Chapter 12

Chemical Logic of Metabolism

A First Look at Metabolism

Metabolism can be subdivided into two major categories:

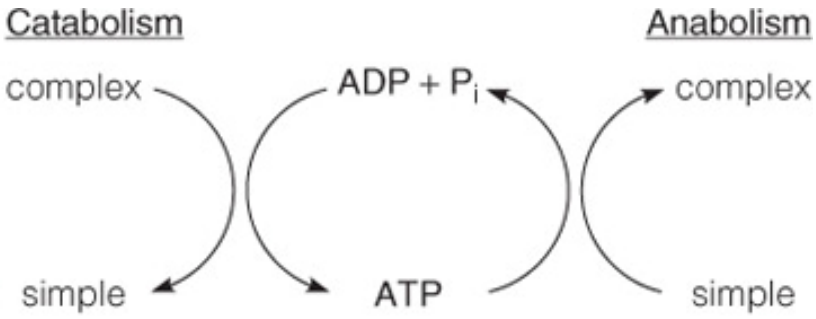
•***Catabolism*** - those processes in which complex substances are degraded to simpler molecules.

•***Anabolism*** - those processes concerned primarily with the synthesis of complex organic molecules.

•Catabolism is generally accompanied by the ***net release*** of chemical energy.

•Anabolism requires a ***net input*** of chemical energy.

•These two sets of reactions are coupled together by ATP.



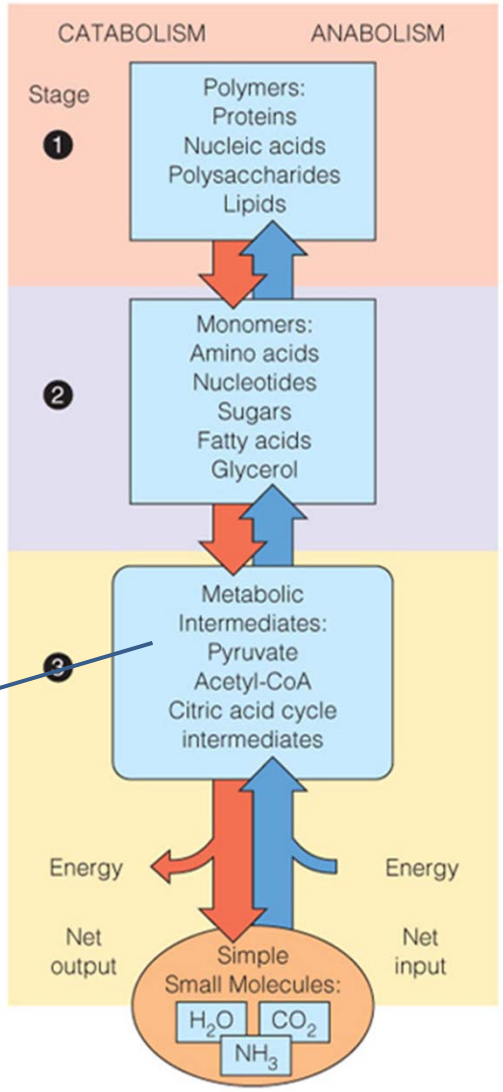
A First Look at Metabolism

Both catabolic and anabolic pathways occur in three stages of complexity:

Stage 1: the interconversion of polymers and complex lipids with monomeric intermediates

Stage 2: the interconversion of monomeric sugars, amino acids, and lipids with still simpler organic compounds

Stage 3: the ultimate degradation to, or synthesis from, inorganic compounds, including CO_2, H_2O and NH_3.



Intermediary metabolism refers primarily to the biosynthesis, utilization, and degradation of low-molecular-weight compound (intermediates).

A First Look at Metabolism

- A fundamental distinction among organisms lies in the source of their fuel molecules.
- ***Autotrophs*** 自營性 (from Greek, “self-feeding”) synthesize glucose and all of their other organic compounds from inorganic carbon, supplied as CO_2.
- ***Heterotrophs*** 異營性 (“feeding on others”) can synthesize their organic metabolites only from other organic compounds, which they must therefore consume.
- A primary difference between plants and animals is that plants are autotrophs and animals are heterotrophs.

mixotrophs

A First Look at Metabolism

- Microorganisms show adaptability with respect to their ability to survive in the absence of oxygen.

- Virtually all multicellular organisms and many bacteria are strictly ***aerobic*** organisms; they depend absolutely upon ***respiration***, the coupling of energy generation to the oxidation of nutrients by oxygen.

- By contrast, many microorganisms either can, or must, grow in ***anaerobic*** environments, deriving their metabolic energy from processes that do not involve molecular oxygen.

Central metabolic pathways and intermediates

Catabolic pathways

Anabolic pathways

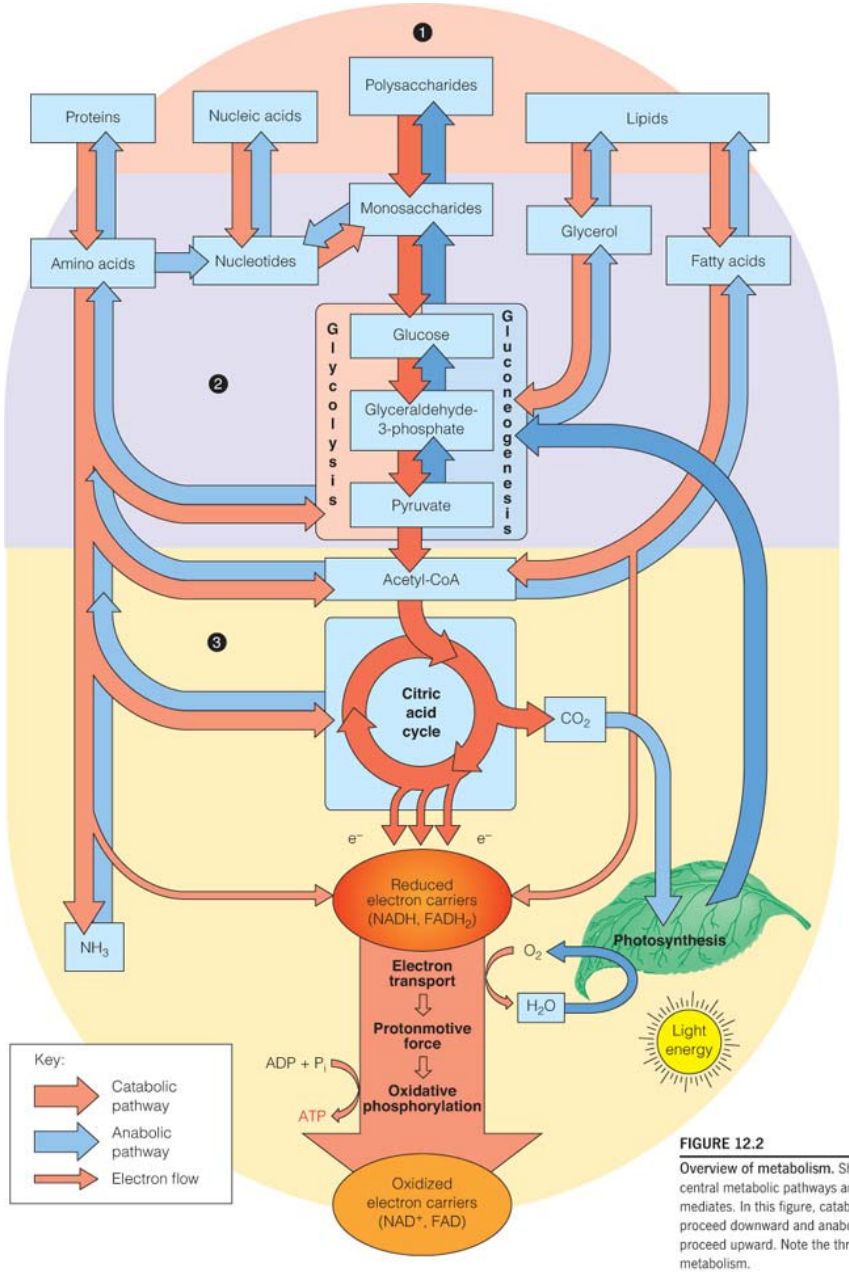
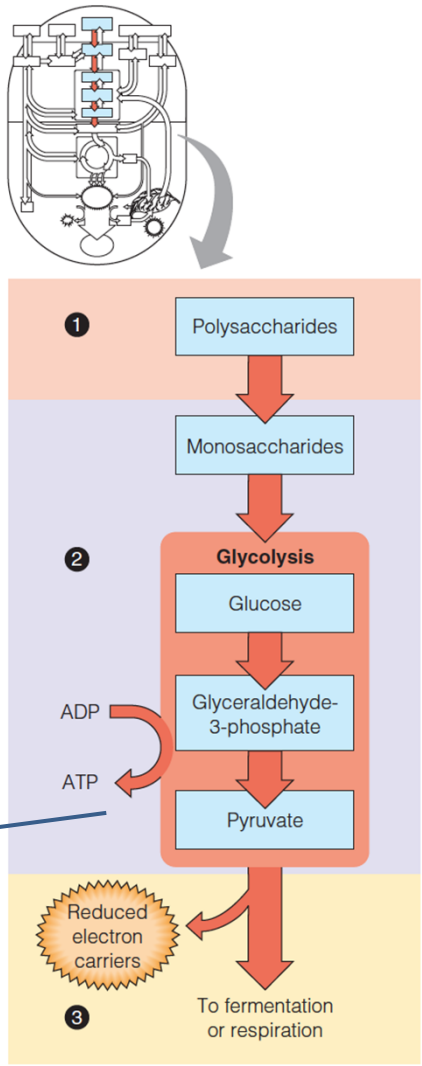


FIGURE 12.2

Overview of metabolism. Shown here are the central metabolic pathways and some key intermediates. In this figure, catabolic pathways (red) proceed downward and anabolic pathways (blue) proceed upward. Note the three stages of metabolism.

Freeways on the Metabolic Road Map

- The first pathway that we study is ***glycolysis*** **(Ch13)**, a stage 2 pathway for degradation of carbohydrates, in either aerobic or anaerobic cells.
- The major input to glycolysis is glucose, usually derived from either energy-storage polysaccharides or dietary carbohydrates.
- This pathway leads to ***pyruvate*** 丙酮酸, a three-carbon α-keto acid.
- Anaerobic organisms reduce pyruvate to a variety of products, for example lactate, or ethanol plus carbon dioxide.
- These processes are called ***fermentations***.



Pyruvate either undergoes reduction in fermentation reactions or enters oxidative metabolism (respiration) via conversion to acetyl-CoA

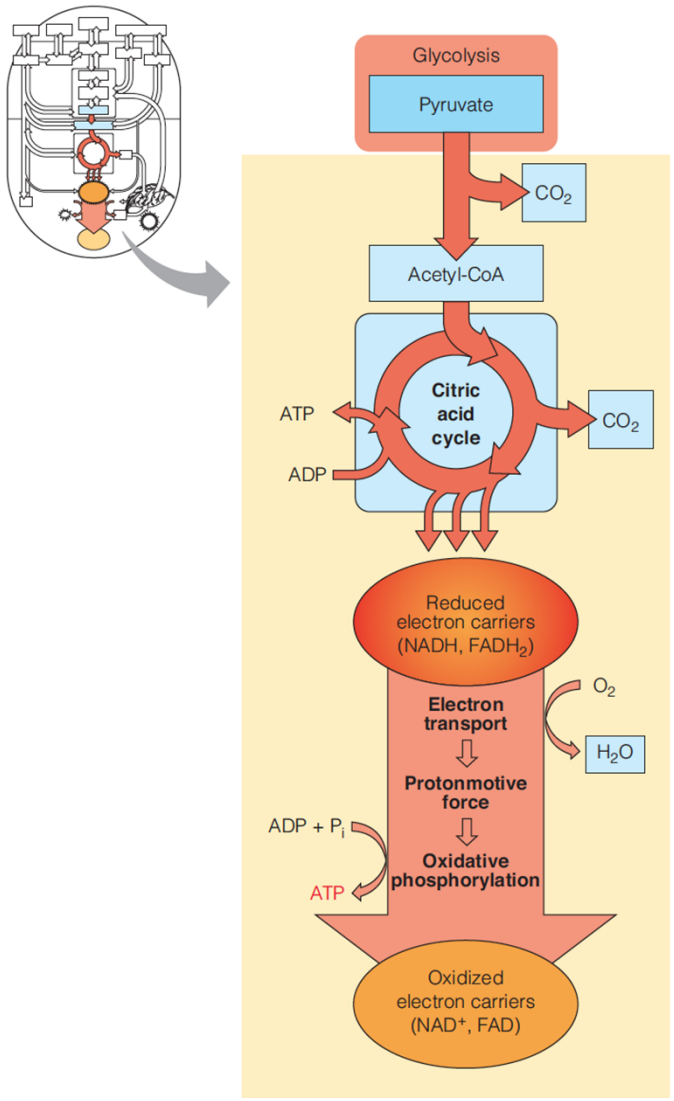
Freeways on the Metabolic Road Map

- In oxidative metabolism (respiration), the major fate of pyruvate is its oxidation to a metabolically activated two-carbon fragment, ***acetyl-CoA***.
- The two carbons in the acetyl group then undergo oxidation in the ***citric acid cycle***.
- In aerobic organisms the citric acid cycle is the principal stage 3 pathway.
- This cyclic pathway accepts simple carbon compounds, derived not only from carbohydrate but also from lipid or protein, and oxidizes them to CO_2.

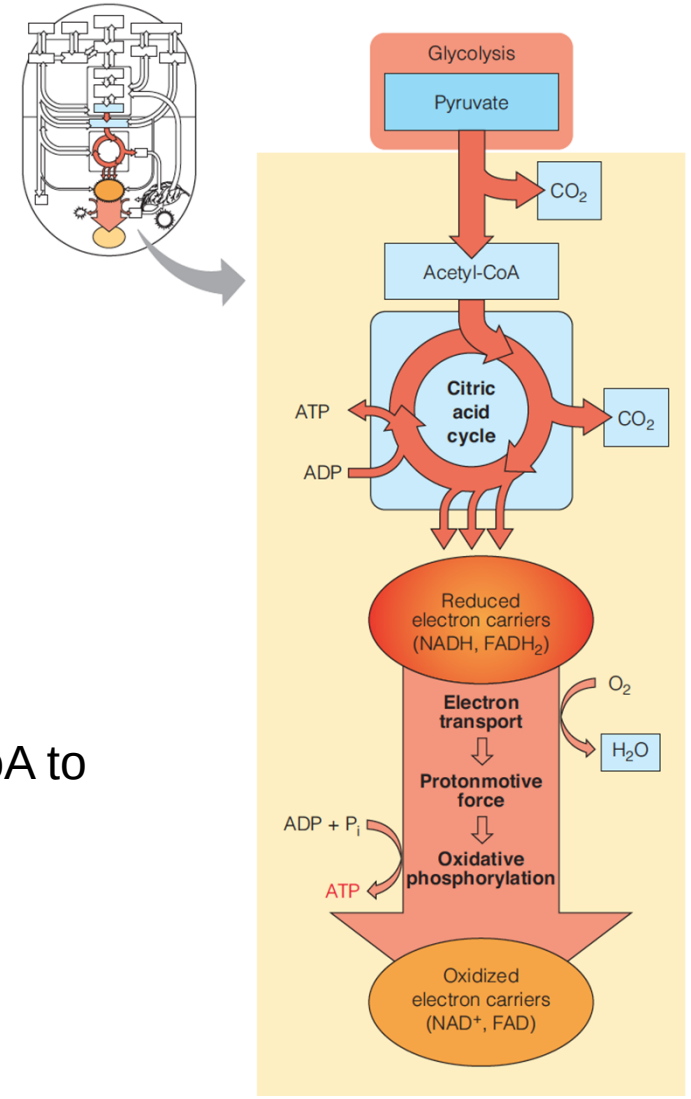
Freeways on the Metabolic Road Map

- Oxidative reactions of the citric acid cycle generate reduced electron carriers whose reoxidation drives ATP biosynthesis, primarily through processes in the mitochondrial respiratory chain—***electron transport*** and ***oxidative phosphorylation***.

- The mitochondrial membrane uses oxidative energy to maintain a transmembrane gradient of hydrogen ion concentration (the ***protonmotive force***), and discharge of this electrochemical potential energy powers the synthesis of ATP from ADP + P_i.



Freeways on the Metabolic Road Map



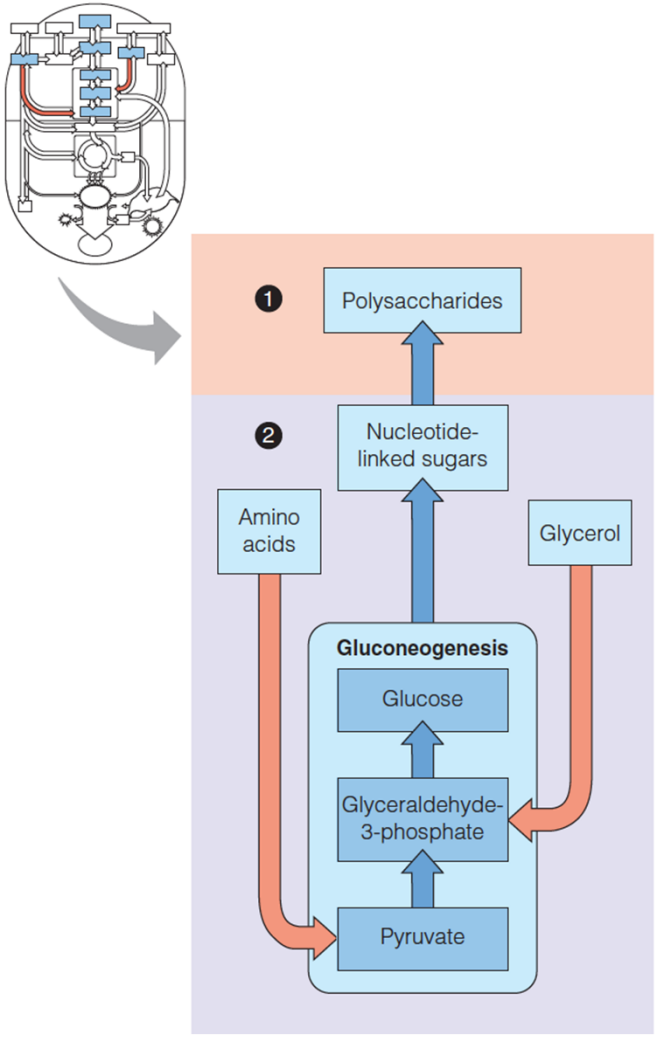
Oxidative metabolism:

•Oxidative metabolism includes

- Pyruvate oxidation
- The citric acid cycle
- Electron transport
- Oxidative phosphorylation

•Pyruvate oxidation supplies acetyl-CoA to the citric acid cycle.

Freeways on the Metabolic Road Map

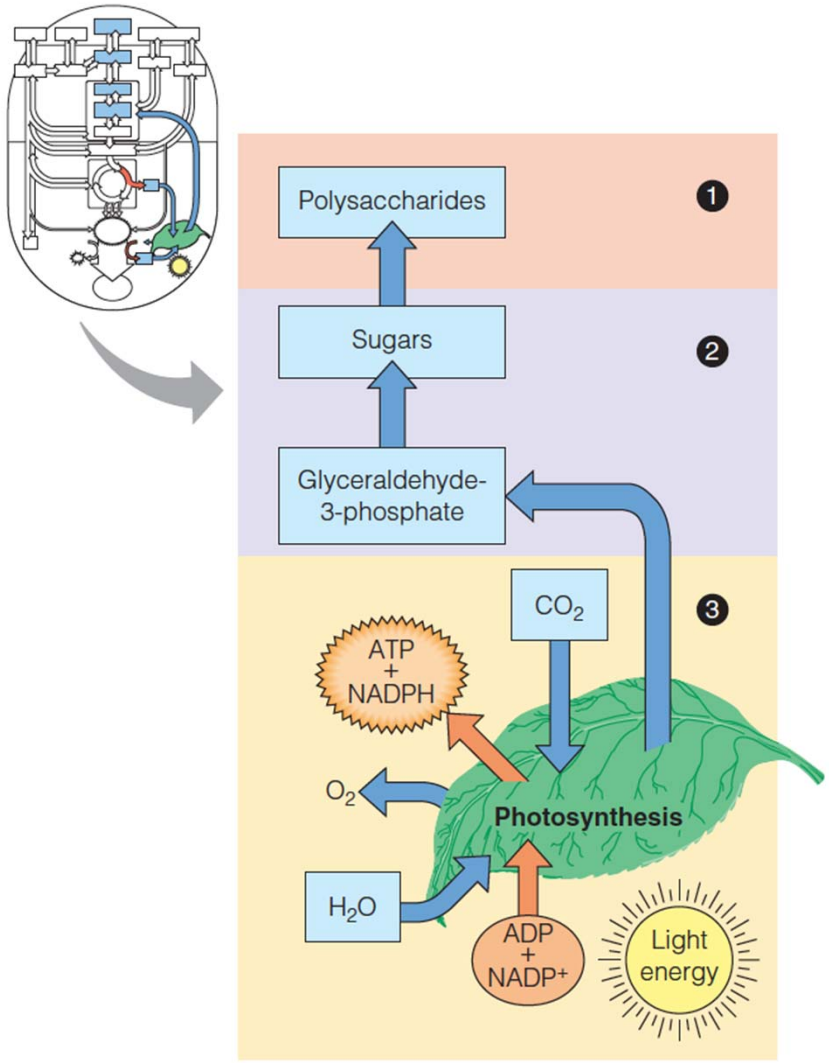


Carbohydrate anabolism:

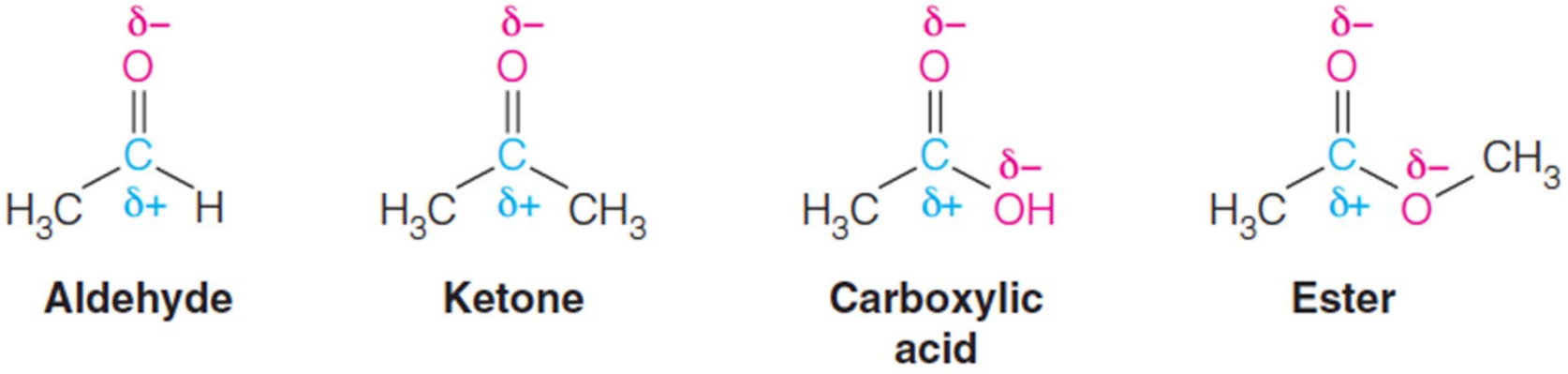
•Biosynthesis of carbohydrates includes gluconeogenesis and polysaccharide synthesis.

Freeways on the Metabolic Road Map

Photosynthesis:



Biochemical Reaction Types

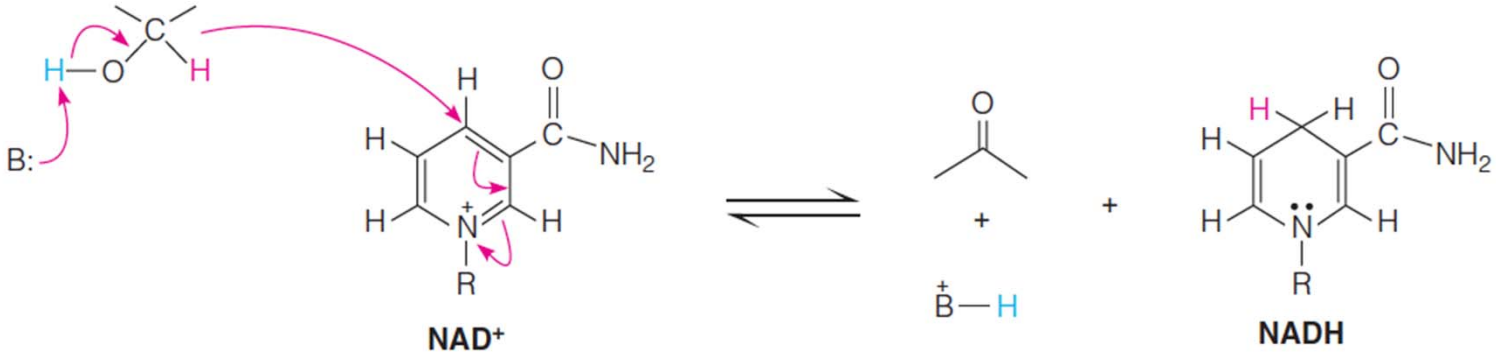


- Much of the chemistry of biological molecules is the chemistry of the carbonyl group because the vast majority of biological molecules contain them.

- Most of the chemistry of carbonyl groups involves ***nucleophiles*** (abbreviated "**Nu:**") and ***electrophiles***.

Biochemical Reaction Types

- Energy production in most cells involves the oxidation of fuel molecules such as glucose.
- Oxidation-reduction, or ***redox***, chemistry thus lies at the core of metabolism.
- Redox reactions involve reversible electron transfer from a donor (the ***reductant***) to an acceptor (the ***oxidant***).
- In this example, because the alcohol has lost a pair of electrons and two hydrogen atoms, this type of oxidation is called ***dehydrogenation***, and enzymes that catalyze this reaction are called ***dehydrogenase*** .



Why redox reaction produces energy?

Basic oxidation-reduction reaction

reductant electron-donating $\quad \mathrm{Fe^{2+}} \rightleftharpoons \mathrm{Fe^{3+}} + e^-$

oxidant electron-accepting $\quad \mathrm{Cu^{2+}} + e^- \rightleftharpoons \mathrm{Cu^{+}}$

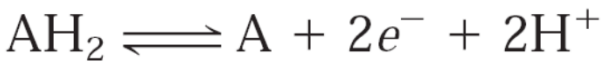
$$\mathrm{Fe^{2+}} + \mathrm{Cu^{2+}} \rightleftharpoons \mathrm{Fe^{3+}} + \mathrm{Cu^{+}}$$

- Biological oxidation-reduction reactions: another kind of electron transfer for bioenergy
- glucose →→→ O2 (higher affinity for electrons)
- Many biochemical oxidation-reduction reactions involve transfer of two electrons
- In order to keep charges in balance, proton transfer often accompanies electron transfer
- In many dehydrogenases, the reaction proceeds by a stepwise transfers of proton (H^+) and hydride ($:H^-$)

Types of electron transfer in cells

- electrons, redox pair

- hydrogen atoms



- hydride ion :H^-

- combination with oxygen

Redox: reduction-oxidation reaction

Reduction Potentials Measure Affinity for Electrons

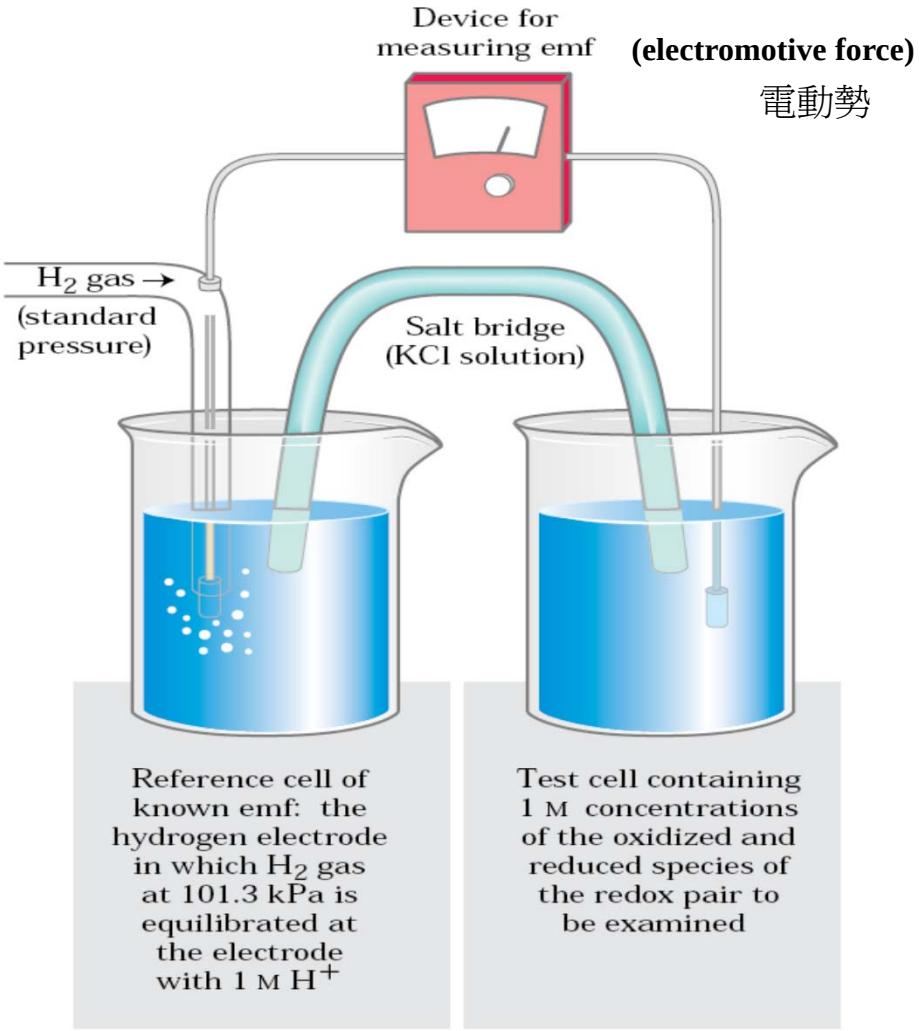
measure of the tendency of a chemical species
to acquire electrons and thereby be reduced

E^0 = standard reduction potential 標準還原電位

$$H^+ + e^- \longrightarrow \tfrac{1}{2}H_2$$

The electrode at which this half-reaction (half cell) occurs → E^0=0.00 V

Measurement of E^0 using H+ half cell 標準氫電極



electrons tend to flow from lower standard reduction potential to higher standard reduction potential

- The half-cell with the stronger tendency to acquire electrons is assigned a positive value of *E*.

Add concentrations…

$$E = E^\circ + \frac{RT}{nF} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

n is the number of electrons transferred per molecule,
and F is the Faraday constant

At 298 K (25°C)…

$$E = E^\circ + \frac{0.026\ \text{V}}{n} \ln \frac{[\text{electron acceptor}]}{[\text{electron donor}]}$$

pH=7…→ E'^0

ΔE and ΔG

the direction in which electrons will tend to flow when the two half-cells are connected can be predicted with *E*

Electrons tend to flow to the half-cell with the more positive *E*

The energy made available by this spontaneous electron flow (the free-energy change for the oxidation-reduction reaction) is proportional to Δ*E*

$$\Delta G = -n\mathcal{F}\,\Delta E \qquad \text{or} \qquad \Delta G'^{\circ} = -n\mathcal{F}\,\Delta E'^{\circ}$$

For example…

$$\text{Acetaldehyde} + \text{NADH} + \text{H}^+ \longrightarrow \text{ethanol} + \text{NAD}^+$$

$$\text{Acetaldehyde} + 2\text{H}^+ + 2e^- \longrightarrow \text{ethanol} \qquad E'^\circ = -0.197\ \text{V}$$

$$\text{NAD}^+ + 2\text{H}^+ + 2e^- \longrightarrow \text{NADH} + \text{H}^+ \qquad E'^\circ = -0.320\ \text{V}$$

$$\Delta E'^\circ = -0.197\ \text{V} - (-0.320\ \text{V}) = 0.123\ \text{V}$$

$$\Delta G'^\circ = -n\mathcal{F}\,\Delta E'^\circ = -2(96.5\ \text{kJ/V} \cdot \text{mol})(0.123\ \text{V})$$
$$= -23.7\ \text{kJ/mol}$$

All concentrations = 1

When concentration is involved…

$$E_{\text{acetaldehyde}} = E^\circ + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{acetaldehyde}]}{[\text{ethanol}]}$$

$$= -0.197\ \text{V} + \frac{0.026\ \text{V}}{2} \ln \frac{1.00}{0.100} = -0.167\ \text{V}$$

$$E_{\text{NADH}} = E^\circ + \frac{RT}{n\mathcal{F}} \ln \frac{[\text{NAD}^+]}{[\text{NADH}]}$$

$$= -0.320\ \text{V} + \frac{0.026\ \text{V}}{2} \ln \frac{1.00}{0.100} = -0.350\ \text{V}$$

$$\Delta E = -0.167\ \text{V} - (-0.350)\ \text{V} = 0.183\ \text{V}$$

$$\Delta G = -n\mathcal{F}\,\Delta E$$

$$= -2(96.5\ \text{kJ/V} \cdot \text{mol})(0.183\ \text{V})$$

$$= -35.3\ \text{kJ/mol}$$

Some Bioenergetic Considerations

Most of the energy source in living systems

- Most biological energy derives from <u>oxidation</u> of reduced metabolites in a series of reactions, with ***oxygen as the final electron acceptor.***

a strong oxidant; attract electrons

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \Delta G^{\circ\prime} = -2870\ \text{kJ/mol}$$

$$C_6H_{12}O_6 + 10NAD^+ + 2FAD + 6H_2O \rightarrow 6CO_2 + 10NADH + 10H^+ + 2FADH_2$$

$$10NADH + 10H^+ + 2FADH_2 + 6O_2 \rightarrow 10NAD^+ + 2FAD + 12H_2O$$

$$\text{Net: } C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

transfer of electrons from these intermediate electron carriers to oxygen is catalyzed by the electron transport chain

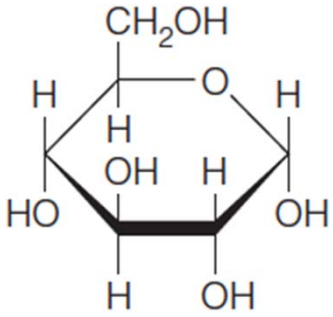
free energy as chemical energy (synthesis of ATP)

Some Bioenergetic Considerations

- Not all metabolic energy comes from oxidation by oxygen.
- Substances other than oxygen can serve as terminal electron acceptors.
- Many microorganisms either can or must live anaerobically.
- For example, *Desulfovibrio* 脱硫弧菌 carry out anaerobic respiration using sulfate as the terminal electron acceptor:

$$SO_4^{2-} + 8e^- + 8H^+ \rightarrow S^{2-} + 4H_2O$$

Some Bioenergetic Considerations



Glucose

$CH_3(CH_2)_{14}COOH$

Palmitic acid

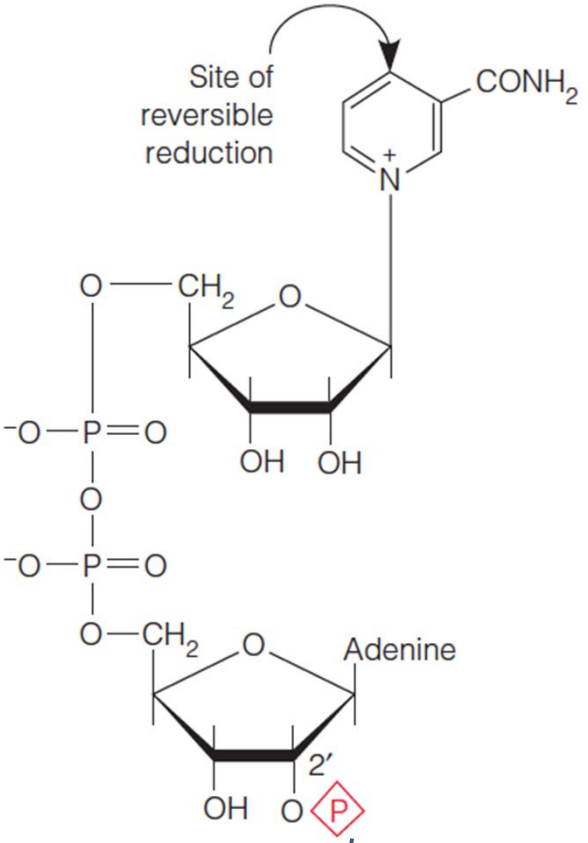
- The combustion of fat provides more heat energy than the combustion of an equivalent mass of carbohydrate.
- In other words, fat has a higher ***caloric content*** than carbohydrate.
- Compare the oxidation of glucose with the oxidation of a typical saturated fatty acid, palmitic acid 棕櫚酸 (十六烷酸).

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \qquad \Delta G^{\circ\prime} = -3.74\,\text{kcal/g}$$

$$C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O \qquad \Delta G^{\circ\prime} = -9.30\,\text{kcal/g}$$

Some Bioenergetic Considerations

- The major source of electrons for ***reductive biosynthesis*** is **NADPH**, nicotinamide adenine dinucleotide phosphate (reduced).
- $NADP^+$ and NADPH are identical to NAD^+ and NADH, respectively, except that the former have an additional phosphate esterified at C-2 on the adenylate moiety.



$NADP^+$
Nicotinamide adenine dinucleotide phosphate (oxidized)

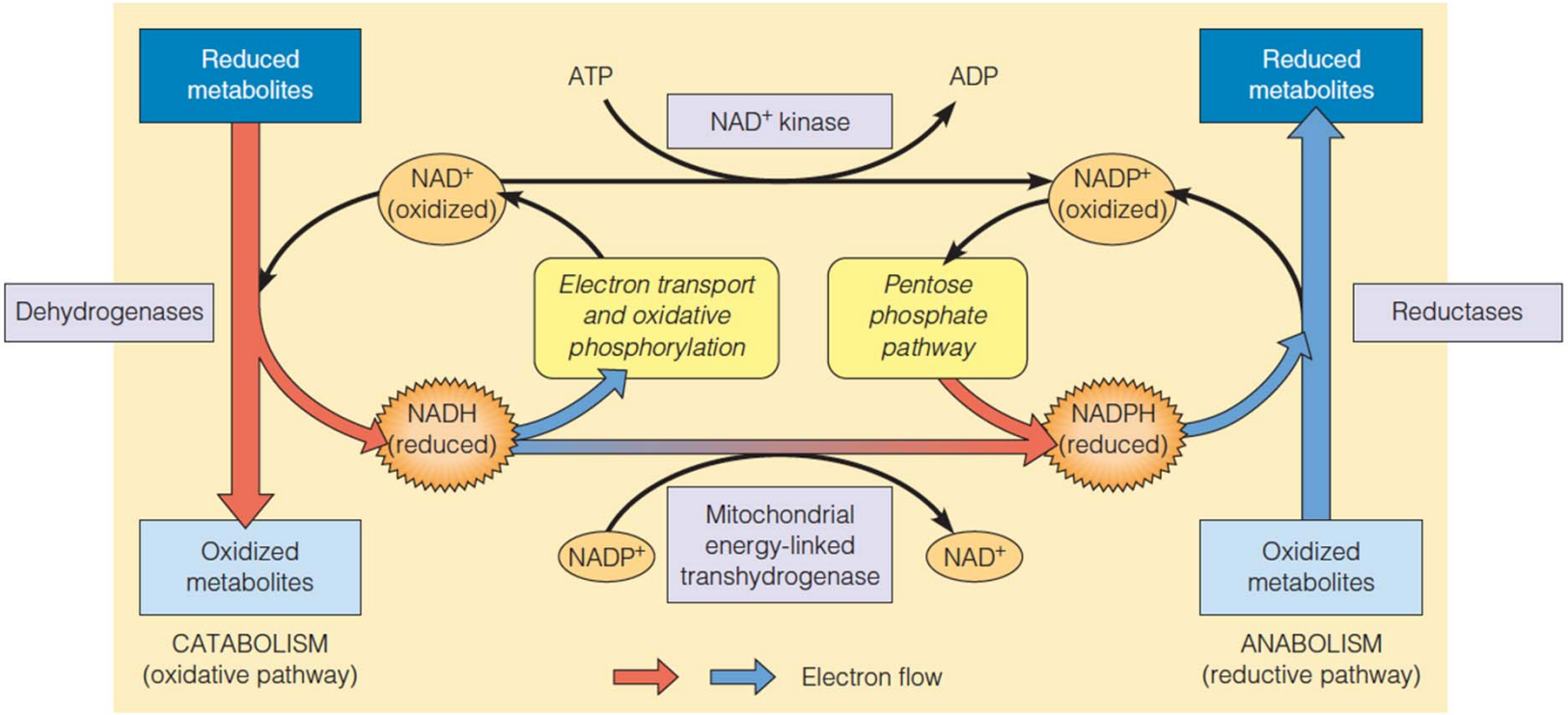
This phosphate is replaced by hydroxyl group in NAD+

Some Bioenergetic Considerations

Nicotinamide nucleotides in catabolism and biosynthesis:

•NAD^+ is the cofactor for most enzymes that act in the direction of substrate oxidation (dehydrogenases).

•NADPH usually functions as a cofactor for reductases, enzymes that catalyze substrate reduction.



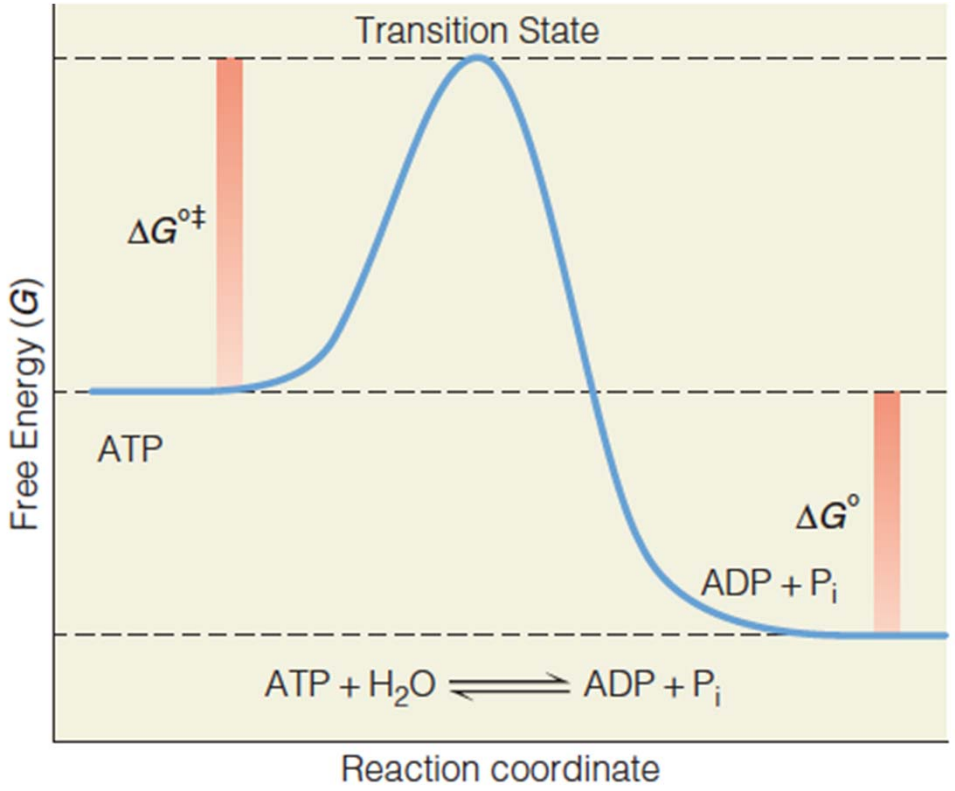
Some Bioenergetic Considerations

- The fundamental biological role of ATP as an energy-coupling compound is to convert thermodynamically unfavorable processes into favorable processes.

- Activated intermediates, such as ATP, allow reactions to occur under physiologically relevant concentrations of metabolic intermediates.

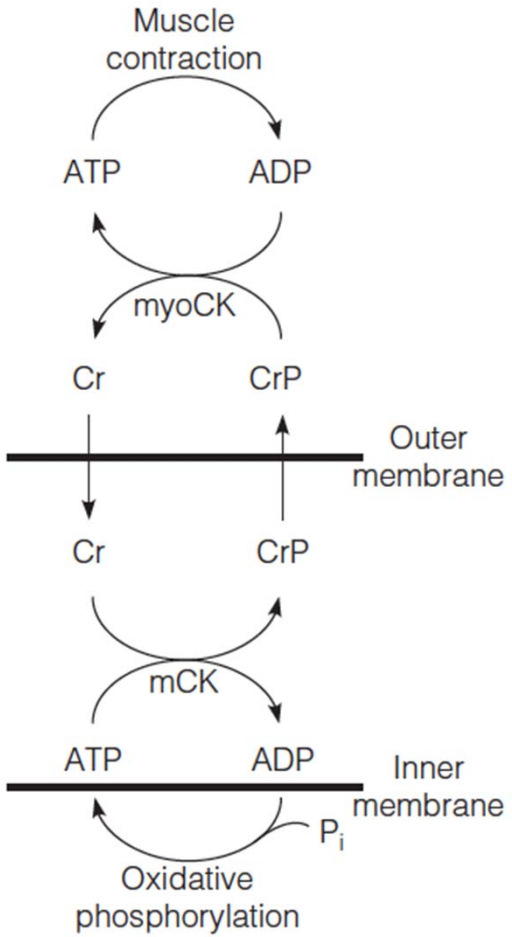
Some Bioenergetic Considerations

- ***Phosphoanhydride*** bonds 酐鍵 are thermodynamically unstable, but kinetically stable—large free energies of activation require enzymes to lower the activation barrier.



Some Bioenergetic Considerations

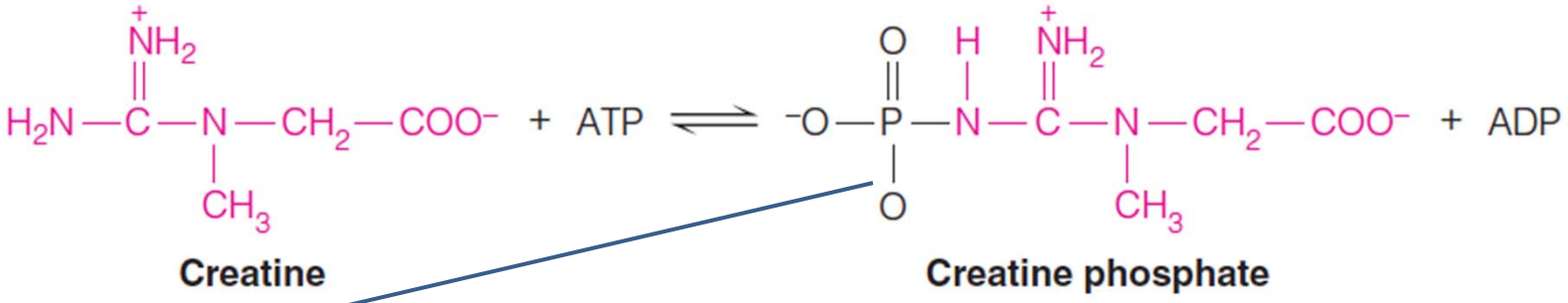
- ATP can drive the synthesis of higher-energy compounds, if nonequilibrium intracellular concentrations make such reactions exergonic.



ATP breakdown is usually coupled with a thermodynamically unfavorable reaction

Some Bioenergetic Considerations

- ATP can also drive the synthesis of compounds of even higher phosphate transfer potential, such as ***creatine phosphate***.
- This compound shuttles phosphate bond energy from ATP in mitochondria to myofibrils, where that bond energy is transduced to the mechanical energy of muscle contraction.
- Creatine phosphate (CrP) is produced from creatine by the enzyme ***creatine kinase***:



the phosphate released from ATP does not become Pj but instead is transferred directly to creatine

Other High-Energy compounds

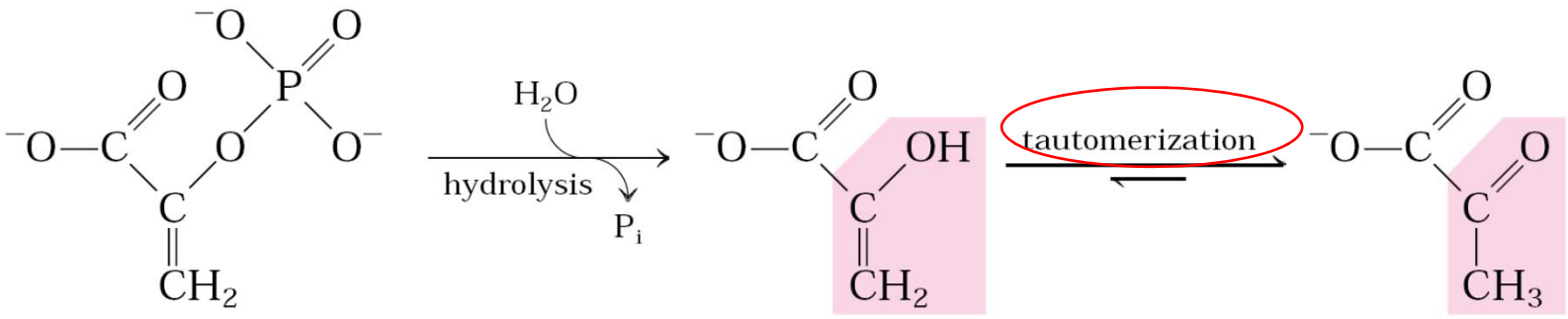
table 14–6

Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

	$\Delta G'^{\circ}$	
	(kJ/mol)	(kcal/mol)
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate (→ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP (→ AMP + P_i)	−32.8	−7.8
ATP (→ ADP + P_i)	−30.5	−7.3
ATP (→ AMP + PP_i)	−45.6	−10.9
AMP (→ adenosine + P_i)	−14.2	−3.4
PP_i (→ $2P_i$)	−19	−4.0
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 1-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

Phosphoenolpyruvate (PEP): phosphate ester bond

(glycolysis and gluconeogenesis)



PEP

Pyruvate
(enol form)

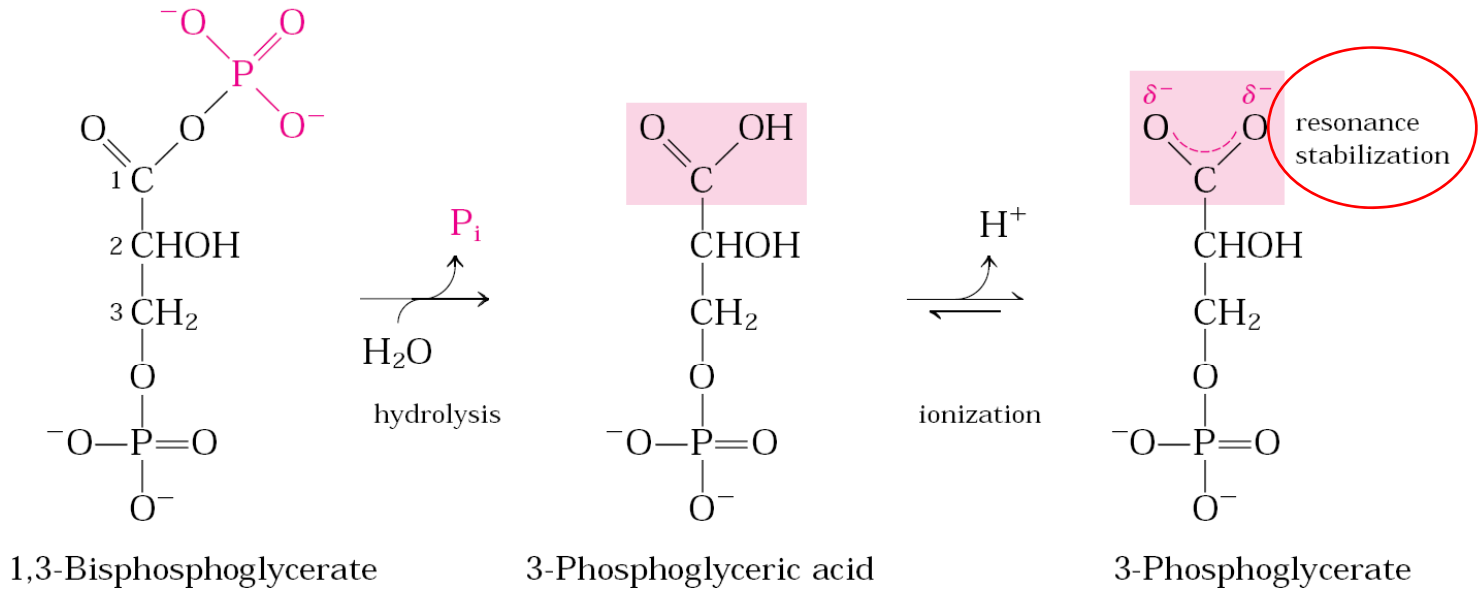
Pyruvate
(keto form)

More stable

$$PEP^{3-} + H_2O \longrightarrow pyruvate^- + P_i^{2-}$$
$$\Delta G'^\circ = -61.9 \text{ kJ/mol}$$

1,3-bisphosphoglycerate (1,3 BPG) : anhydride bond

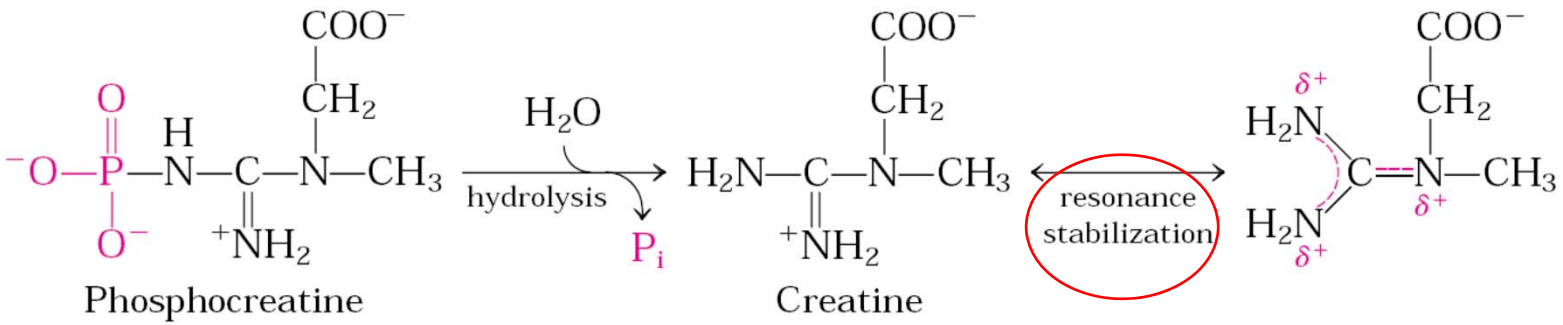
(glycolysis, photosynthesis)



1,3-Bisphosphoglycerate 3-Phosphoglyceric acid 3-Phosphoglycerate

$$1,3\text{-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow 3\text{-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -49.3 \text{ kJ/mol}$$

Phosphocreatine: the P-N bond



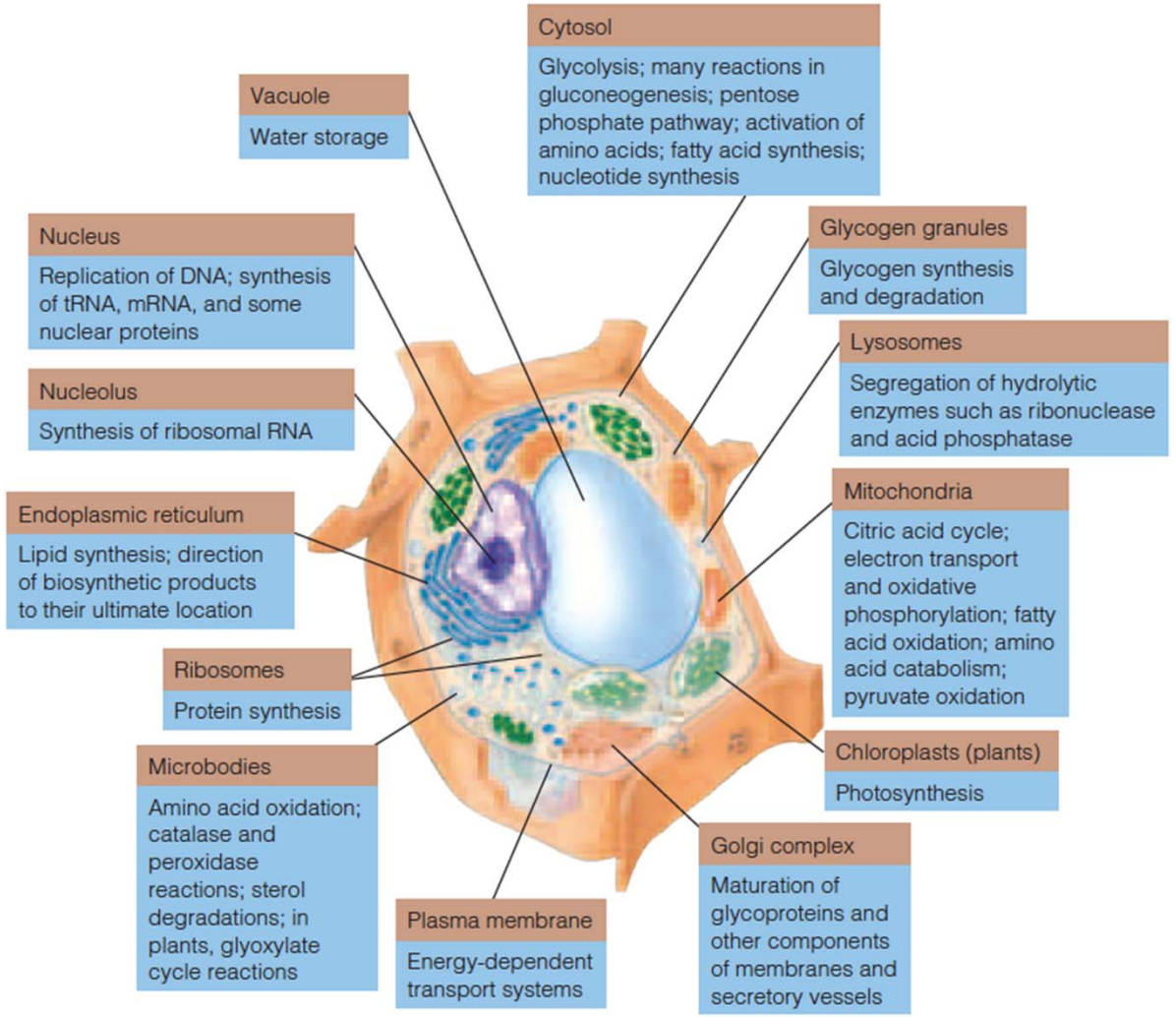
$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + P_i^{2-}$$

$$\Delta G'^{\circ} = -43.0 \text{ kJ/mol}$$

Major Metabolic Control Mechanisms

Locations of major metabolic pathways within a eukaryotic cell:

•This hypothetical cell combines features of a plant cell and an animal cell.

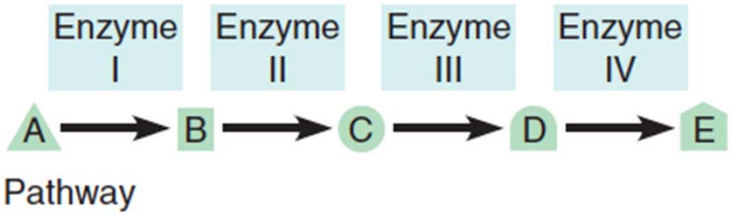


Experimental Analysis of Metabolism

- By inactivating individual enzymes, mutations and enzyme inhibitors help identify the metabolic roles of enzymes.
- The steps of a hypothetical metabolic pathway are identified by analysis of mutants defective in individual steps of the pathway.
- We can identify metabolite C as the substrate for enzyme III by the absence of this enzyme in mutants that accumulate C.
- We know that D and E follow C in the pathway because feeding either D or E to mutants defective in enzyme III bypasses the genetic block and allows the cells to grow.

A mutant defective in enzyme:	Accumulates metabolite in culture medium:	Requires an external source of:	Culture filtrate allows the growth of another mutant, defective in enzyme:
I	A	B, C, D, or E	—
II	B	C, D, or E	I
III	C	D or E	I or II
IV	D	E	I, II, or III

Analysis of mutants



Pathway

Metabolomics

- The development of these new technologies has driven the “-omics” revolution:
    - Genomics
    - Transcriptomics
    - Proteomics
    - Metabolomics
- Hundreds or even thousands of specific components are measured simultaneously in a biological sample.
- Thus, it is now feasible to measure the full set of transcripts (transcriptome), proteins (proteome), or metabolites (metabolome) in a particular cell or tissue.
- The metabolome represents the ultimate molecular phenotype of a cell under a given set of conditions because all the changes in gene expression and enzyme activity eventually lead to changes in cellular metabolite levels (the metabolic state or profile).

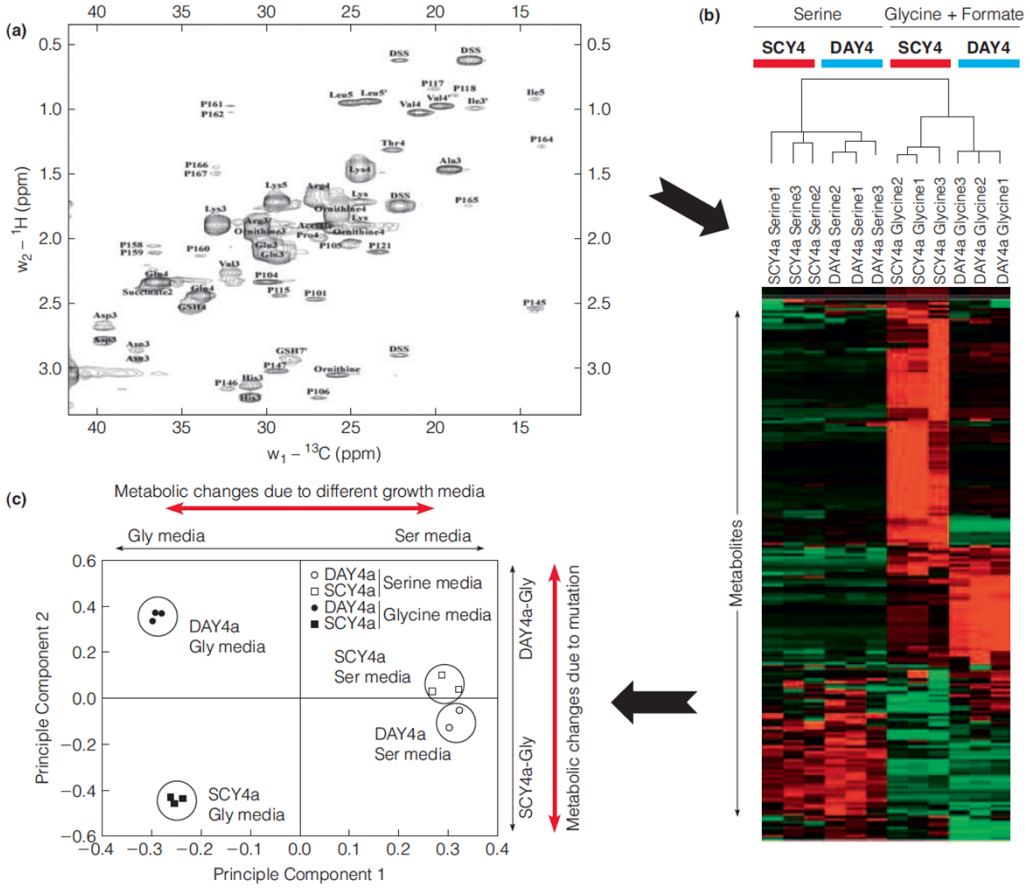
Metabolomics

Basic process of metabolic profiling:

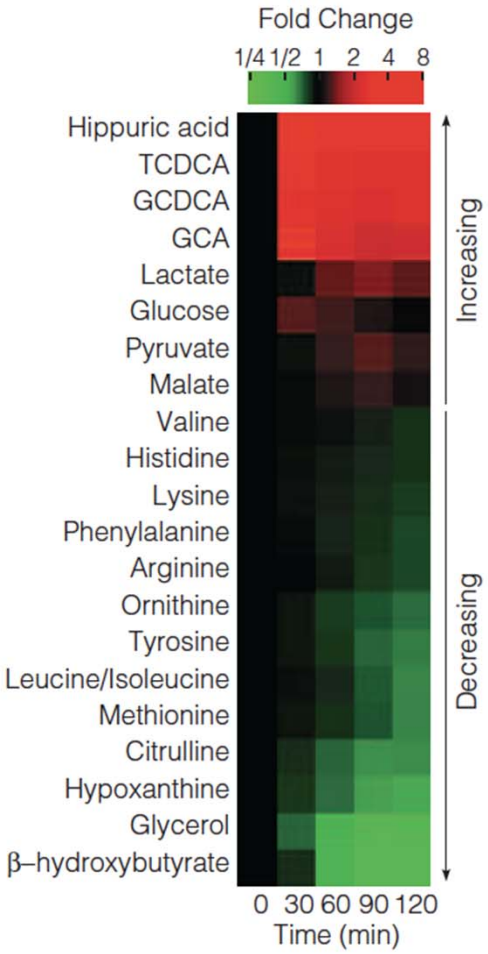
a)Metabolites are identified and quantified by an analytical method.

b)Data are collected and visualized by informatics approaches.

c)Informatics approaches are then used to reveal relationships and patterns among the samples.



Metabolomics



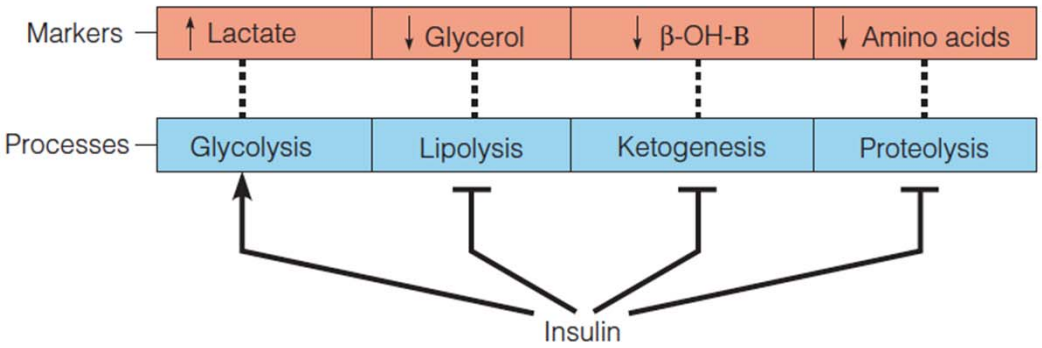


FIGURE 12B.3

Changes in plasma metabolites following glucose ingestion. Color intensity in the heat map reflects the median fold change compared to the fasting levels. Metabolites are ordered according to the magnitude of change. This set of metabolites reflects four distinct arms of insulin action in response to glucose ingestion.

Courtesy of Vamsi Mootha