Biochemistry

Metabolic pathways

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Objectives of the course:

Energy metabolism

- high-energy donors
- coupled reactions
- co-factors: NAD^+/NADH; FAD/$FADH_2$; TPP; PLP; CoA; Biotin
- glycolysis/glyconeogenesis
- citric acid cycle: regulation (tissue-dependent)
  GABA-shunt;
  glyoxylate cycle
- respiratory chain and oxidative phosphorylation
- glycogen metabolism (activated glucose)
- pentose phosphate pathway (tissue specificity)
- photosynthesis: RUBISCO
  C4-plants

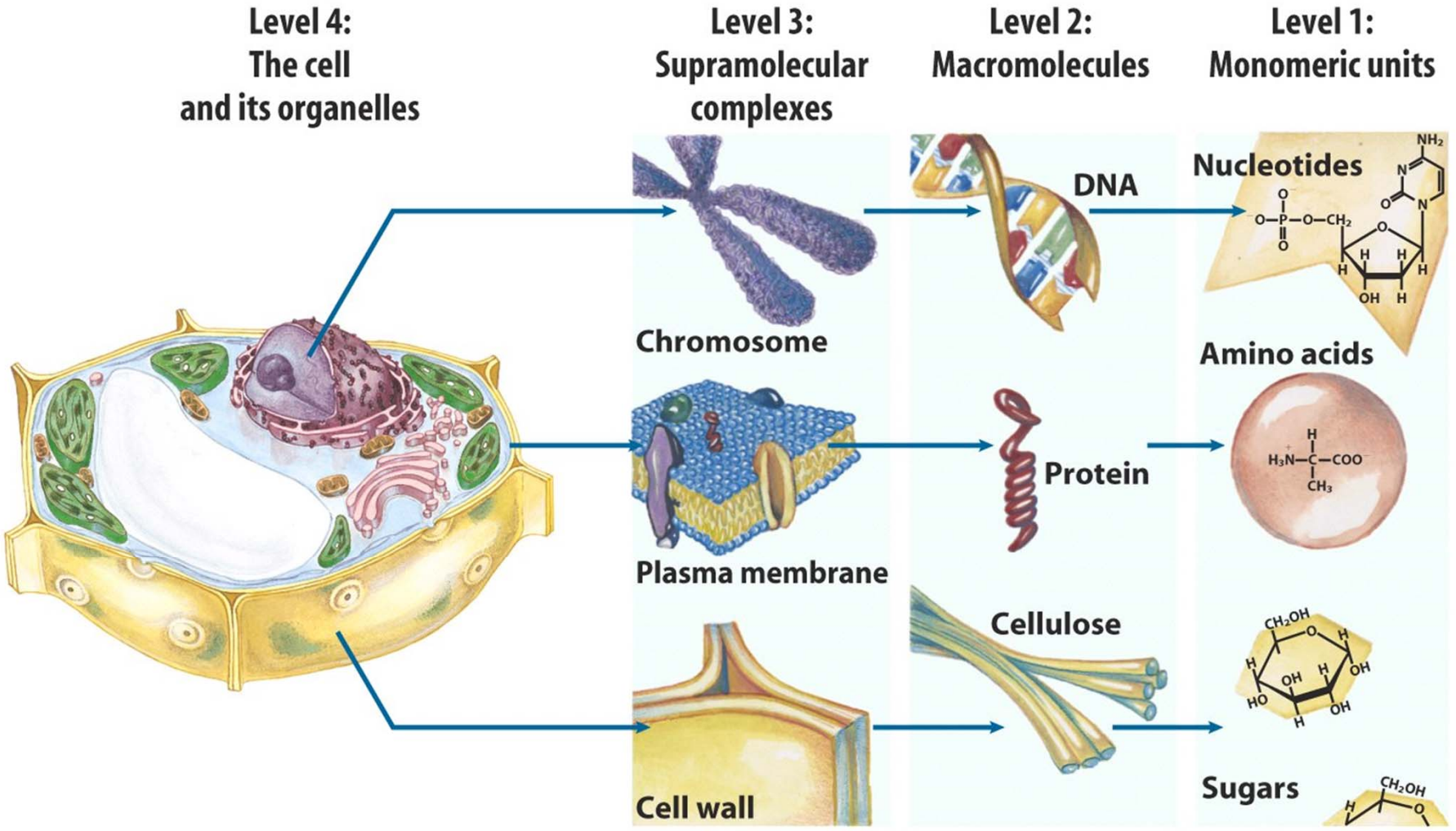
Nitrogen metabolism

- N_2 assimilation via reduction to NH_3 (nitrogenase complex)
- NH_3 metabolism: glutamate-dehydrogenase
  glutamate synthase
  glutamine synthetase
  glutamine amidotransferase
- urea cycle
- C_1 metabolism (PLP, THF, SAM, homocystein)
- nucleotide metabolism: biosynthesis of purines and pyrimidines
  from RNA to DNA (NDP reductase)
  salvage pathway, HGPRT deficiency
  cytostatic drugs
  catabolism (ADA-deficiency, urate)

Signal transduction

- GPCR – glucagon signalling
- RTK – insulin signalling
- G-proteins

Structural hierarchy in the molecular organization of cells



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Essential Questions?

- What are the properties of **regulatory** enzymes?
- How do regulatory enzymes sense the momentary needs of cells?
- What molecular mechanisms are used to regulate enzyme activity?
- Factors that influence enzymatic activity
- General features of **allosteric regulation**
- The kind of **covalent modification** that regulates the activity of enzymes
- Is the activity of some enzymes controlled by both allosteric regulation and covalent modification?
- Special focus: is there an example in nature that exemplifies the relationship between quaternary structure and the emergence of allosteric properties? hemoglobin and myoglobin – paradigms of protein structure and function

What Factors Influence Enzymatic Activity?

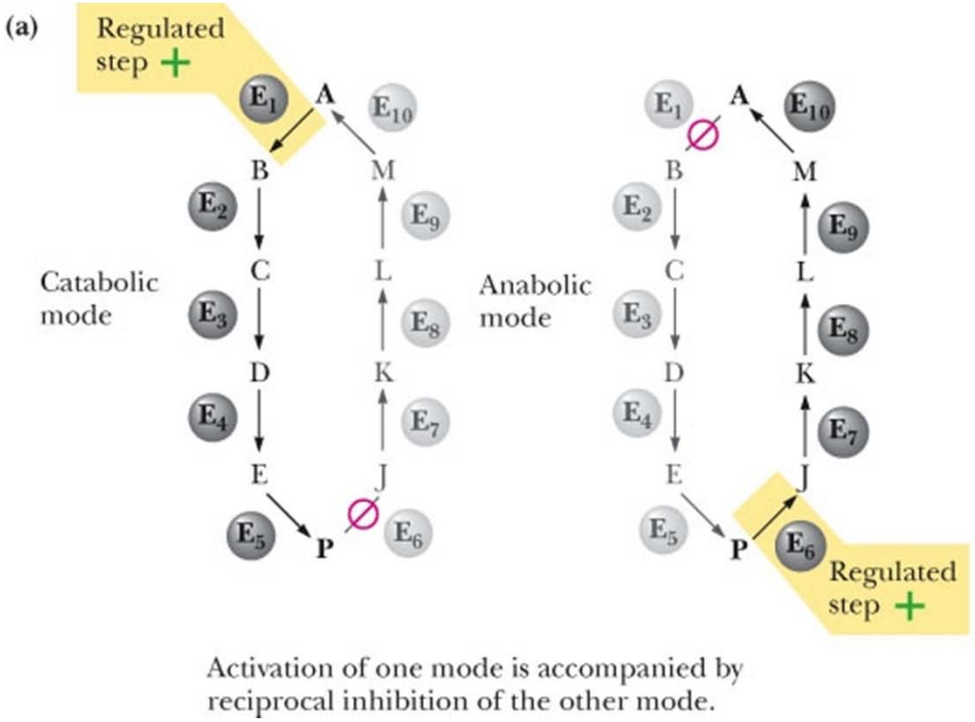
- The **availability of substrates and cofactors** usually determines how fast the reaction goes.
- **Km** values ~ the prevailing *in vivo* concentration of the substrates
- As product accumulates, the apparent rate of the enzymatic reaction will decrease as equilibrium is approached

- **Genetic regulation of enzyme synthesis and decay** determines the amount of enzyme present at any moment
  - **Induction** = activation of enzyme synthesis
  - **Repression** = shutdown of enzyme synthesis
  - **By controlling the [E], cells can activate or terminate metabolic routes.**
  - Other factors: **Zymogens, isozymes, and modulator** proteins may play a role

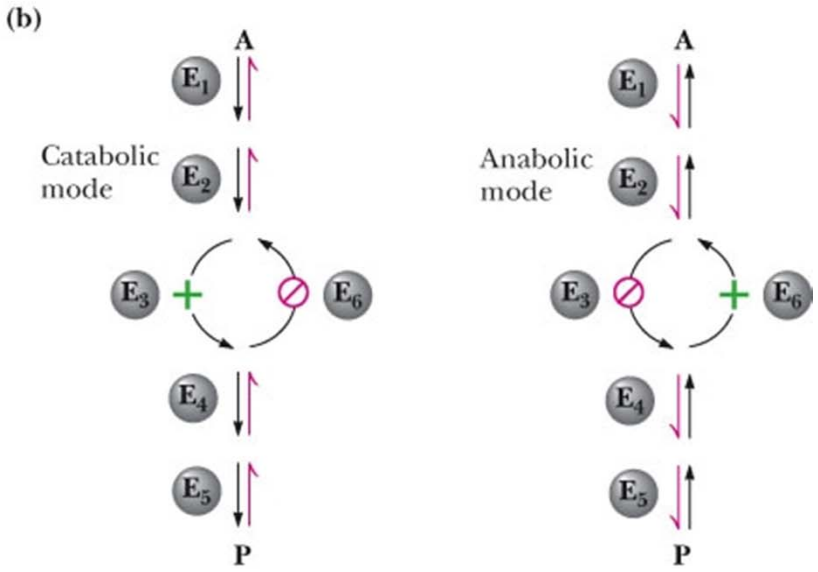
Principal characteristics of metabolic pathways

1. Metabolic pathways are irreversible.
2. Catabolic and anabolic pathways must differ.
3. Every metabolic pathway has a first committed step.
4. All metabolic pathways are regulated.
5. M.p. in eukaryotic cells occur in specific subcellular compartments.

Metabolic Regulation Requires Different Pathways for Oppositely Directed Metabolic Sequences



Activation of one mode is accompanied by reciprocal inhibition of the other mode.



Parallel pathways of catabolism and anabolism must **differ in at least one metabolic step** in order that they can be regulated independently. Shown here are two possible arrangements of opposing catabolic and anabolic sequences between **A and P**. (a) Parallel sequences proceed by independent routes. (b) Only one reaction has two different enzymes.

Metabolic Pathways are Compartmentalized within Cells

- Eukaryotic cells are compartmentalized by an endomembrane system – advantageous for metabolism
- Each organelle (compartment) – dedicated to specialized metabolic functions and contains appropriate enzymes – confined together.

- **Advantages:**
  - Allow analyses of respective functions because they can be separated
  - Enzymes are isolated from competing pathways
  - Temporal compartmentalization
  - Intermediates are spatially and chemically segregated
  - Genes of metabolism show a circadian pattern of regulated expression
  - Example: glucose-6 phosphatase that converts glucose-6-phosphate to glucose is localized in the ER.

Where metabolic processes occur at the organ level

Liver

- Liver is the **center of metabolism** – maintains blood glucose levels and regulates the concentration of metabolites in the blood.
- Stores **glycogen** that can be made into glucose-6-phosphate, then glucose.
- Makes glucose by gluconeogenesis (from pyruvate, *de novo*).
- Synthesizes **FA, cholesterol and bile salts**.
- Produces **ketone bodies** but cannot use them (no CoA transferase in the liver)
- Only the liver and kidneys contain glucose-6-phosphatase

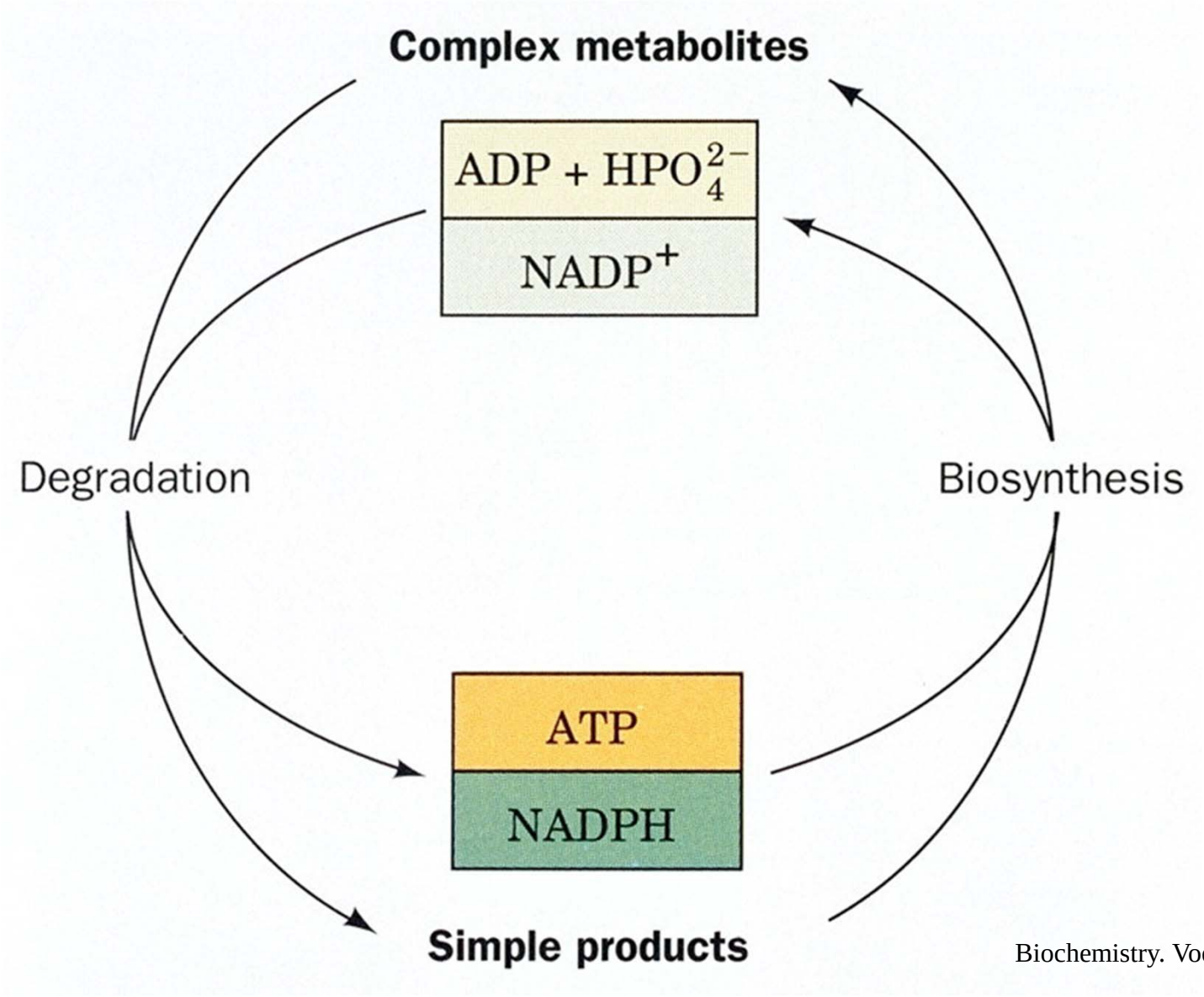
Glucose regulation in the liver

- When blood glucose is high (fed state), FA are synthesized by the liver, converted into triacylglycerols and packaged into VLDL that are secreted into the blood.
- When blood glucose is low (fasting state), the liver produces ketone bodies to fuel the heart and muscle to preserve glucose for the brain. Eventually brain is fed by ketone bodies.

Other organs in metabolism

- Brain - Glucose is the primary fuel; only after prolonged fasting (not eating) does the brain use ketone bodies for fuel (last resort).
  - Brain has no capacity to store fuel – needs a constant supply
  - Consumes a lot of energy – 120 g of glucose per day.
  - Glucose is transported into the brain by GLU3 glucose transporter (crosses the membrane).
  - [glucose] in brain – maintained around 5 mM so glucose is saturated under normal conditions. If drops to 2.2 mM the brain is in trouble.
- **Muscle** - uses glucose, FA and ketone bodies for fuel; have **stores of glycogen** that is converted to glucose when needed for bursts of activity.
- **Intestines**
- **Kidney**
- **Adipose tissue**
- **Heart**

Metabolic principle: Degradation is coupled to the formation of **ATP** (energy store**)** and **NADPH** (reduction equivalents), that represent sources of free energy for biosynthetic reactions.



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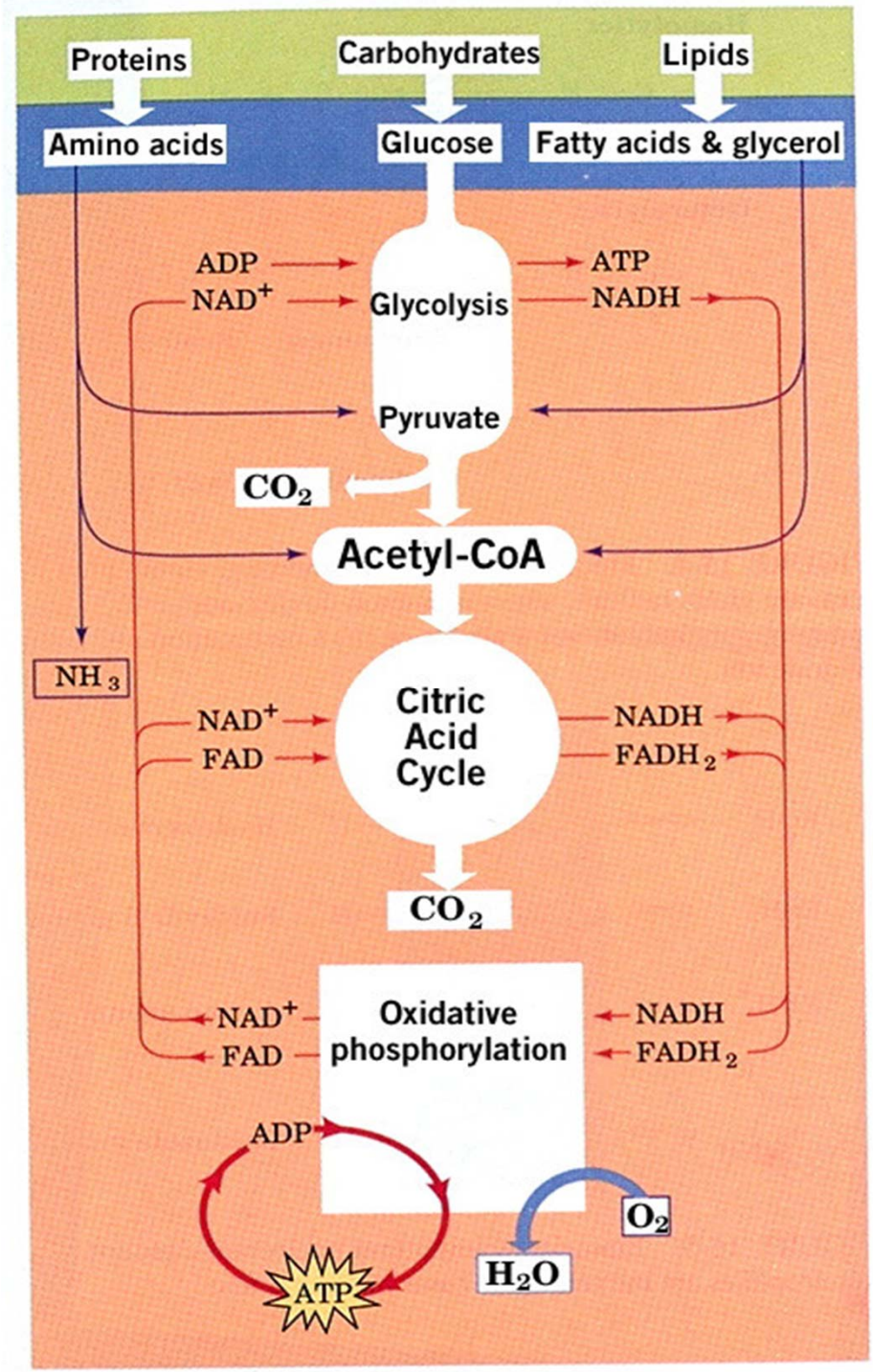

high-enthalpy, low-entropy

Overview of catabolism

low-enthalpy, high-entropy

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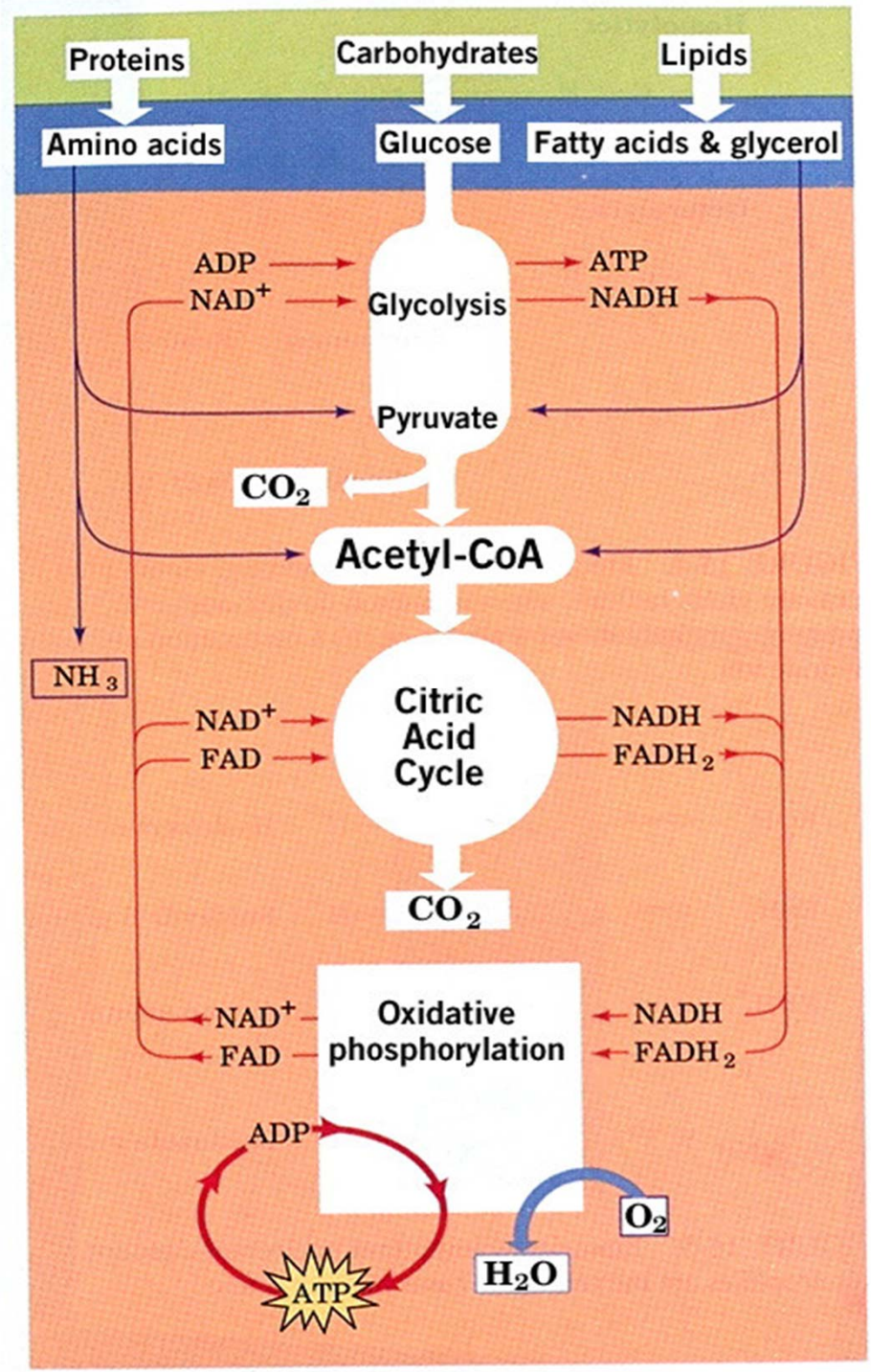

Stage 1: Proteins, polysaccharides and lipids are broken down into their component building blocks.

Stage 2: The building blocks are degraded into the **common product**, generally the **acetyl groups of acetyl-CoA**.

Stage 3: Catabolism converges to three principal end products: **water, carbon dioxide, and ammonia**.

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The c**entral role of ATP** for energy exchange in biological systems was discovered in 1941 by Fritz Lipmann und Herman Kalckar.

ATP-turnover: 40 kg/day

Energy rich compounds: the *triphosphate subunit*, contains 2 *phosphoric acid anhydride linkages*, which upon hydrolysis release high amounts of energy:

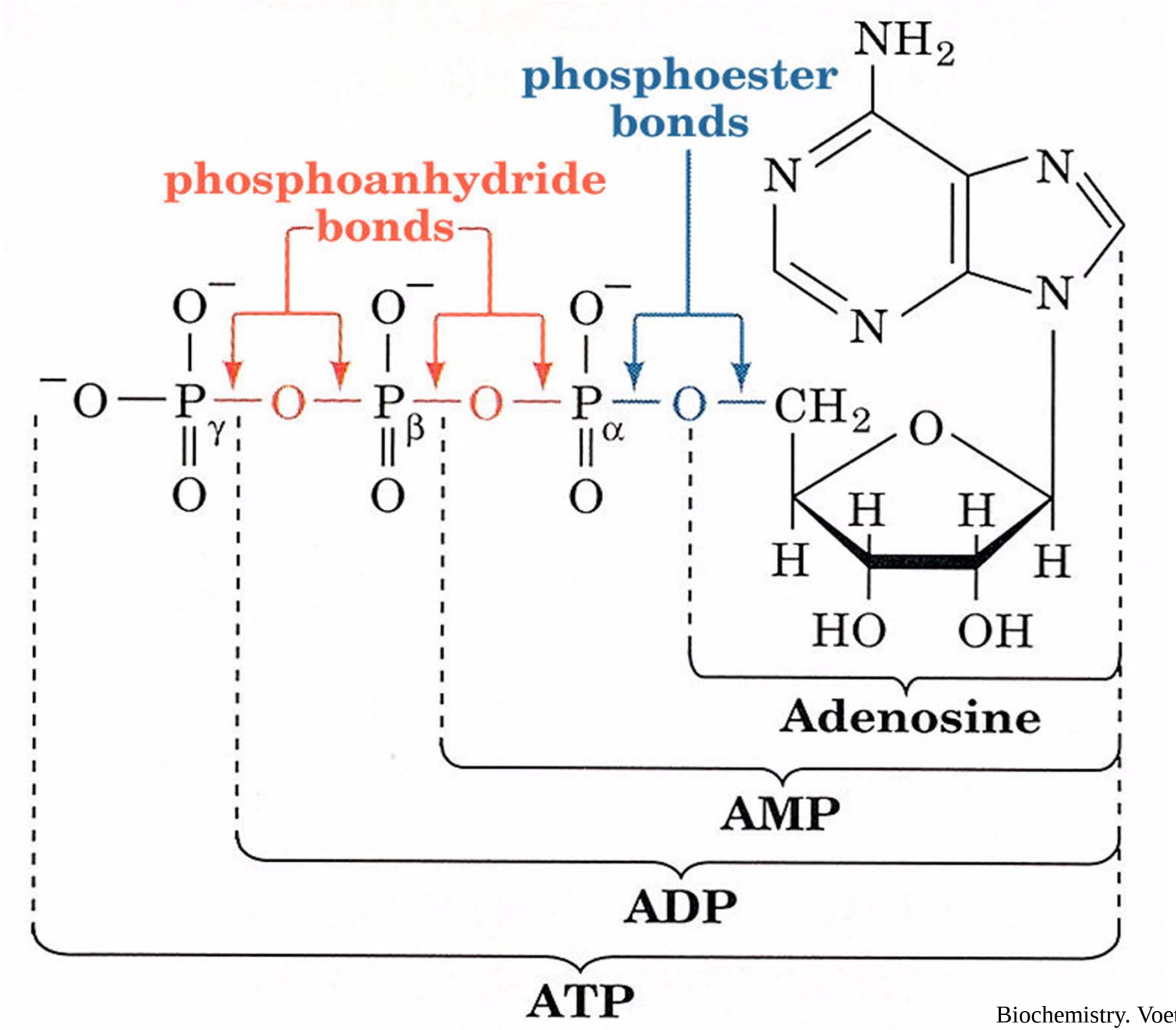
$ATP + H_2O \rightleftharpoons ADP + Pi + H^+$ $\quad \Delta G°' = -30{,}5$ kJ/mol

$ATP + H_2O \rightleftharpoons AMP + PPi + H^+$ $\quad \Delta G°' = -30{,}5$ kJ/mol

ATP, ADP, AMP, interconversionable: *adenylate-kinase:*

$ATP + AMP \rightleftharpoons ADP + ADP$

ATP is **continuously** formed and hydrolysed.



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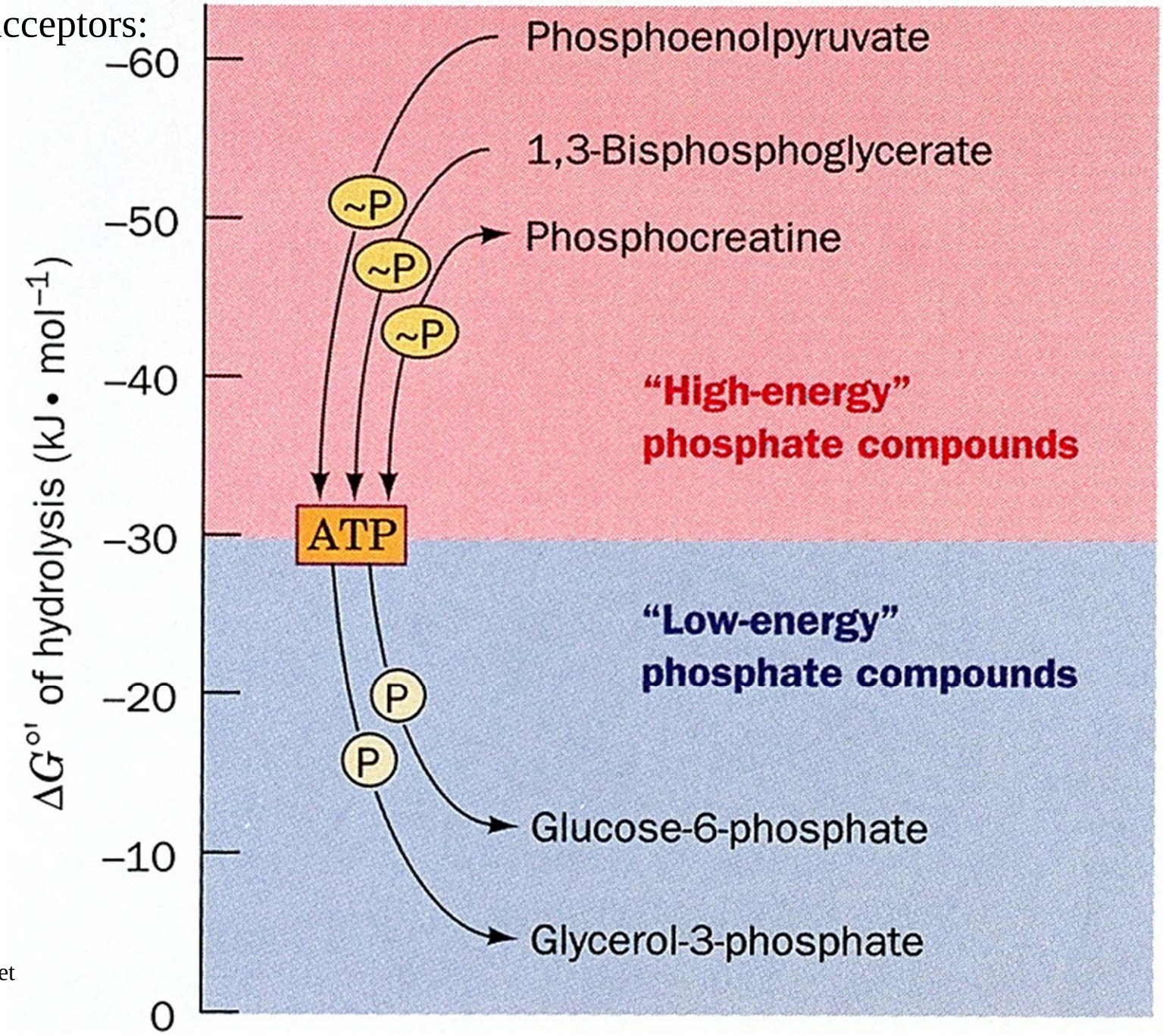
Transfer of the phosphate group from “high-energy” donors via ATP to “low-energy” acceptors:



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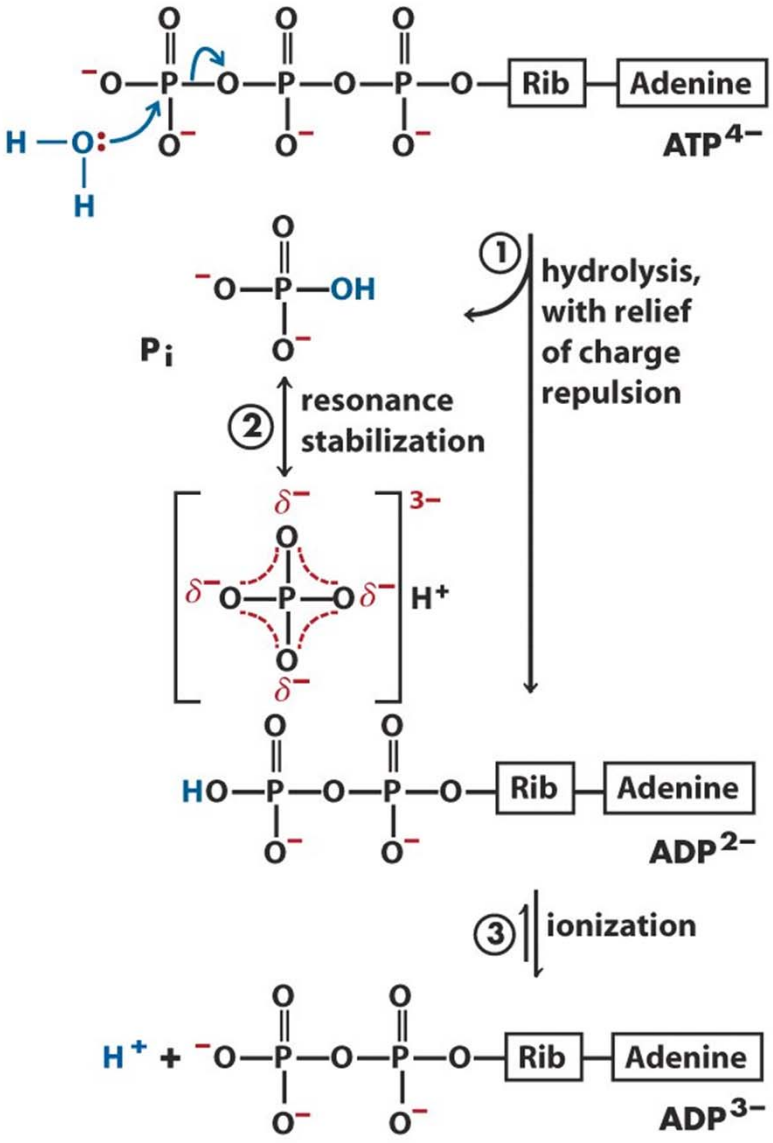
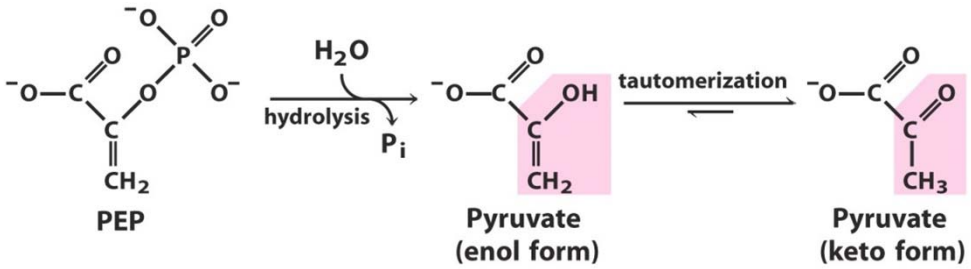
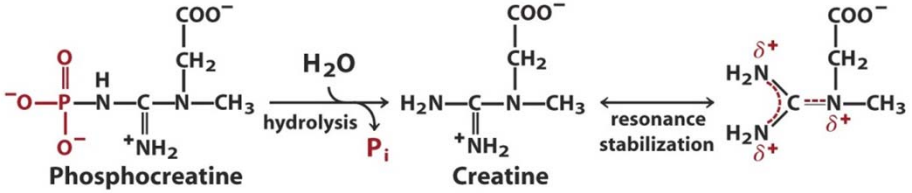


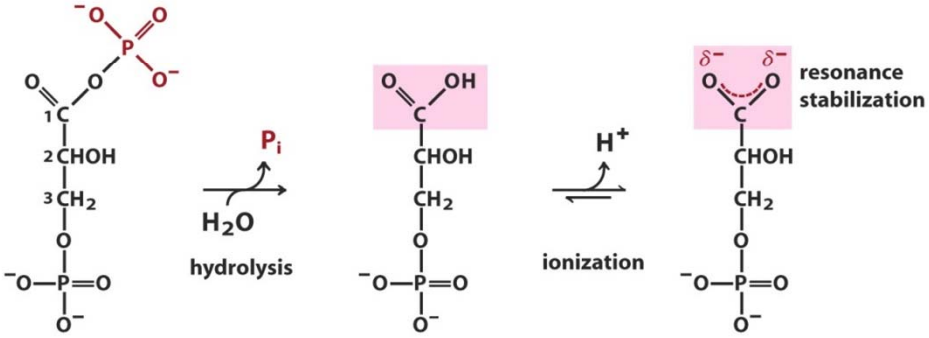
$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$
$$\Delta G'^{\circ} = -30.5\ kJ/mol$$



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



$$Phosphocreatine^{2-} + H_2O \longrightarrow creatine + P_i^{2-}$$
$$\Delta G'^{\circ} = -43.0\ kJ/mol$$



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

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TABLE 13–5 Adenine Nucleotide, Inorganic Phosphate, and Phosphocreatine Concentrations in Some Cells

	Concentration (mM)*				
	ATP	*ADP*†	*AMP*	P_i	*PCr*
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	28
Rat neuron	2.59	0.73	0.06	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	1.65	0
E. coli cell	7.90	1.04	0.82	7.9	0

*For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 505.

†This value reflects total concentration; the true value for free ADP may be much lower (see Box 13–1).

When a sudden demand of energy depletes ATP, the PCr reservoir is used to replenish ATP at a rate considerably faster than possible via catabolism.
Vice versa, when demand of energy is reduced, ATP produced by catabolism is used to replenish the PCr reservoir.

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Structure of Acetyl-CoA:

a „high-energy" thioester of the nucleotide cofactor Coenzyme A

$\Delta G^{0'} = -31$ kJ/mol

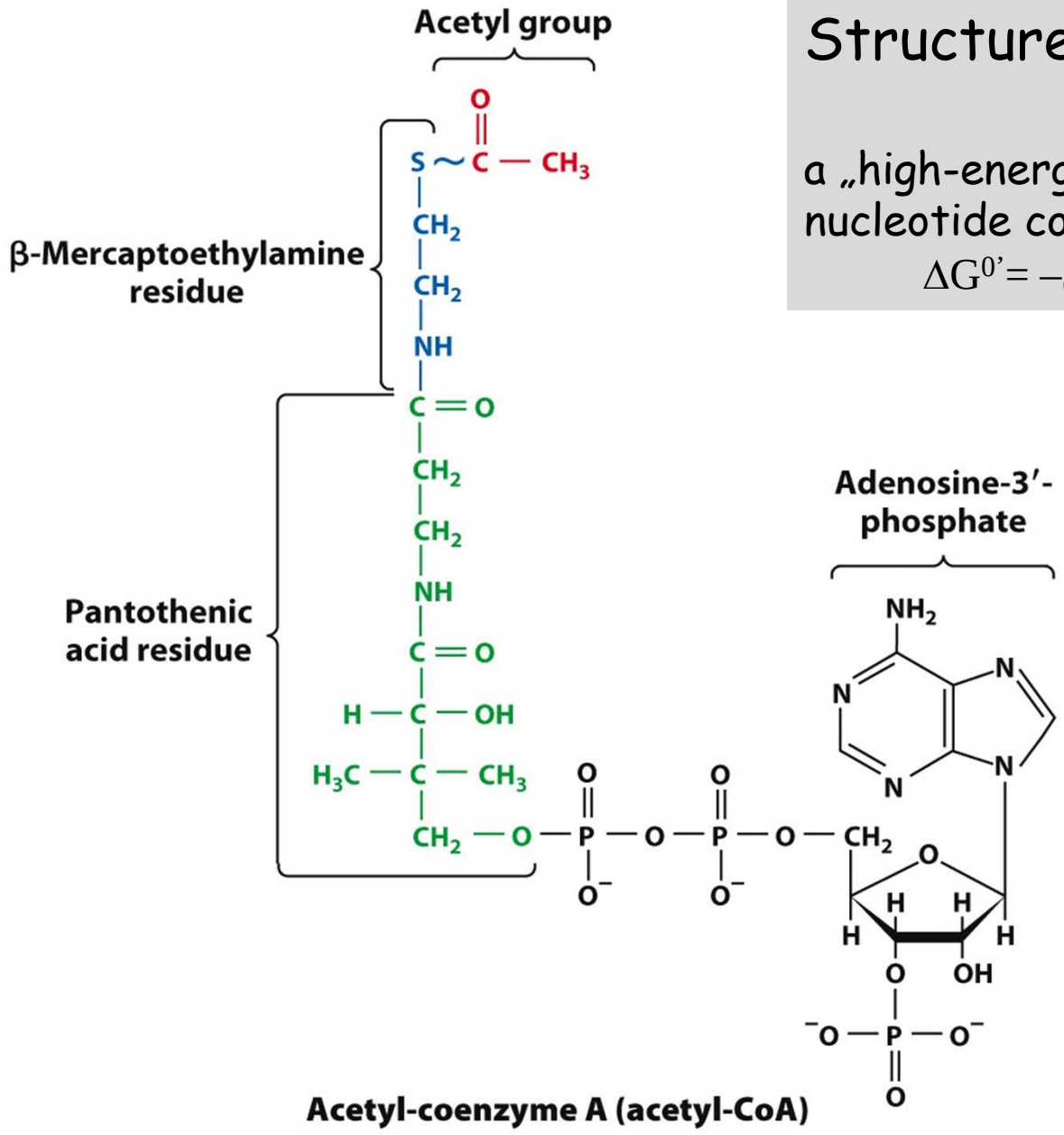
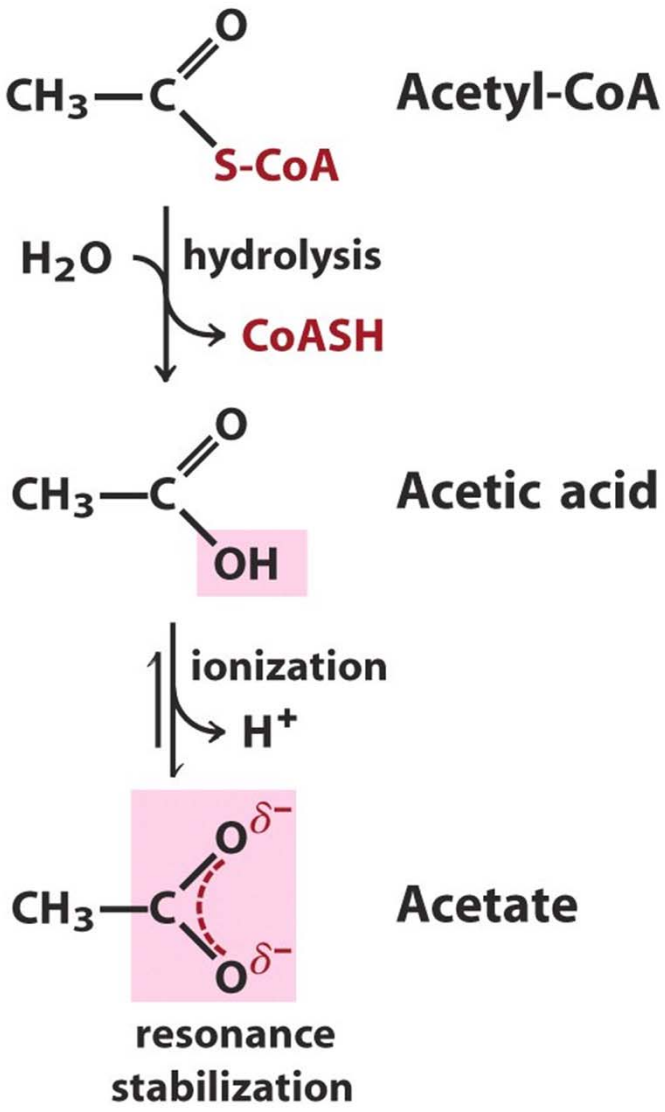


Figure 21-2



$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^\circ = -31.4\ \text{kJ/mol}$$

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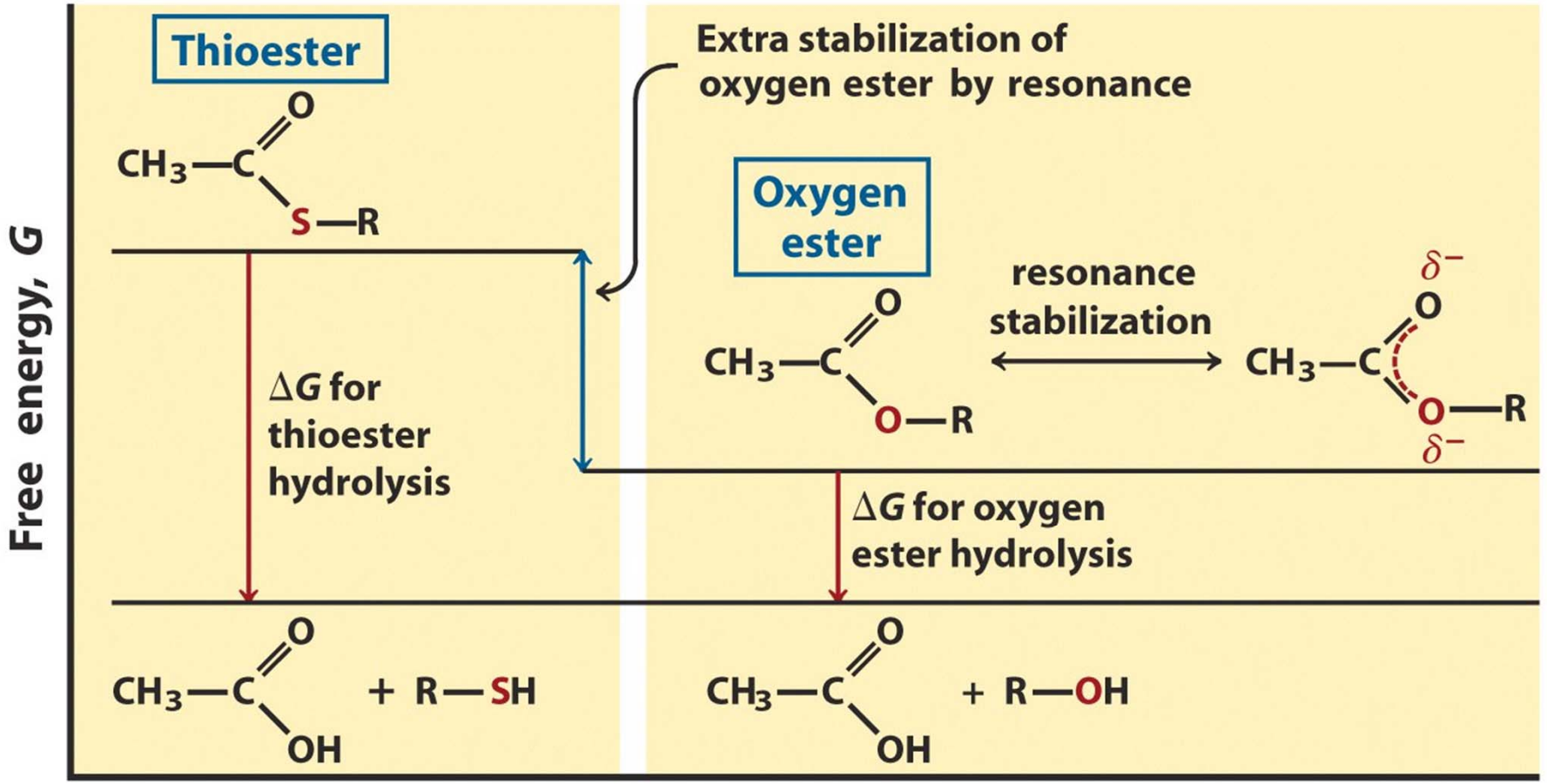

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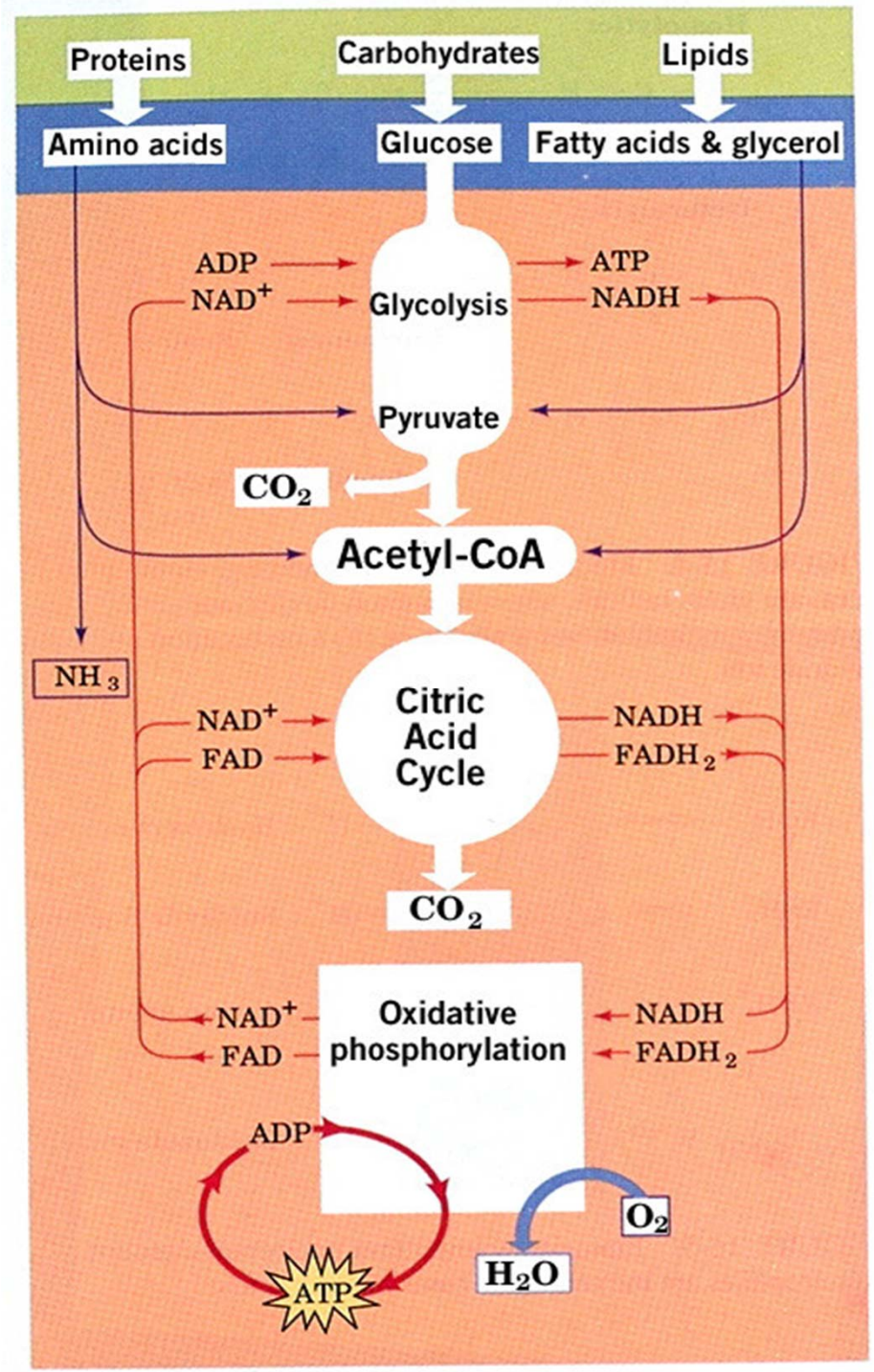

The central role of Acetyl-CoA in metabolism:

Citric acid cycle

Metabolic intermediate:

Fatty acid metabolism

Carbohydrate metabolism

Amino acid metabolism

Precursor of cholesterol and steroid hormones

Acetyl-group donor:

Choline: Acetylcholine (neurotransmitter)

Lys of histones

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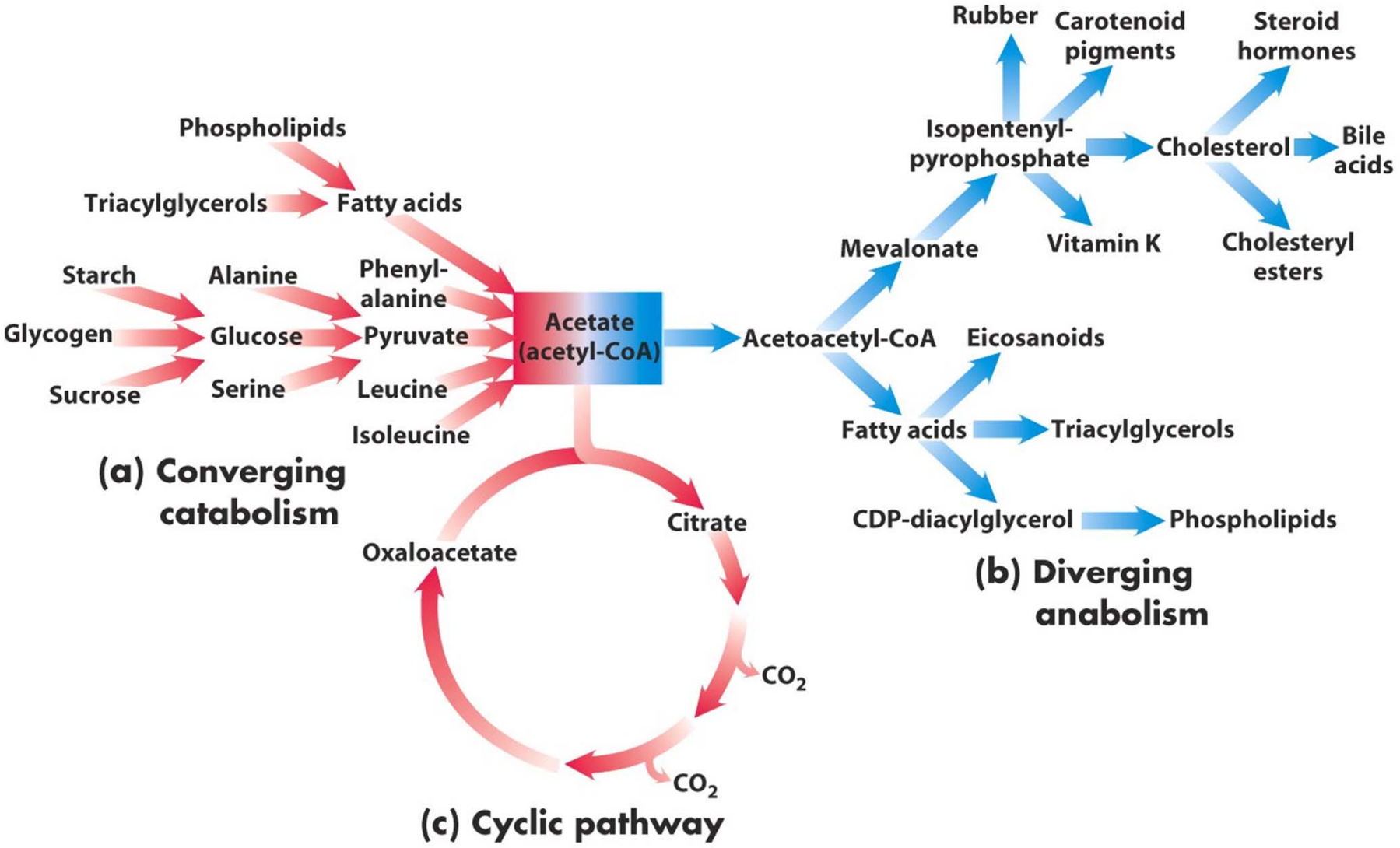
Three types of nonlinear metabolic pathways



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NAD^+ and $NADP^+$ accept e^- only pairwise („parking“ an hydride ion)

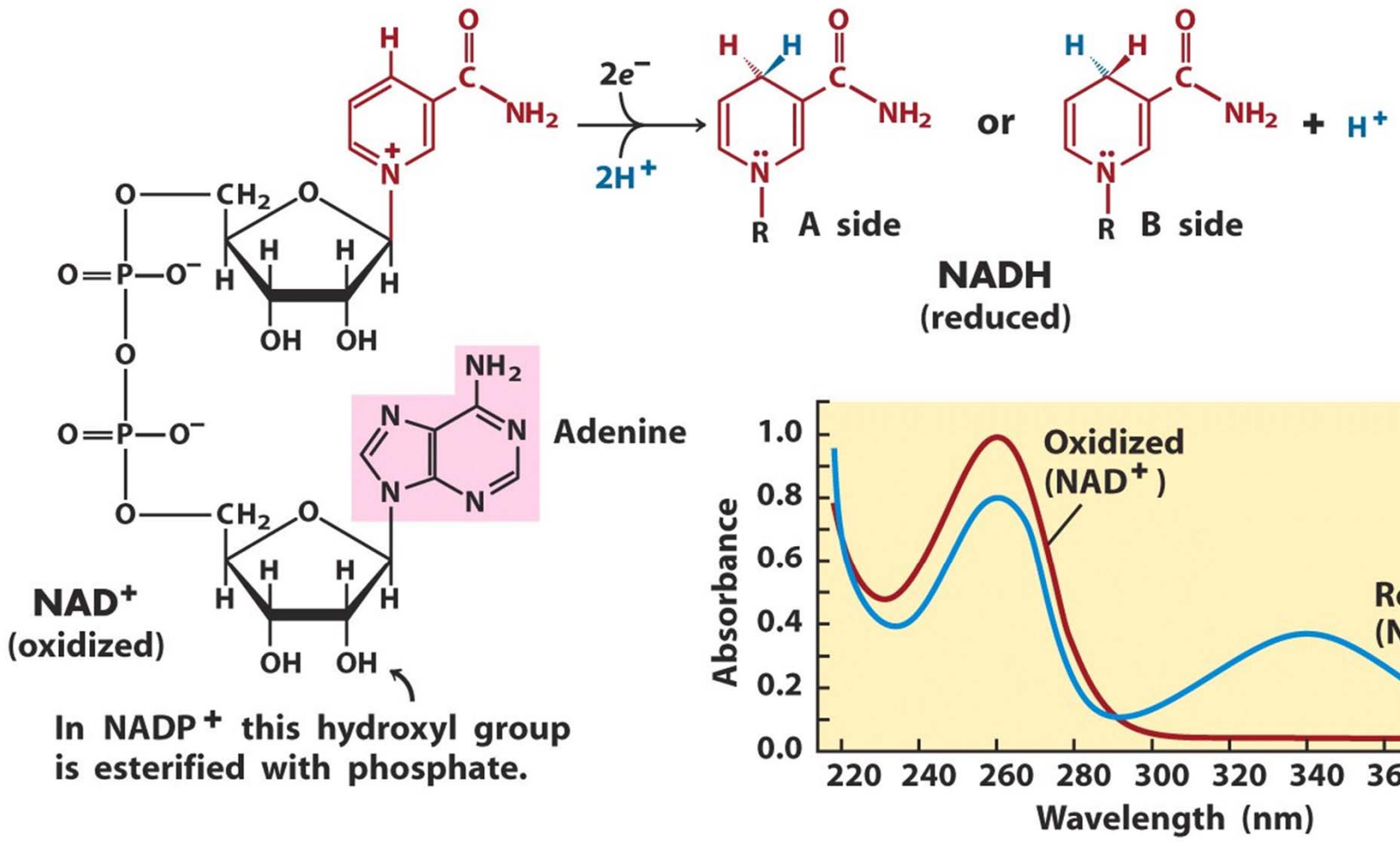

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TABLE 13–8 Stereospecificity of Dehydrogenases That Employ NAD^+ or $NADP^+$ as Coenzymes

Enzyme	*Coenzyme*	*Stereochemical specificity for nicotinamide ring (A or B)*	*Text page(s)*
Isocitrate dehydrogenase	NAD^+	A	610
α-Ketoglutarate dehydrogenase	NAD^+	B	610
Glucose 6-phosphate dehydrogenase	$NADP^+$	B	540
Malate dehydrogenase	NAD^+	A	612
Glutamate dehydrogenase	NAD^+ or $NADP^+$	B	665
Glyceraldehyde 3-phosphate dehydrogenase	NAD^+	B	530
Lactate dehydrogenase	NAD^+	A	538
Alcohol dehydrogenase	NAD^+	A	540

NAD^+ and $NADP^+$, NADH and NADPH are cofactors in more than **200 reactions** (type **electron transfer**):

$$\mathbf{AH_2 + NAD(P)^+ \rightleftharpoons A + NAD(P)H + H^+}$$

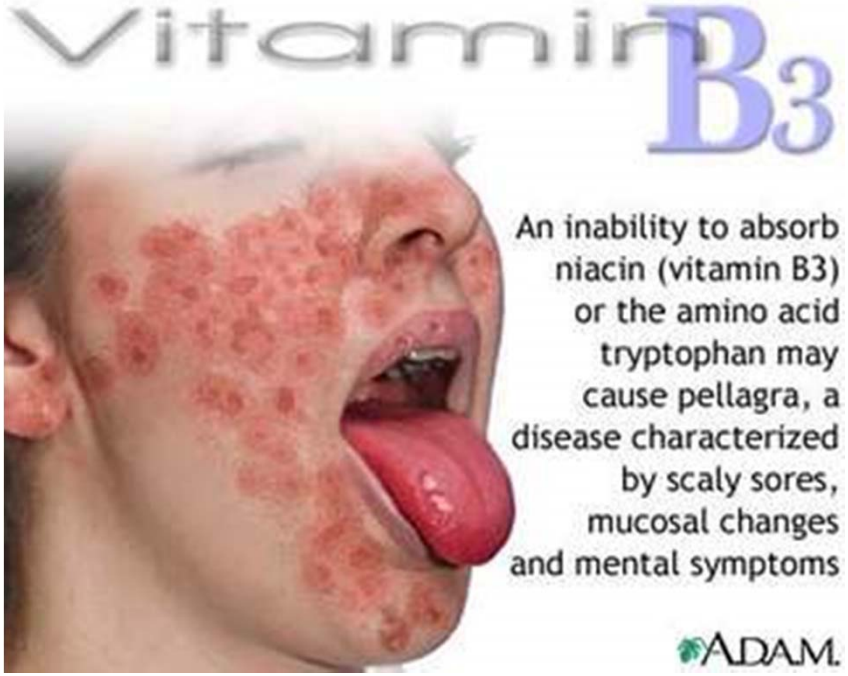
Enzymes: **Oxidoreductases/Dehydrogenases**

Alcohol-dedydrogenase

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3CHO + NADH + H^+$$

$[NAD^+] >> [NADH]$	Total concentration ≅ $[10^{-5}$ M]
Enables catabolic oxidations	
$[NADP^+] << [NADPH]$	Total concentration ≅ $[10^{-6}$ M]
Enables anabolic reductions	

Niacin deficiency causes Pellagra (3 Ds: **D**ermatitis
Diarrhoea
Dementia).



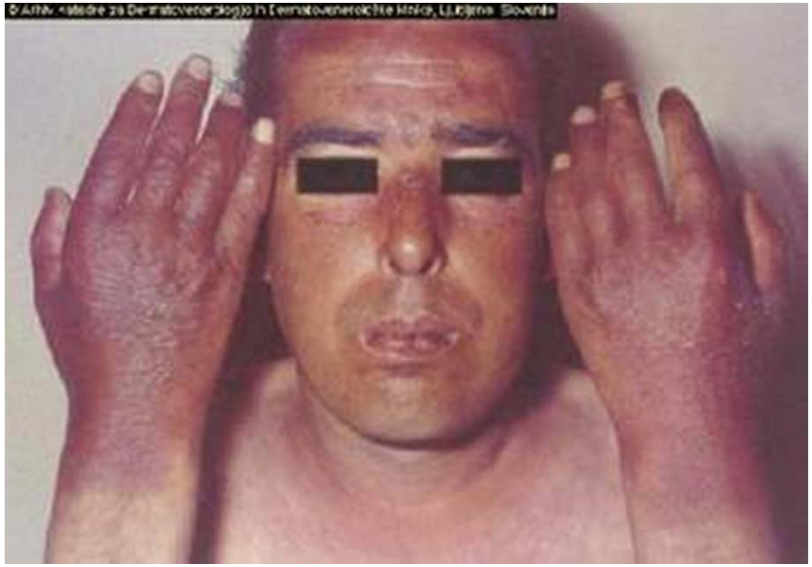
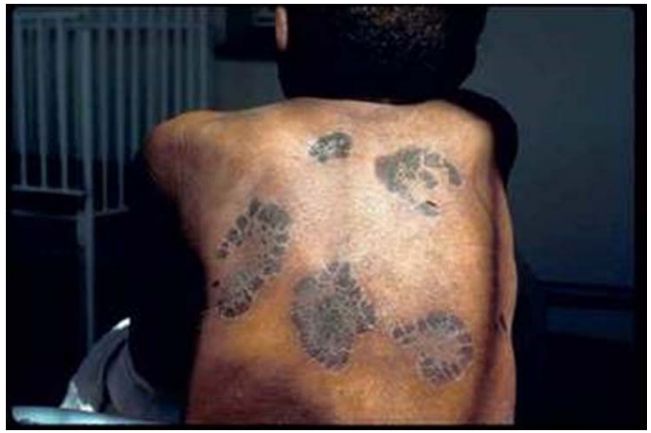


Fig. 6-8 Clinical findings of niacin deficiency before (A) and after (B) therapy in an alcoholic patient.



FMN and FAD are able to sequentially transfer one e^-

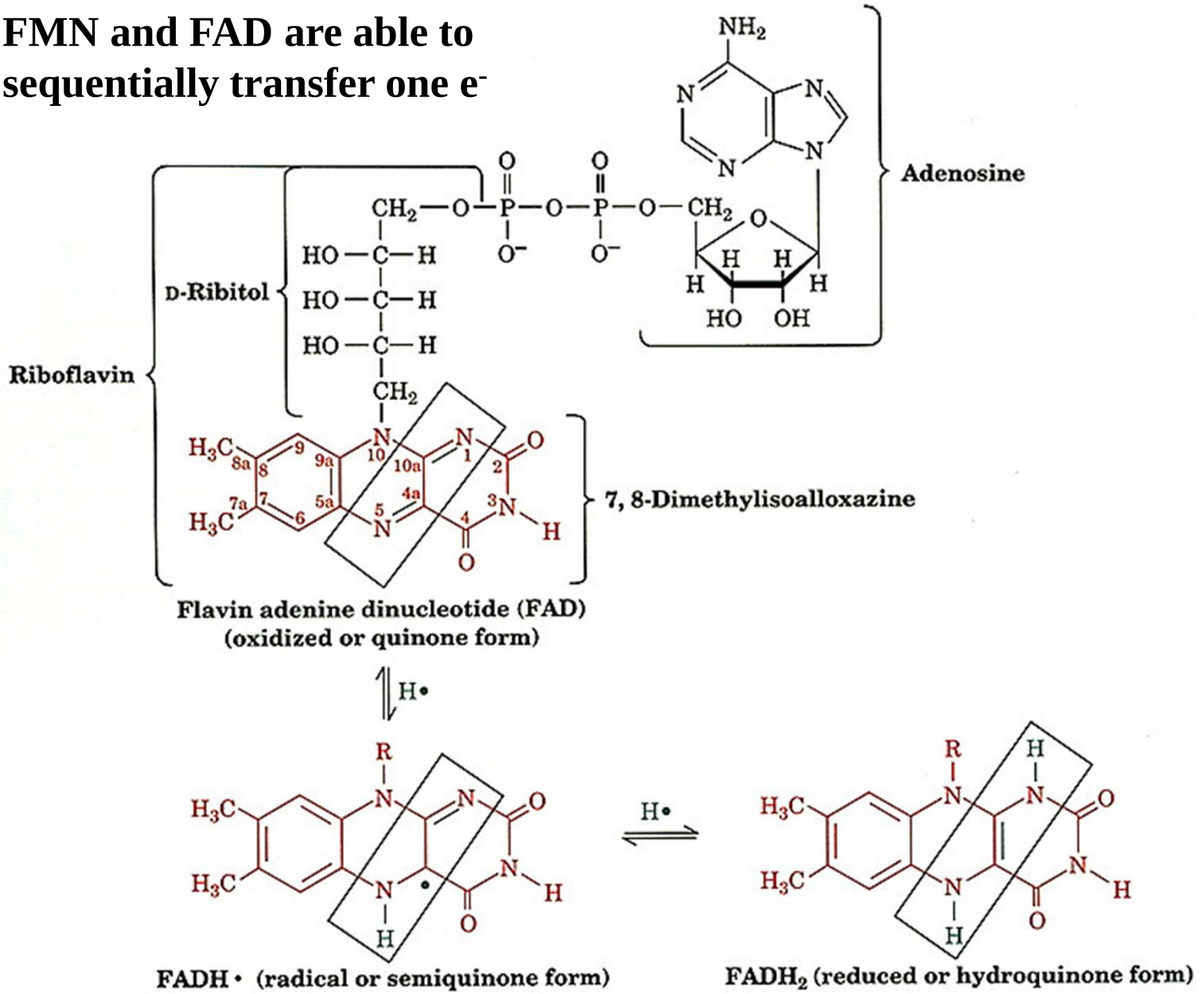


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TABLE 13–9 Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	*Flavin nucleotide*	*Text page(s)*
Acyl–CoA dehydrogenase	FAD	638
Dihydrolipoyl dehydrogenase	FAD	605
Succinate dehydrogenase	FAD	612
Glycerol 3-phosphate dehydrogenase	FAD	714–715
Thioredoxin reductase	FAD	869
NADH dehydrogenase (Complex I)	FMN	696–697
Glycolate oxidase	FMN	767

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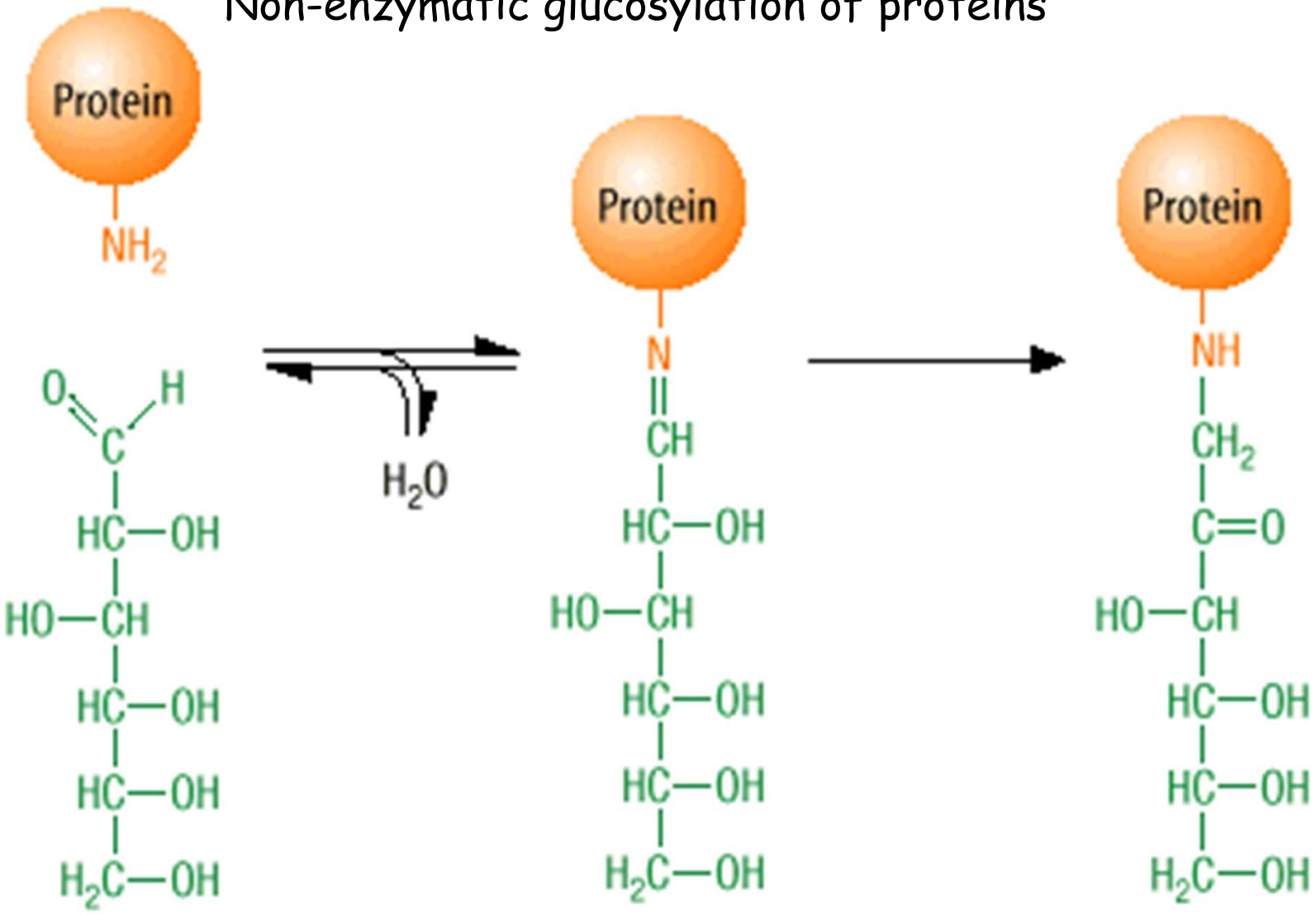
Non-enzymatic glucosylation of proteins



Reversible generation of a Schiff base

Irreversible Amadori-rearrangement