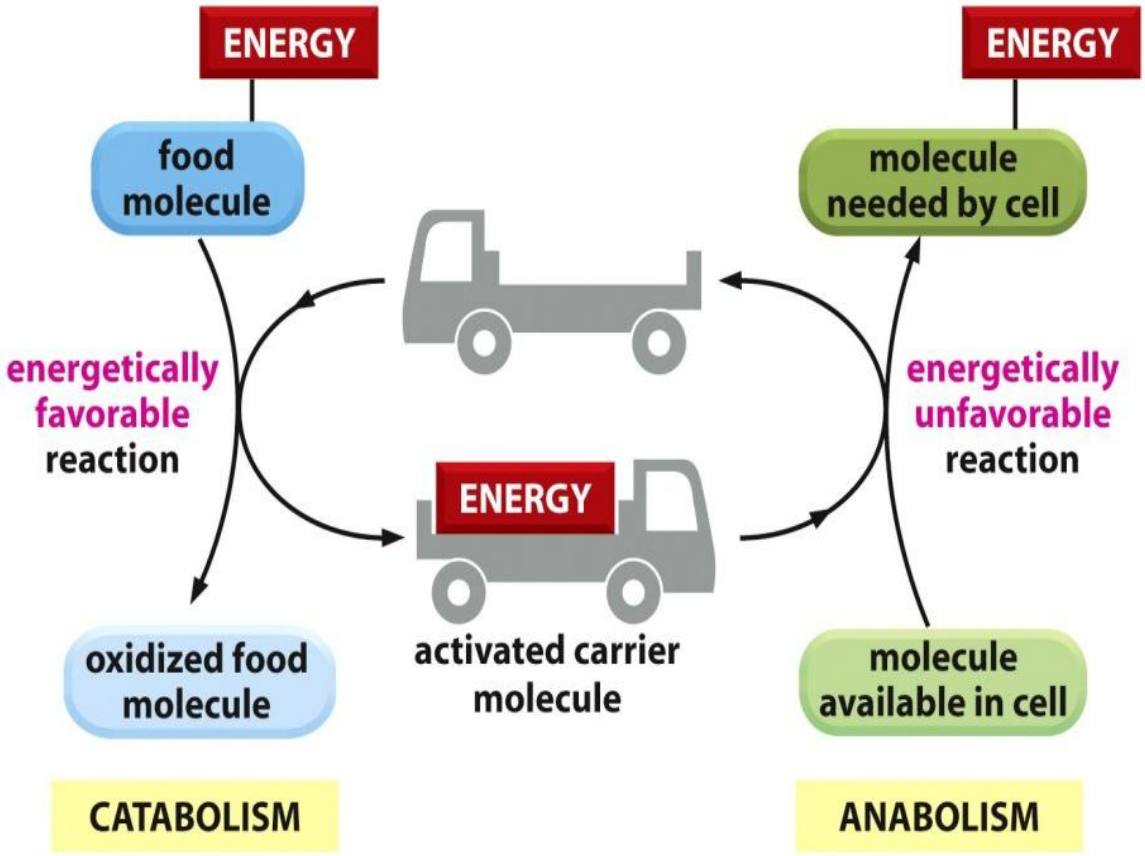
The cell does not oxidize organic molecules in one step



The electron transfer occures from one atom to another.

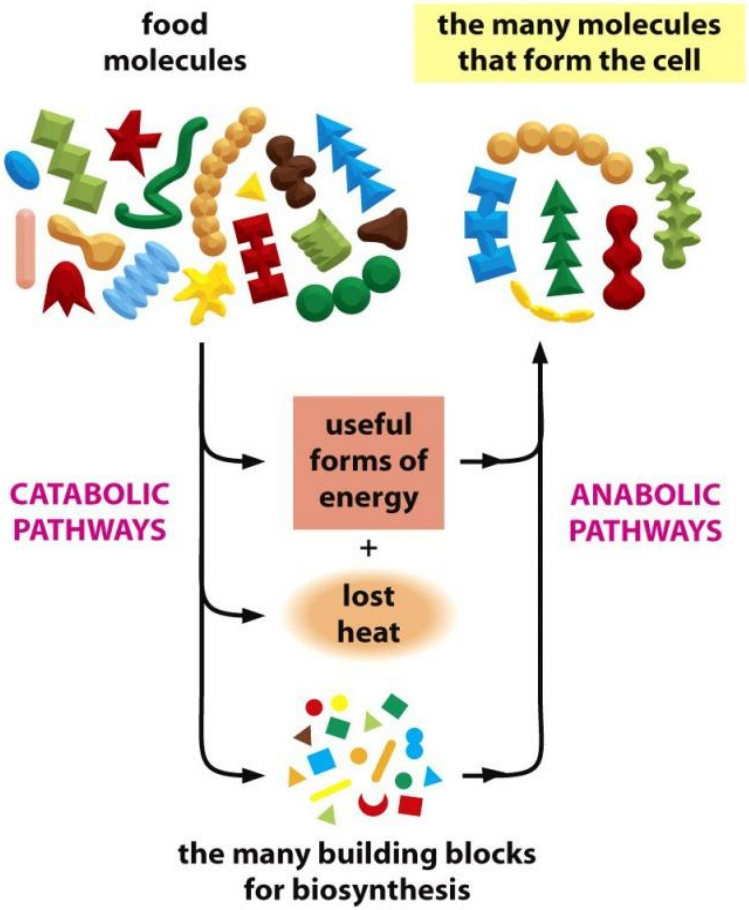
Oxidation in this sense refers to the removal of electrons, and reduction the converse of oxidation means the addition of electrons.

Oxidation and reduction always occur simultaneously: that is, if one molecule gains an electron in a reaction (reduction), a second molecule loses the electron (oxidation).



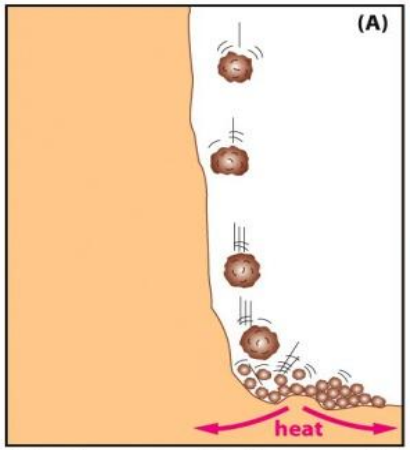
The energy released by the oxidation of food molecules must be stored temporarilyI

The energy is stored as chemical bond energy in a small set of activated "carrier molecules," which contain one or more energy-rich covalent bonds.

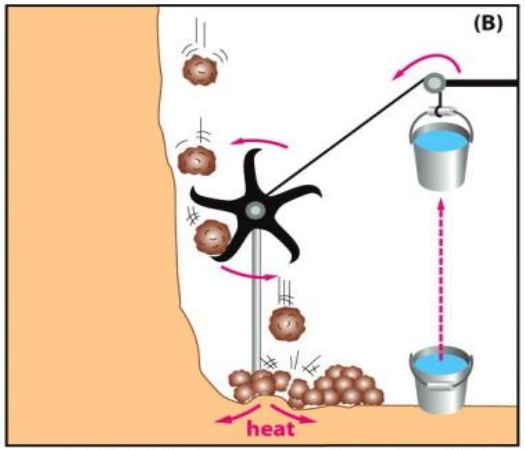


Metabolism = catabolism + anabolism

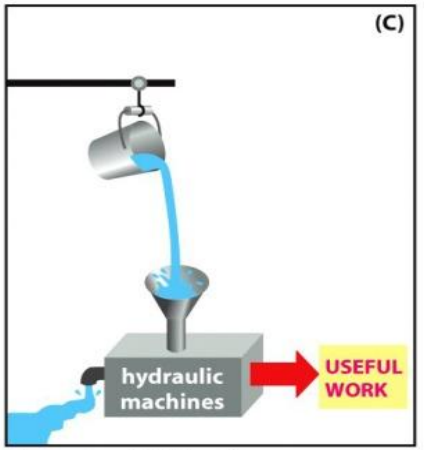
The most important of the activated carrier molecules are ATP and two molecules that are closely related to each other, NADH and NADPH



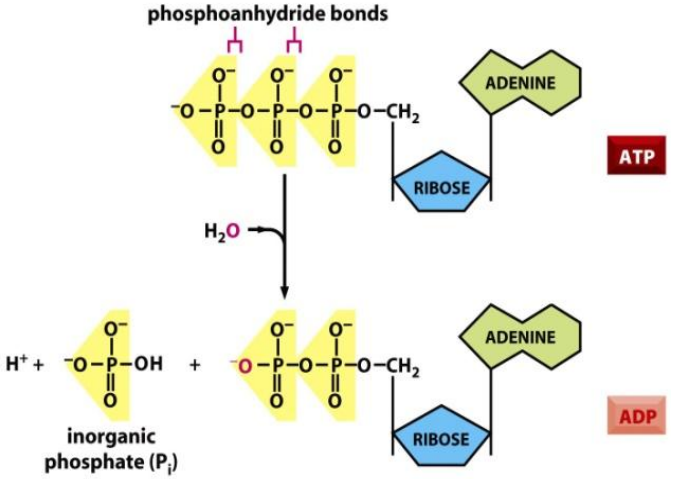
kinetic energy of falling rocks is transformed into heat energy only

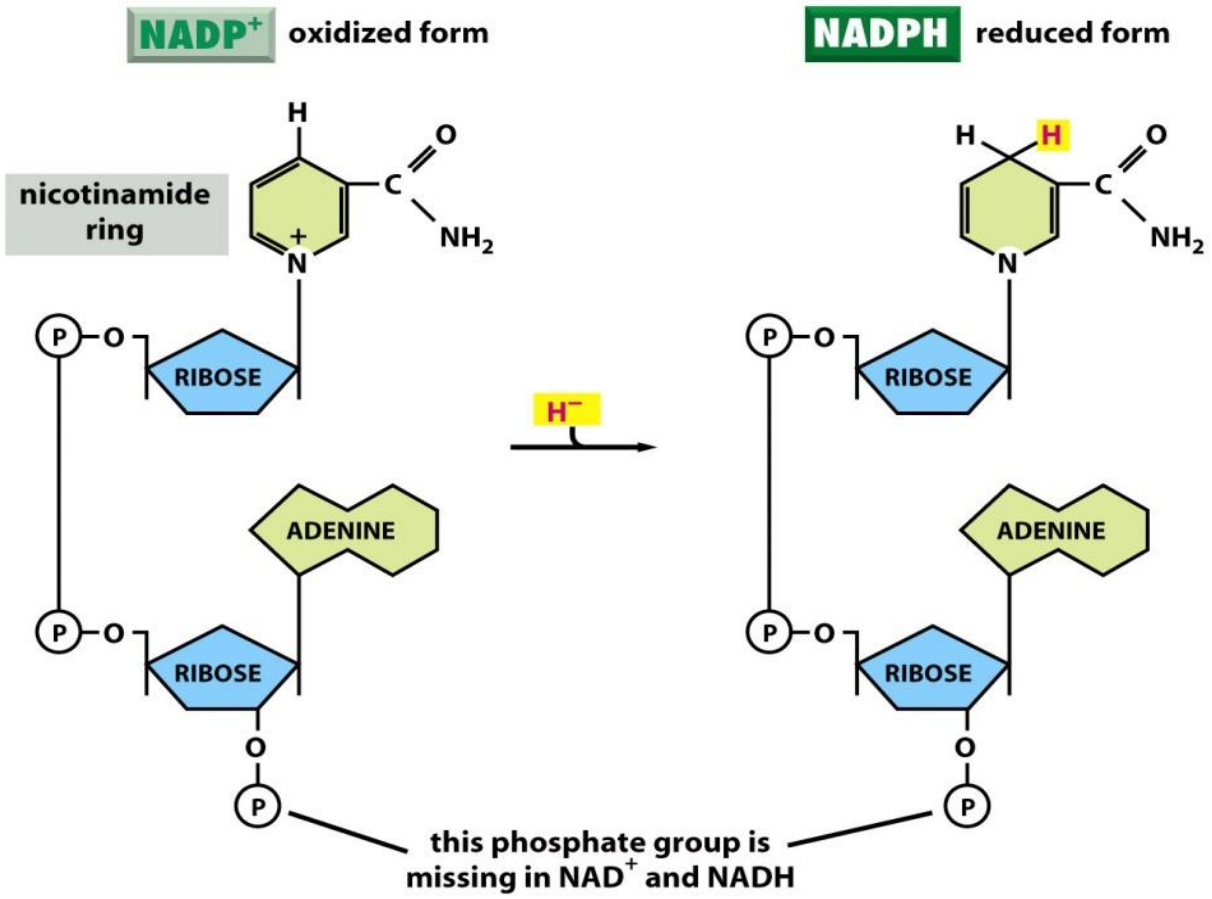


part of the kinetic energy is used to lift a bucket of water, and a correspondingly smaller amount is transformed into heat

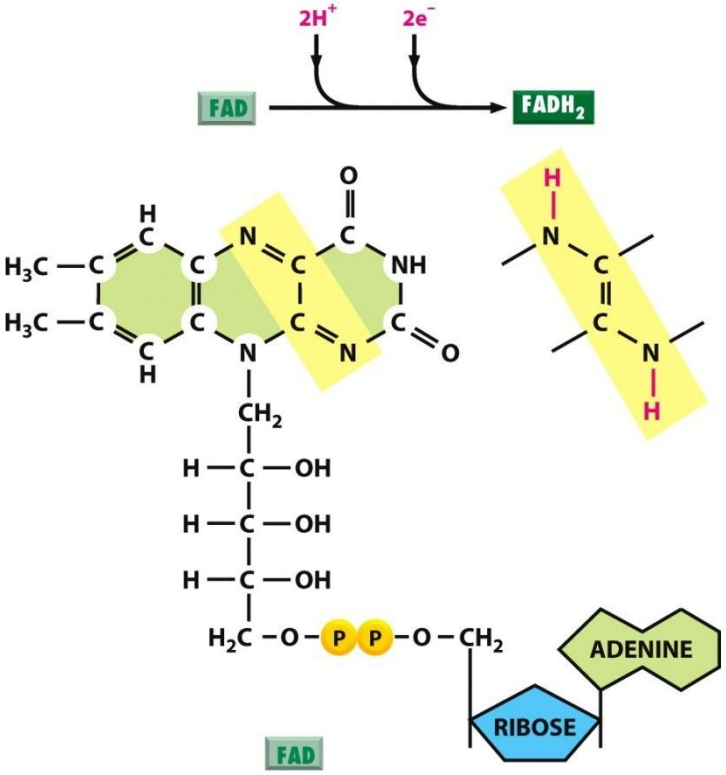


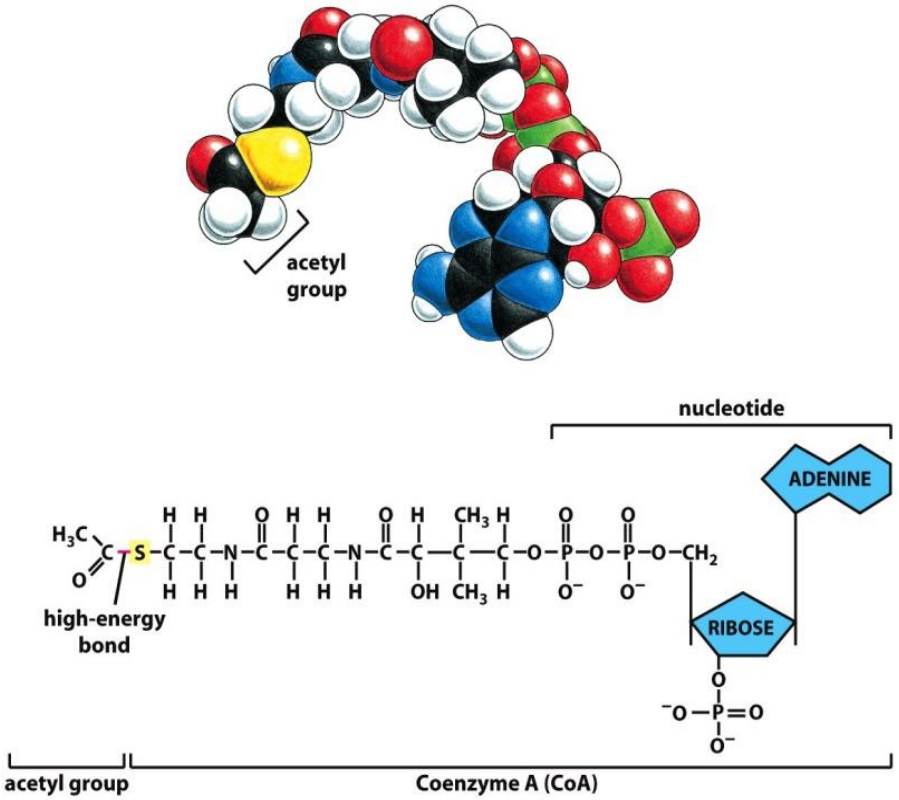
the potential kinetic energy stored in the raised bucket of water can be used to drive hydraulic machines that carry out a variety of useful tasks





NADPH, an important carrier of electrons





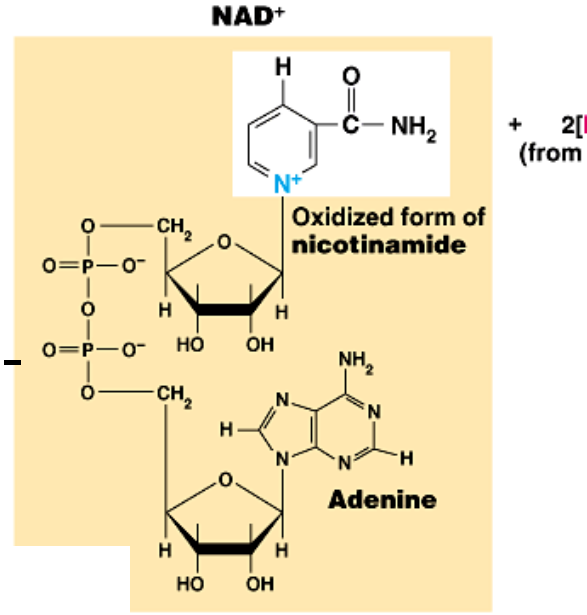
The structure of $FADH_2$

The structure of the important activated carrier molecule acetyl CoA

Electrontransfer, the important electron carriers

NAD: nikotinamide adenine-dinucleotide

FAD: flavin adenine-dinucleotide



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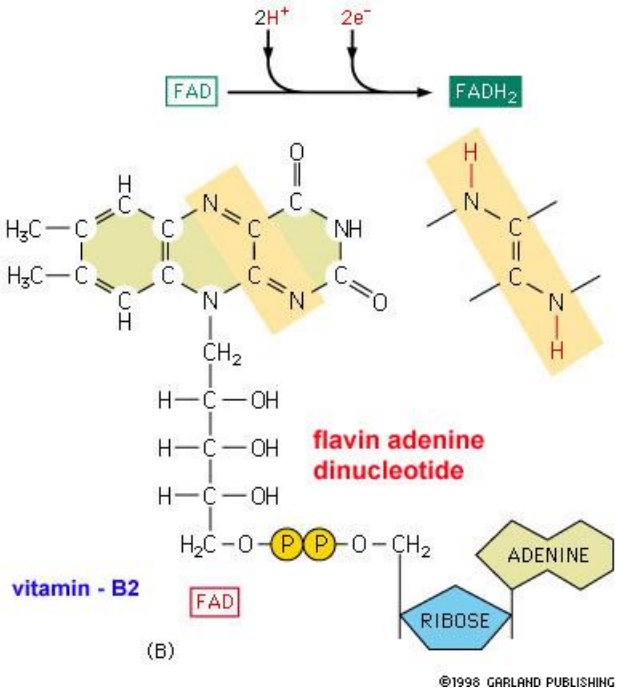
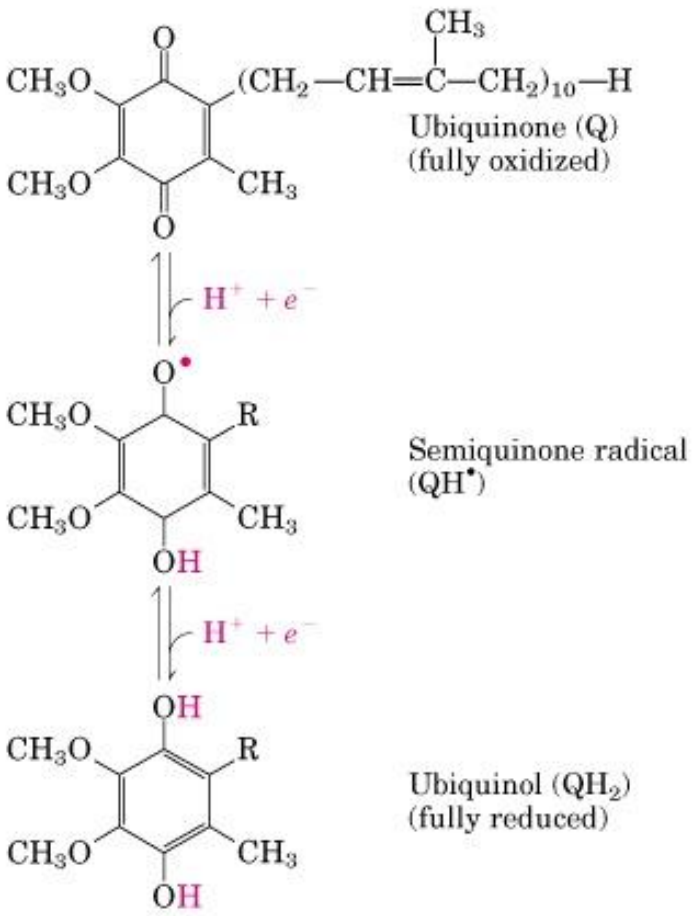
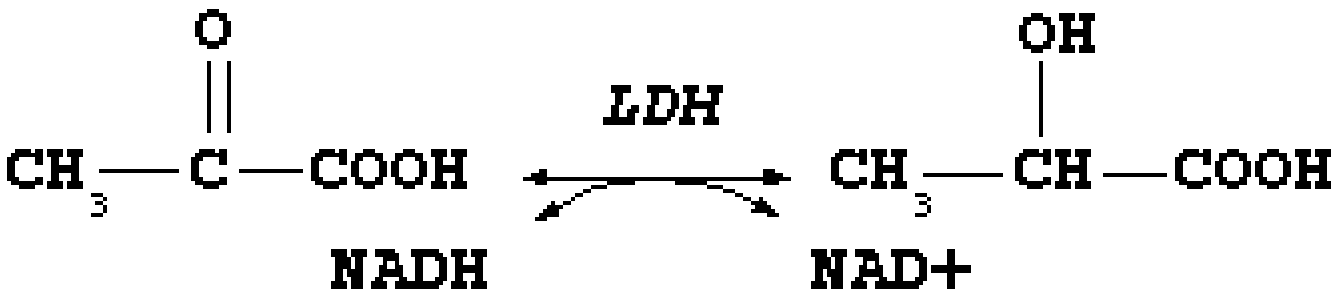


Ubiquinone





Pyruvate

Lactate