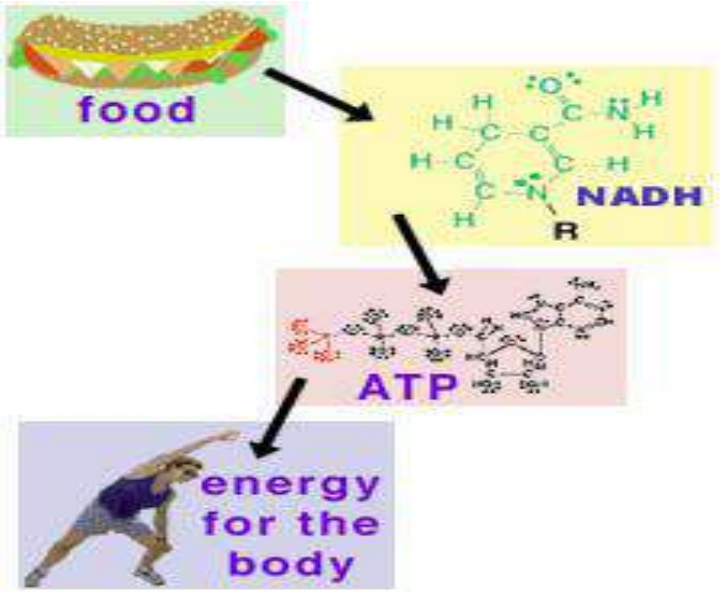
Biochemistry (2)

الكيمياء الحيويّة (2)

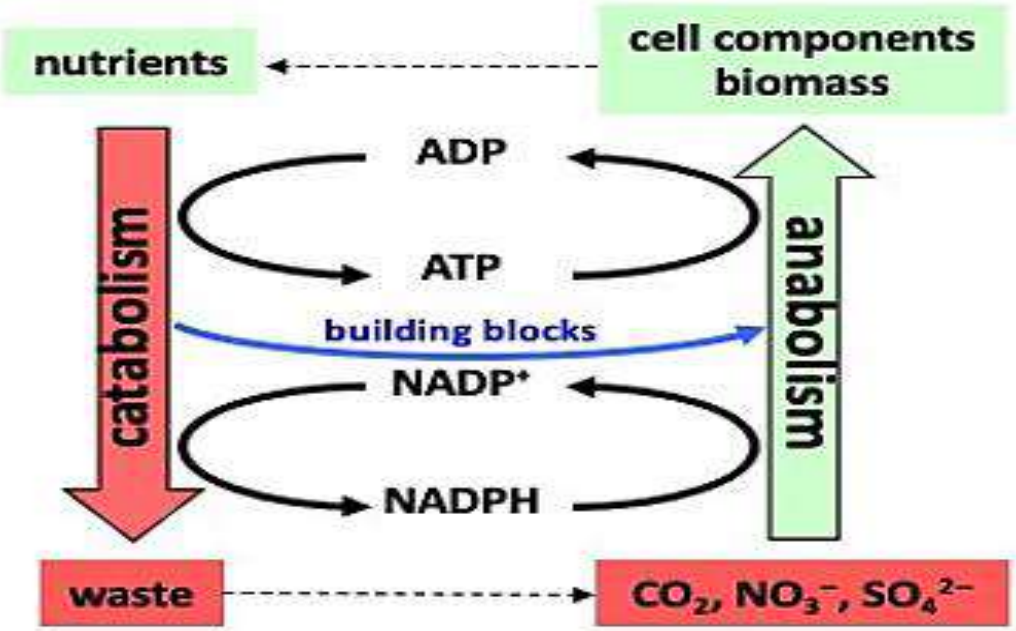
د. غسان أبـوشامة

Introduction to metabolic biochemistry

What is metabolism?



- ***Metabolism*** refers to:
  - the chemical machinery that allows cells to **transform** specific sources of **chemical elements** **into** **chemical energy** (**ATP**, **NADH** Or **NADPH**) and **building blocks** (small organic molecules, activated coenzymes) for **biomass production**.

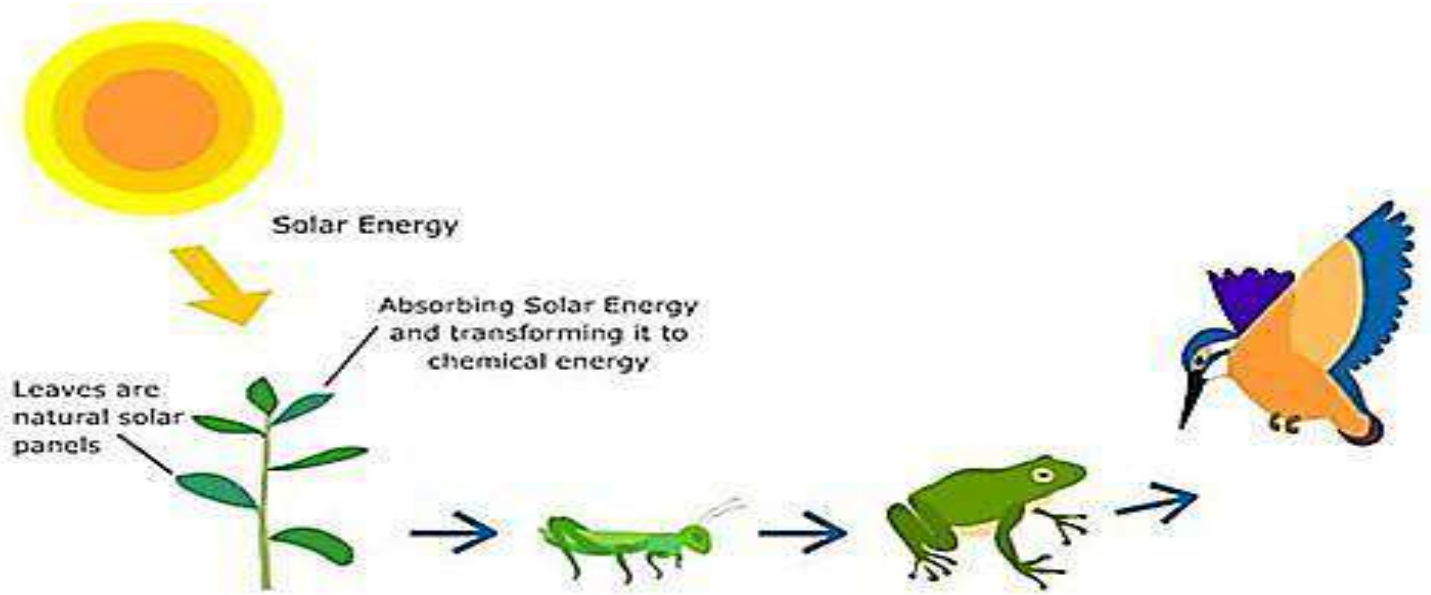


Peretó *et al.* (2005), fig. 12.1.

Introduction to metabolic biochemistry

Why should I study metabolism?

- ***Metabolism*** **concerned with managing cellular material and energy**
- **Biologic systems** are **isothermal** and **use chemical energy to power living processes**.
- **The way in which an animal obtains suitable fuel from its food to provide this energy is basic to the understanding of normal nutrition and metabolism.**

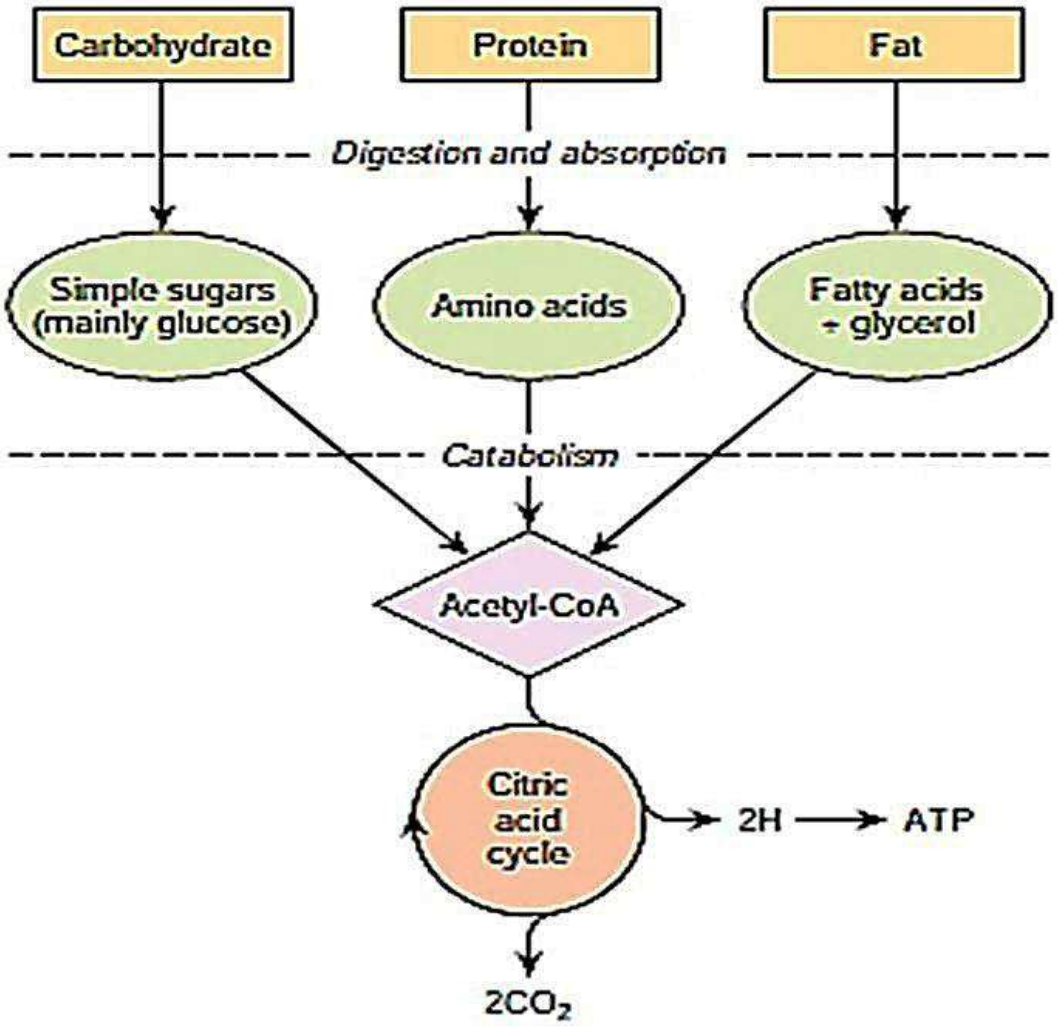


Plant is eaten by grasshoper is eaten by frog is eaten by bird.
Stored chemical energy is transferred from the plant to the grasshopper, to the frog, to the bird, enabling each in turn to function as a living organism.

Introduction to metabolic biochemistry

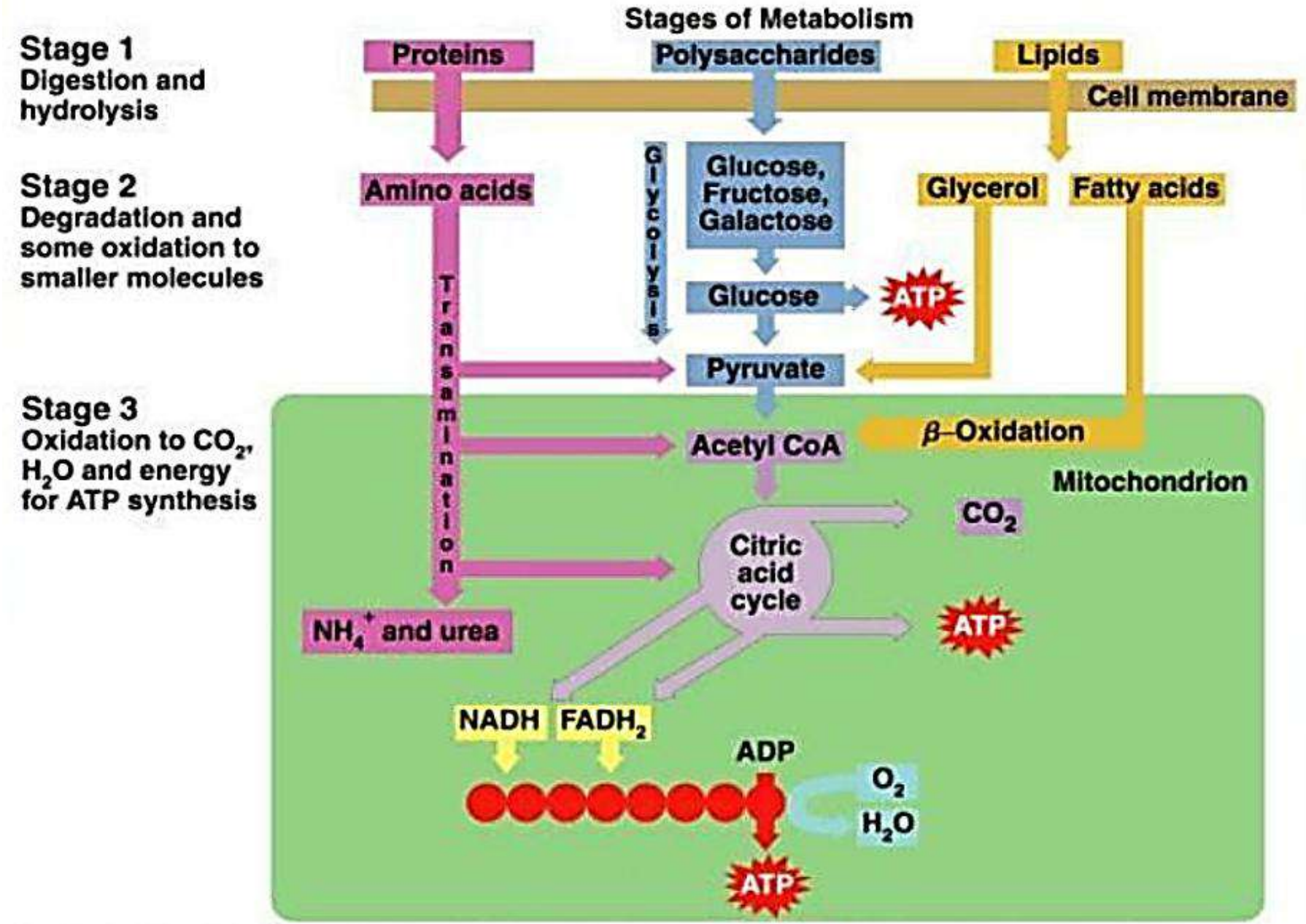
Importance of Metabolism

- **Knowledge of normal metabolism is essential for an understanding of abnormalities that underlie disease.**
- **Normal metabolism includes adaptation to periods of fasting, starvation, and exercise, as well as pregnancy and lactation.**
- **Abnormal metabolism may result from nutritional deficiency, enzyme deficiency, abnormal secretion of hormones, or the actions of drugs and toxins.**



- **The mix of carbohydrate, lipid, and protein being oxidized varies**, depending on whether the subject is in the fed or fasting state, and on the duration and intensity of physical work.[4]

Introduction to metabolic biochemistry

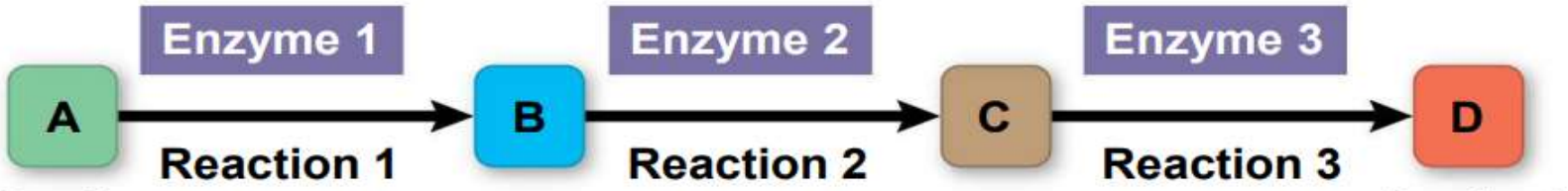


Concept of cellular metabolism

Biomedical importance

- **Metabolism** is the term used to describe:
  - the **interconversion of chemical compounds** in the body, the **pathways taken by individual molecules**, **their interrelationships**, and **the mechanisms that regulate the flow of metabolites** through the **pathways**.

- **Metabolic Pathways** are **chemical reactions organized in multi-steps sequences** where: The **product** of **one reaction of the pathway serves as substrate** of the **subsequent reaction**.

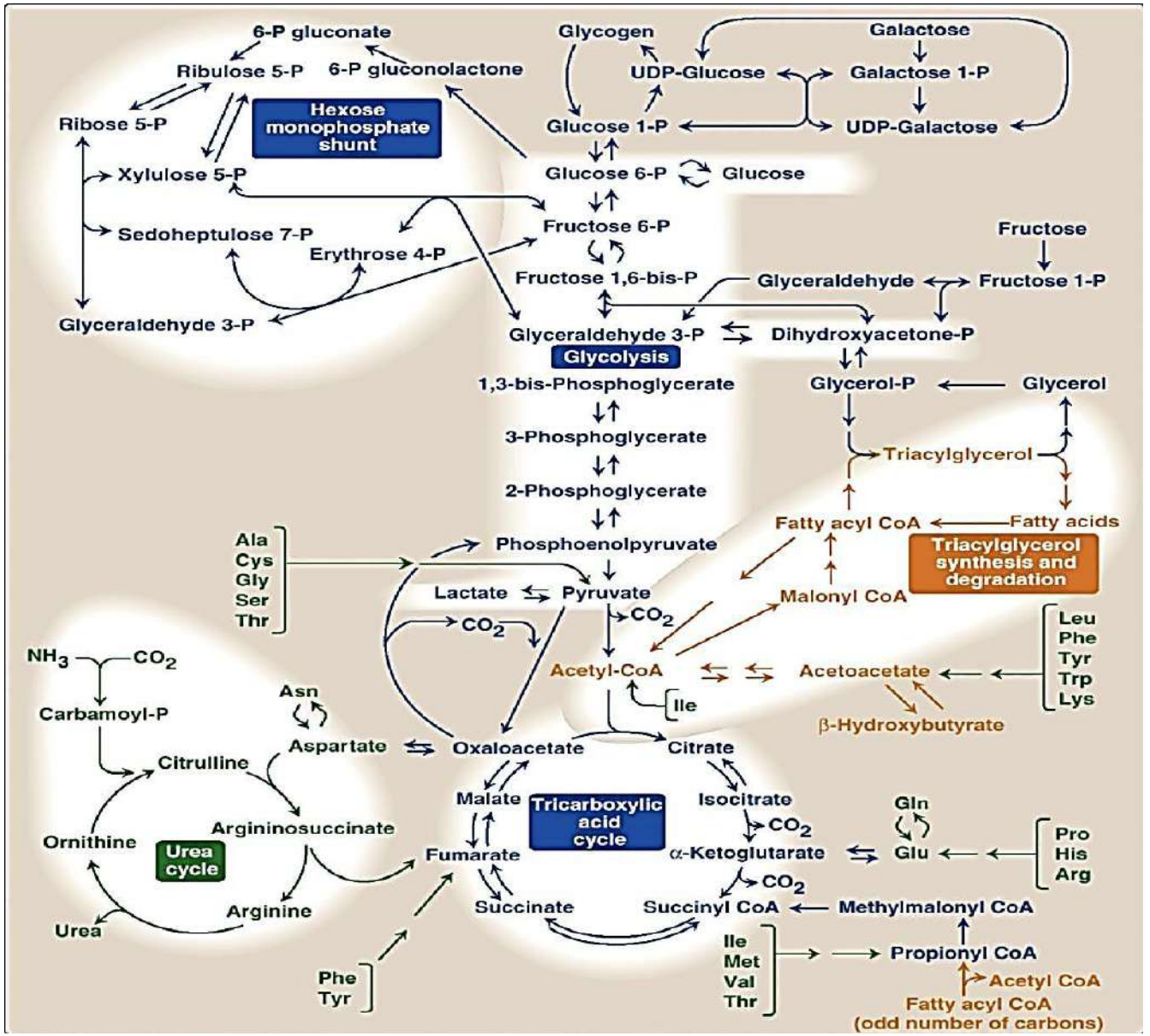


substrate **product**

Concept of cellular metabolism

Biomedical importance

- **Metabolic Map:**
- **integrated and purposeful network of chemical reactions**
- **Different pathways can intersect**, forming an Metabolic Map
- **metabolic map**
- shows how all pathways come together, it **helps us understand the effect of each path on the entire metabolism**



Concept of cellular metabolism

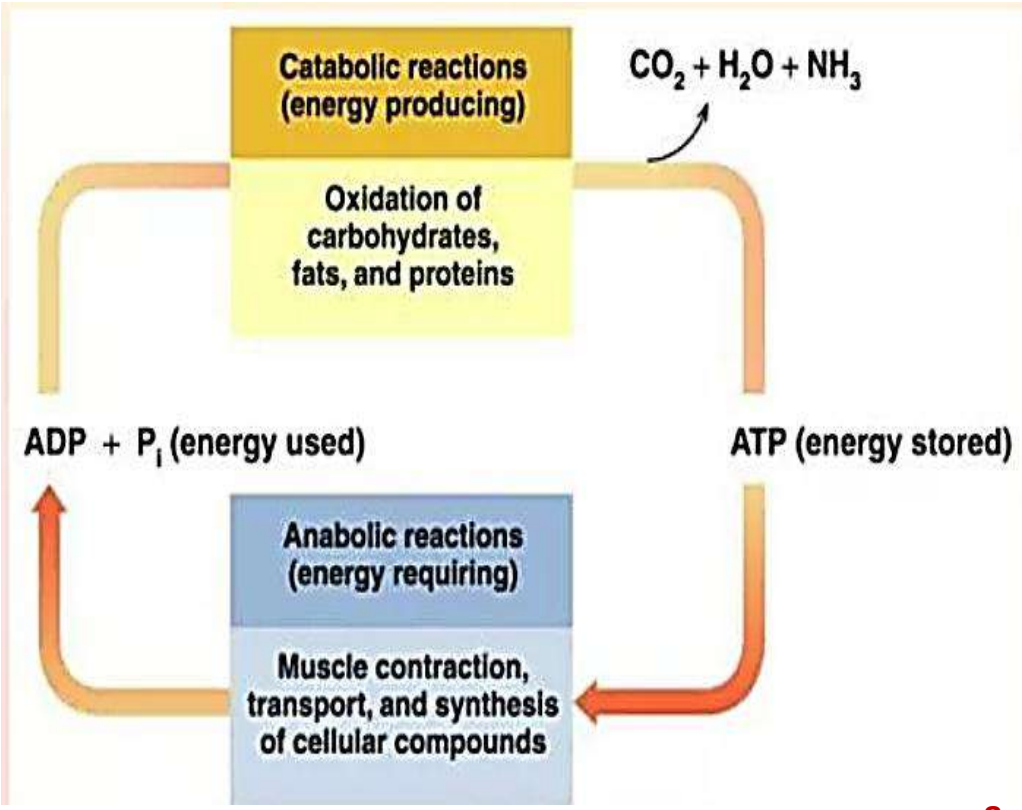
Biomedical importance

❑ **Metabolic pathways** fall into **three categories**:

1) **Anabolic pathways**, which are those involved in the **synthesis of larger and more complex compounds from smaller precursors**—for **example,** the **synthesis of protein from amino acids** and the **synthesis of reserves of triacylglycerol** and **glycogen. Anabolic pathways are endothermic**.

2) **Catabolic pathways**, which are involved in the **breakdown of larger molecules**, commonly involving **oxidative reactions**; **Catabolic pathways are exothermic**, **producing reducing equivalents,** and, **mainly via the respiratory chain**, **ATP**.

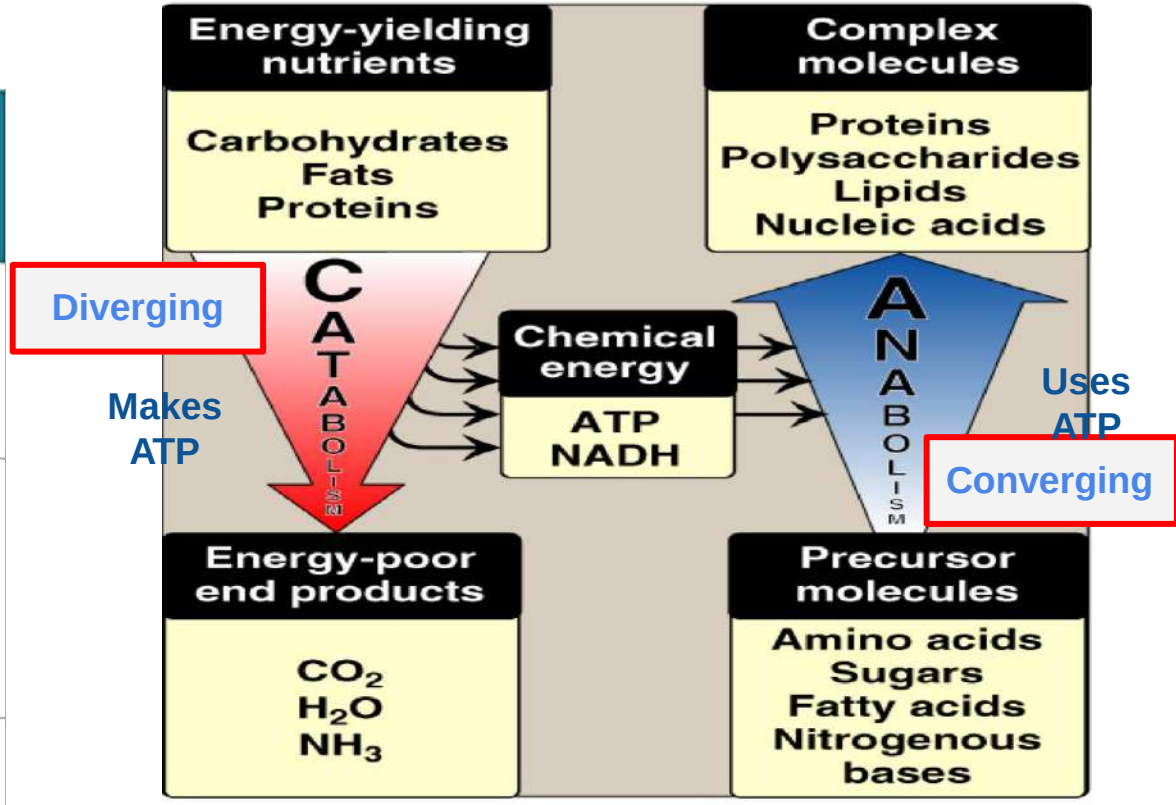
3) **Amphibolic pathways**, which occur at the "crossroads" of metabolism, acting as **links between the anabolic and catabolic pathways**, for **example**, the **citric acid cycle**.

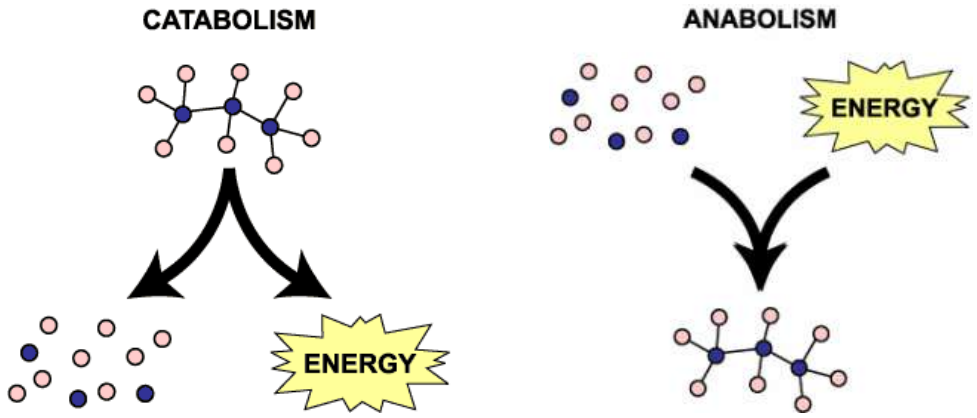


Concept of cellular metabolism

Catabolism Vs Anabolism

Catabolic	Anabolic
Complex to simple molecules	Simple to complex molecules
Exergonic (energy producing) (provides energy in form of ATP)	Endergonic (energy consuming)
Involves oxidations	Involves reductions
Requires NAD^+	Requires NADPH
Convergent process	Divergent process



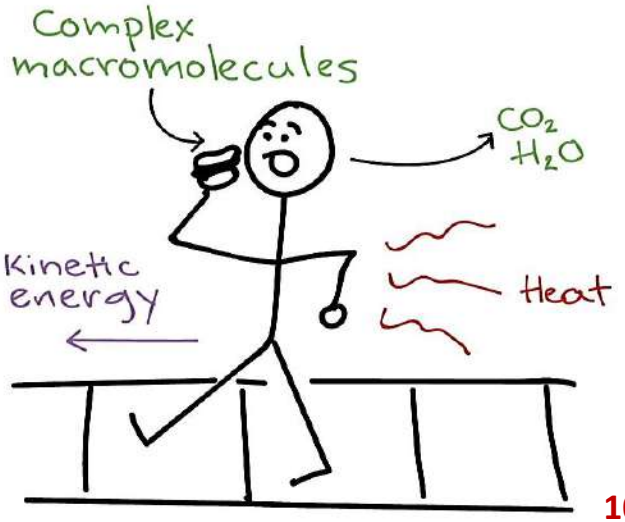


Concept of cellular metabolism

various forms of Chemical Energy

- ***Energy*** is the **capacity to cause change**
- Energy exists in **various forms**, some of which can perform work:
  - ***Kinetic energy*** is energy associated with **motion**
  - ***Thermal energy*** is kinetic energy associated with random movement of atoms or molecules
  - ***Potential energy*** is energy that matter possesses because of its **location** or **structure**
  - ***Chemical energy*** is **potential energy** available for release in a chemical reaction

- ***Energy*** is stored in **chemical bonds** and can be **released and transformed** by **metabolic pathways**.
- **Chemical energy available to do work** is termed **free energy (G).**
- **Biologic Systems Conform to the General Laws of Thermodynamics**



Concept of cellular metabolism

General Laws of Thermodynamics

1) **The first law of thermodynamics** states that **the total energy of a system, including its surroundings, remains constant**.
   - ❑ It implies that **within the total system**, **energy is neither lost nor gained during any change**. However, **energy may be transferred** from one part of the system to another, or **may be transformed** into another form of energy.
   - ❑ **In living systems**, **chemical energy** may be transformed into **heat** or into **electrical**, **radiant**, or **mechanical energy**.

2) **The second law of thermodynamics** states that **the total entropy of a system must increase if a process is to occur spontaneously**.
   - ❑ **Entropy** is the **extent of disorder or randomness of the system** and **becomes maximum** as **equilibrium is approached**.
   - ❑ Under conditions of **constant temperature and pressure**, the relationship between the **free-energy change** (**ΔG**) of a reacting system and the **change in entropy** (**ΔS**) is expressed by the following equation, which **combines the two laws of thermodynamics**:

Concept of cellular metabolism

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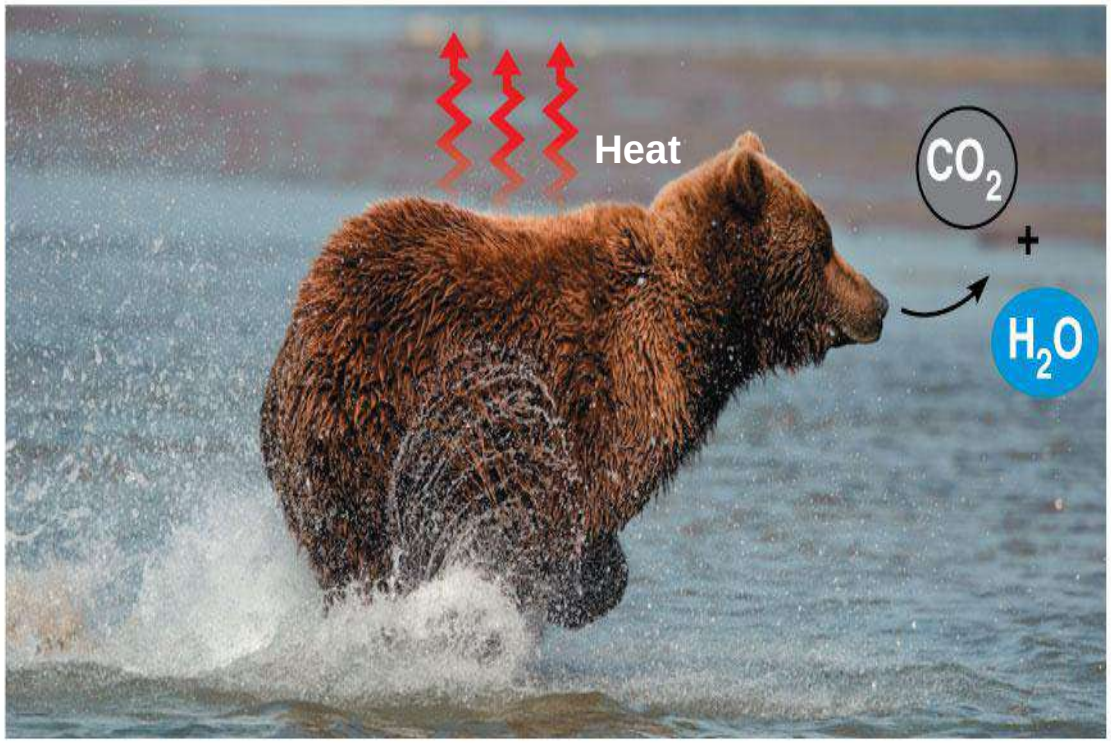
$$\Delta G = \Delta H - T\Delta S$$

where ΔH is the change in enthalpy (heat) and T is the absolute temperature.

Concept of cellular metabolism

General Laws of Thermodynamics

(a) First law of thermodynamics

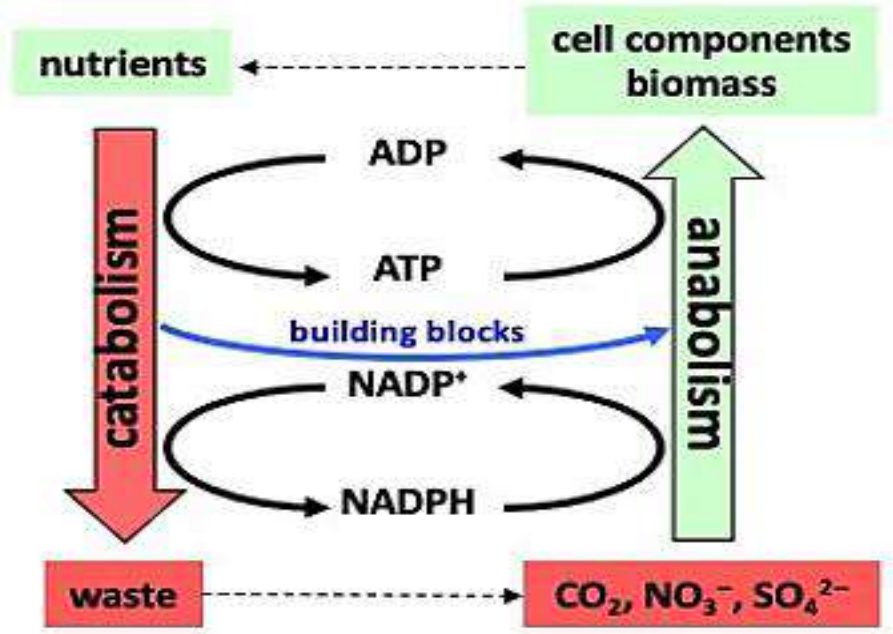


(b) Second law of thermodynamics

Concept of cellular metabolism

Endergonic processes proceed by coupling to exergonic processes

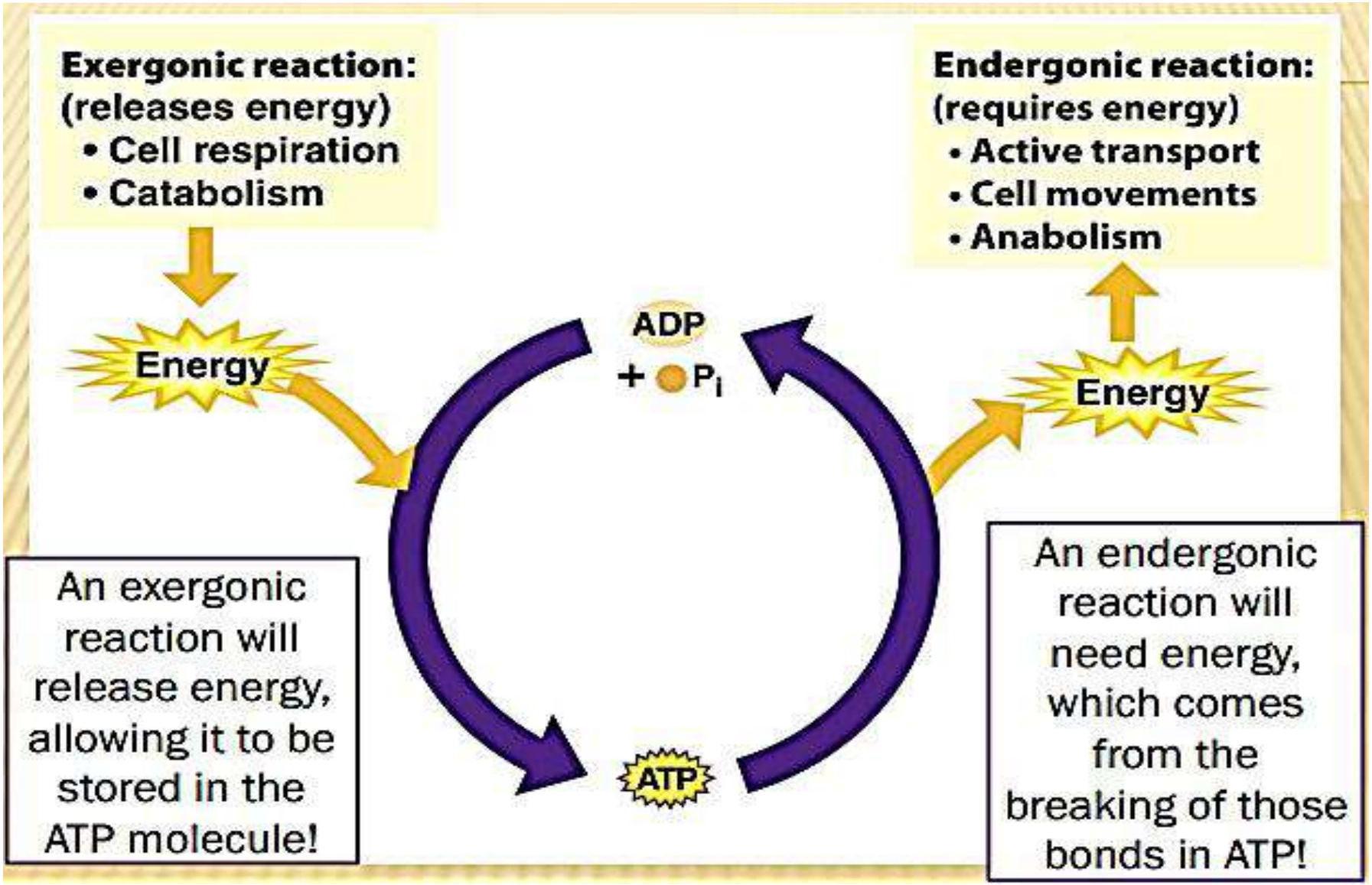
- **cell** does **three main kinds of work**
  - Chemical
  - Transport
  - Mechanical
- **To do work**, cells manage energy resources by **energy coupling**



- In cells, **energy-transforming reactions** are often **coupled**: An **energy-releasing** (**exergonic**) **reaction** is **coupled** to an **energy-requiring** (**endergonic**) **reaction**.
- **Energy can be transferred by tow strategies in cell**:
  1) **Coupling agent AS ATP**
  2) **Electrons** in **redox reactions** in the presence of **electron carriers** as **Coenzyme NAD+** or **FAD.**

Concept of cellular metabolism

Energy Currency: ATP



Concept of cellular metabolism

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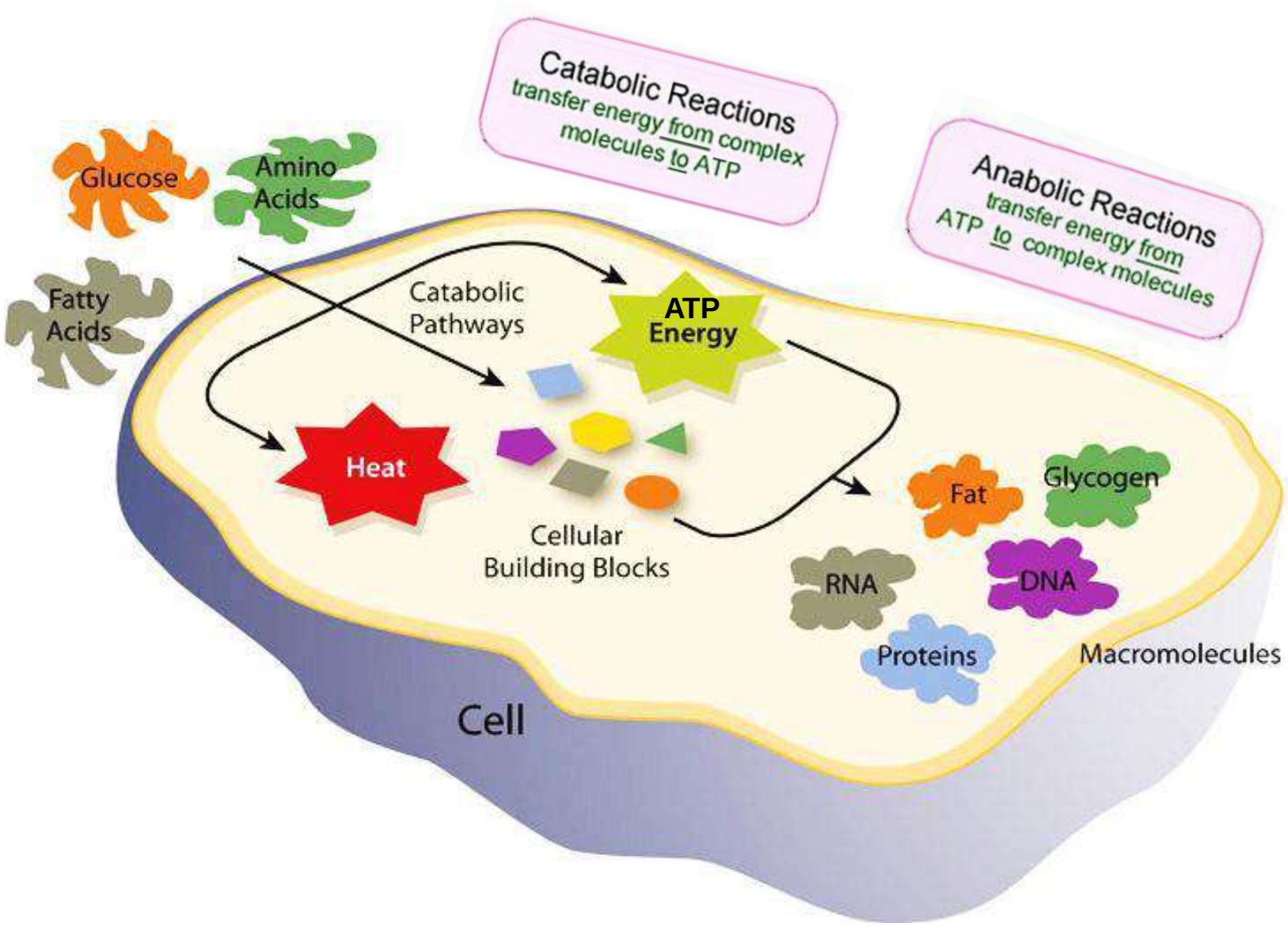
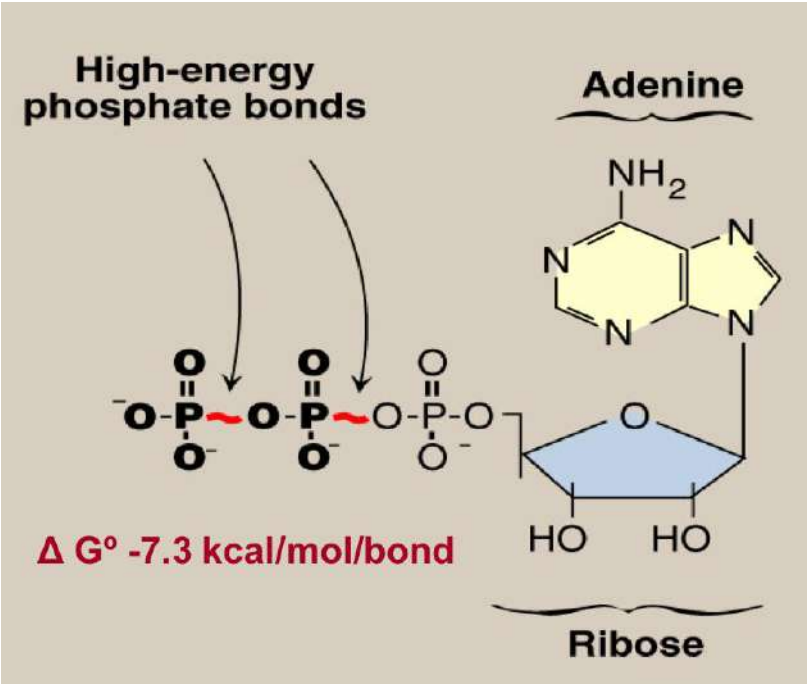


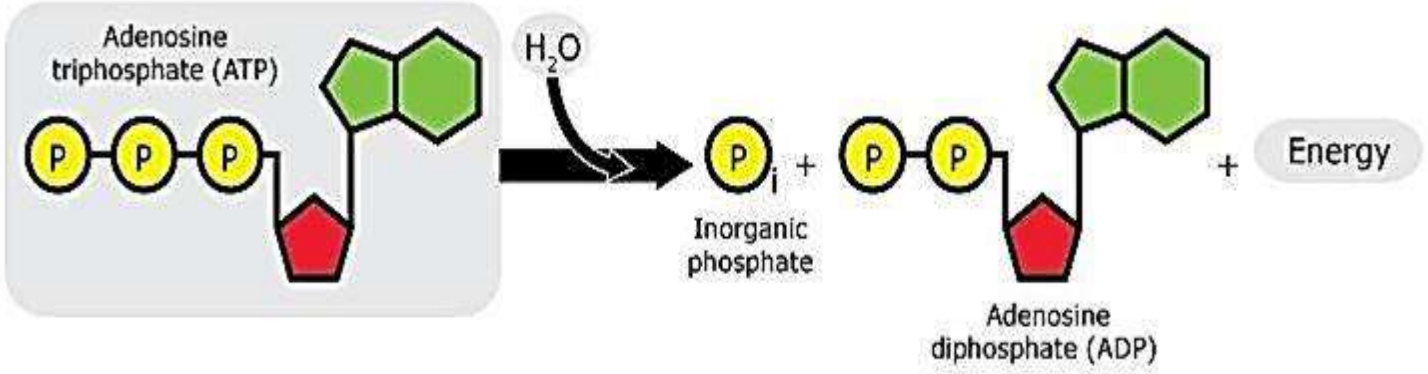
Concept of cellular metabolism

Energy Currency: ATP

- The **free energy liberated** in the **hydrolysis of ATP is used to drive** the **endergonic reactions**
- **ATP** is formed from **ADP and Pi** when **fuel molecules are oxidized**
- This **ATP-ADP cycle** is the **fundamental mode** of **energy exchange** in **biological systems**
- **ATP is the energy currency of the cells**



$$ATP + H_2O \rightarrow P_i + ADP$$



Concept of cellular metabolism

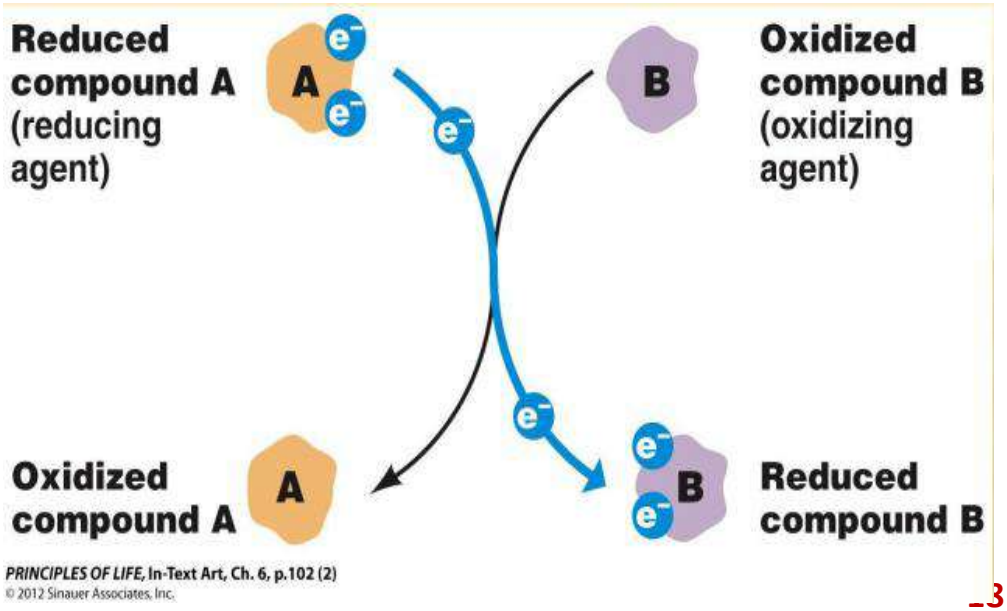
Energy Currency: ATP

- **Energy** can also be transferred by the transfer of electrons in oxidation–reduction, or redox reactions.
  - **Oxidation** is the **loss** of one or more **electrons**.
  - **Reduction** is the **gain** of one or more **electrons**.

Oxidation and reduction always occur together

Transfers of hydrogen atoms involve transfers of electrons ($H = H^+ + e^-$).

- When a **molecule loses a hydrogen** atom, it becomes **oxidized**.
- The **more reduced a molecule** is, the **more energy is stored in its bonds**
- **Coenzyme NAD^+** is a key **electron carrier** in **redox reactions**.
  - **NAD^+** (oxidized form)
  - **NADH** (reduced form)



Concept of cellular metabolism

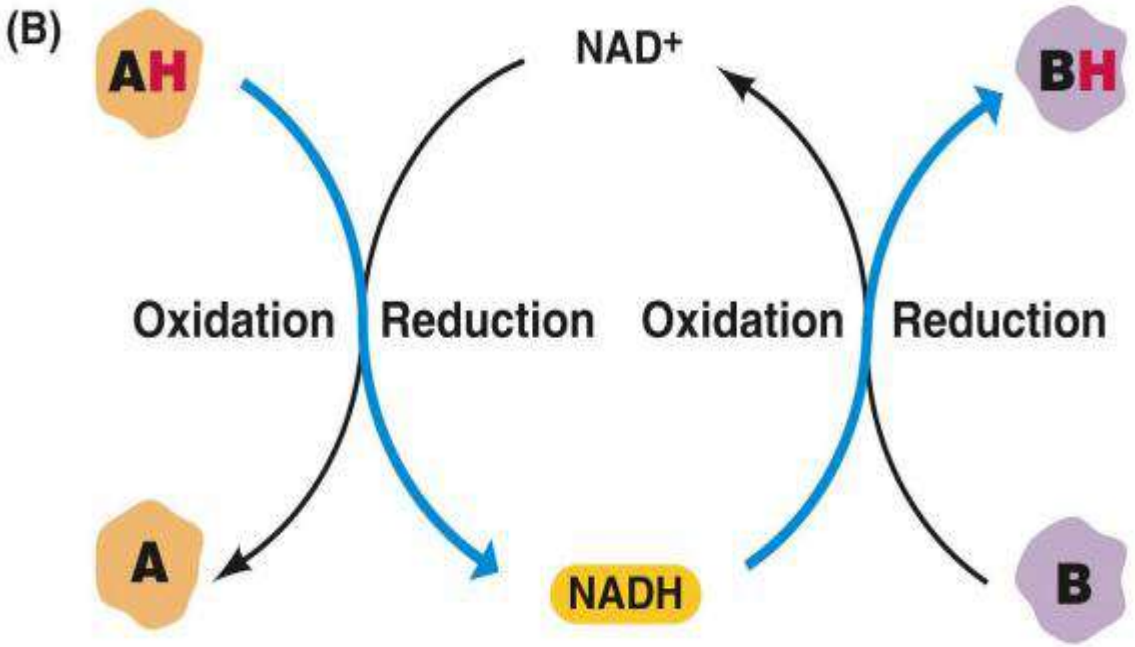
Energy Currency: ATP

Reduction of NAD+ is highly endergonic:

$$NAD^+ + H^+ + 2e^- \rightarrow NADH$$

Oxidation of NADH is highly exergonic:

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O$$

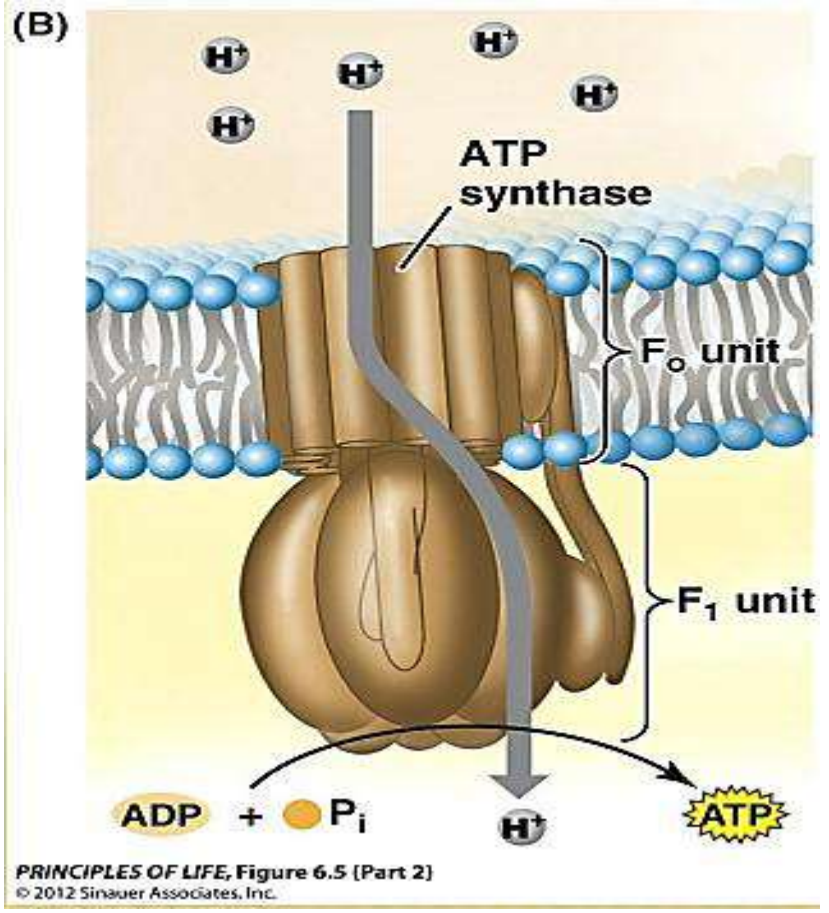


PRINCIPLES OF LIFE, Figure 6.4 (Part 2)

Concept of cellular metabolism

Energy Currency: ATP

- In cells, energy is released in **catabolism** (**breaking bonds**) by **oxidation**…
- **Energy** is then **trapped** by **reduction** of **coenzymes** such as **NADH**…
- BUT, energy for **anabolic** (**building bonds**)processes is supplied by **ATP**, not **NADH**!
- So, **oxidative phosphorylation** transfers energy from **NADH** to **ATP**.



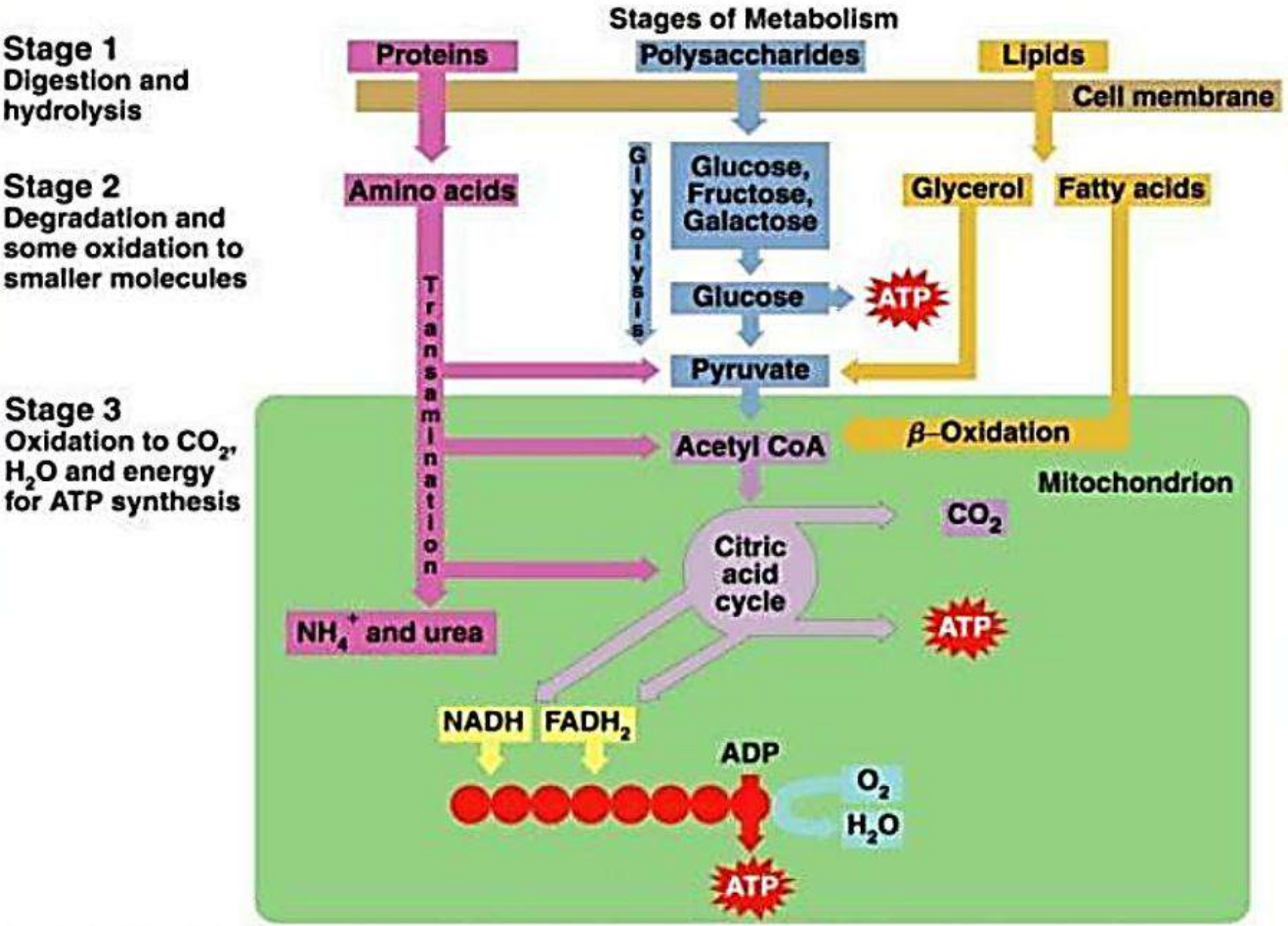
PRINCIPLES OF LIFE, Figure 6.5 (Part 2)

Oxidative phosphorylation couples:

oxidation of NADH: $NADH \rightarrow NAD^+ + H^+ + 2e^- + energy$

with production of ATP: $energy + ADP + P_i \rightarrow ATP$

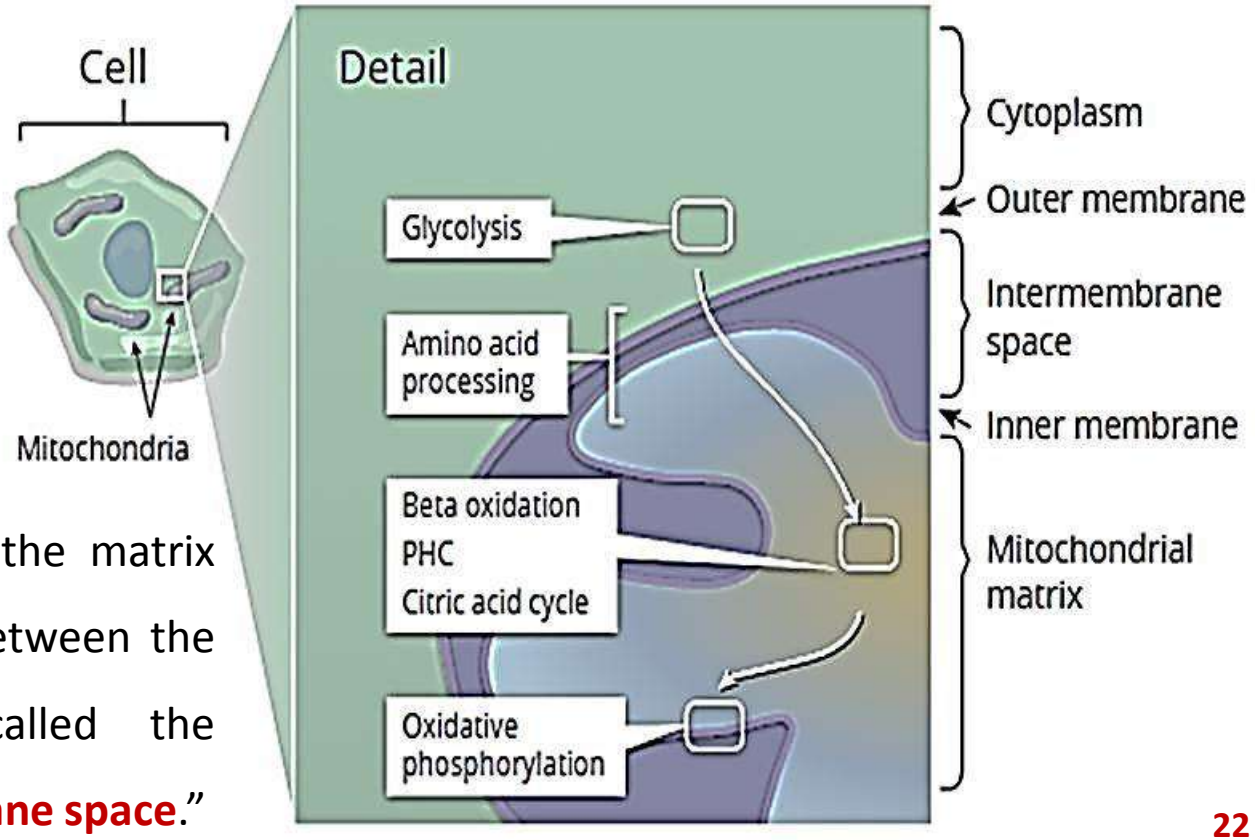
Concept of cellular metabolism



Concept of cellular metabolism

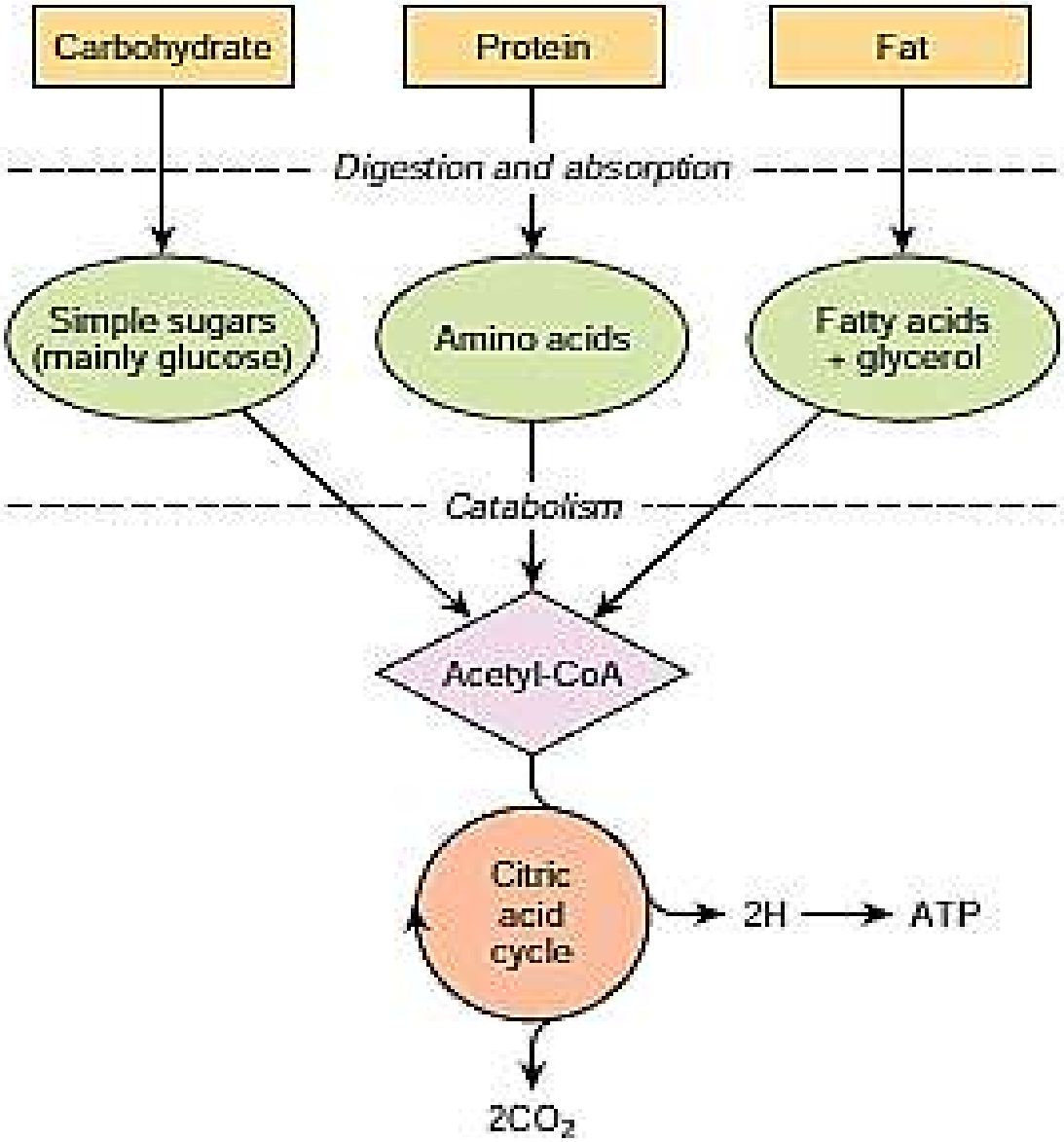
Metabolic reactions happen in specific locations in the cell

- **Glycolysis**, **fatty acid synthesis**, and **glycogen synthesis** **happen in the cytosol**, along with some steps of **amino acid breakdown**
- The **matrix of Mitochondria** , which is inside of both membranes, is home to **beta oxidation**, the **citric acid cycle**, and some steps of **amino acid breakdown**.
- Components of **oxidative phosphorylation** (the **electron transport chain and ATP synthase**)are **embedded within the inner mitochondrial membrane**.
- **Protons** are pumped from the matrix into the fluid-filled space between the two membranes, also called the "**mitochondrial intermembrane space**."



Glycolysis & the oxidation of Pyruvate

Biomedical importance



Glycolysis & the oxidation of Pyruvate

Biomedical importance

- **Most tissues have** at least **some requirement for glucose**. **For brain**, requirement is **substantial**.
- **Glycolysis**, the **major pathway for glucose metabolism**, occurs in the **cytosol** of all cells.
- It is unique, in that **it can function** either **aerobically** or **anaerobically**, **depending** on the **availability of oxygen and the electron transport chain**.
- **Erythrocytes**, which **lack mitochondria**, are completely **reliant on glucose** as their metabolic fuel, and metabolize it by **anaerobic glycolysis**.
- However, **to oxidize glucose beyond pyruvate** (the end product of glycolysis) **requires both oxygen and mitochondrial enzyme systems**: the **pyruvate dehydrogenase complex**, the **citric acid cycle**, and the **respiratory chain**.
- **Glycolysis** is both the principal route for **glucose metabolism** and also the main pathway for the **metabolism of** fructose, galactose, and **other carbohydrates** derived from the diet.
- The **ability of glycolysis to provide ATP in the absence of oxygen** is especially important, because this allows **skeletal muscle to perform** at very high levels **when oxygen supply is insufficient**, and it allows tissues to survive anoxic episodes (**نوبات نقص الأكسجين**).

Glycolysis & the oxidation of Pyruvate

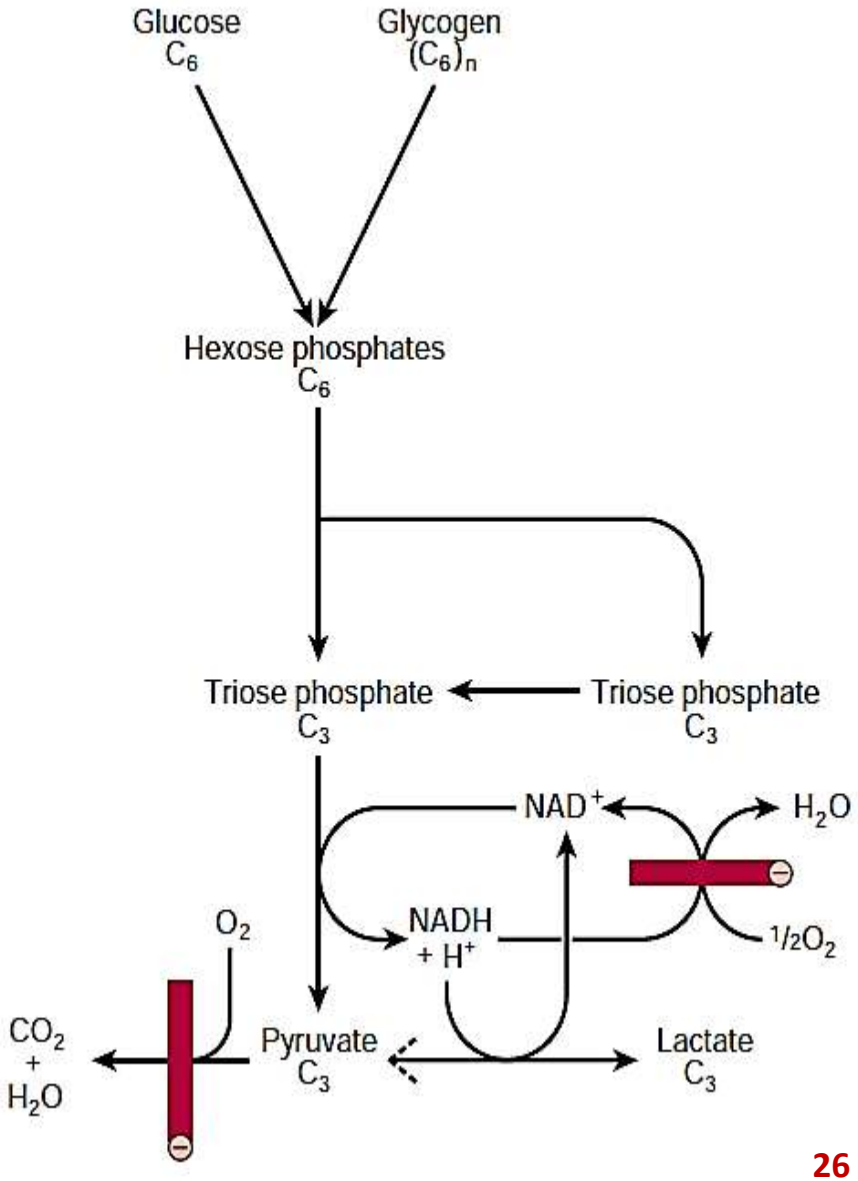
Biomedical importance

- However, **heart muscle**, which is **adapted for aerobic performance**, has **relatively low glycolytic activity and poor survival under conditions of ischemia(نقص التروية)**.
- **Diseases** in which **enzymes** of glycolysis (eg, **pyruvate kinase**) are **deficient** are mainly seen as **hemolytic anemias (فقر دم انحلالي)** or, if the **defect affects skeletal muscle** (eg, **phosphofructokinase**), as **fatigue (وهن/ تعب)**.
- In **fast-growing cancer cells**, **glycolysis proceeds** at a **high rate**, forming **large amounts** of **pyruvate**, which is **reduced to lactate** and **exported**. This **produces** a relatively **acidic local environment** in the **tumor (الورم)**, which may have **implications for cancer therapy**.
- The **lactate** is used for **gluconeogenesis** in the **liver**, an **energy-expensive process**, which is responsible for much of the **hypermetabolism** seen in cancer cachexia (**سرطان الدنف**).
- **Lactic acidosis** results from several causes, including **impaired activity of pyruvate dehydrogenase**.

Glycolysis & the oxidation of Pyruvate

Glycolysis can function under anaerobic conditions

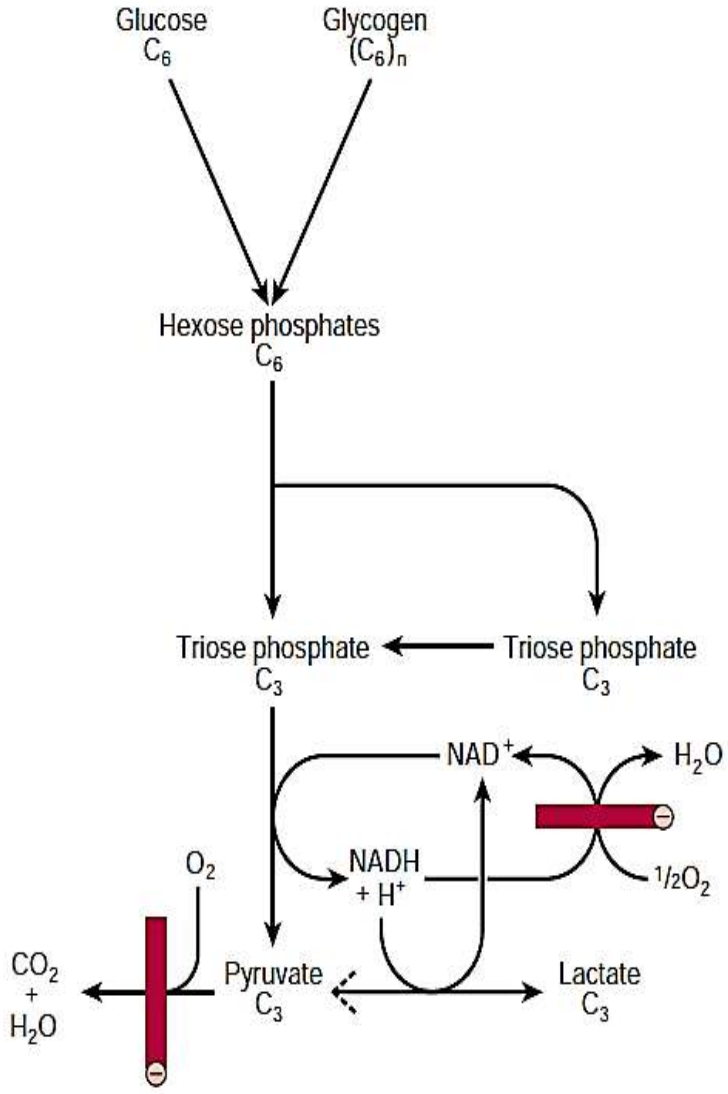
- Early in the investigations of glycolysis it was realized that **fermentation in yeast** was **similar** to the **breakdown of glycogen in muscle**.
- It was noted that when a **muscle contracts** in an **anaerobic medium**, ie, one from which **oxygen is excluded**, **glycogen disappears** **and** **lactate appears**.
- When **oxygen is admitted**, **aerobic recovery takes place** and **lactate is no longer produced**.
- However, if **contraction occurs under aerobic conditions**, **lactate does not accumulate** and **pyruvate is the major end product of glycolysis**.
- **Pyruvate is oxidized further to CO_2 and water**.



Glycolysis & the oxidation of Pyruvate

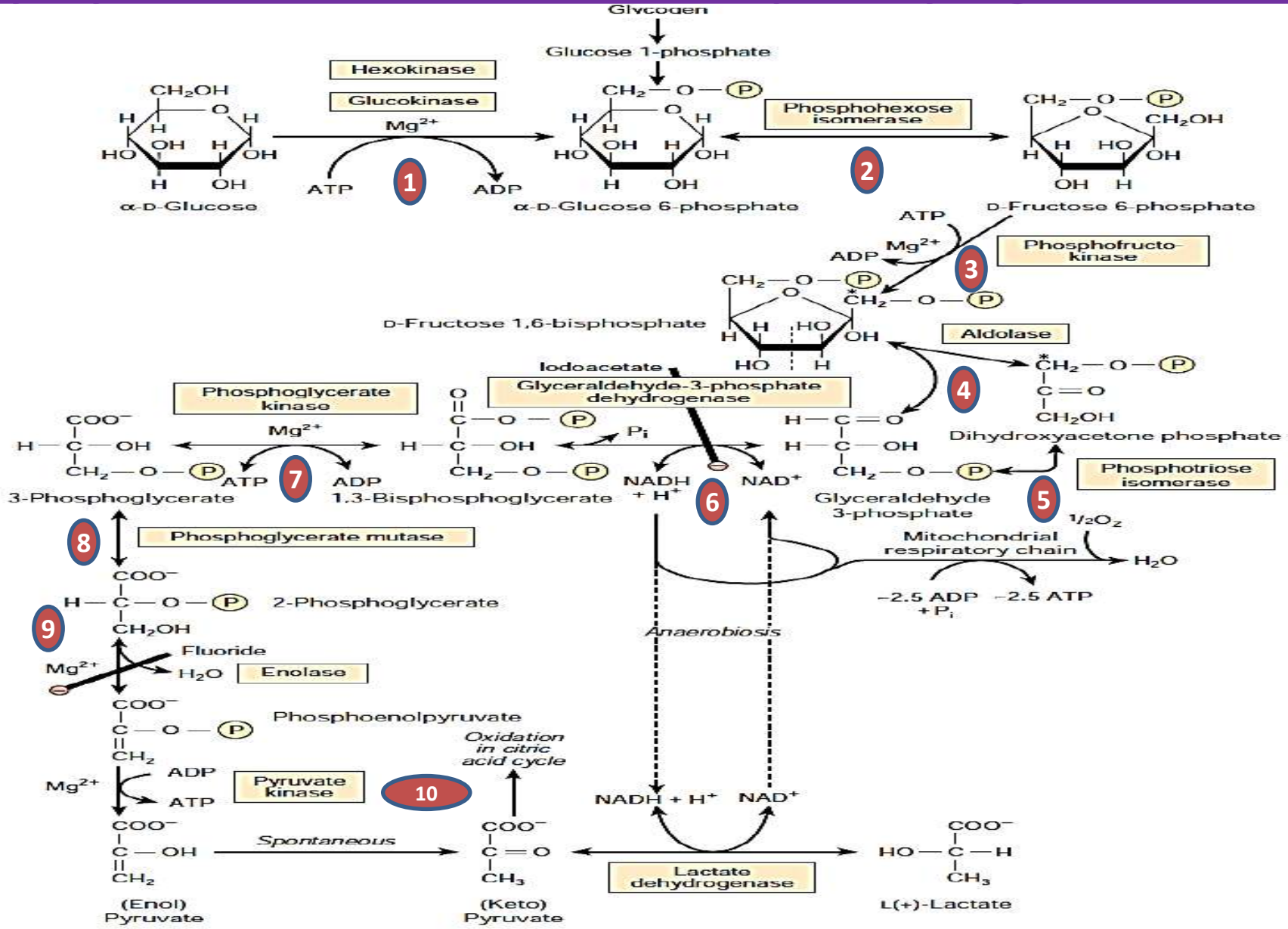
Glycolysis can function under anaerobic conditions

- When **oxygen is in short supply**, **mitochondrial reoxidation of NADH formed during glycolysis is impaired (reaction 6)**, and **NADH is reoxidized by reducing pyruvate to lactate**, so **permitting glycolysis to proceed**.
- While **glycolysis** can occur **under anaerobic conditions**, this **has a price**, for it **limits the amount of ATP formed per mole of glucose oxidized**, so that **much more glucose must be metabolized under anaerobic than aerobic conditions**.
- In **yeast** and **some other microorganisms**, **pyruvate formed in anaerobic glycolysis is not reduced to lactate**, but is **decarboxylated and reduced to ethanol**.



Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization



Glycolysis & the oxidation of Pyruvate

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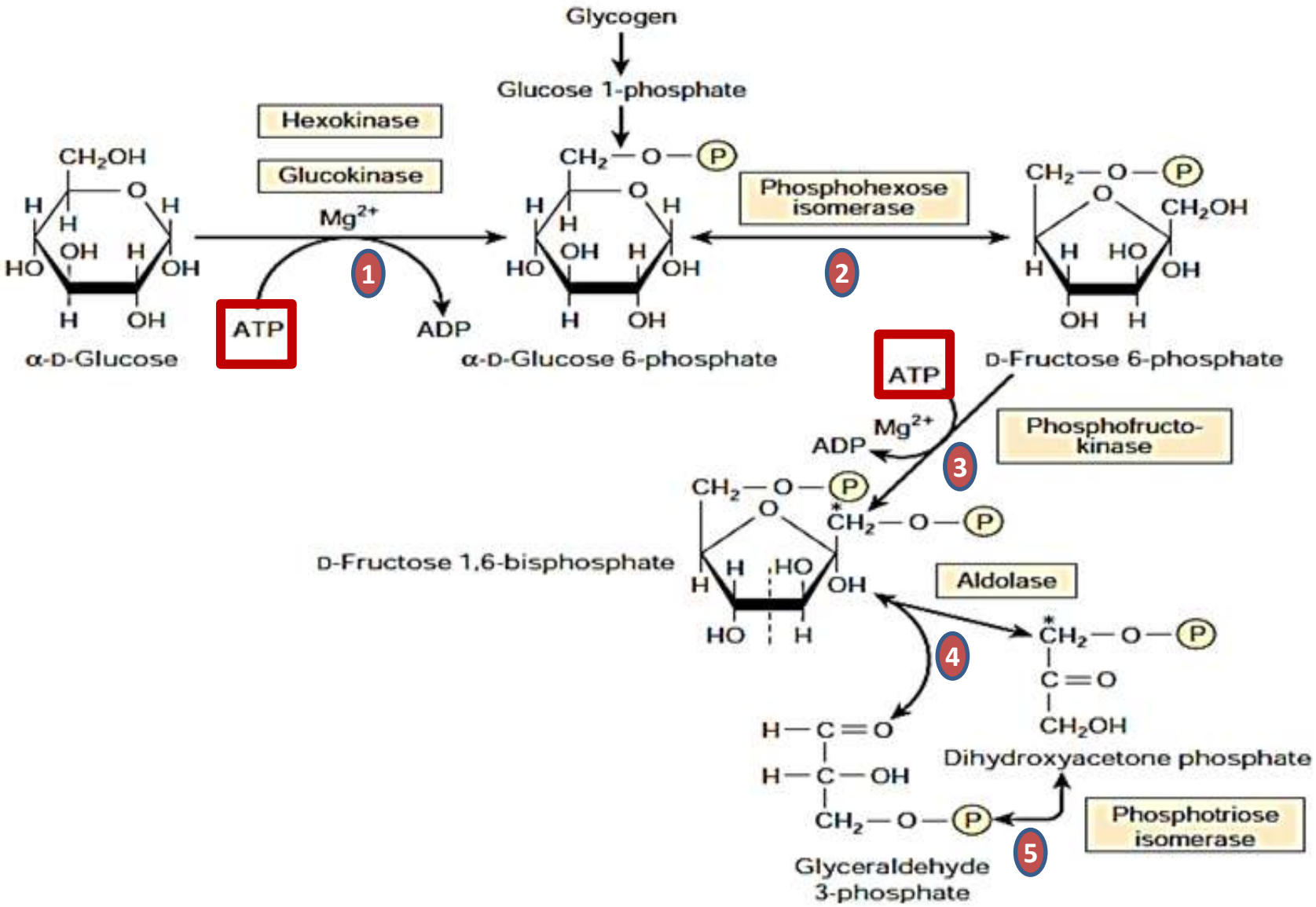

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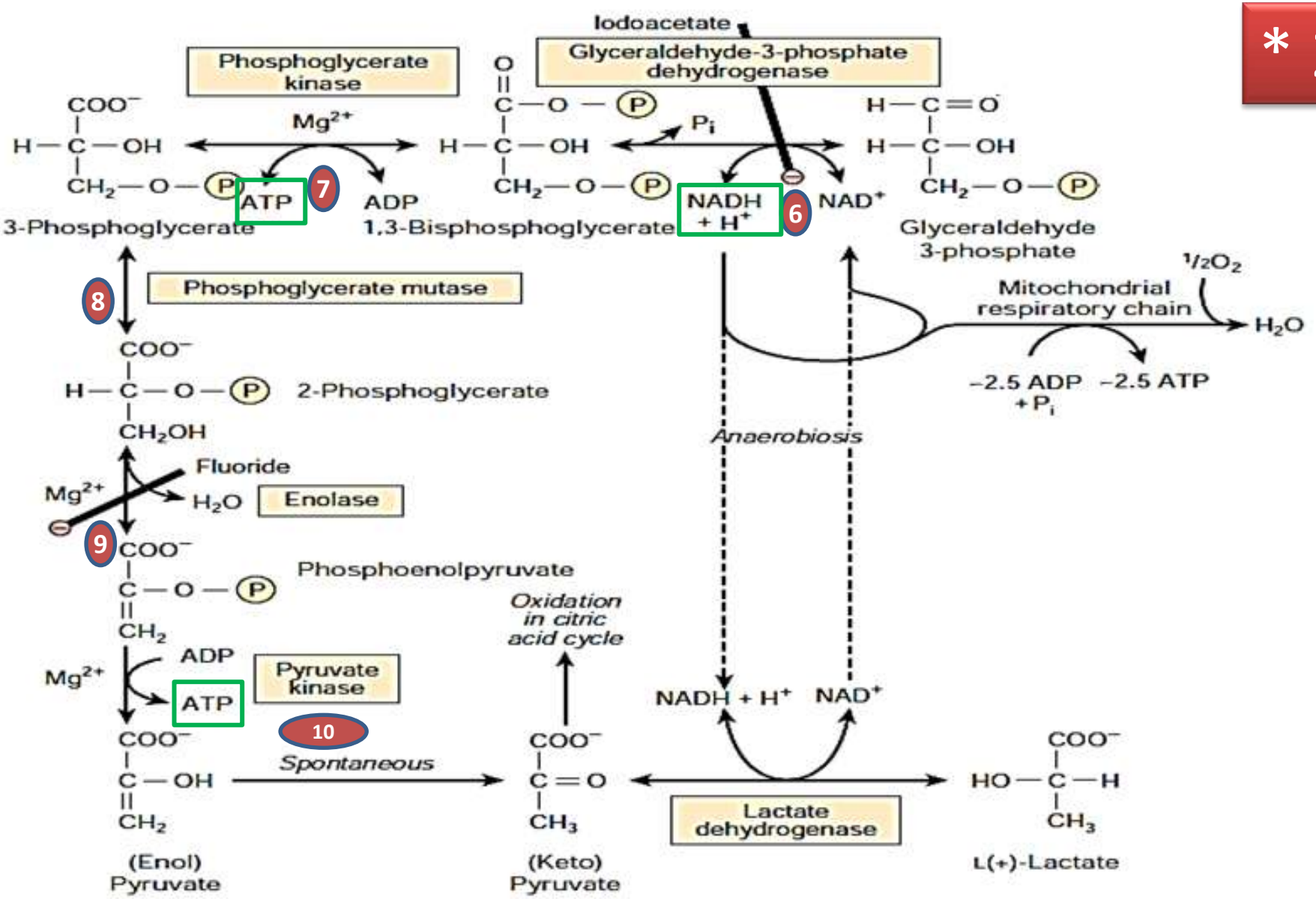


Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

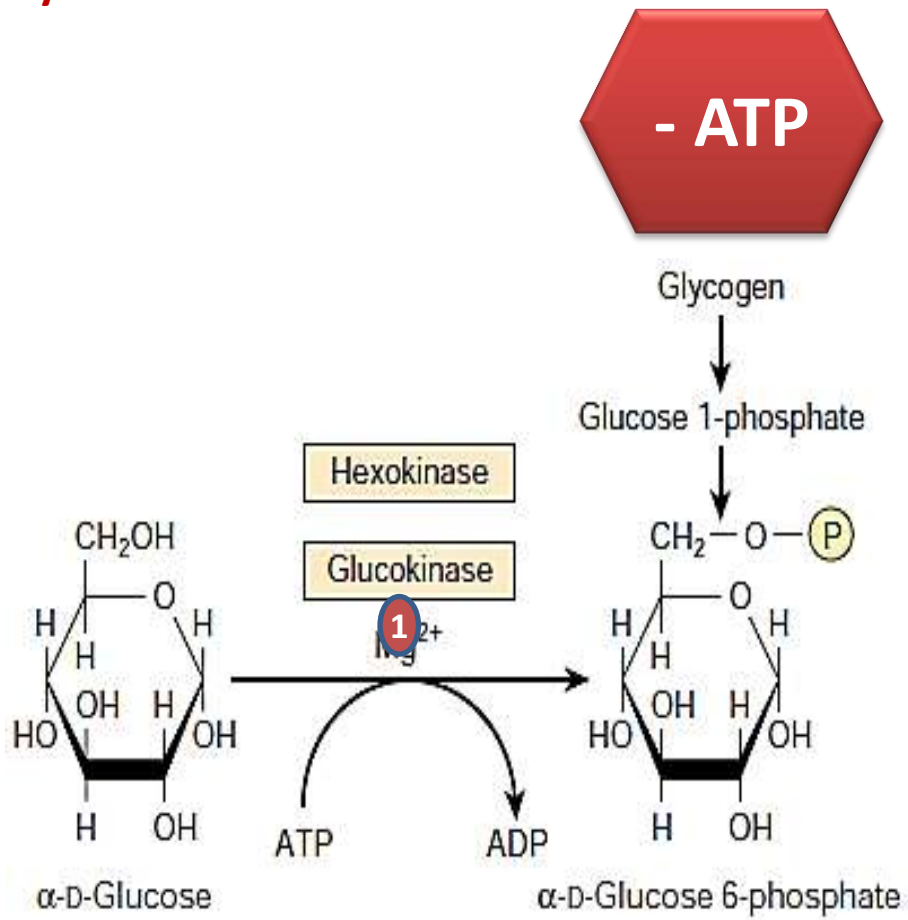
- The overall equation for **glycolysis** from **glucose to lactate** is as follows:

Glucose + 2 ADP + 2 Pi → 2 Lactate + 2 ATP + 2 H_2O

- All of the **enzymes of glycolysis** are found in the **cytosol**.

Reaction (1)
(*Phosphorylation*)

- **Glucose** enters glycolysis by **phosphorylation** to **glucose 6-phosphate**, catalyzed by **hexokinase**, using **ATP** as the phosphate donor.
- Under physiologic conditions, the **phosphorylation of glucose** to glucose 6-phosphate can be regarded as **irreversible**.
- **Hexokinase** is **inhibited allosterically** by **its product, glucose 6-phosphate**.



Glycolysis & the oxidation of Pyruvate

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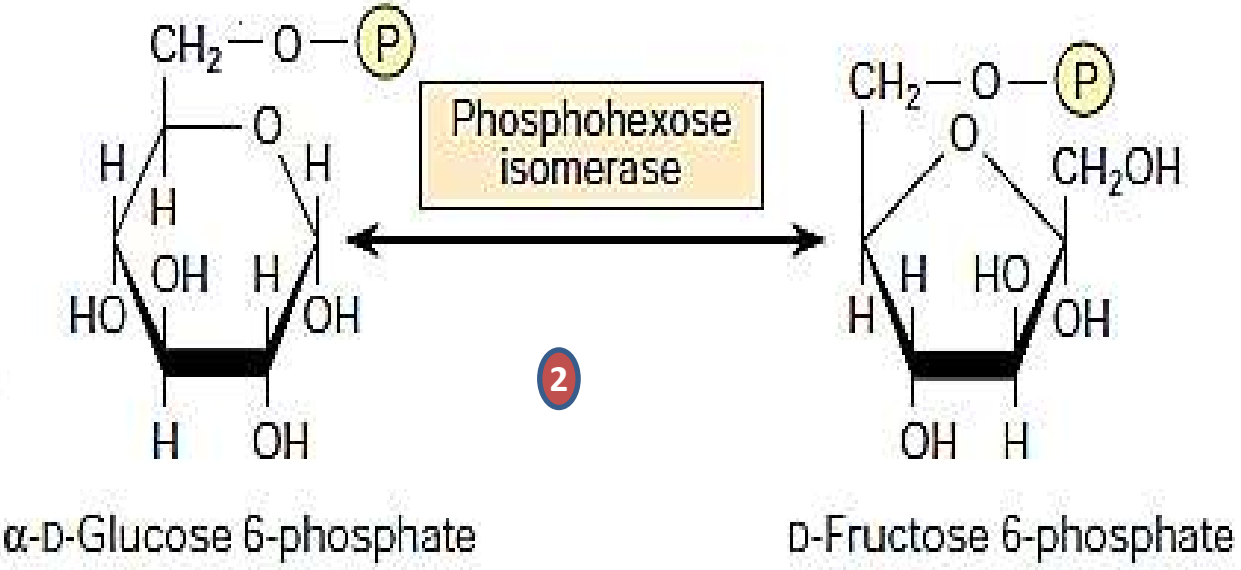
Reaction (1)
(*Phosphorylation*)

- **In tissues other than the liver** and **pancreatic β-islet cells**, **the availability of glucose for glycolysis** or (**glycogen synthesis in muscle**, and **lipogenesis in adipose tissue**), **is controlled by transport into the cell**, which in turn is **regulated by insulin**.
- **Hexokinase** has a **high affinity** (**low K_m**) for **glucose**, and **in the liver** it **is saturated under normal conditions**, and so **acts at a constant rate to provide glucose 6-phosphate to meet the cell's need**.
- **Liver cells** also contain an **isoenzyme of hexokinase**, **glucokinase**, which has a **K_m very much higher than the normal intracellular concentration of glucose**.
- The **function of glucokinase** in the liver is to **remove glucose from the blood following a meal**, **providing glucose 6-phosphate in excess of requirements for glycolysis**, **which is used for glycogen synthesis and lipogenesis**.
- **Glucose 6-phosphate** is an **important** compound at the **junction** of **several metabolic pathways: glycolysis**, **gluconeogenesis**, the **pentose phosphate pathway**, **glycogenesis**, and **glycogenolysis.**

Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (2)
(*Isomerization*)

- In **glycolysis: Glucose 6-phosphate** is **converted to fructose 6-phosphate** by **phosphohexose isomerase**, which involves an aldose-ketose isomerization.

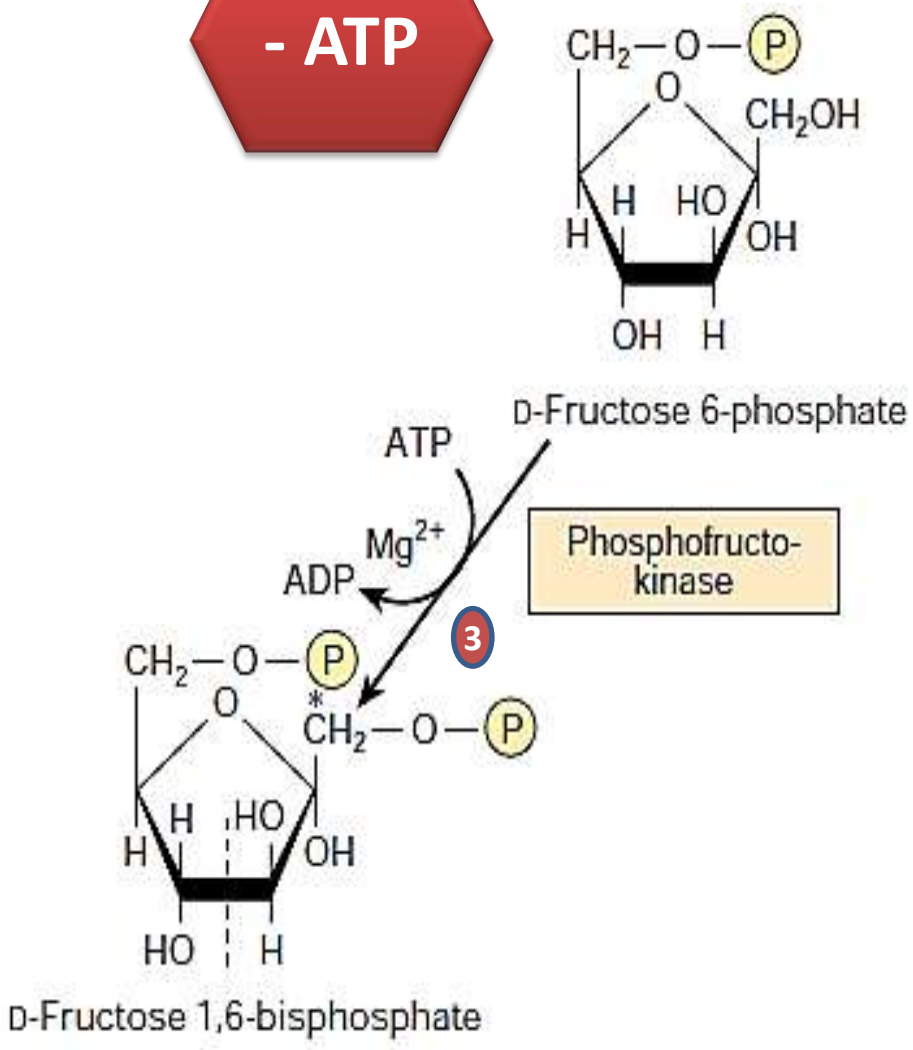


Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (3)
(*Phosphorylation*)

- ATP

- Isomeration reaction is **followed by another phosphorylation** catalyzed by the enzyme **phosphofructokinase** (**phosphofructokinase-1/ PFK-1**) **forming fructose 1,6-bisphosphate**.
- The **phosphofructokinase reaction** may be considered to be functionally **irreversible** under physiologic conditions; it is both **inducible and subject to allosteric regulation**, and has a major role in **regulating the rate of glycolysis**.



Glycolysis & the oxidation of Pyruvate

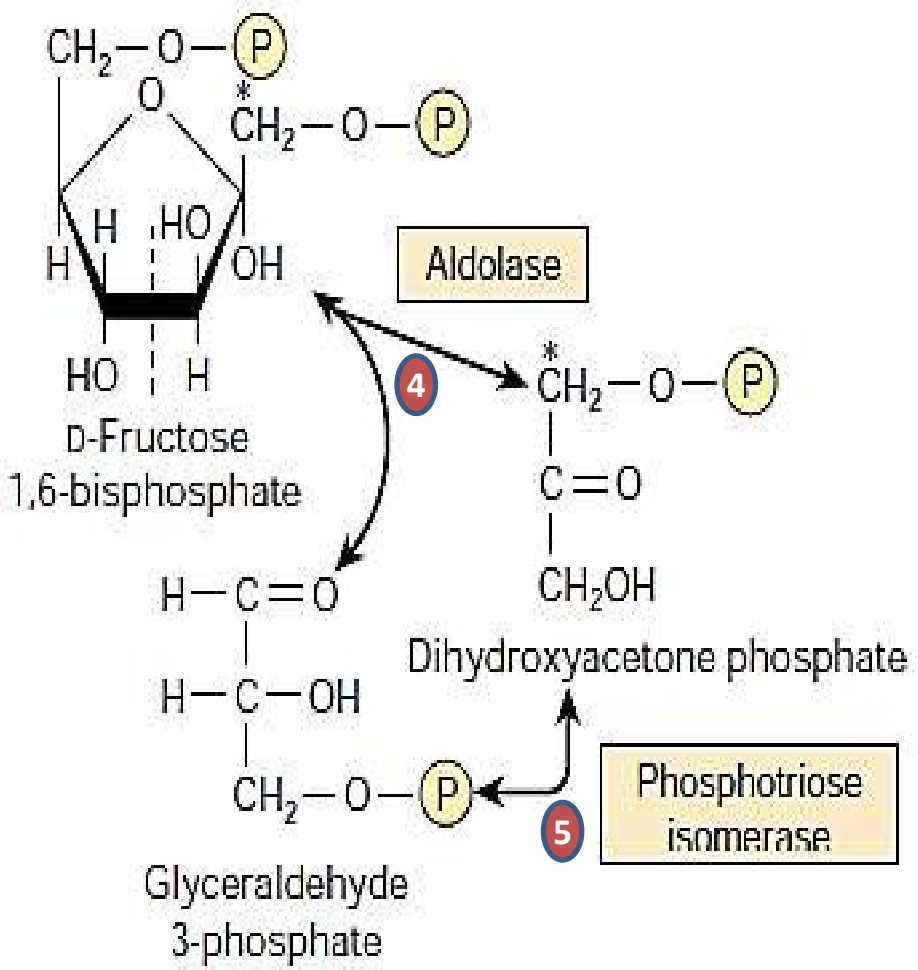
Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (4)
(*Cleavage*)

- **Fructose 1,6-bisphosphate** is **cleaved** by **aldolase** (**fructose 1,6-bisphosphate aldolase**) into two triose phosphates, **glyceraldehyde 3-phosphate** and **dihydroxyacetone phosphate**.

Reaction (5)
(*Isomerization*)

- **Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate** are **interconverted** by the enzyme **phosphotriose isomerase**.



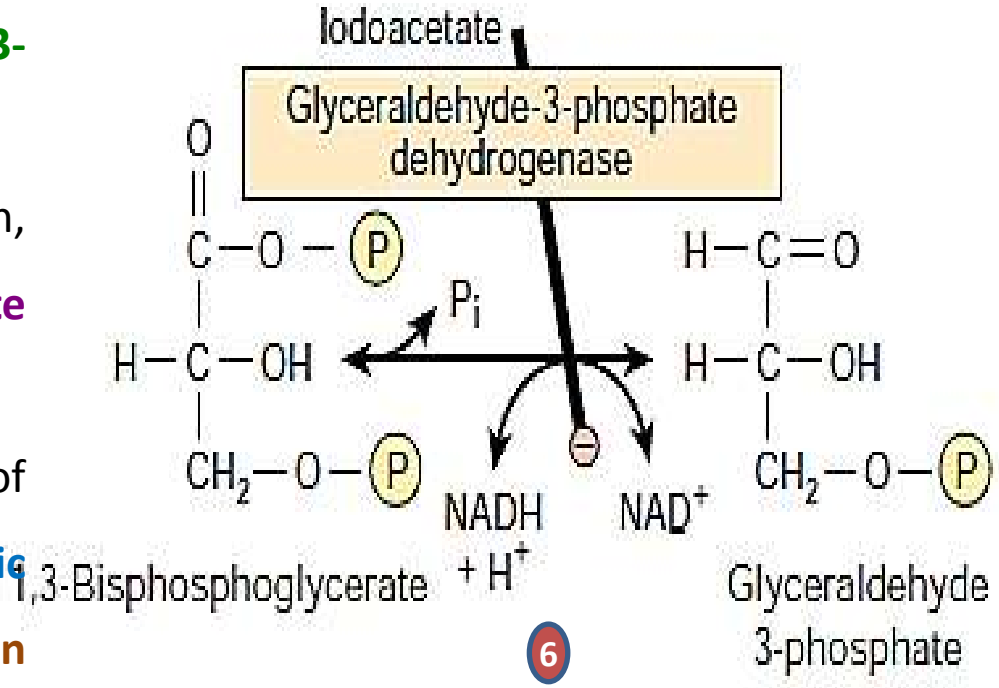
Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (6)
(*Oxidation and phosphorylation*)

+ NADH
*** 2**

- Glycolysis continues with the **oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate**.
- The enzyme catalyzing this oxidation, **glyceraldehyde 3-phosphate dehydrogenase**, is **NAD⁺-dependent**.
- The **toxicity of arsenic** is the result of **competition of arsenate with inorganic phosphate (Pi)** in this **above reaction (6)**to give **1-arseno-3-phosphoglycerate**, **which hydrolyzes spontaneously to 3-phosphoglycerate without forming ATP.**

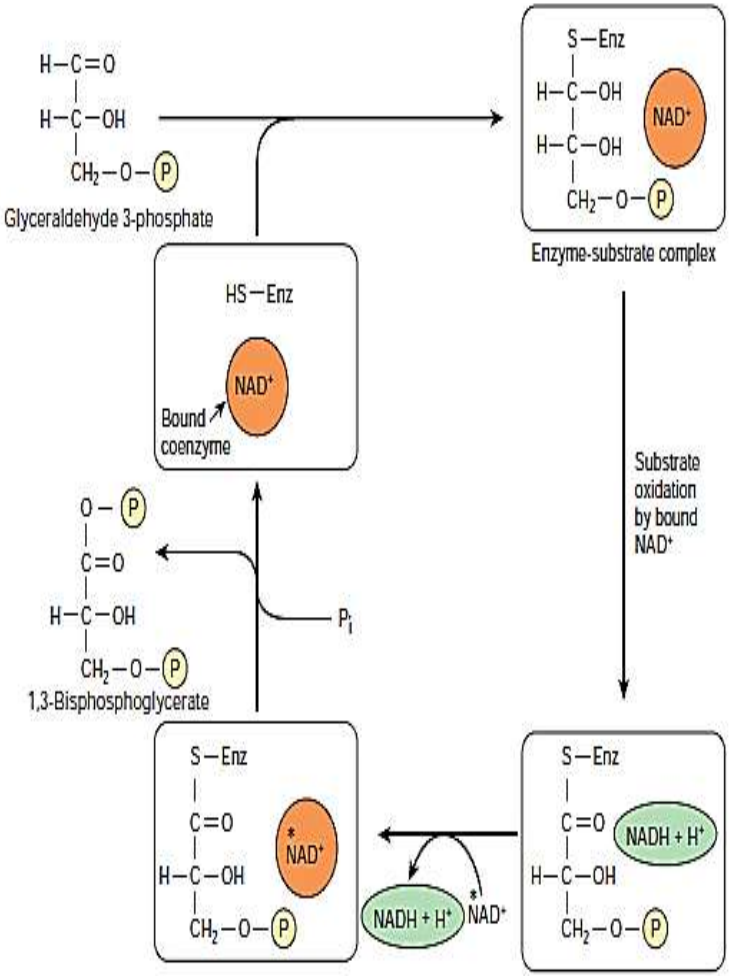


Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (6)
(*Oxidation and phosphorylation*)

- Structurally, **glyceraldehyde 3-phosphate dehydrogenase** consists of **four identical polypeptides** (**monomers**) forming a **tetramer**.
- **Four —SH** groups are present on each polypeptide, derived from **cysteine** residues within the polypeptide chain. One of the **—SH** groups is found at the active site of the enzyme.
- The **substrate** initially combines with this **—SH** group, forming a **thiohemiacetal** that is oxidized to a **thiol ester**; the hydrogens removed in this oxidation are transferred to **NAD^+**. The thiol ester then undergoes phosphorolysis; **inorganic phosphate** (**Pi**) is added, **forming 1,3-bisphosphoglycerate**, and the **—SH** group is reconstituted.



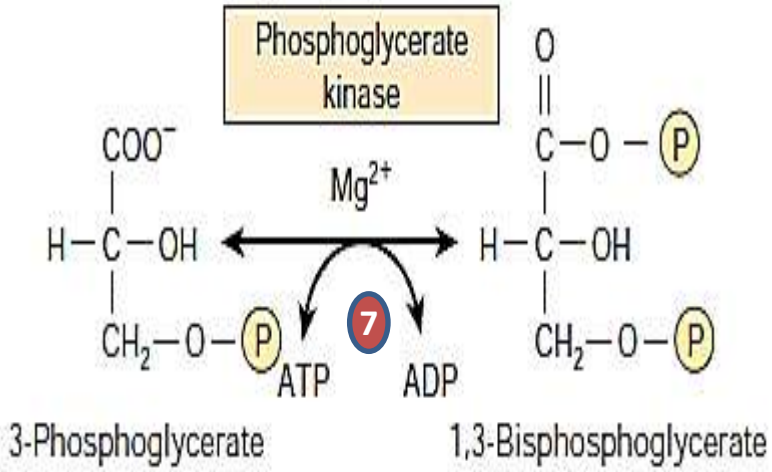
Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (7)
(*Substrate level phosphorylation*)

+ ATP * 2

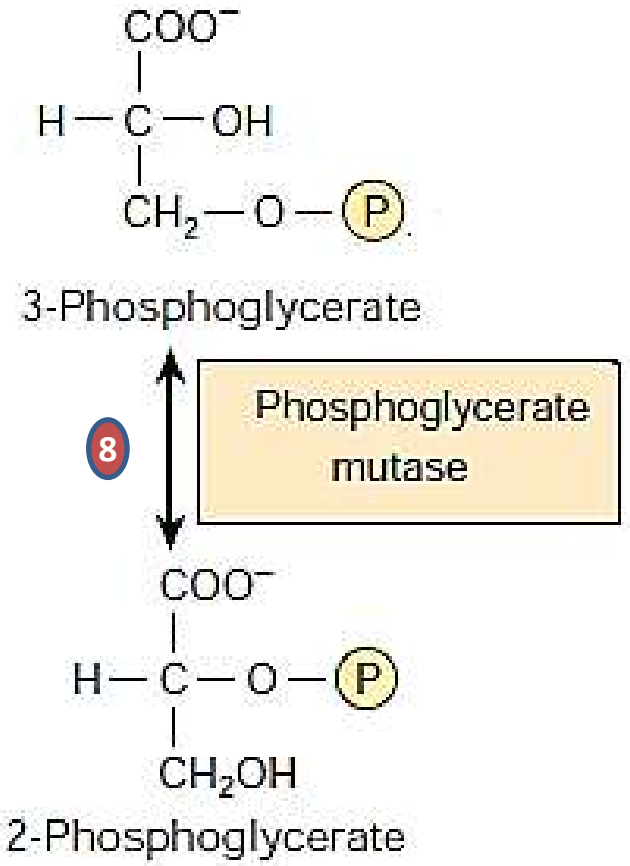
- In the next reaction, catalyzed by **phosphoglycerate kinase**, **phosphate is transferred from 1,3-bisphosphoglycerate onto ADP**, **forming ATP** (**substrate-level phosphorylation**) and **3-phosphoglycerate**.
- Since two molecules of triose phosphate are formed per molecule of glucose undergoing glycolysis, **two molecules of ATP are formed at this stage per molecule of glucose** undergoing glycolysis.
- It is likely that **2,3-bisphosphoglycerate** (**diphosphoglycerate**, **DPG**) is an **intermediate in this reaction**.



Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (8)
(*Isomerization*)

- **3-Phosphoglycerate** is **isomerized** to **2-phosphoglycerate** by **phosphoglycerate mutase**.

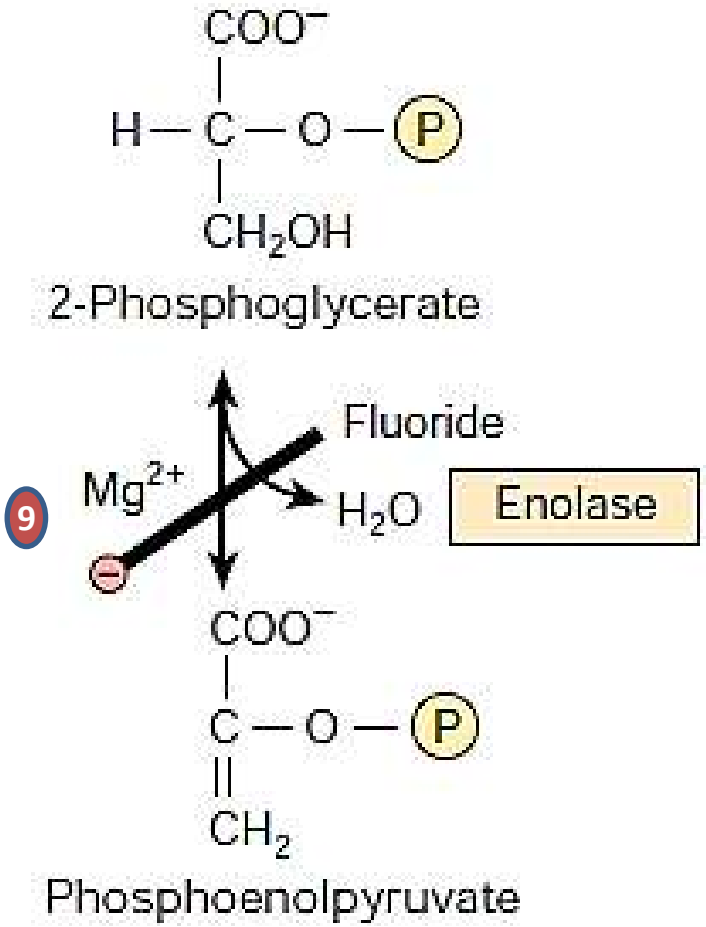


Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (9)
(*dehydration*)

- The subsequent step is catalyzed by **enolase** and involves a **dehydration**, forming **phosphoenolpyruvate**.
- **Enolase** is **inhibited by fluoride**, and **when blood samples are taken for measurement of glucose**, **it is collected in tubes containing fluoride to inhibit glycolysis**.
- The **enzyme** is also dependent on the presence of either **Mg^{2+} or Mn^{2+}.**



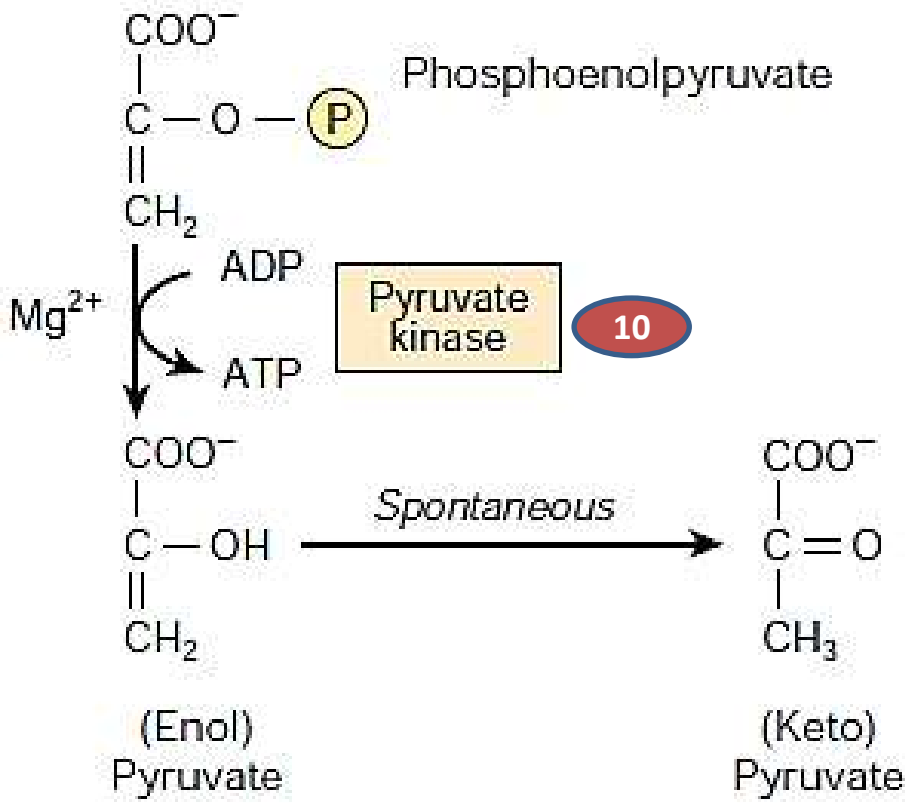
Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

Reaction (10)
(*Substrate level phosphorylation*)

+ ATP
*** 2**

- The phosphate of **phosphoenolpyruvate** is transferred to **ADP** by **pyruvate kinase** to form **two molecules** of **ATP** per molecule of glucose oxidized.



Glycolysis & the oxidation of Pyruvate

Glycolysis reactions constitute the main pathway of glucose utilization

The redox state of the tissue now determines which of two pathways is followed:

Under anaerobic conditions

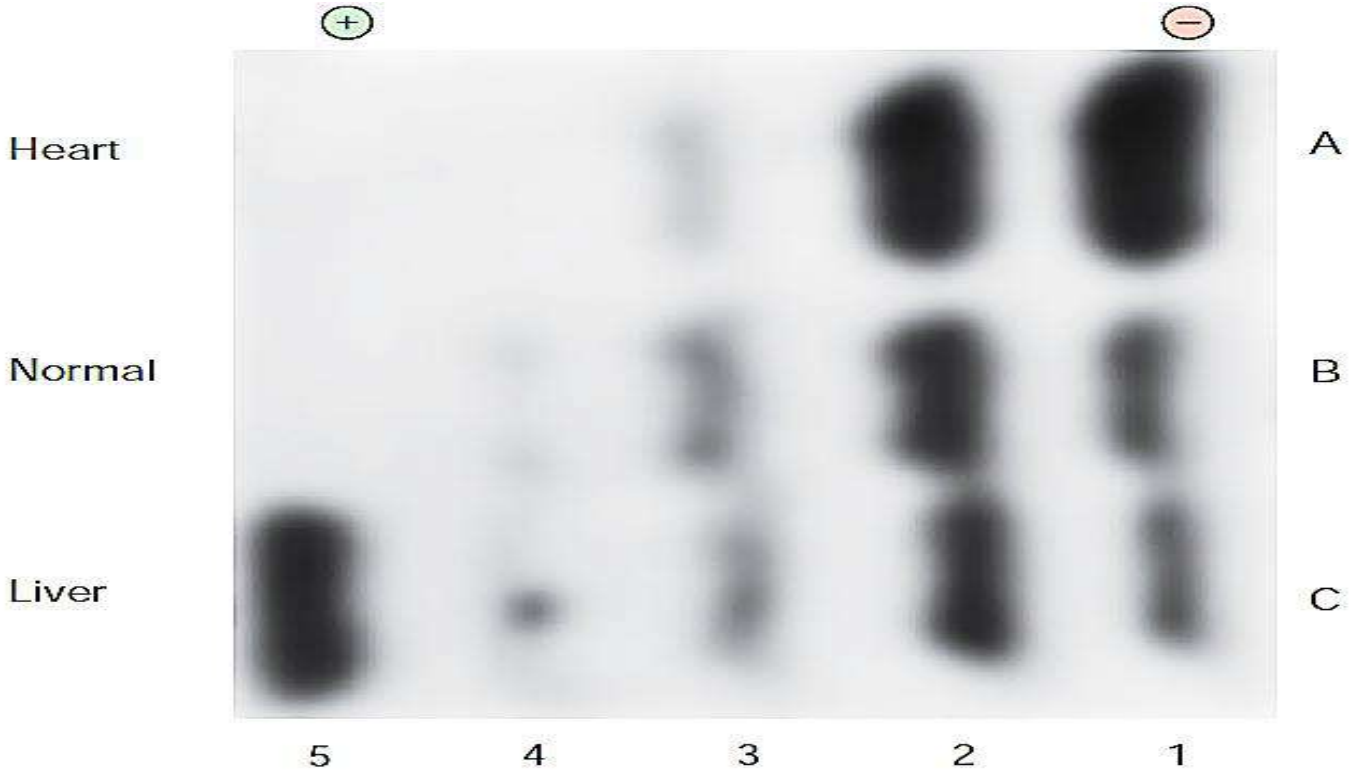
- The NADH cannot be reoxidized through the **respiratory chain** to NAD^+.
- **Pyruvate is reduced by the NADH to lactate**, catalyzed by **lactate dehydrogenase**.
- **There are different tissue specific isoenzymes lactate dehydrogenase that have clinical significance**.
- The **reoxidation of NADH via lactate formation allows glycolysis to proceed in the absence of oxygen by regenerating sufficient NAD^+ for another cycle of the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase (reaction 6).**

Under aerobic conditions

- pyruvate is taken up into **mitochondria**, and **after oxidative decarboxylation to acetyl-CoA is oxidized to CO2 by the citric acid cycle**.
- The reducing equivalents from the **NADH formed in glycolysis** are taken up **into mitochondria** for oxidation **via one of the two shuttles:** the **glycerophosphate** (Figure 13–12) and **malate** (Figure 13–13) shuttles

Glycolysis reactions constitute the main pathway of glucose utilization

The redox state of the tissue now determines which of two pathways is followed:



Pattern

A is serum from a patient with a myocardial infarct(احتشاء عضلة القلب);

B is normal serum;and

C is serum from a patient with liver disease (أمراض الكبد).

Arabic numerals denote specific LDH isozymes

Glycolysis & the oxidation of Pyruvate

Glycolysis Reactions Constitute The Main Pathway Of Glucose Utilization

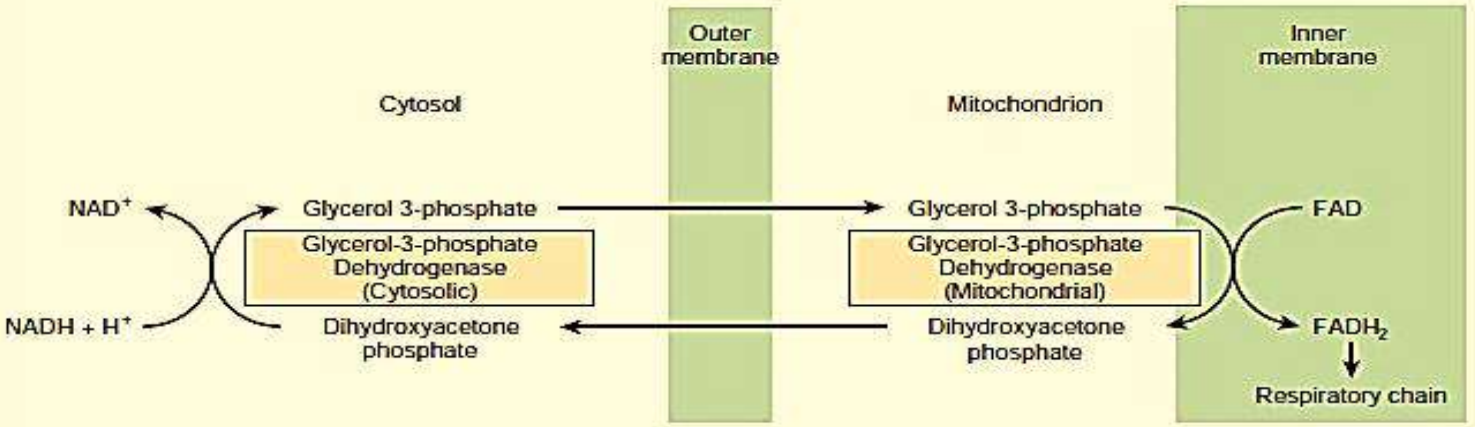
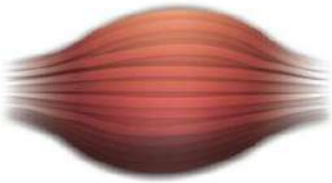


FIGURE 13–12 Glycerophosphate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion.

skeletal muscle



brain

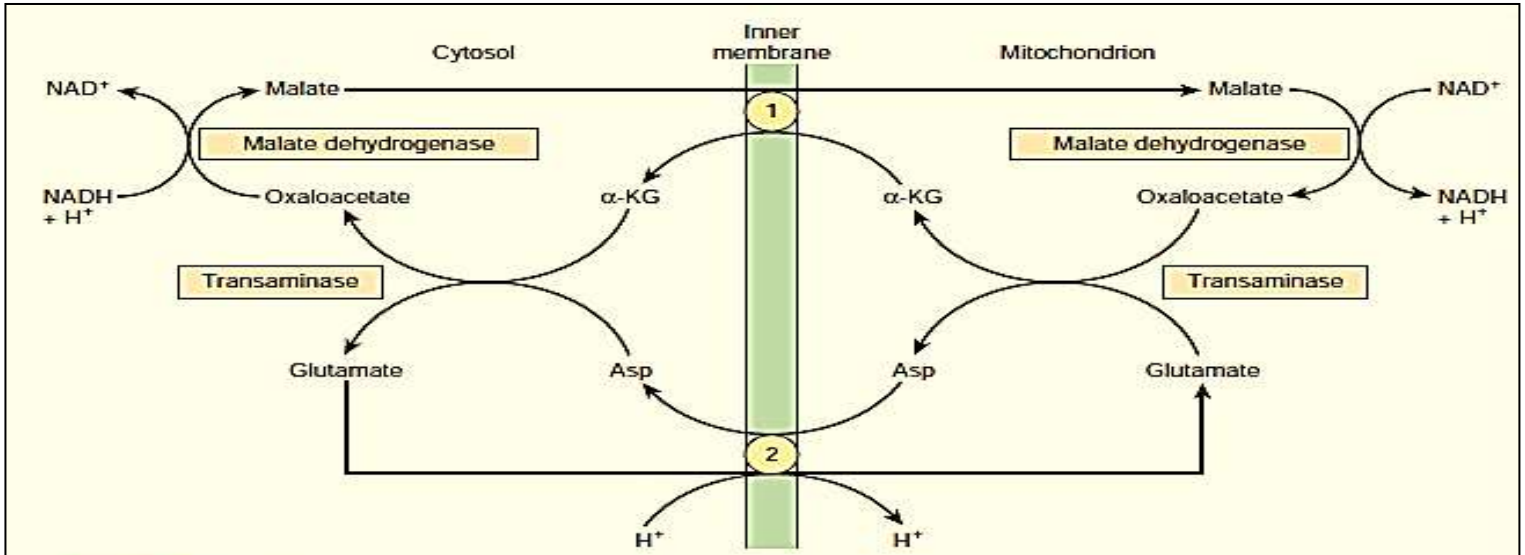


FIGURE 13–13 Malate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion. ① α-Ketoglutarate transporter, ② glutamate/aspartate transporter (note the proton symport with glutamate).

HEART

KIDNEYS

LIVER

Glycolysis & the oxidation of Pyruvate

Tissues That Function Under Hypoxic (نقص الأكسجة) Conditions Produce Lactate

- **Skeletal muscle**, particularly the white fibers, where the rate of work output, and hence the need for ATP formation, may exceed the rate at which oxygen can be taken up and utilized.

- Glycolysis in **erythrocytes** always terminates in lactate, because the subsequent reactions of pyruvate oxidation are mitochondrial, and **erythrocytes lack mitochondria**.

- Other tissues that normally derive much of their energy from glycolysis and produce lactate include **brain**, **gastrointestinal tract (**الجهاز الهضمي**) , renal medulla (**لب الكلية**) , retina (** شبكية العين**), and skin**.

- Lactate production is also increased in **septic shock** (الصدمة الإنتانية), and many **cancers** also produce lactate.

- **The liver, kidneys, and heart usually take up lactate and oxidize it, but produce it under hypoxic conditions**.

Glycolysis & the oxidation of Pyruvate

Tissues That Function Under Hypoxic (نقص الأكسجة) Conditions Produce Lactate

- When **lactate production is high**, as in **vigorous exercise, septic shock, and cancer cachexia**, much **is used in the liver for gluconeogenesis** (استحداث السكر), leading to an **increase in metabolic rate to provide the ATP and GTP needed**. The resultant increase in oxygen consumption is seen as oxygen debt after vigorous exercise.

- Under some conditions **lactate** may be formed **in the cytosol, but then enter the mitochondrion to be oxidized to pyruvate for onward metabolism**. **This provides a pathway for the transfer of reducing equivalents from the cytotol into the mitochondrion for the electron transport chain** in addition to the glycerophosphate (Figure 13–12) and malate (Figure 13–13) shuttles.

Glycolysis & the oxidation of Pyruvate

Glycolysis is regulated at three steps involving nonequilibrium reactions

- Although most of the reactions of glycolysis are reversible, **three** are markedly **exergonic** and must therefore be considered **physiologically irreversible**. These reactions, catalyzed by:

  1) **hexokinase (and glucokinase) (reaction 1)**
  2) **phosphofructokinase, (reaction 3)**
  3) **and pyruvate kinase (reaction 10) , are the major sites of regulation of glycolysis.**

Glycolysis & the oxidation of Pyruvate

Glycolysis is regulated at three steps involving nonequilibrium reactions

- **Phosphofructokinase** is significantly **inhibited** at **normal** intracellular concentrations of **ATP**; as discussed in Chapter 20, this **inhibition can be rapidly relieved by 5'AMP that is formed as ADP begins to accumulate, signaling the need for an increased rate of glycolysis.**
- **Cells that are capable of gluconeogenesis** (reversing the glycolytic pathway, Chapter 20) have different enzymes that catalyze reactions to reverse these irreversible steps;
  1) **glucose 6-phosphatase (reaction 1),**
  2) **fructose 1,6-bisphosphatase (reaction 3)**
  3) **pyruvate carboxylase** and **phosphoenolpyruvate carboxykinase**. to reverse the reaction of **pyruvate kinase (reaction 10)**
- **Fructose** enters glycolysis by phosphorylation to **fructose 1-phosphate**, and **bypasses** the main **regulatory steps**, so **resulting in formation of more pyruvate (and acetyl-CoA) than is required for ATP formation** (Chapter 21).
- In the **liver and adipose tissue, this leads to increased lipogenesis,** and a high intake of fructose may be a factor in the development of **obesity.**

Glycolysis & the oxidation of Pyruvate

In erythrocytes, the first site of ATP formation in glycolysis may be bypassed

- In **erythrocytes**, the reaction catalyzed by **phosphoglycerate kinase (reaction 7)** may be **bypassed** to some extent by the reaction of **bisphosphoglycerate mutase**, which catalyzes the conversion of **1,3-bisphosphoglycerate to 2,3-bisphosphoglycerate**, followed by hydrolysis to **3-phosphoglycerate** and **Pi**, catalyzed by **2,3-bisphosphoglycerate phosphatase** (Figure 18–4).
- This **alternative pathway involves no net yield of ATP from glycolysis**.
- However, it does serve to **provide 2,3-bisphosphoglycerate, which binds to hemoglobin, decreasing its affinity for oxygen, and so making oxygen more readily available to tissues**.

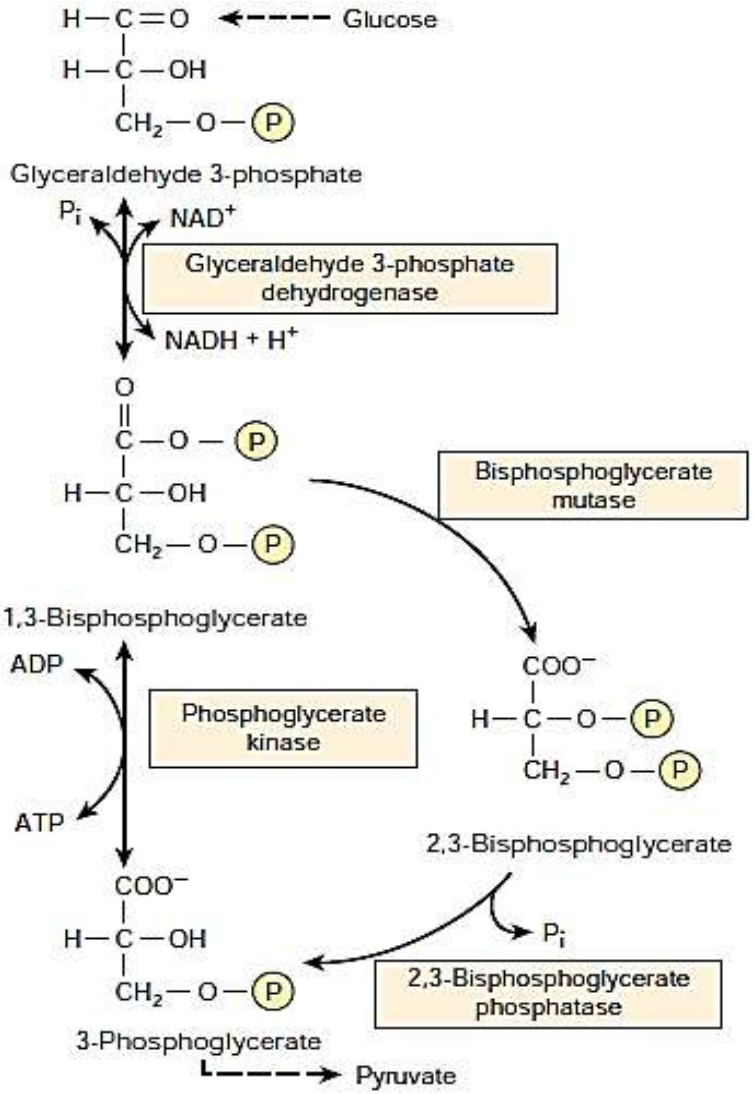
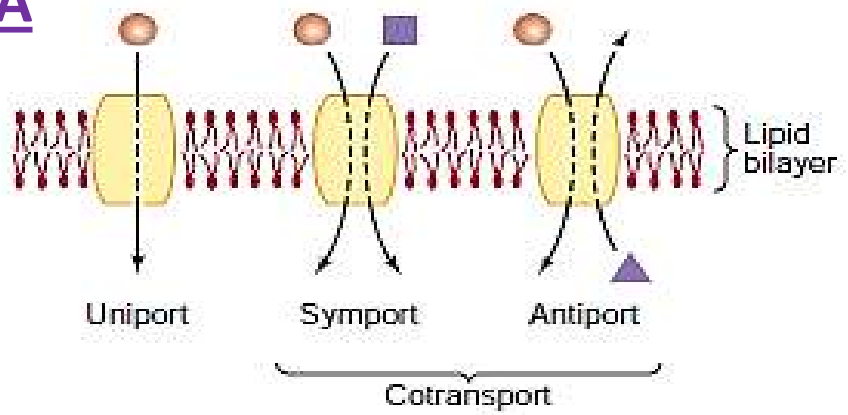


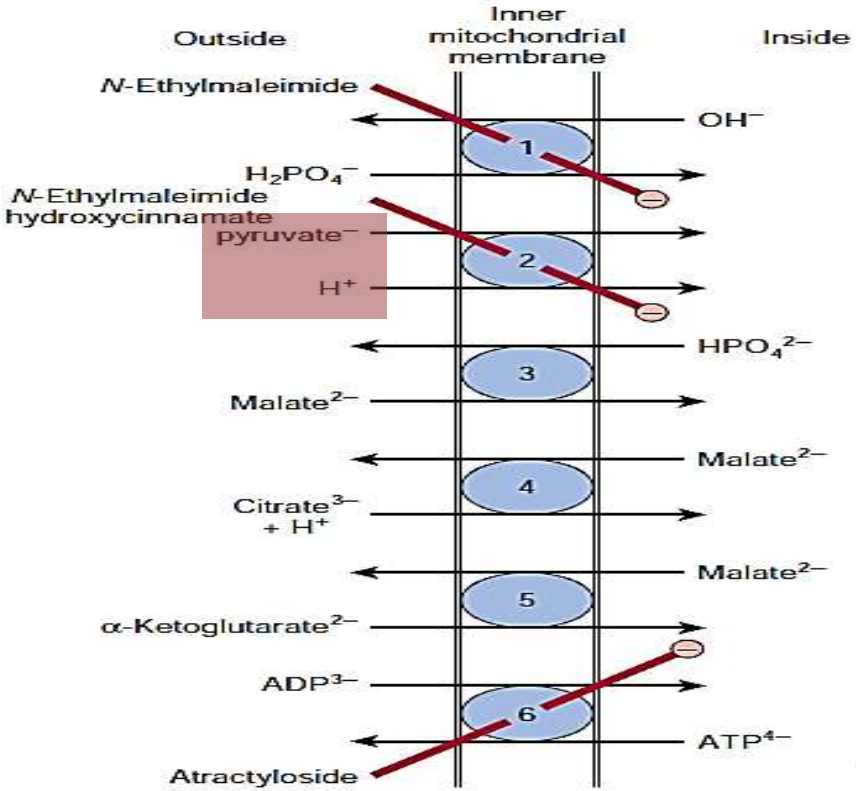
FIGURE 18–4 2,3-Bisphosphoglycerate pathway in erythrocytes.

Glycolysis & the oxidation of Pyruvate

The oxidation of pyruvate to Acetyl-CoA

- The **oxidation of pyruvate** to **acetyl-CoA** is the **irreversible route from glycolysis to the citric acid cycle**.
- **Pyruvate**, formed in the **cytosol**, is **transported into the mitochondrion** by a **proton symporter** (Figure 13–10).
- **Inside the mitochondrion**, it is **oxidatively decarboxylated** to **acetyl-CoA** by a **multienzyme complex** that is associated with the inner mitochondrial membrane.
- This **pyruvate dehydrogenase complex** is **analogous** to the **α-ketoglutarate dehydrogenase complex** of the **citric acid cycle** .



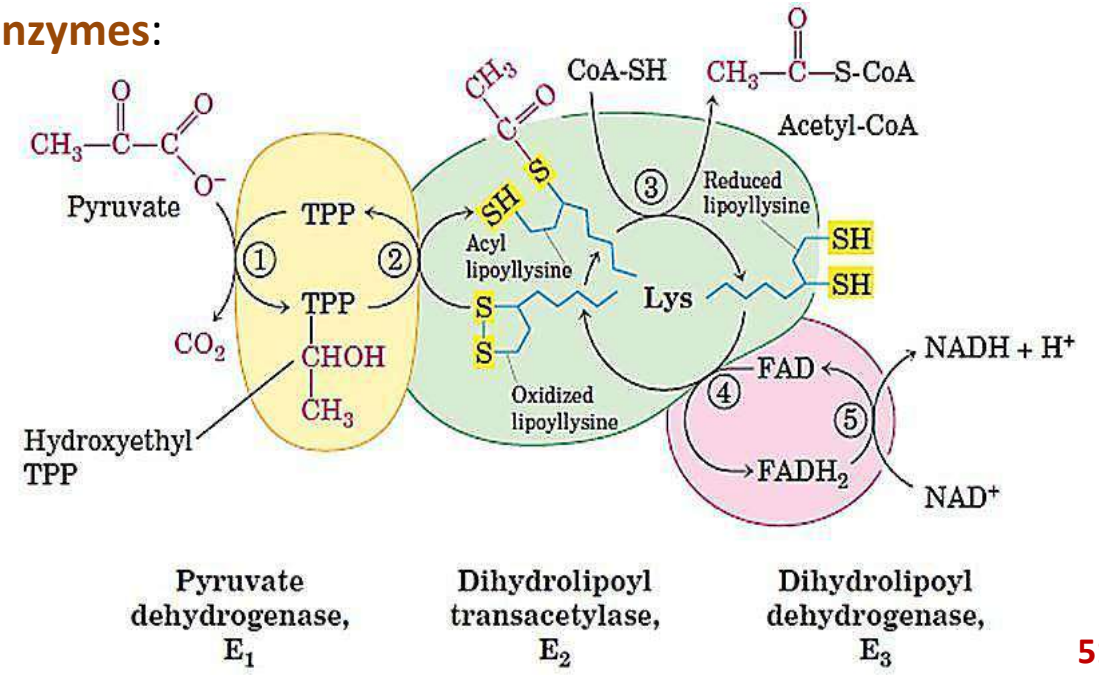


Glycolysis & the oxidation of Pyruvate

THE OXIDATION OF PYRUVATE TO ACETYL-CoA

- **Pyruvate Dehydrogenase Complex** (**PDH**) is a multiple enzyme complex made up of **three enzymes**.
  - **These Three enzymes are:**
    - **1- pyruvate dehydrogenase (E1),**
    - **2- dihydrolipoyl transacetylase (E2)**
    - **3- dihydrolipoyl dehydrogenase (E3).**

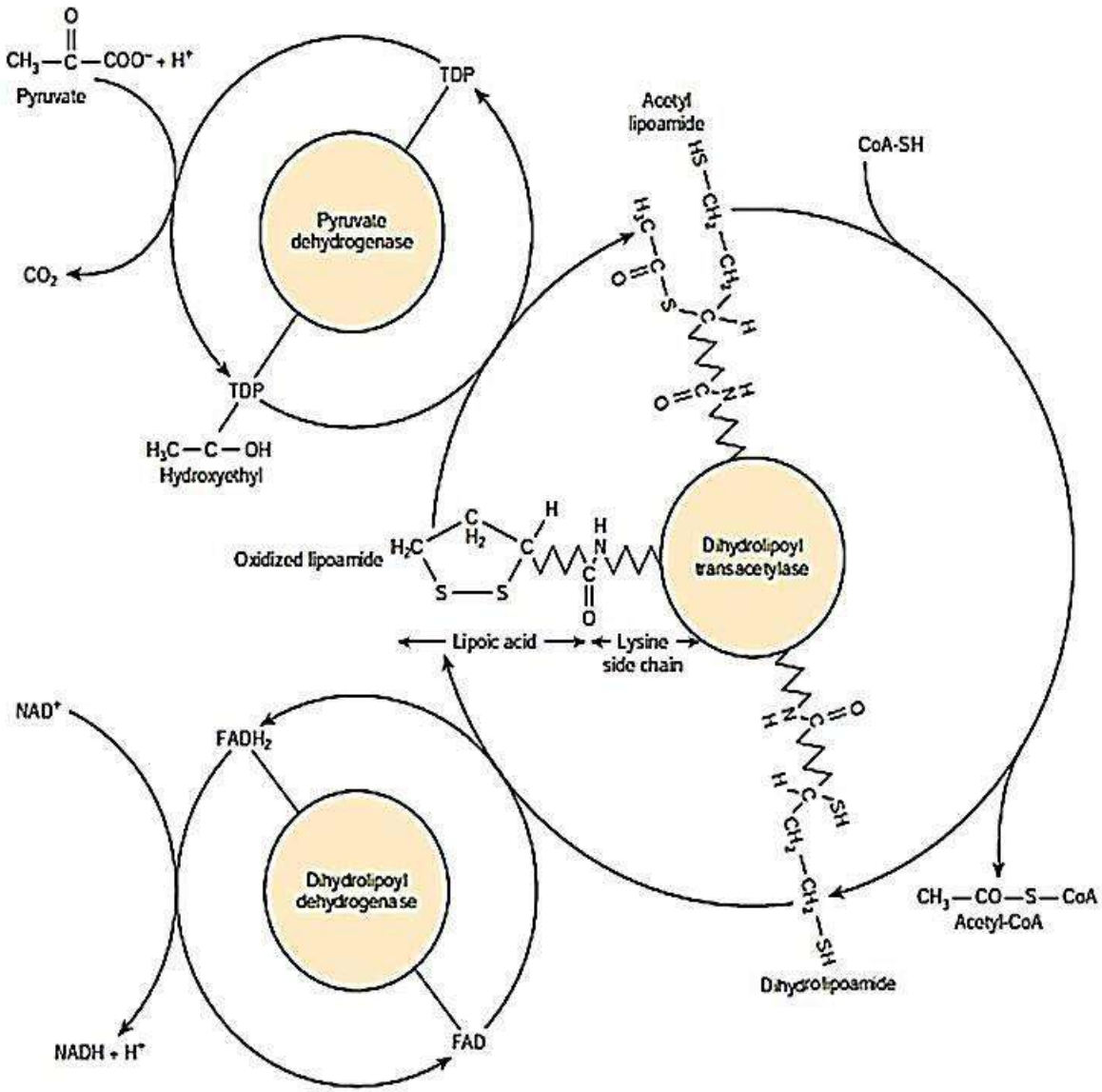
- **PDH complex also requires five coenzymes:**
  1) thiamine pyrophosphate,
  2) lipoate,
  3) coenzyme A,
  4) FAD
  5) NAD^+



Glycolysis & the oxidation of Pyruvate

THE OXIDATION OF PYRUVATE TO ACETYL-CoA

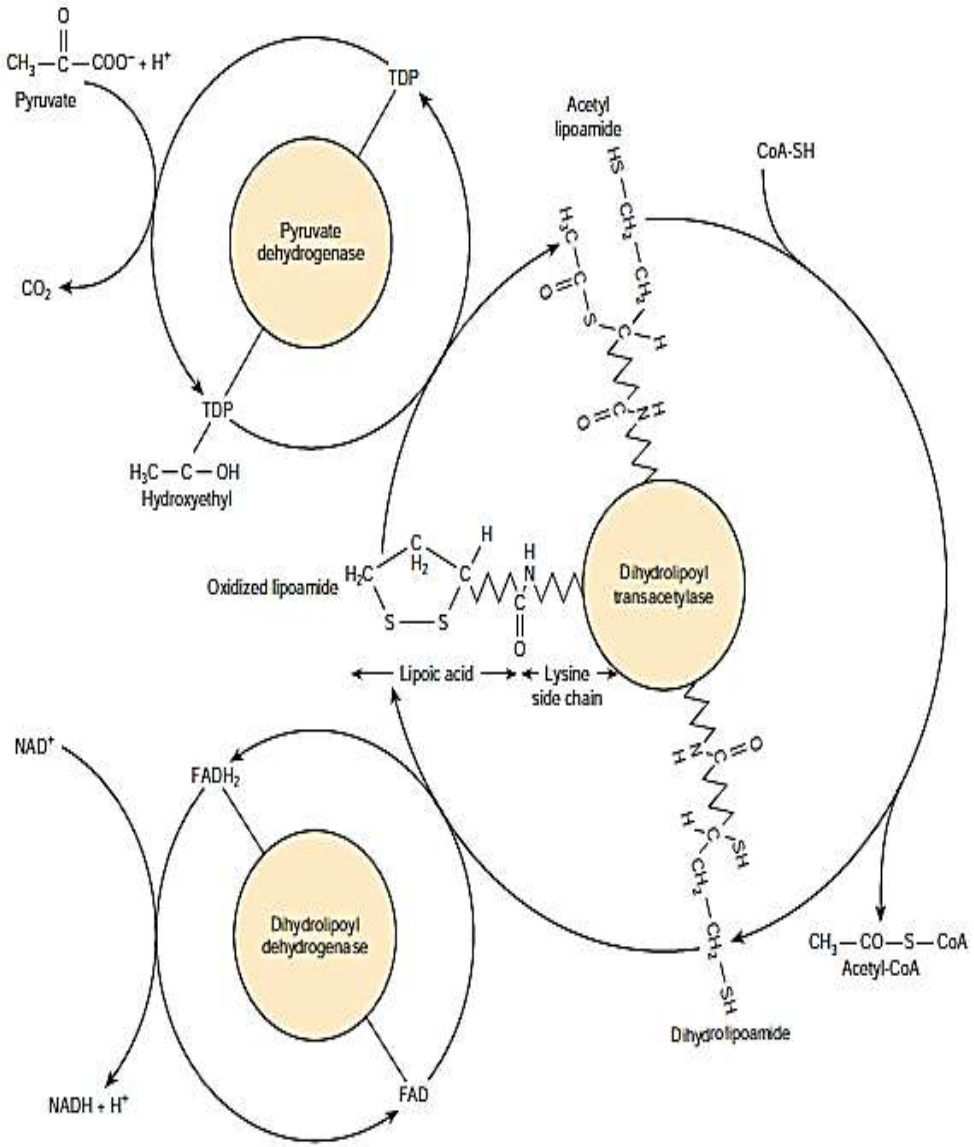
- **Pyruvate is decarboxylated** by the **pyruvate dehydrogenase (**component of the enzyme complex) **to a hydroxyethyl derivative of the thiazole ring of enzyme-bound thiamin diphosphate (TDP),**
- which in turn **reacts** with **oxidized lipoamide**, the **prosthetic group** of **dihydrolipoyl transacetylase**, to **form acetyl lipoamide**



Glycolysis & the oxidation of Pyruvate

THE OXIDATION OF PYRUVATE TO ACETYL-CoA

- **Thiamin is vitamin B1** and in **deficiency**, **glucose metabolism is impaired**, and there is **significant** (and potentially life-threatening) **lactic and pyruvic acidosis**.
- **Acetyl lipoamide reacts with coenzyme A** to **form acetyl-CoA** and **reduced lipoamide**.
- The reaction is completed when the **reduced lipoamide** is **reoxidized** by a **flavoprotein, dihydrolipoyl dehydrogenase**, containing FAD.
- Finally, the **reduced flavoprotein** is **oxidized by NAD^+,**
- which in turn **transfers reducing equivalents to the respiratory chain**.



Glycolysis & the oxidation of Pyruvate

The oxidation of pyruvate to Acetyl-CoA

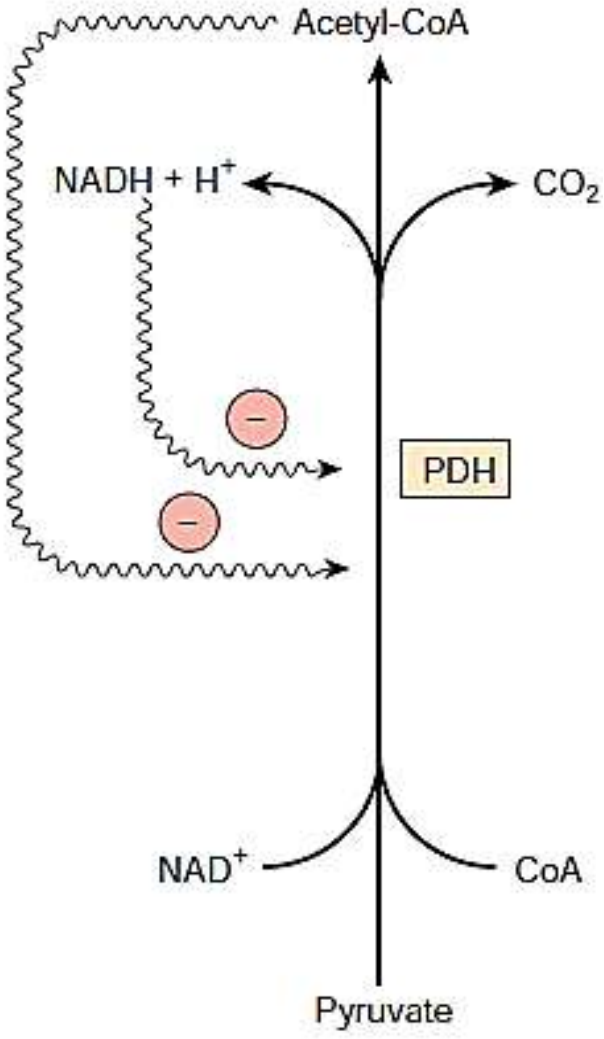
- The **pyruvate dehydrogenase complex** **consists of a number of polypeptide chains** of each of the **three component enzymes**, and **the intermediates do not dissociate, but remain bound to the enzymes**.
- **Such a complex of enzymes**, in which **the substrates are handed on from one enzyme** to the next is called:
- **Substrate channeling** is a process by which two or more sequential enzymes in a pathway interact to transfer a metabolite (or intermediate) from one enzyme active site to another without allowing free diffusion of the metabolite into the bulk solution and this provides:
  1) **increases the reaction rate**
  2) **eliminates side reactions,**
  3) **increasing overall efficiency.**

$$\text{Pyruvate} + NAD^+ + CoA \rightarrow \text{Acetyl-CoA} + NADH + H^+ + CO_2$$

Glycolysis & the oxidation of Pyruvate

Pyruvate Dehydrogenase Is Regulated by End-Product Inhibition & Covalent Modification

- **Pyruvate dehydrogenase** is **inhibited by its products:**
  1) **acetyl CoA and**
  2) **NADH**

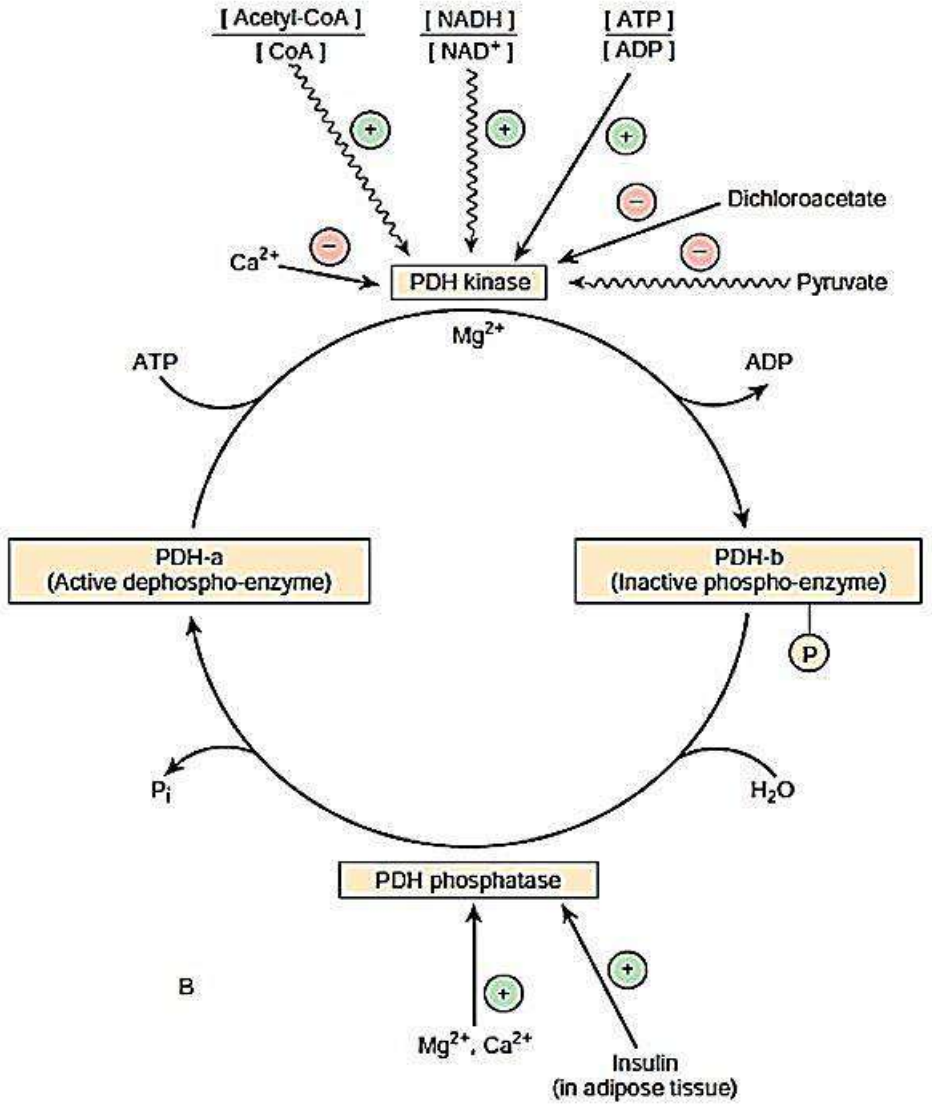


(A) Regulation by end-product inhibition

Glycolysis & the oxidation of Pyruvate

Pyruvate Dehydrogenase Is Regulated by End-Product Inhibition & Covalent Modification

- It is also **regulated Covalently** by:
  1) **Inhibition by phosphorylation** by a **kinase of three serine residues on the pyruvate dehydrogenase component** of the **multienzyme complex**, **resulting in decreased activity.**
  2) **Activation by dephosphorylation by a phosphatase** that **causes an increase in activity.**
- The **kinase** is **activated (So pyruvate dehydrogenase is inhibited) by increases** in the **[ATP]/[ADP], [acetyl-CoA]/[CoA], and [NADH]/[NAD$^+$] ratios.**



(B) Regulation by interconversion of active and inactive forms.

Glycolysis & the oxidation of Pyruvate

Pyruvate Dehydrogenase Is Regulated by End-Product Inhibition & Covalent Modification

- Thus, **pyruvate dehydrogenase**, **and therefore glycolysis**, is **inhibited both when:**
  1) **there is adequate ATP** (**and reduced coenzymes (NADH, $FADH_2$) for ATP formation**) **available**,
  2) and also when **fatty acids are being oxidized (β Oxidation of fatty acids produces both acetyl-CoA and NADH)**
- In **fasting**, when **free fatty acid concentrations increase**, **there is a decrease in the proportion of the enzyme in the active form**, **leading to a sparing of carbohydrate**.
- In **adipose tissue**, where **glucose provides acetyl-CoA for lipogenesis**, **the enzyme is activated in response to insulin**.

Glycolysis & the oxidation of Pyruvate

ATP From Oxidation of Glucose:

- **Oxidation of glucose yields up to 32 mol of ATP under aerobic conditions**, but **only 2 mol when O_2 is absent**.
- When **1 mol of glucose** is combusted **in a calorimeter to CO_2 and water**, approximately **2870 kJ** are liberated as heat.
- When **oxidation** occurs in the **tissues**, approximately **32 mol of ATP** are generated per **molecule of glucose** oxidized to CO_2 and water.
- **In vivo**, **ΔG for the ATP synthase reaction** has been calculated as approximately **51.6 kJ**.
- It follows that the total energy captured in **ATP per mole of glucose oxidized is 1651 kJ**, or approximately **58% of the energy of combustion**.
- **Most of the ATP is formed by oxidative phosphorylation resulting from the reoxidation of reduced coenzymes by the respiratory chain**.
- **The remainder is formed by substrate-level phosphorylation** (Table 18–1).

Glycolysis & the oxidation of Pyruvate

ATP From Oxidation of Glucose:

Pathway	Reaction Catalyzed by	Method of ATP Formation	ATP per Mol of Glucose
Glycolysis	Glyceraldehyde 3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH (6)	5*
	Phosphoglycerate kinase	Substrate level phosphorylation (7)	2
	Pyruvate kinase	Substrate level phosphorylation (10)	2
			1 X (9)
	Consumption of ATP for reactions of hexokinase and phosphofructokinase (1-3)		−2
			Net 7
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH (pyruvat oxidation)	5
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH (3)	5
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH (4)	5
	Succinate thiokinase	Substrate level phosphorylation (5)	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $FADH_2$ (6)	3
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH (8)	5
			Net 25
	Total per mol of glucose under aerobic conditions		32
	Total per mol of glucose under anaerobic conditions		2

*This assumes that NADH formed in glycolysis is transported into mitochondria by the malate shuttle (Figure 13–13). If the glycerophosphate shuttle is used, then only 1.5 ATP will be formed per mol of NADH. Note that there is a considerable advantage in using glycogen rather than glucose for anaerobic glycolysis in muscle, since the product of glycogen phosphorylase is glucose 1-phosphate (Figure 19–1), which is interconvertible with glucose 6-phosphate. This saves the ATP that would otherwise be used by hexokinase, increasing the net yield of ATP from 2 to 3 per glucose.

Glycolysis & the oxidation of Pyruvate

Clinical aspects (inhibition of pyruvate metabolism leads to lactic acidosis)

- Arsenite and mercuric ions react with the —SH groups of lipoic acid and inhibit pyruvate dehydrogenase, as does a dietary deficiency of thiamin, allowing pyruvate to accumulate.
- Many alcoholics are thiamin-deficient (both because of a poor diet and also because alcohol inhibits thiamin absorption), and may develop potentially fatal pyruvic and lactic acidosis.
- Patients with inherited pyruvate dehydrogenase deficiency, which can be the result of defects in one or more of the components of the enzyme complex, also present with lactic acidosis, particularly after a glucose load. Because of the dependence of the brain on glucose as a fuel, these metabolic defects commonly cause neurologic disturbances.
- Inherited aldolase A (reaction 4) deficiency and pyruvate kinase (reaction 10) deficiency in erythrocytes cause hemolytic anemia (فقر دم انحلالي).
- The exercise capacity of patients with muscle phosphofructokinase (reaction 3) deficiency is low, particularly if they are on high-carbohydrate diets. By providing lipid as an alternative fuel, eg, during starvation, when blood free fatty acid and ketone bodies are increased, work capacity is improved.

Glycolysis & the oxidation of Pyruvate

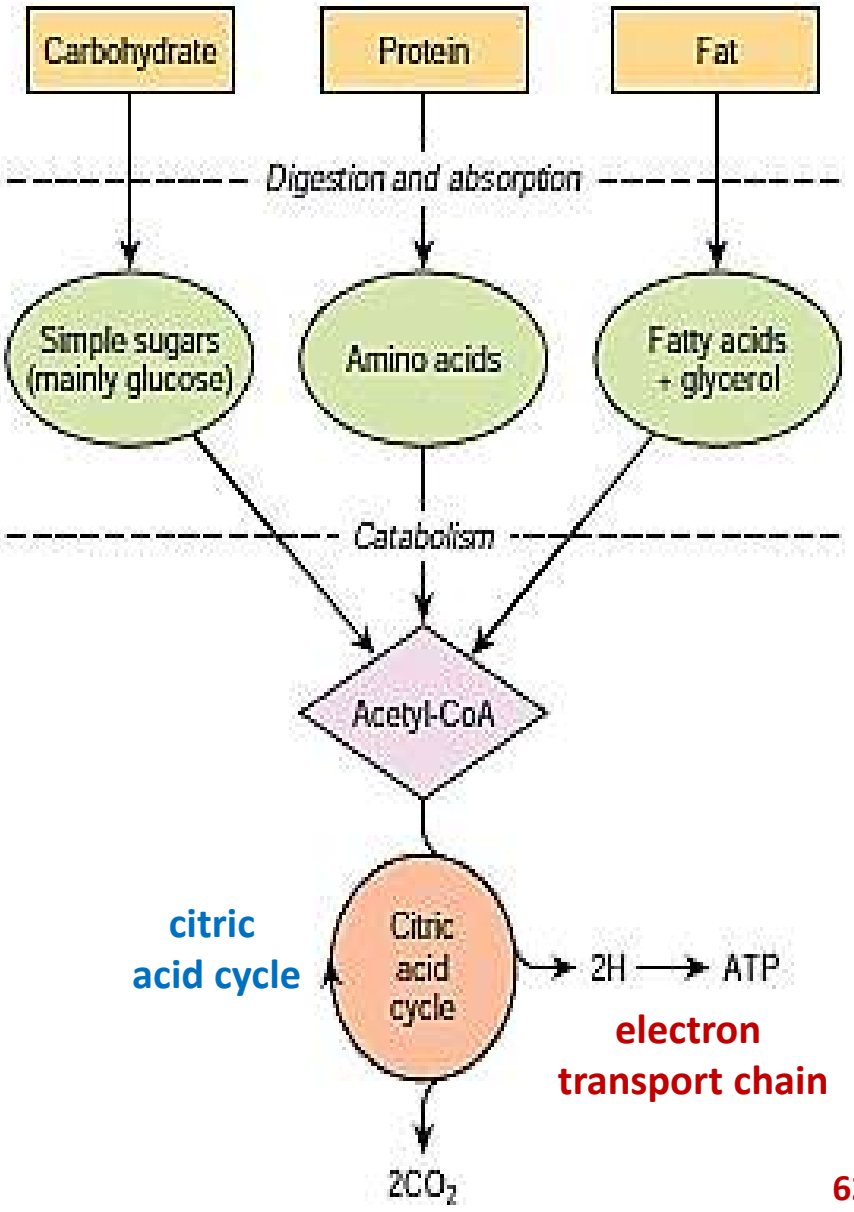
SUMMARY

1) **Glycolysis is the cytosolic pathway** of all mammalian cells **for the metabolism of glucose (or glycogen) to pyruvate and lactate.**
2) **It can function anaerobically by regenerating oxidized NAD^+** (required in the glyceraldehyde-3-phosphate dehydrogenase reaction /**6**), **by reducing pyruvate to lactate**.
3) **Lactate is the end product of glycolysis under anaerobic conditions** (eg, in exercising muscle) and in erythrocytes, where there are **no mitochondria to permit further oxidation of pyruvate**.
4) **Glycolysis is regulated by three enzymes** catalyzing nonequilibrium reactions: **hexokinase (1)**, **phosphofructokinase (3)**, and **pyruvate kinase (10)**.
5) **In erythrocytes**, **the first site in glycolysis for generation of ATP may be bypassed**, leading to the **formation of 2,3-bisphosphoglycerate**, which is important in **decreasing the affinity of hemoglobin for O_2.**
6) **Pyruvate is oxidized to acetyl-CoA** by a multienzyme complex, **pyruvate dehydrogenase**, which is **dependent on the vitamin derived cofactor thiamin diphosphate**.
7) Conditions that involve an **impairment of pyruvate metabolism** frequently **lead to lactic acidosis.**

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Biomedical importance

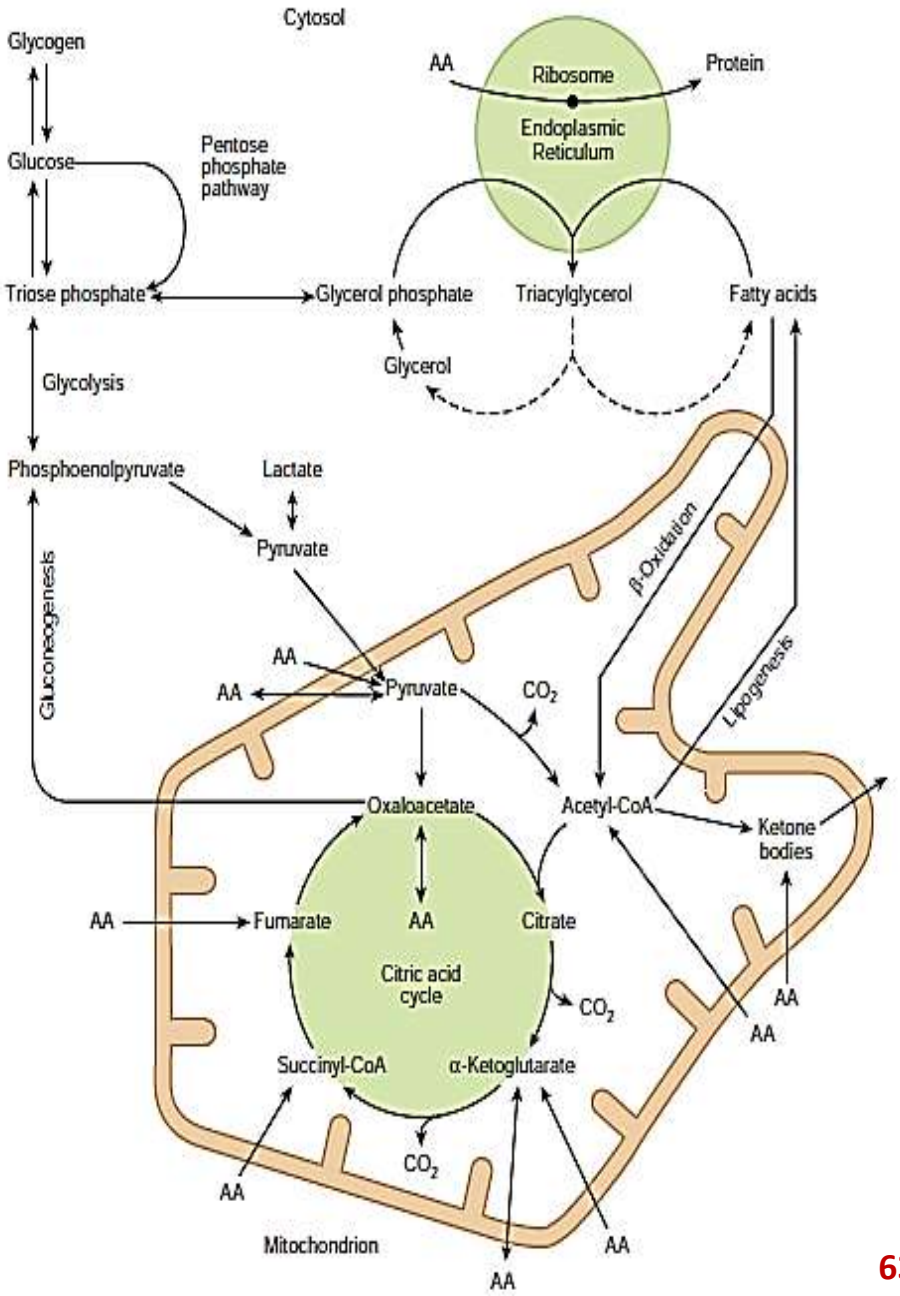
- **The citric acid cycle (Krebs cycle, tricarboxylic acid cycle)** is a sequence of reactions (**8 reactions**) in mitochondria that **oxidizes the acetyl moiety of acetyl-CoA** and **reduces coenzymes** (**NAD^+, FAD**)that are **reoxidized through the electron transport chain**, linked to the **formation of ATP**.
- The citric acid cycle is the **final common pathway for the oxidation of carbohydrate, lipid, and protein** because **glucose, fatty acids, and most amino acids are metabolized to acetyl- CoA or intermediates of the cycle**.



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Biomedical importance

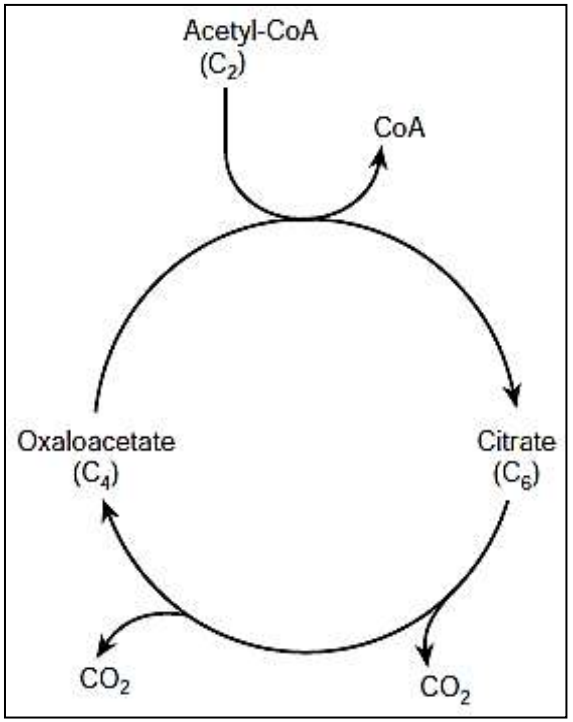
- The citric acid cycle also **has a central role in gluconeogenesis, lipogenesis, and interconversion of amino acids**.
- Many of these processes occur in most tissues, but **the liver is the only tissue in which all occur to a significant extent**.
- The **repercussions** are therefore profound when, for example, **large numbers of hepatic cells are damaged** as in **acute hepatitis (التهاب كبد حاد) or replaced by connective tissue (نسيج ضام)** (as in **cirrhosis/تليف الكبد**).
- The few **genetic defects of citric acid cycle enzymes** that have been reported are associated with **severe neurological damage** as a result of very considerably **impaired ATP formation in the central nervous system.**

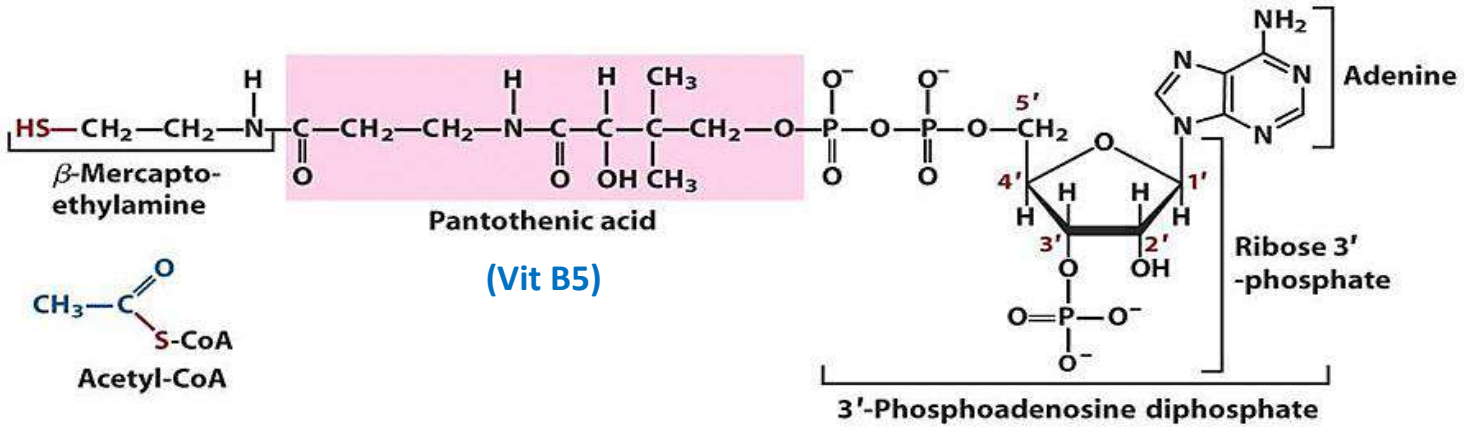


The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The citric acid cycle provides substrate for the respiratory chain

- The cycle starts with reaction between the **acetyl moiety of acetyl-CoA** and the **four-carbon** dicarboxylic acid **oxaloacetate**, forming a **six-carbon** tricarboxylic acid, **citrate**.
- In the subsequent reactions, **two molecules of CO_2 (reaction 3-4) are released** and **oxaloacetate is regenerated**
- Only a **small quantity of oxaloacetate** is needed for the oxidation of a large quantity of acetyl-CoA; it can be considered as **playing a catalytic role**.
- **The citric acid cycle** is an integral part of the process by which much of the **free energy liberated** during the oxidation of fuels **is made available**.

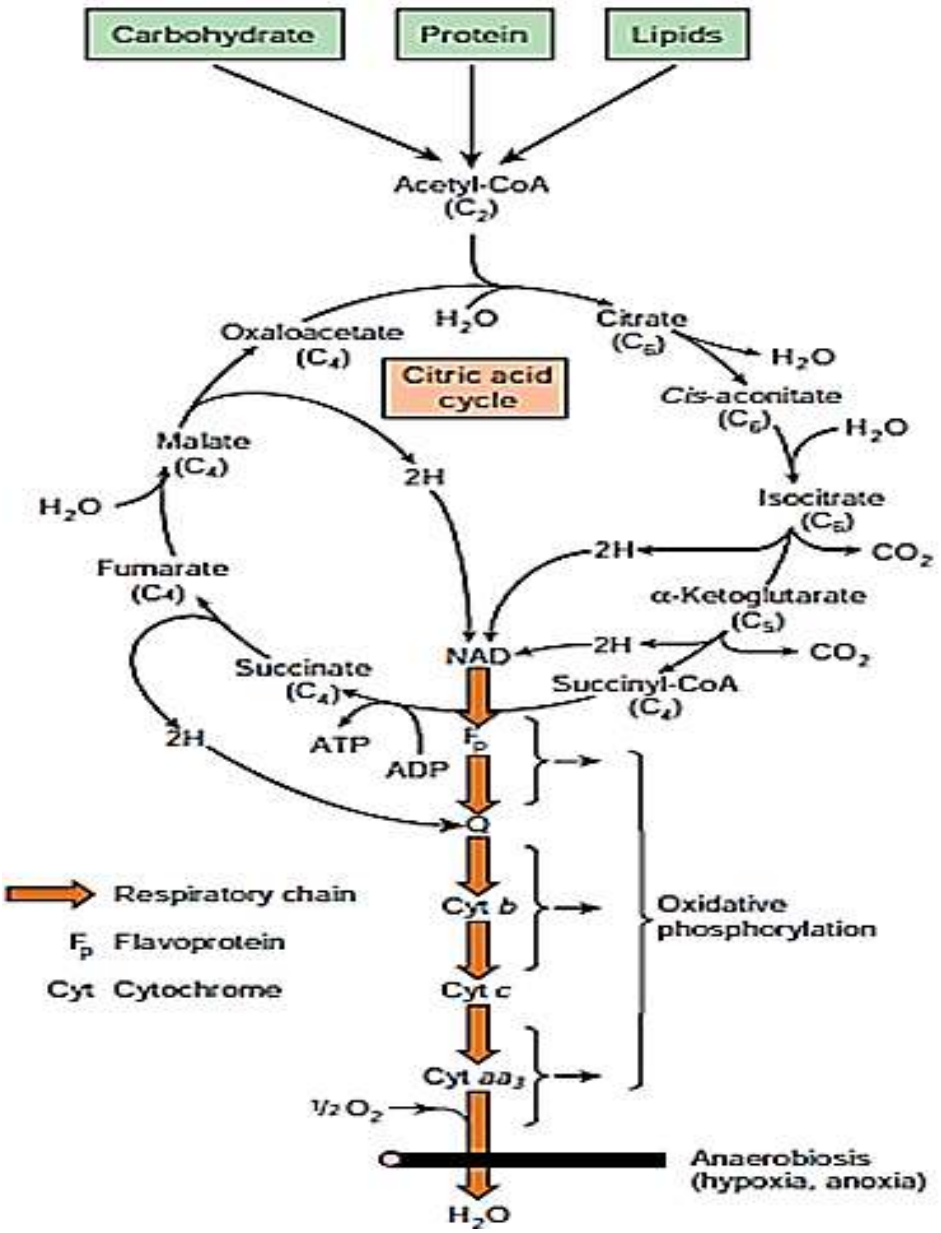


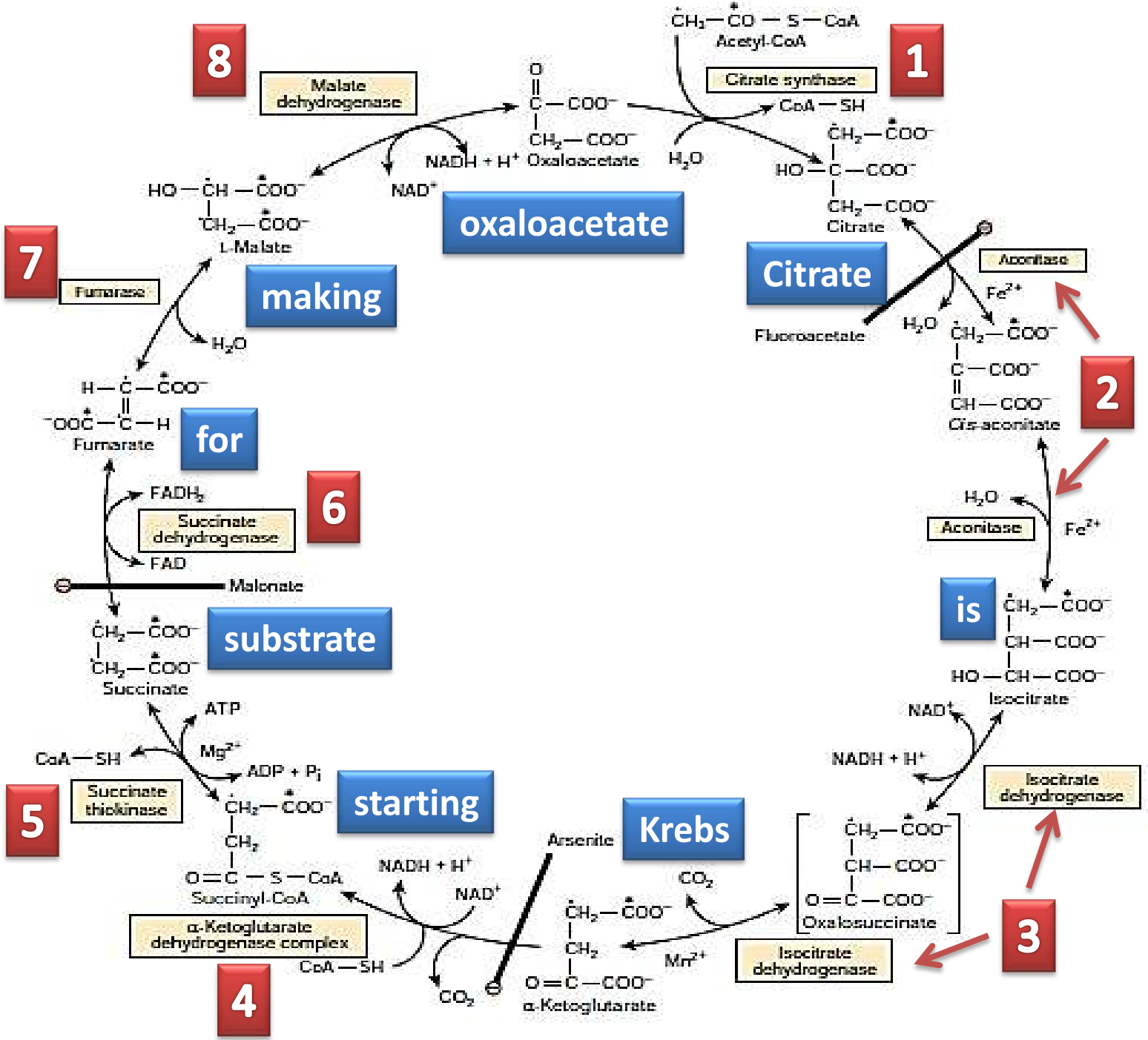


The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The citric acid cycle provides substrate for the respiratory chain

- During the **oxidation of acetyl-CoA**, **coenzymes are reduced** and subsequently **reoxidized in the respiratory chain,** linked to the **formation of ATP** (**oxidative phosphorylation**).
- This process is **aerobic**, requiring **oxygen** as the final oxidant of the reduced coenzymes.
- The **enzymes of the citric acid cycle are located in the mitochondrial matrix**, **either free or attached to the inner mitochondrial membrane and the crista (أعراف) membrane**, where **the enzymes and coenzymes of the respiratory chain are also found**.



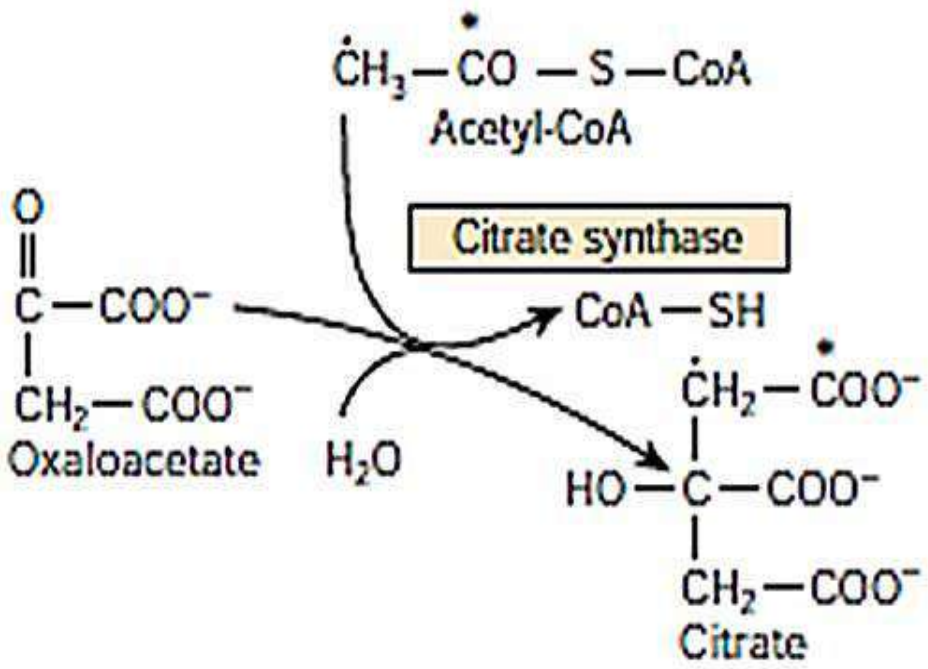

8
Malate dehydrogenase
Oxaloacetate
NADH + H+
NAD+
L-Malate
oxaloacetate
Acetyl-CoA
1
Citrate synthase
CoA — SH
H2O
Citrate
Citrate
7
Fumarase
making
H2O
Fumarate
for
Fluoroacetate
Aconitase
Fe2+
H2O
Cis-aconitate
2
FADH2
Succinate dehydrogenase
FAD
6
Malonate
H2O
Aconitase
Fe2+
is
substrate
Succinate
Isocitrate
ATP
CoA — SH
Mg2+
ADP + Pi
5
Succinate thiokinase
starting
NAD+
NADH + H+
Isocitrate dehydrogenase
Arsenite
Krebs
Succinyl-CoA
NADH + H+
NAD+
CO2
Oxalosuccinate
α-Ketoglutarate dehydrogenase complex
CoA — SH
Mn2+
Isocitrate dehydrogenase
3
4
CO2
α-Ketoglutarate

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co_2

Reaction (1)

- The initial reaction between **acetyl-CoA** and **oxaloacetate** to form **citrate** is catalyzed by **citrate synthase**, which forms a carbon-carbon bond between the methyl carbon of acetyl CoA and the carbonyl carbon of oxaloacetate .
- The thioester bond of the resultant citryl CoA is hydrolyzed, releasing **citrate** and CoASH (an **exothermic reaction)**.

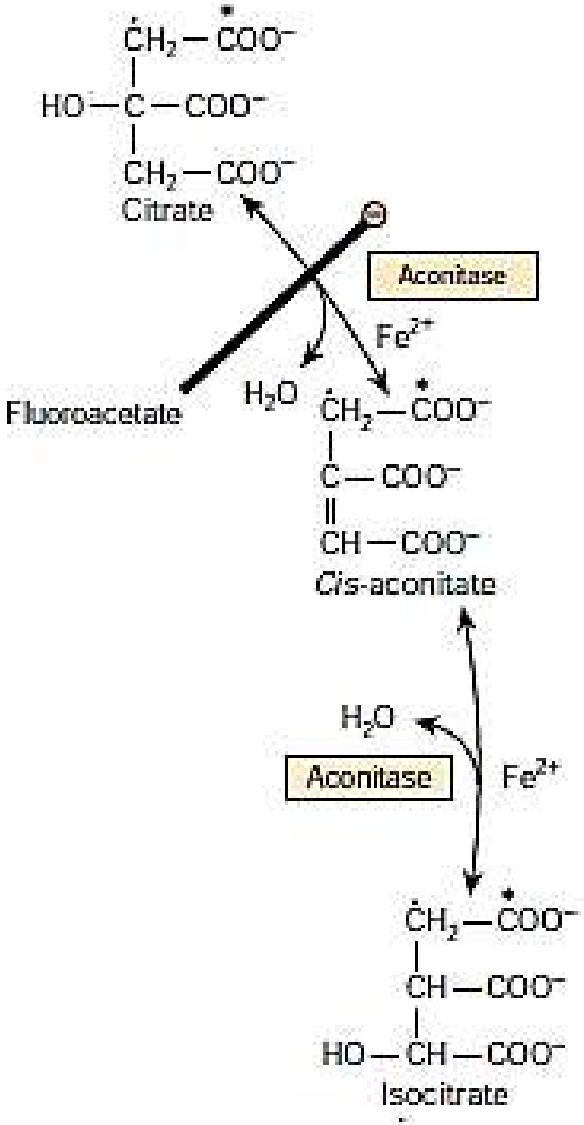


The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co2

Reaction (2)

- **Citrate** is **isomerized** to **isocitrate** by the enzyme **aconitase (aconitate hydratase)**; the reaction occurs in **two steps**: **dehydration** to cis-aconitate and **rehydration** to isocitrate.
- Although citrate is a symmetric molecule, aconitase reacts with citrate asymmetrically, so that **the two carbon atoms that are lost in subsequent reactions of the cycle are not those that were added from acetyl-CoA.** This asymmetric behavior is the result of channeling—transfer of the product of **citrate synthase** directly onto the active site of **aconitase**, without entering free solution.
- This provides **integration of citric acid cycle activity** and **the provision of citrate in the cytosol as a source of acetyl- CoA for fatty acid synthesis.**
- The poison **fluoroacetate is toxic**, because **fluoroacetyl-CoA** condenses with **oxaloacetate** to form **fluorocitrate**, which **inhibits aconitase, causing citrate to accumulate**.

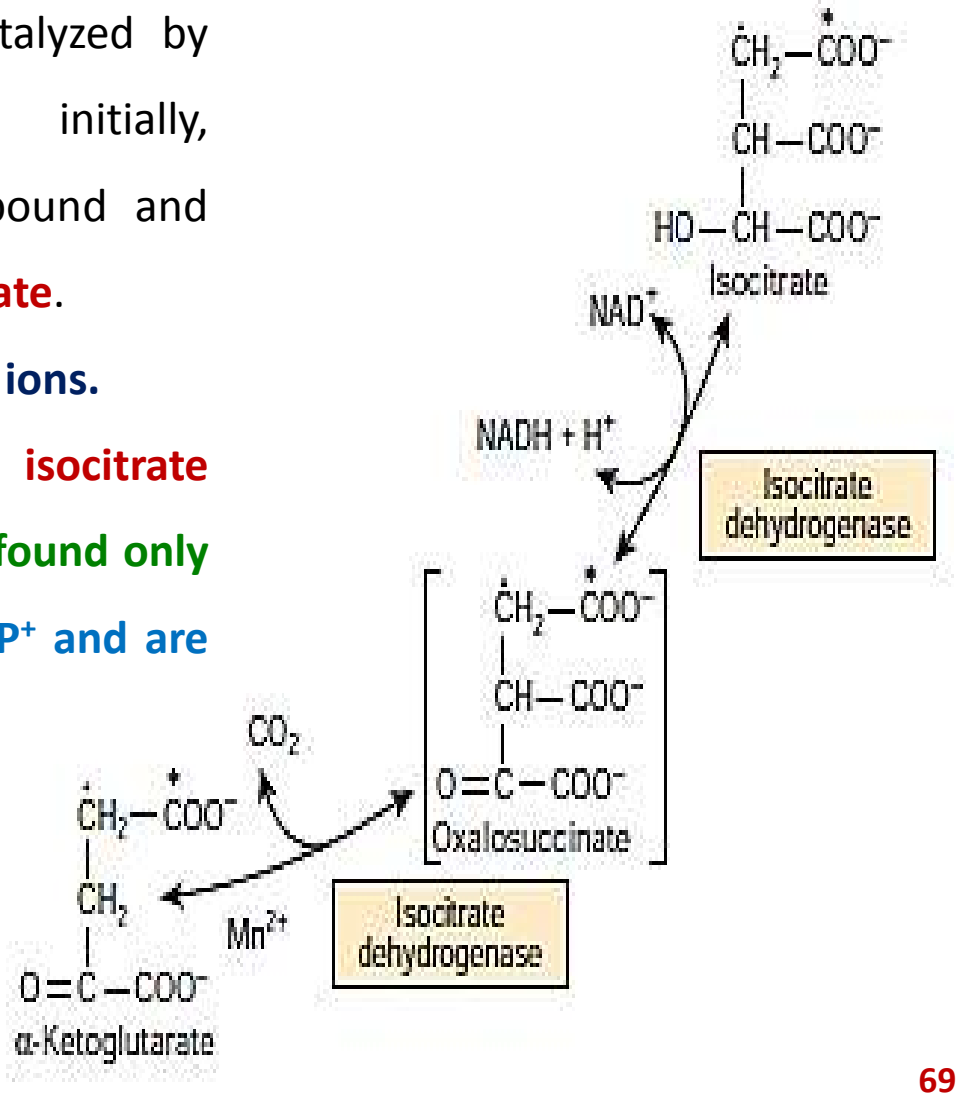


The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co2

Reaction (3)

- **Isocitrate** undergoes **dehydrogenation** catalyzed by **isocitrate dehydrogenase** to form, initially, **oxalosuccinate**, which remains enzyme bound and undergoes **decarboxylation** to **α-ketoglutarate**.
- **The decarboxylation requires Mg^{++} or Mn^{++} ions.**
- There are **three isoenzymes of isocitrate dehydrogenase**. **One, which uses NAD^+, is found only in mitochondria**. The other **two use $NADP^+$ and are found in mitochondria and the cytosol**.
- **Respiratory-chain-linked oxidation of isocitrate proceeds almost completely through the NAD^+-dependent enzyme.**

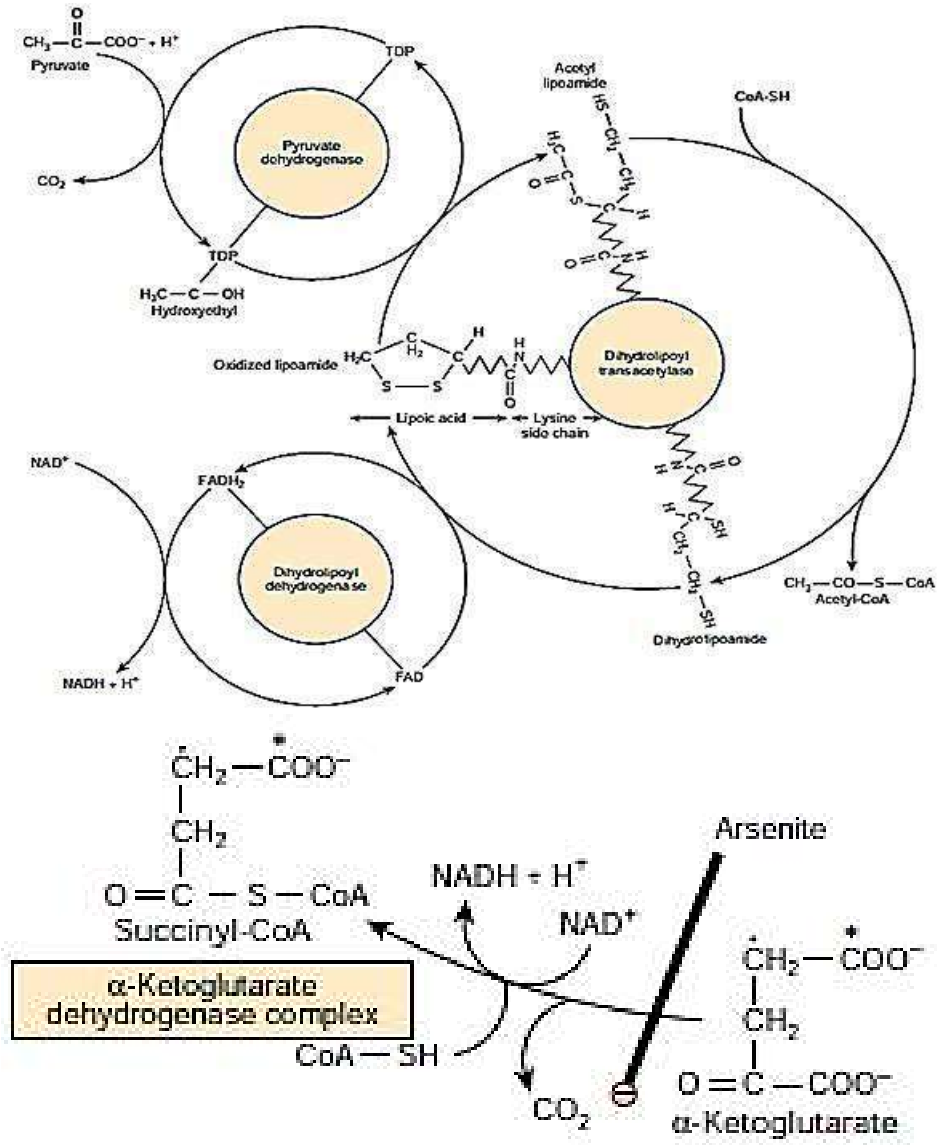


The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co2

Reaction (4)

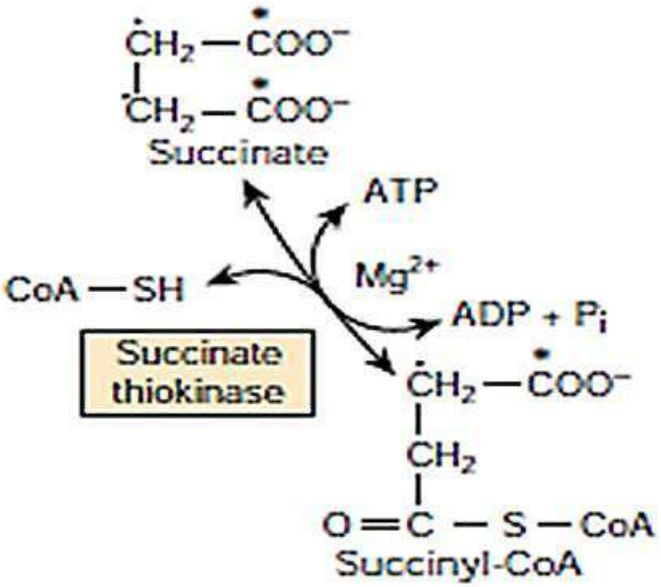
- **α-Ketoglutarate** undergoes **oxidative decarboxylation** in a reaction catalyzed by a **multi-enzyme complex** similar to that involved in the **oxidative decarboxylation of pyruvate**.
- The **α-ketoglutarate dehydrogenase complex** requires the same cofactors as the **pyruvate dehydrogenase complex**— **thiamin diphosphate, lipoate, NAD^+, FAD, and CoA** and results in the formation of **succinyl-CoA**.
- **The equilibrium of this reaction is so much in favor of succinyl-CoA formation that it must be considered to be physiologically unidirectional**.
- As in the case of pyruvate oxidation, **arsenite inhibits** the reaction, causing the substrate, **α-ketoglutarate**, to **accumulate**.



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co2

Reaction (5)



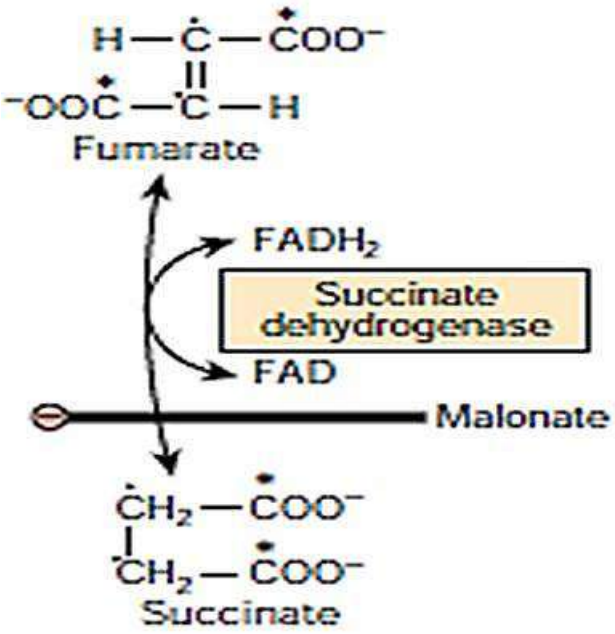
- **Succinyl-CoA** is converted to **succinate** by the enzyme **succinate thiokinase (succinyl-CoA synthetase)**.
- **This is the only example in the citric acid cycle of substrate-level phosphorylation**.
- Tissues in which **gluconeogenesis** occurs (**the liver and kidney**) contain **two isoenzymes of succinate thiokinase**, one specific for **GDP** and the other for **ADP**.
- The **GTP** formed is **used** for the **decarboxylation of oxaloacetate** to **phosphoenolpyruvate** in **gluconeogenesis**, and **provides** a **regulatory link between citric acid cycle activity** and the **withdrawal** of **oxaloacetate for gluconeogenesis**.
- **Nongluconeogenic tissues have only the isoenzyme that uses ADP**.
- When **ketone bodies** are being **metabolized** in **extrahepatic tissues (أنسجة خارج الكبد)**there is an alternative reaction catalyzed by **succinyl-CoA-acetoacetate-CoA transferase (thiophorase)**, involving **transfer of CoA from succinyl-CoA to acetoacetate, forming acetoacetyl-CoA**.

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co2

Reaction (6)

- The onward metabolism of **succinate**, leading to the regeneration of **oxaloacetate**, is the same sequence of chemical reactions as occurs in the β-oxidation of fatty acids: **dehydrogenation** to form a carbon-carbon double bond (**fumarate**), **addition of water** to form a hydroxyl group (**malate**), and a further **dehydrogenation** to yield the oxo-group of **oxaloacetate**.
- The **first dehydrogenation** reaction, forming **fumarate**, is catalyzed by **succinate dehydrogenase**, which is bound to the inner surface of the inner mitochondrial membrane.
- **The enzyme** contains **FAD** and **iron-sulfur (Fe:S) protein,** and directly **reduces ubiquinone** in the **electron transport chain.**

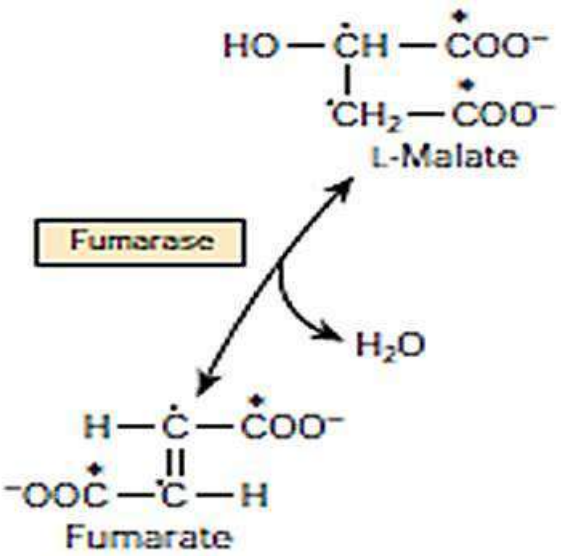


The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Reactions of the citric acid cycle liberate reducing equivalents & co2

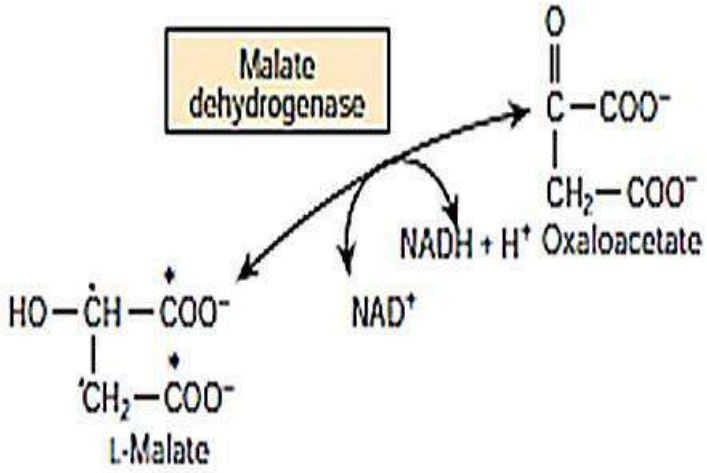
Reaction (7)

- **Fumarase (fumarate hydratase)** catalyzes the addition of water across the double bond of **fumarate**, yielding **malate**.



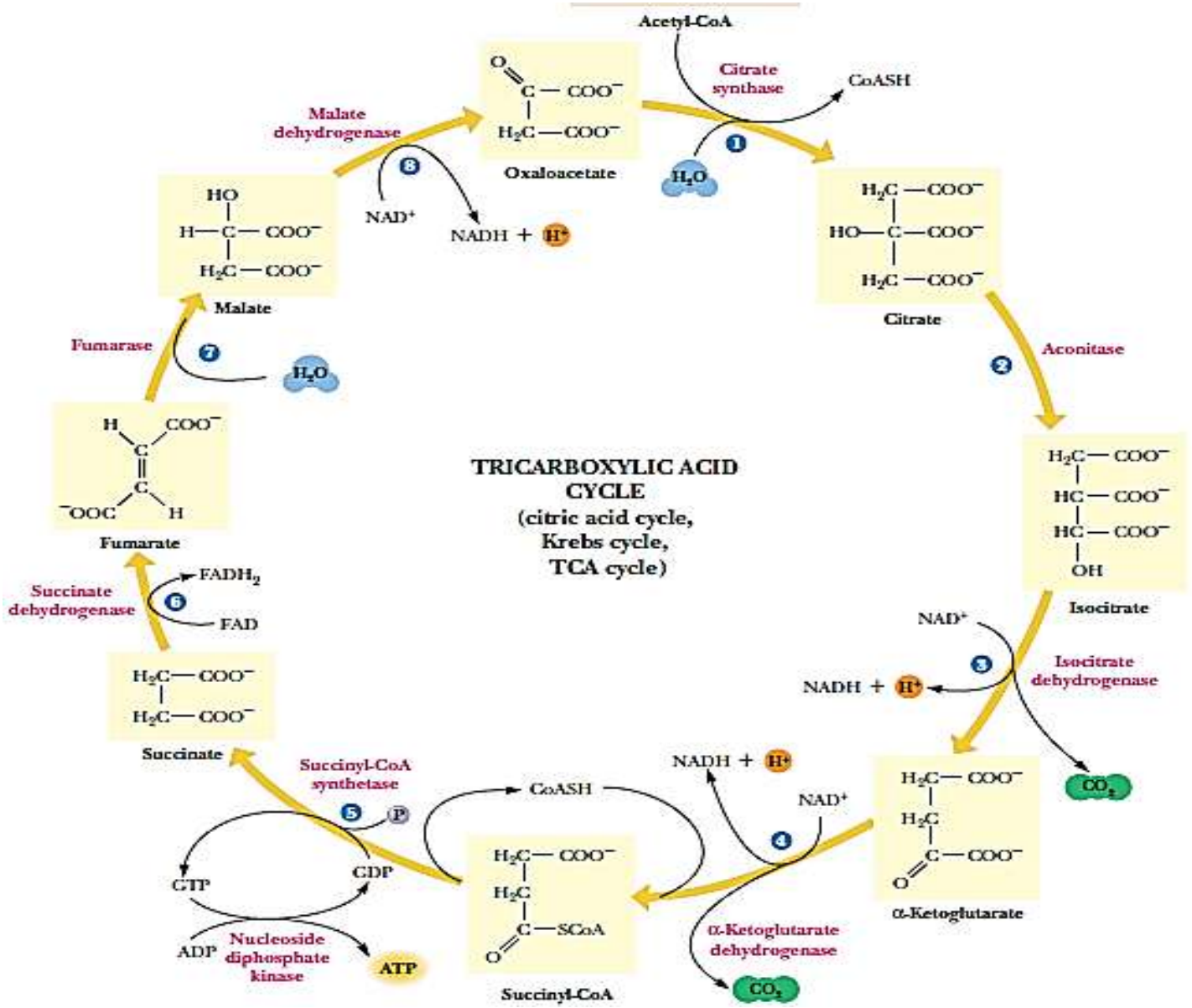
Reaction (8)

- **Malate** is converted to **oxaloacetate** by **malate dehydrogenase**, a reaction **requiring NAD^+**.
- Although **the equilibrium** of this reaction strongly **favors malate**, the net flux is to **oxaloacetate** because of the continual **removal of oxaloacetate** (to form citrate, as a **substrate for gluconeogenesis**, or to undergo **transamination to aspartate**) and also the continual **reoxidation of NADH**.



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

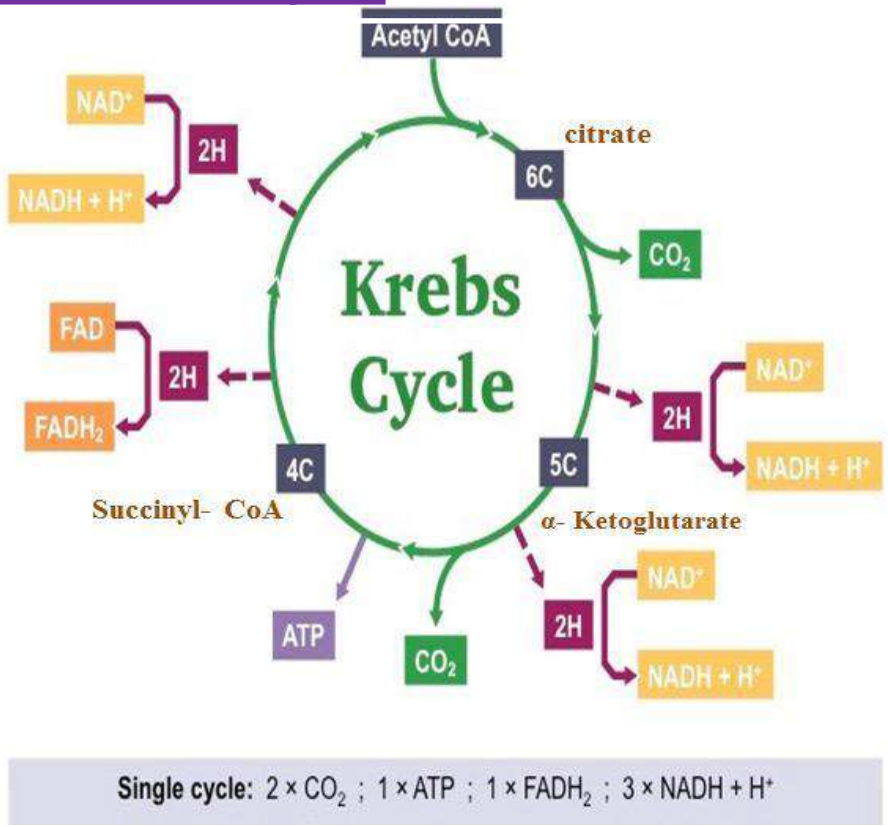
Reactions of the citric acid cycle liberate reducing equivalents & co2



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Ten ATP are formed per turn of the citric acid cycle

- As a result of oxidations catalyzed by the **dehydrogenases** of the citric acid cycle, **three molecules of NADH** (**reactions 3-4-8**) and **one** of **FADH2** (**reaction 6**) are produced for **each molecule of acetyl-CoA** catabolized in one turn of the cycle.
- These reducing equivalents are transferred to the **respiratory chain**, where reoxidation of each **NADH results in formation of ~2.5 ATP**, and of **FADH2, ~1.5 ATP**.
- In addition, **1 ATP (or GTP)** (**reaction 5**) is formed by **substrate-level phosphorylation** catalyzed by **succinate thiokinase**.



Reaction (3+4+8)	=>	3NADH = 3*2.5 ATP	= 7.5 ATP
Reaction (6)	=>	$FADH_2$ =	1.5 ATP
Reaction (5)	=>	GTP =	1 ATP
		Sum	10 ATP

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

ATP From Oxidation of Glucose:

- **Oxidation of glucose yields up to 32 mol of ATP under aerobic conditions**, but **only 2 mol when O_2 is absent**.
- When **1 mol of glucose** is combusted **in a calorimeter to CO_2 and water**, approximately **2870 kJ** are liberated as heat.
- When **oxidation** occurs in the **tissues**, approximately **32 mol of ATP** are generated per **molecule of glucose** oxidized to CO_2 and water.
- **In vivo**, **ΔG for the ATP synthase reaction** has been calculated as approximately **51.6 kJ**.
- It follows that the total energy captured in **ATP per mole of glucose oxidized is 1651 kJ**, or approximately **58% of the energy of combustion**.
- **Most of the ATP is formed by oxidative phosphorylation resulting from the reoxidation of reduced coenzymes by the respiratory chain**.
- **The remainder is formed by substrate-level phosphorylation** (Table 18–1).

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

ATP From Oxidation of Glucose:

Pathway	Reaction Catalyzed by	Method of ATP Formation	ATP per Mol of Glucose
Glycolysis	Glyceraldehyde 3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH (6)	5*
	Phosphoglycerate kinase	Substrate level phosphorylation (7)	2
	Pyruvate kinase	Substrate level phosphorylation (10)	2
			1 X (9)
	Consumption of ATP for reactions of hexokinase and phosphofructokinase (1-3)		−2
			Net 7
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2 NADH (pyruvat oxidation)	5
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2 NADH (3)	5
	α-Ketoglutarate dehydrogenase	Respiratory chain oxidation of 2 NADH (4)	5
	Succinate thiokinase	Substrate level phosphorylation (5)	2
	Succinate dehydrogenase	Respiratory chain oxidation of 2 $FADH_2$ (6)	3
	Malate dehydrogenase	Respiratory chain oxidation of 2 NADH (8)	5
			Net 25
	Total per mol of glucose under aerobic conditions		32
	Total per mol of glucose under anaerobic conditions		2

*This assumes that NADH formed in glycolysis is transported into mitochondria by the malate shuttle (Figure 13–13). If the glycerophosphate shuttle is used, then only 1.5 ATP will be formed per mol of NADH. Note that there is a considerable advantage in using glycogen rather than glucose for anaerobic glycolysis in muscle, since the product of glycogen phosphorylase is glucose 1-phosphate (Figure 19–1), which is interconvertible with glucose 6-phosphate. This saves the ATP that would otherwise be used by hexokinase, increasing the net yield of ATP from 2 to 3 per glucose.

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

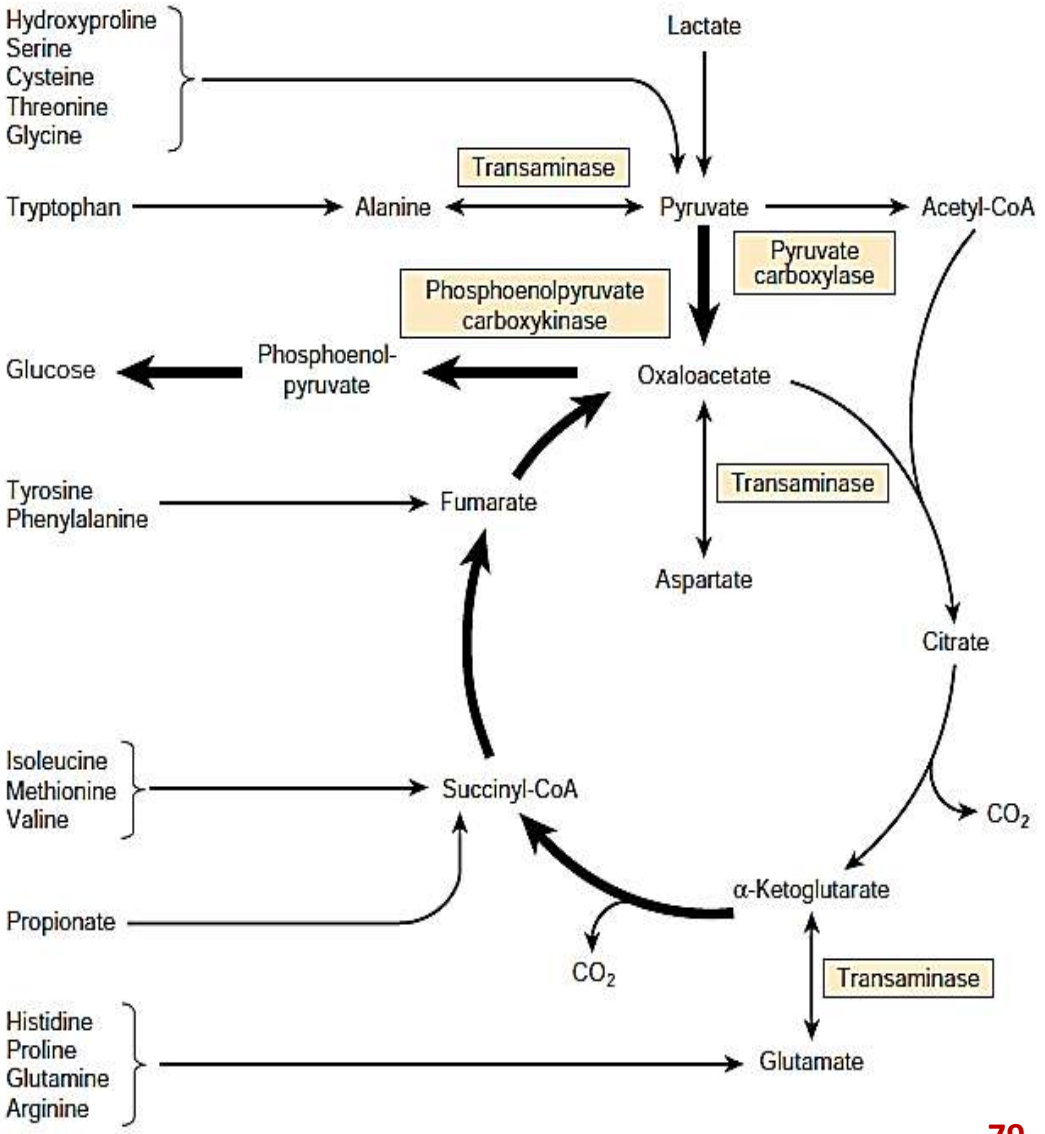
Vitamins play key roles in the citric acid cycle

- **Four of the B vitamins** are essential in the citric acid cycle and hence energy-yielding metabolism:
  1) **Riboflavin** (**vitamin B2**), in the form of **flavin adenine dinucleotide** (**FAD**), a cofactor for **succinate dehydrogenase** (**reaction 6**).
  2) **Niacin** (**vitamin B3**), in the form of **nicotinamide adenine dinucleotide** (**NAD^+**), the electron acceptor for **isocitrate dehydrogenase**, **α-ketoglutarate dehydrogenase**, and **malate dehydrogenase** (**reaction 3+4+8**).
  3) **Thiamin** (**vitamin B1**), as **thiamin diphosphate**, the coenzyme for decarboxylation in the **α-ketoglutarate dehydrogenase** reaction (**reaction 4**).
  4) **Pantothenic acid** (**vitamin B5**), as part of **coenzyme A,** the cofactor attached to "active" carboxylic acid residues such as a**cetyl-CoA** and **succinyl-CoA**.

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The citric acid cycle plays a pivotal role in metabolism

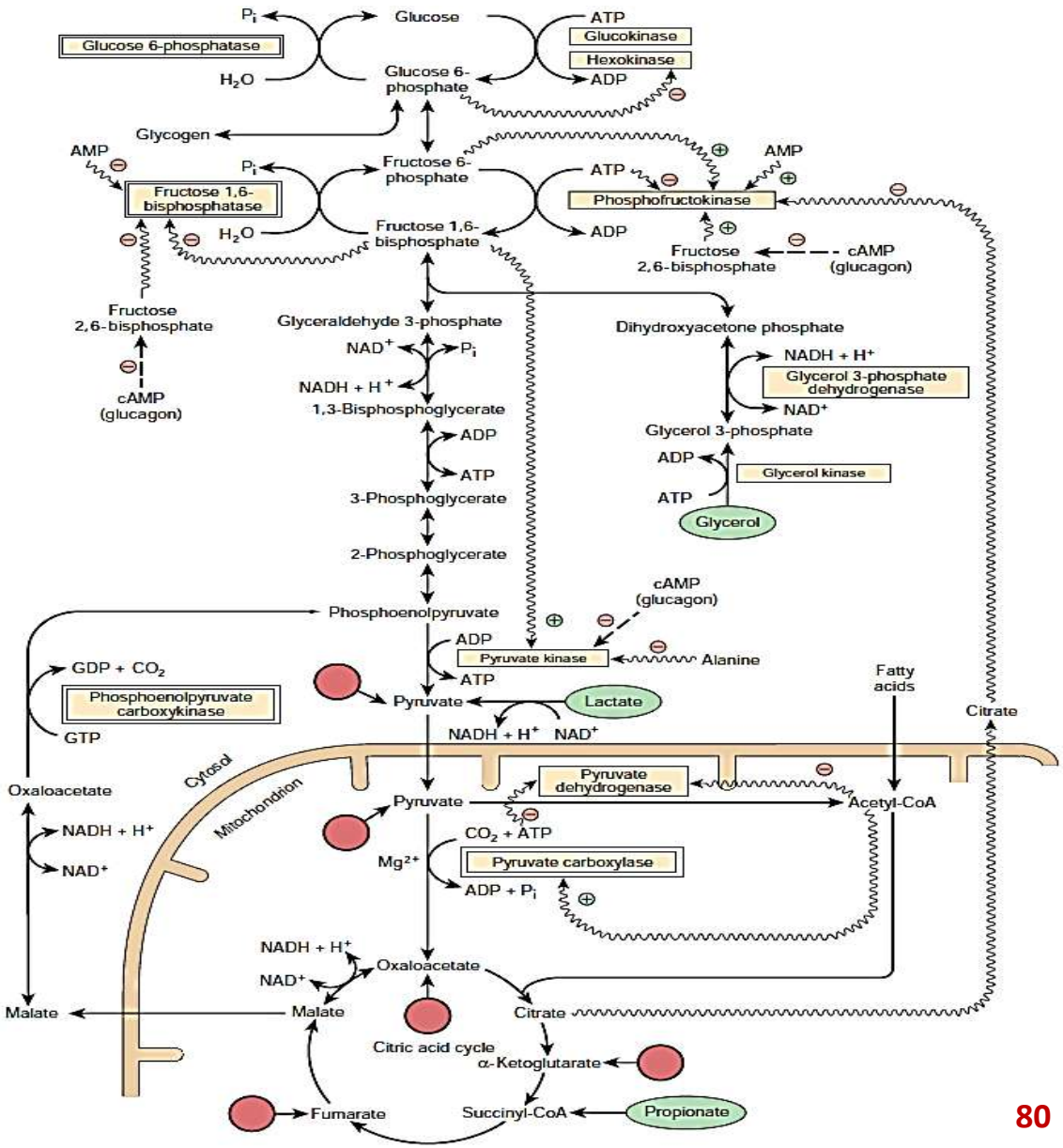
- The citric acid cycle is not only a pathway for oxidation of two carbon units, but is also a major pathway for:
  1. interconversion of metabolites arising from transamination and deamination of amino acids.
  2. providing the substrates for amino acid synthesis by transamination
  3. gluconeogenesis
  4. fatty acid synthesis.
- Because it functions in both oxidative and synthetic processes, it is amphibolic.



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The citric acid cycle takes part in gluconeogenesis, transamination, & Deamination

- **All the intermediates of the cycle** are potentially **glucogenic**, since they can **give** rise to **oxaloacetate**, and hence net production of **glucose** (in the **liver** and **kidney**, the organs that carry out gluconeogenesis /**small intestine in fasting/**).
- **The key enzyme** that catalyzes net transfer **out of the cycle** into gluconeogenesis is **phosphoenolpyruvate carboxykinase**, which catalyzes the **decarboxylation of oxaloacetate** to **phosphoenolpyruvate**, with **GTP acting as the phosphate donor**.



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The citric acid cycle takes part in gluconeogenesis, transamination, & Deamination

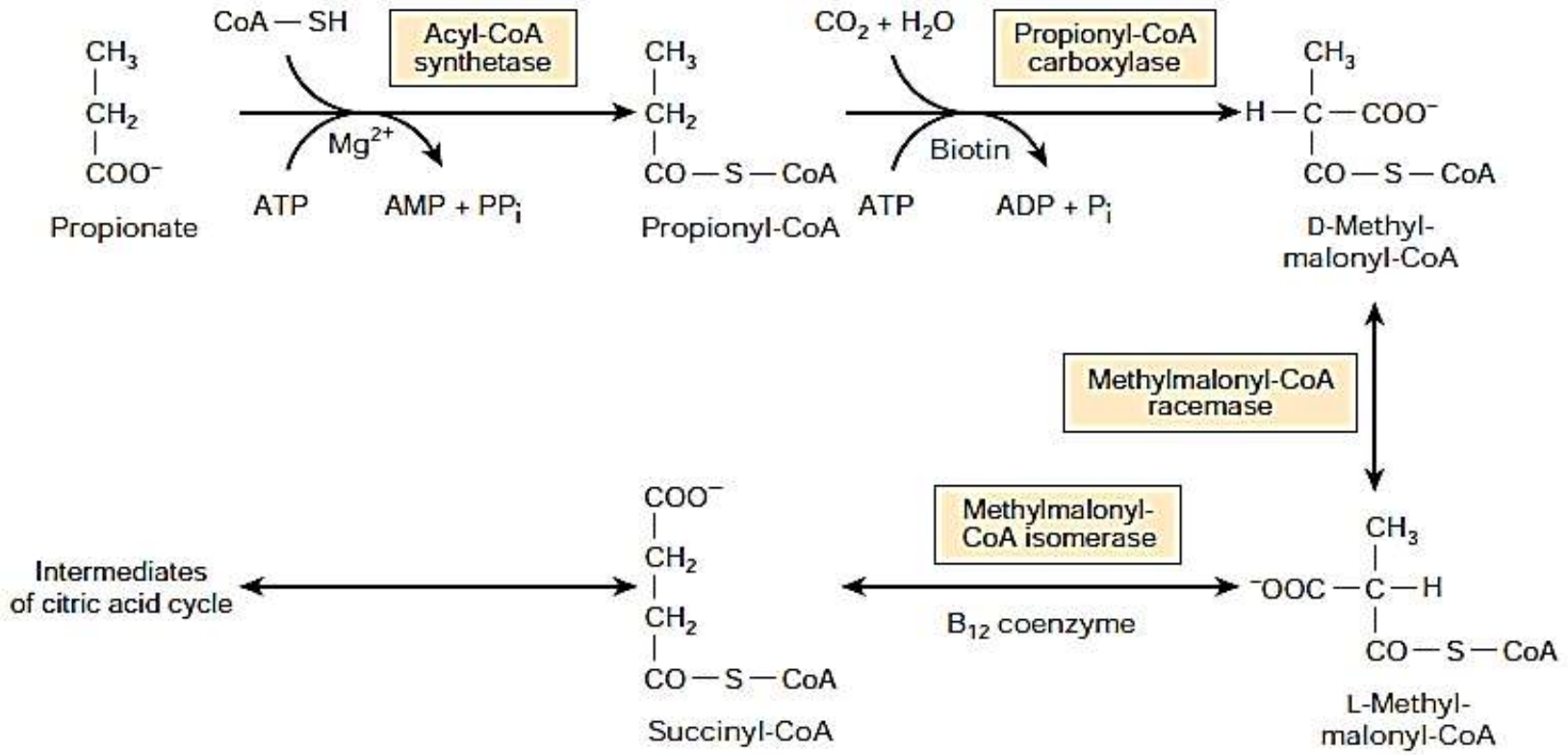
- Net transfer into the cycle occurs as a result of several reactions. Among the most important of such anaplerotic reactions is the **formation** of **oxaloacetate** by:

1. the **carboxylation** of **pyruvate** to **oxaloacetate** , catalyzed by **pyruvate carboxylase**. This reaction is important in maintaining an adequate concentration of **oxaloacetate** for the condensation reaction with acetyl-CoA. **If acetyl-CoA accumulates**, it acts as both an **allosteric activator of pyruvate carboxylase** and an **inhibitor of pyruvate dehydrogenase**, thereby ensuring a supply of oxaloacetate.
2. **Lactate** enters the cycle via **oxidation to pyruvate** , and then **carboxylation to oxaloacetate**.
3. **Aminotransferase (transaminase) reactions** form **pyruvate from alanine**, **oxaloacetate from aspartate**, and **α-ketoglutarate from glutamate**. Because these reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of these amino acids.
4. **Other amino acids (18 glucogenic amino acid)** contribute to gluconeogenesis because **their carbon skeletons give rise to citric acid cycle intermediates**. Alanine, cysteine, glycine, hydroxyproline, serine, threonine, and tryptophan yield **pyruvate**; arginine, histidine, glutamine, and proline yield **α-ketoglutarate**; isoleucine, methionine, and valine yield **succinyl-CoA**; tyrosine and phenylalanine yield **fumarate** . (**the exceptions are leucine and lysine)**

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The citric acid cycle takes part in gluconeogenesis, transamination, & Deamination

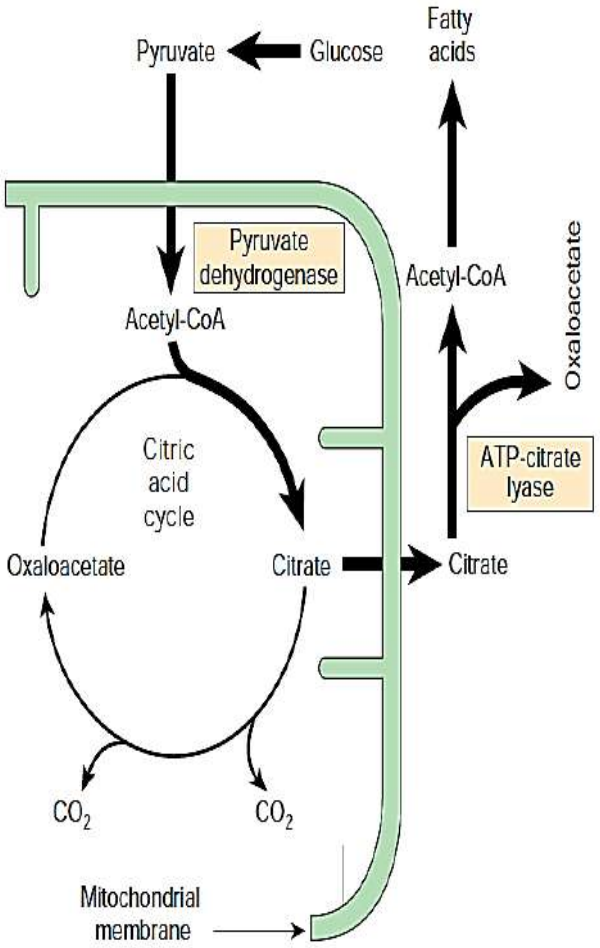
5) **In ruminants,** whose main metabolic fuel is short-chain fatty acids formed by bacterial fermentation, (including **human beings**, propionate arises from the **β-oxidation of odd-chain and branched-chain fatty acids** is a (relatively minor) substrate for gluconeogenesis)the conversion of **propionate,** the major glucogenic product of rumen fermentation, **to succinyl-CoA** via the **methylmalonyl-CoA pathway.**



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

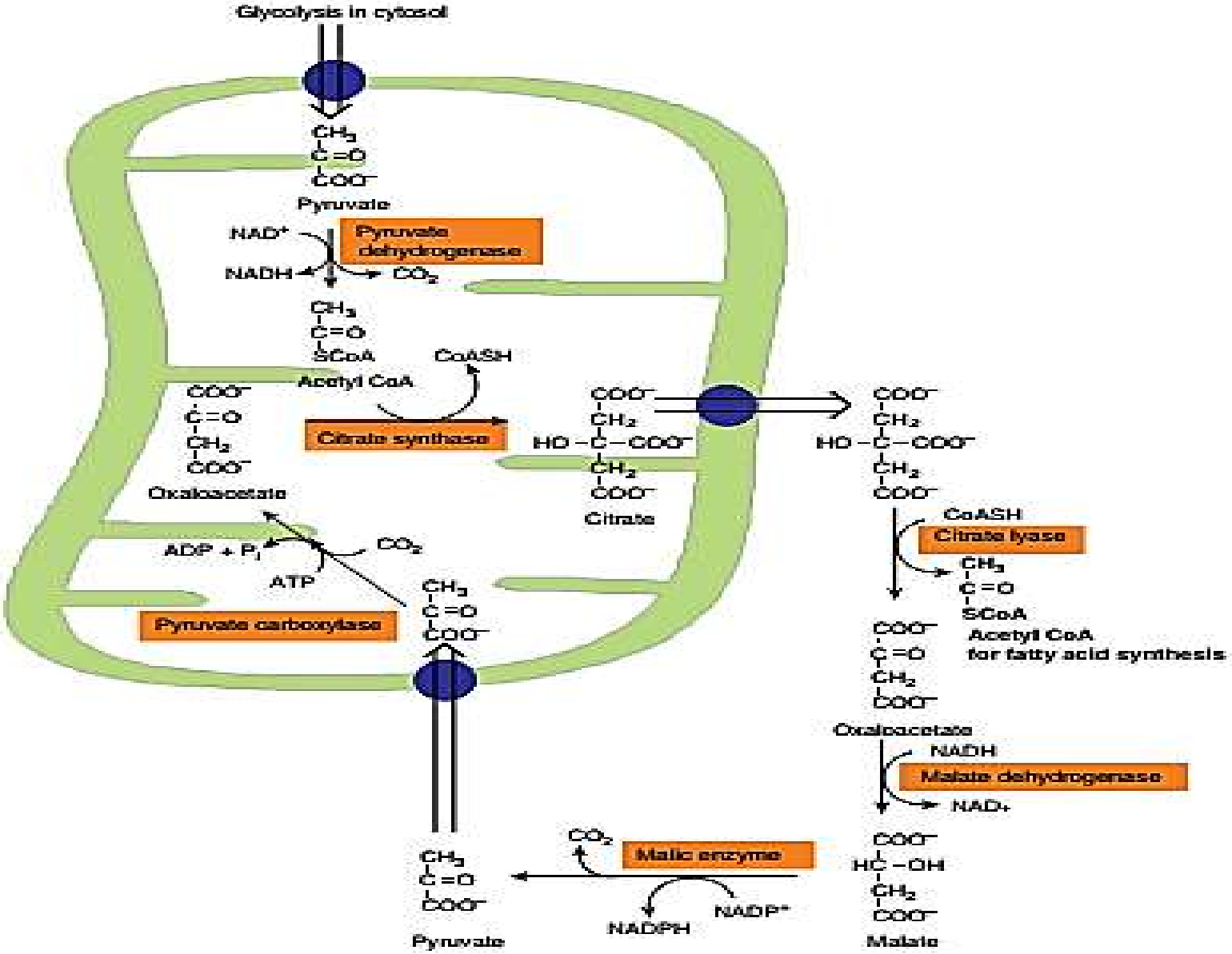
The Citric Acid Cycle Takes Part in Fatty Acid Synthesis

- **Acetyl-CoA**, formed **from pyruvate** by the action of **pyruvate dehydrogenase**, is the **major substrate for long-chain fatty acid synthesis in non ruminants.** (**In ruminants, acetyl-CoA is derived directly from acetate**).
- **Pyruvate dehydrogenase is a mitochondrial enzyme**, and **fatty acid synthesis is a cytosolic pathway**; the **mitochondrial membrane is impermeable to acetyl-CoA**.
- **Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol**, and **cleaved in a reaction catalyzed** by **ATP-citrate lyase**.
- **Citrate is only available for transport out of the mitochondrion when aconitase is saturated with its substrate**, and **citrate cannot be channeled directly from citrate synthase onto aconitase**.
- This ensures that **citrate is used for fatty acid synthesis only when there is an adequate amount to ensure continued activity of the cycle**.



The Citric Acid Cycle: The Catabolism of Acetyl-CoA

The Citric Acid Cycle Takes Part in Fatty Acid Synthesis



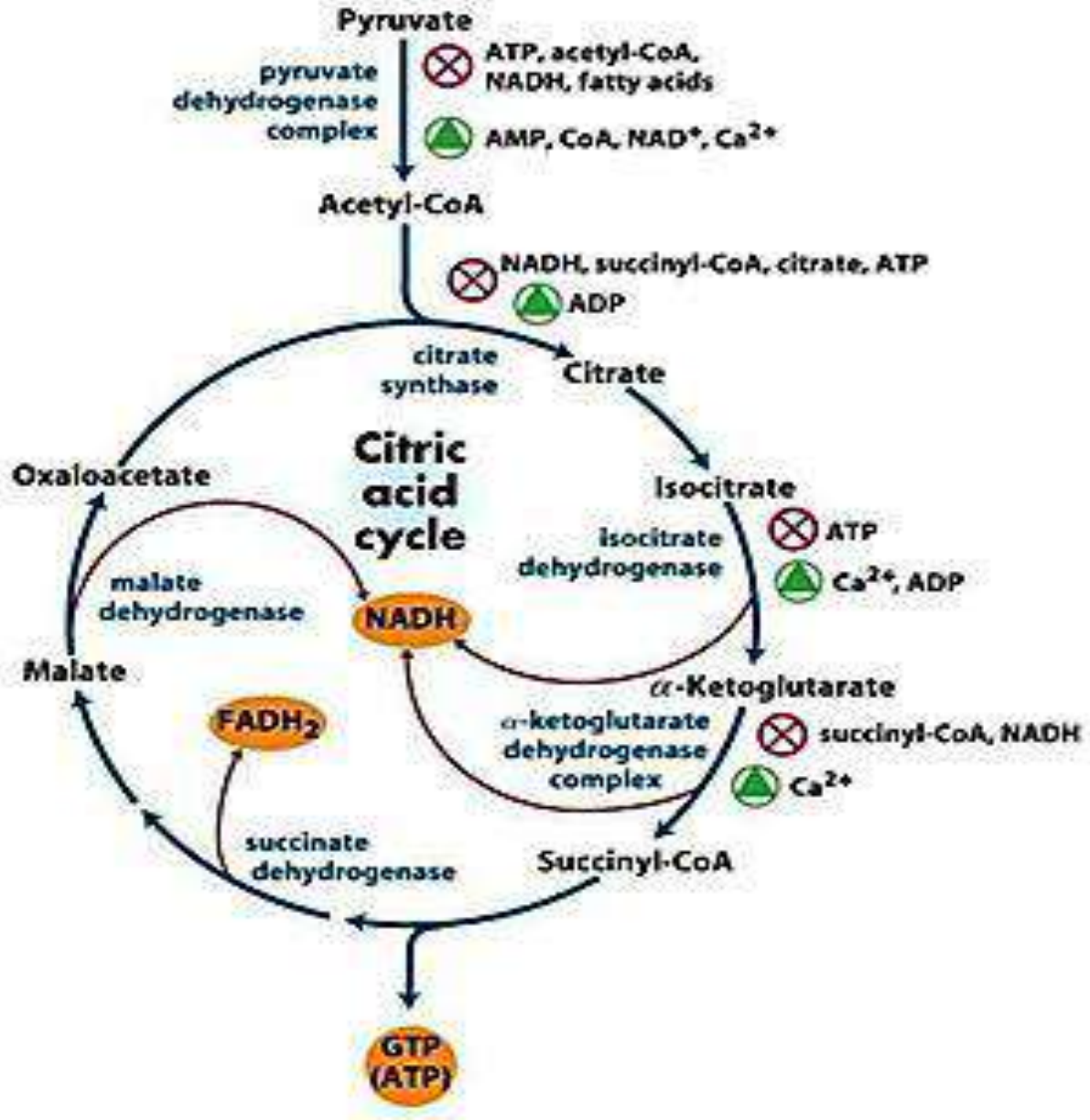
The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

- **In most tissues**, where the primary role of the citric acid cycle is in **energy-yielding metabolism**, **respiratory control** via the **respiratory chain and oxidative phosphorylation regulates citric acid cycle activity** . Thus, **activity** is immediately **dependent on** the **supply of** NAD^+, which in turn, because of the tight coupling between oxidation and phosphorylation, **is dependent on** the **availability of ADP** and hence, ultimately on **the rate of utilization of ATP in chemical and physical work**.
- In addition, **individual enzymes** of the cycle are regulated.
- The most likely sites for regulation are the **nonequilibrium reactions** catalyzed by **pyruvate dehydrogenase**, **citrate synthase (1)**, **isocitrate dehydrogenase (3)**, and **α-ketoglutarate dehydrogenase (4).**
- The **dehydrogenases** are activated by Ca^{2+}, which increases in concentration during **muscular contraction** and **secretion**, when there is **increased energy demand**.
- In a tissue such as **brain**, which is largely **dependent on carbohydrate** to supply **acetyl-CoA**, **control of the citric acid cycle may occur at pyruvate dehydrogenase**.

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

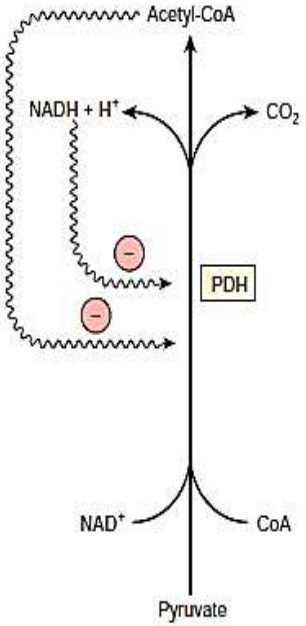
Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors



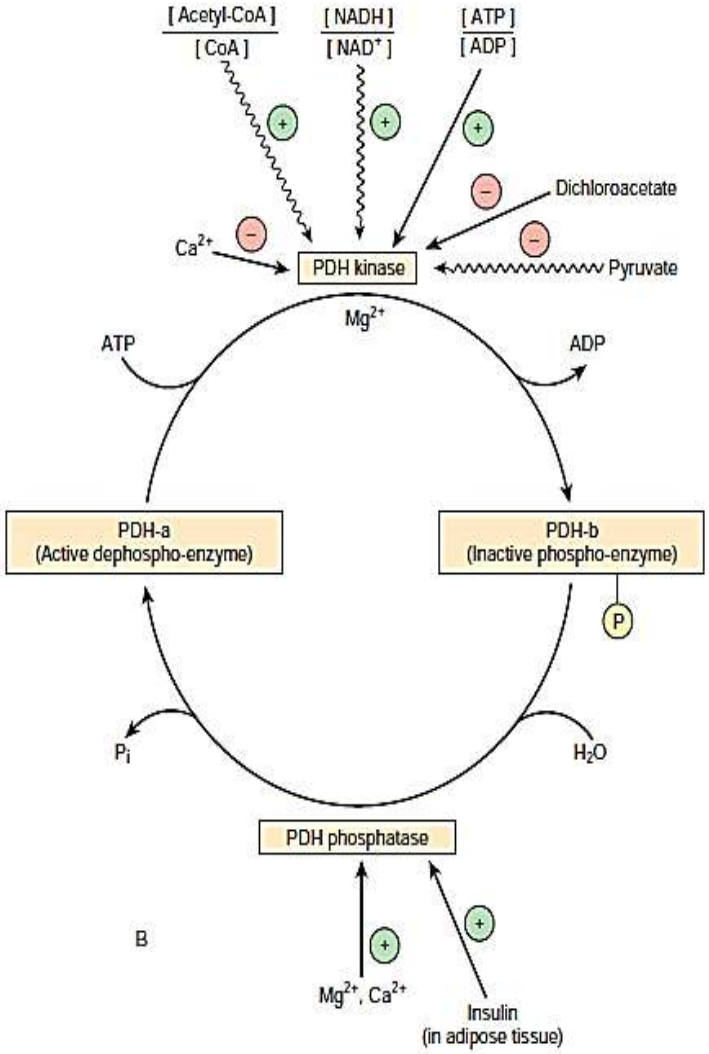
The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

- **Several enzymes are responsive to the energy status** as shown by the **[ATP]/[ADP]** and **[NADH]/[NAD+]** ratios.
- Thus, there is **allosteric inhibition of citrate synthase (Reaction 1)** by **ATP** and **long-chain fatty acyl-CoA**.
- **Allosteric activation of mitochondrial NAD-dependent isocitrate dehydrogenase (Reaction 3)** by **ADP** is **counteracted** by **ATP** and **NADH**.
- **The α-ketoglutarate dehydrogenase complex is regulated in the same way as is pyruvate dehydrogenase**.



A



B

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

- **Succinate dehydrogenase (Reaction 6)** is **inhibited by oxaloacetate**, and the availability of oxaloacetate, as controlled by **malate dehydrogenase (Reaction 8)** , **depends on the [NADH]/[NAD+] ratio.**
- Since the **Km** for oxaloacetate of **citrate synthase** is of the same order of magnitude as the intramitochondrial concentration, it is likely that **the concentration of oxaloacetate controls the rate of citrate formation**. Which of these mechanisms are important in vivo is still to be resolved.
- **Hyperammonemia (فرط أمونيا الدم)**, as occurs in **advanced liver disease** and a number of (rare) **genetic diseases of amino acid metabolism**, leads to **loss of consciousness**, **coma** and **convulsions (تشنجات)**, and may be **fatal**. This is largely because of the withdrawal of **α-ketoglutarate** to form glutamate (catalyzed by **glutamate dehydrogenase**) and then glutamine (catalyzed by **glutamine synthetase**), leading to lowered concentrations of all citric acid cycle intermediates, and hence reduced generation of **ATP**.
- In addition, **ammonia** inhibits **α-ketoglutarate dehydrogenase**, and possibly also **pyruvate dehydrogenase**.

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

SUMMARY

1) **The citric acid cycle is the final pathway for the oxidation of carbohydrate, lipid, and protein**. Their common end metabolite, **acetyl-CoA, reacts with oxaloacetate to form citrate**. By a **series of dehydrogenations and decarboxylations**, **citrate is degraded, reducing coenzymes, releasing two CO2, and regenerating oxaloacetate**.

2) **The reduced coenzymes are oxidized by the respiratory chain linked to formation of ATP**. Thus, the cycle is the major pathway for the formation of ATP and is **located in the matrix of mitochondria** adjacent to the enzymes of the respiratory chain and oxidative phosphorylation.

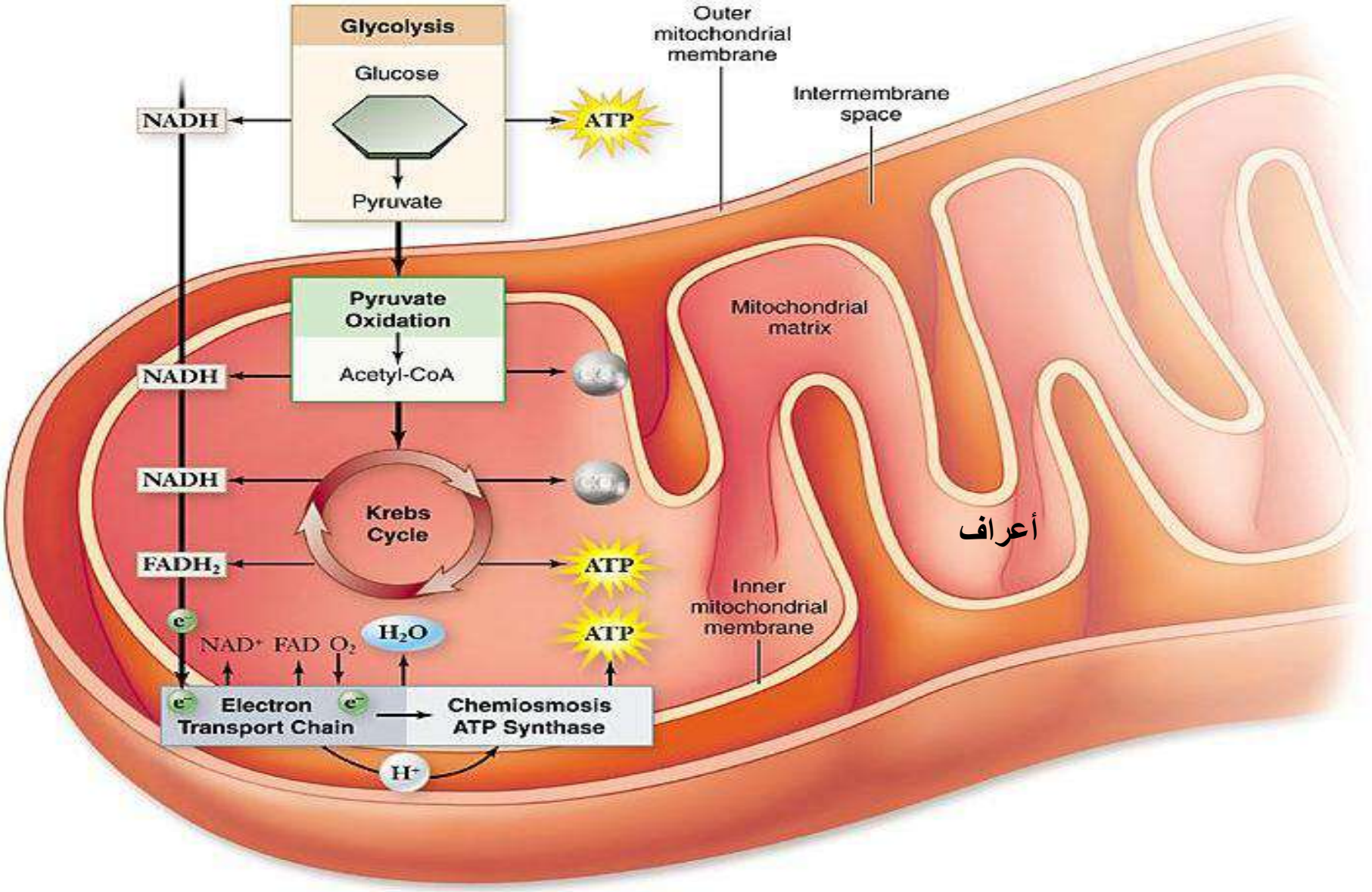
3) **The citric acid cycle is amphibolic**, since in addition to **oxidation** it is important in the **provision of carbon skeletons** for **gluconeogenesis**, **acetyl CoA** for **fatty acid synthesis**, and **interconversion of amino acids**.

The Respiratory Chain & Oxidative Phosphorylation

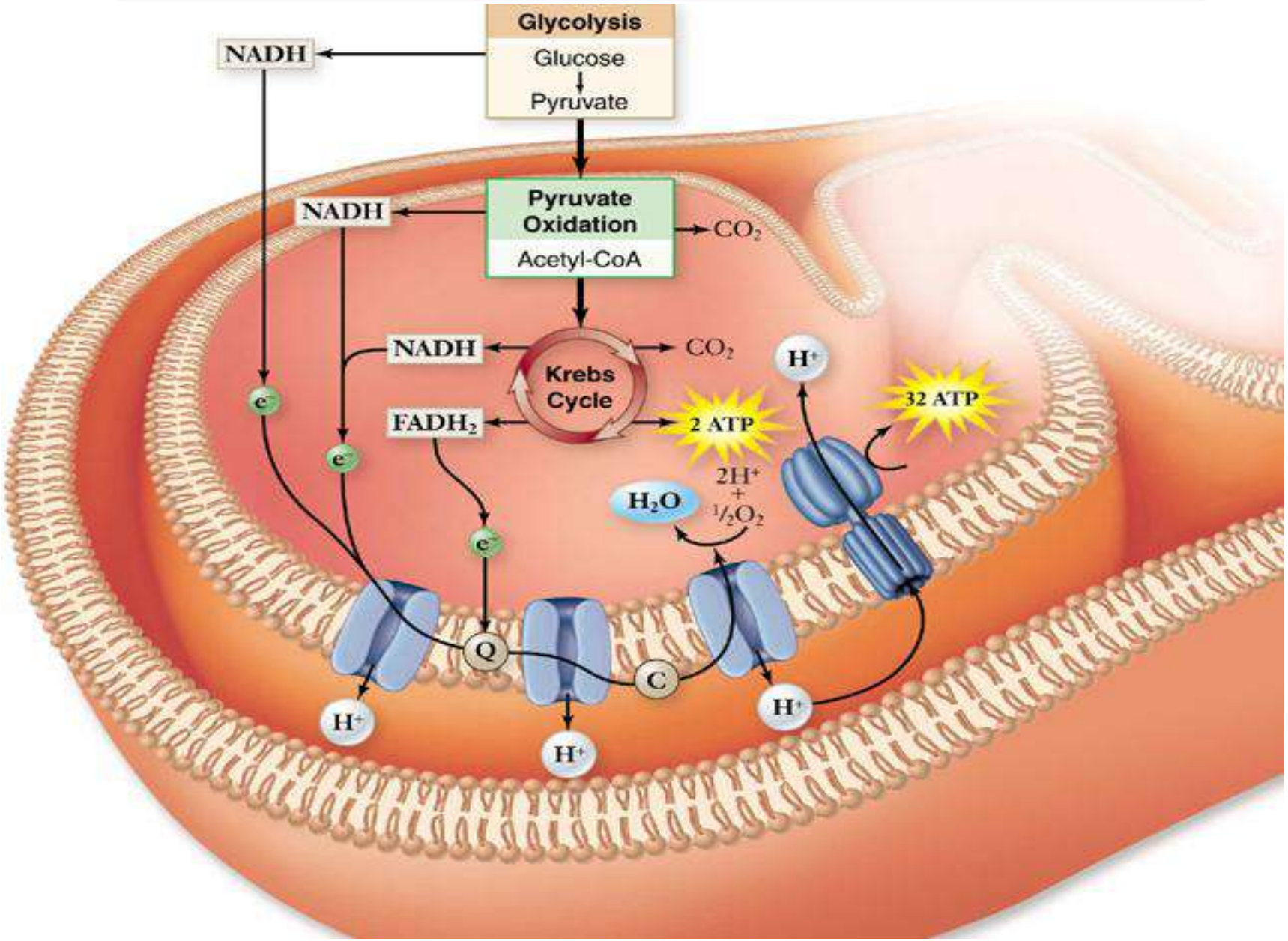
Biomedical importance

- **Aerobic organisms** are able to **capture a far greater proportion of the available free energy of respiratory substrates than anaerobic organisms**.
- Most of this takes place **inside mitochondria**, which have been termed the "**powerhouses**" of the cell.
- **Respiration is coupled to the generation of the high-energy intermediate, ATP by oxidative phosphorylation.**
- **A number of drugs** (eg, **amobarbital**) **and poisons** (eg, **cyanide**, **carbon monoxide**) **inhibit oxidative phosphorylation**, usually with **fatal consequences**.
- **Several inherited defects of mitochondria** involving components of the **respiratory chain and oxidative phosphorylation** have been reported.
- Patients present with **myopathy** (اعتلال عضلي) and **encephalopathy** (اعتلال دماغي) and often have **lactic acidosis**.

The Respiratory Chain & Oxidative Phosphorylation



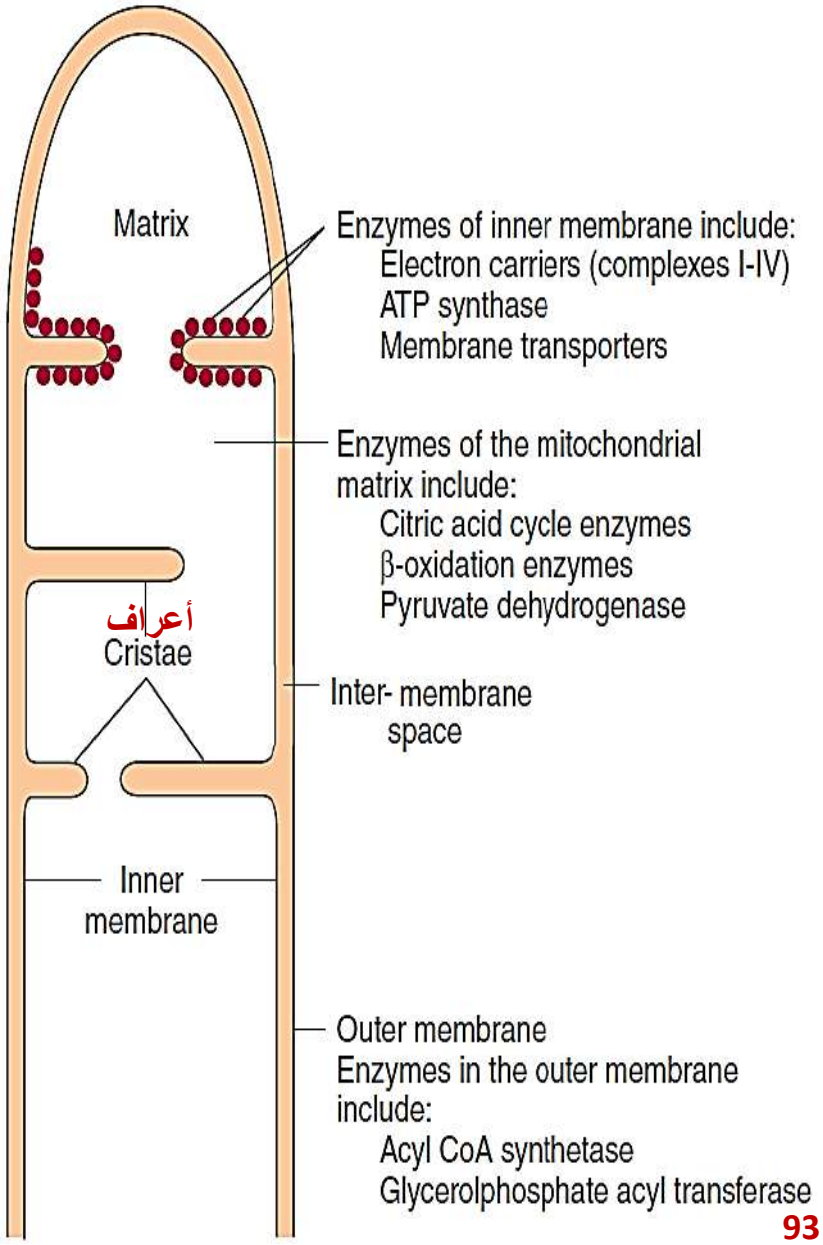
The Respiratory Chain & Oxidative Phosphorylation



The Respiratory Chain & Oxidative Phosphorylation

Specific enzymes are associated with compartments separated by the mitochondrial membranes

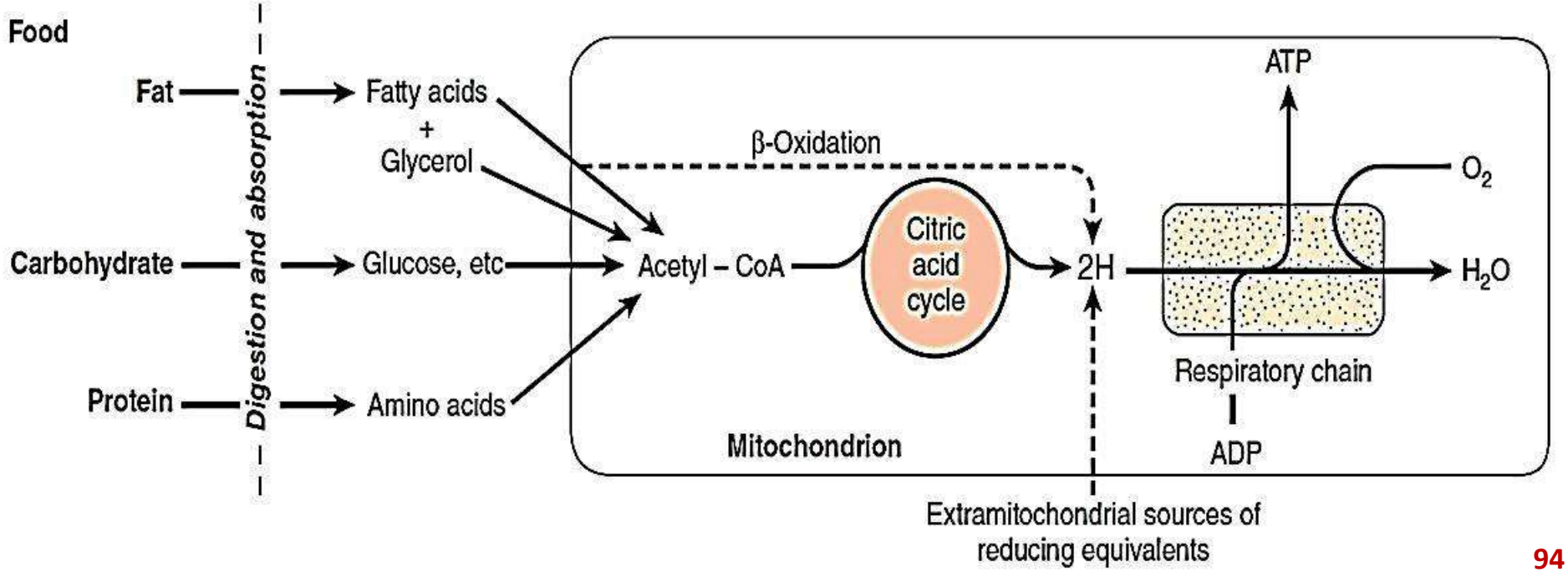
- The **Mitochondrial matrix** is **enclosed by a double membrane**.
- **The outer membrane is permeable to most metabolites** and the **inner membrane is selectively permeable**.
- The **outer membrane** is characterized by the presence of various enzymes, including **acyl-CoA synthetase** and **glycerolphosphate acyltransferase**.
- Other enzymes, including **adenylyl kinase** and **creatine kinase** are found in the **intermembrane space**.
- The **phospholipid cardiolipin** is concentrated in the **inner membrane** together with the **enzymes** of **the respiratory chain**, **ATP synthase**, and **various membrane transporters**.



The Respiratory Chain & Oxidative Phosphorylation

The respiratory chain oxidizes reducing equivalents & acts as A proton pump

- Most of the **energy liberated during the oxidation of carbohydrate**, **fatty acids**, and **amino acids** is **made available within mitochondria as reducing equivalents** (**-H** or **electrons**).
- The **enzymes** of the **citric acid cycle** and **β-oxidation**, **the respiratory chain complexes**, and **the machinery for oxidative phosphorylation** are **all found in mitochondria**.
- The **respiratory chain collects** and **transports reducing equivalents**, directing them to their **final reaction with oxygen to form water**, and **oxidative phosphorylation** is the process by which the **liberated free energy** is trapped as **high-energy phosphate**.



The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Components of the Respiratory Chain Are Contained in Four Large Protein Complexes Embedded in the Inner Mitochondrial Membrane

- **Electrons flow through** the **respiratory chain** through a **redox span** of **1.1 V** from **NAD^+/NADH** to **O_2/$2H_2O$**, **passing through three large protein complexes**:

1) **NADH-Q oxidoreductase** (**Complex I**), **where electrons are transferred from NADH** to **coenzyme Q** (**Q**) (also called **ubiquinone**)
2) **Q-cytochrome c oxidoreductase** (**Complex III**), which passes the electrons on to **cytochrome c**; and
3) **cytochrome c oxidase** (**Complex IV**), which completes the chain, passing the **electrons** to **O_2** and causing it to be **reduced** to **H_2O**

- Some substrates with **more positive redox potentials** than **NAD^+/NADH** (eg, **succinate (FADH$_2$)**) pass **electrons** to **Q** via a **fourth complex,**

4) **succinate-Q reductase** (**Complex II**), rather than **Complex I**.

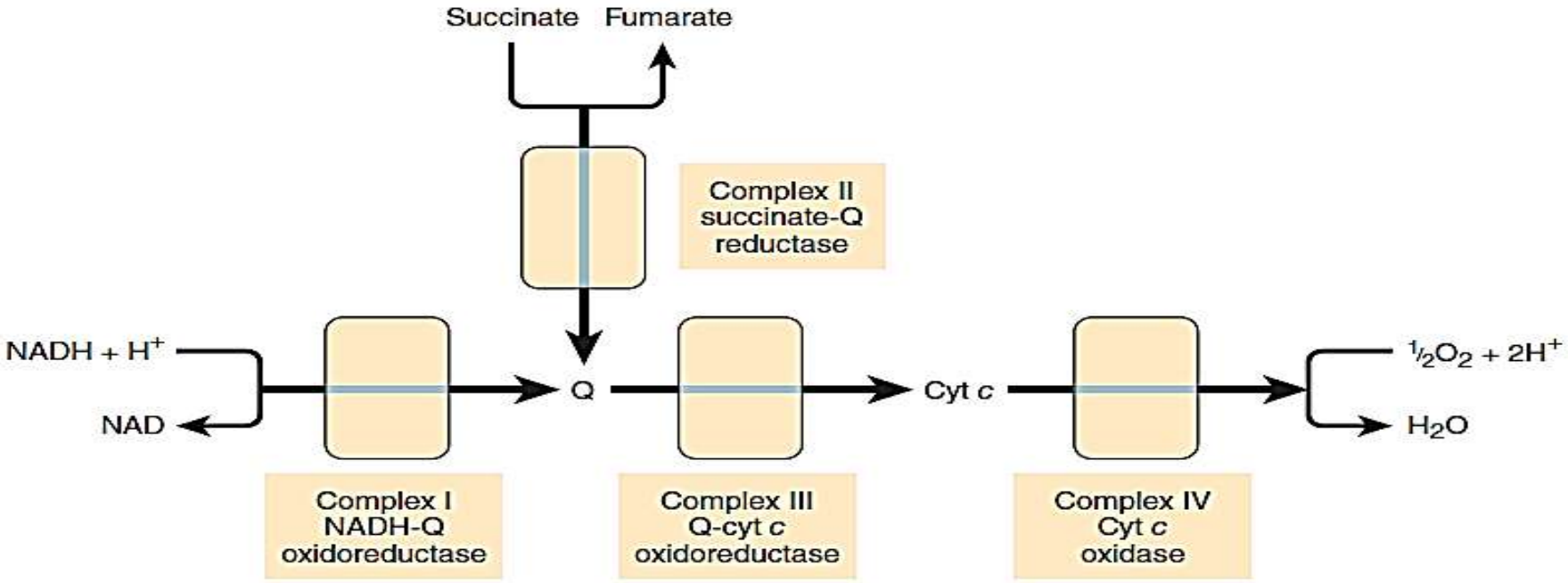
System	E'_0 Volts
H^+/H_2	−0.42
NAD^+/NADH	−0.32
Lipoate; ox/red	−0.29
Acetoacetate/3-hydroxybutyrate	−0.27
Pyruvate/lactate	−0.19
Oxaloacetate/malate	−0.17
Fumarate/succinate	+0.03
Cytochrome *b*; Fe^{3+}/Fe^{2+}	+0.08
Ubiquinone; ox/red	+0.10
Cytochrome c_1; Fe^{3+}/Fe^{2+}	+0.22
Cytochrome *a*; Fe^{3+}/Fe^{2+}	+0.29
Oxygen/water	+0.82

The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Components of the Respiratory Chain Are Contained in Four Large Protein Complexes Embedded in the Inner Mitochondrial Membrane

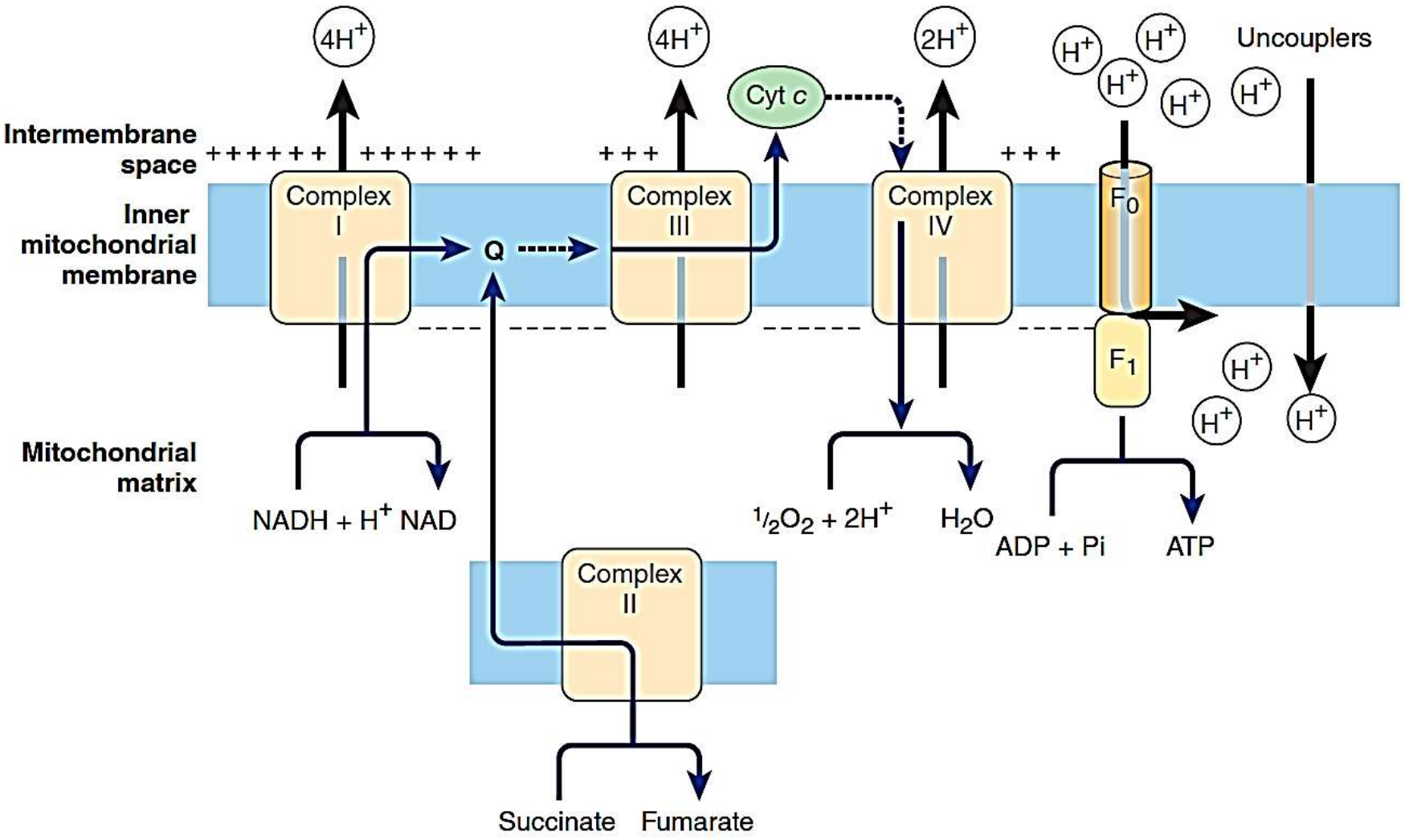
- **The four complexes are embedded in the inner mitochondrial membrane**, but **Q** and **cytochrome c** are mobile.
- **Q diffuses rapidly within the membrane**, while **cytochrome c is a soluble protein**.
- The flow of electrons through **Complexes I**, **III**, and **IV** results in the **pumping of protons from the matrix across the inner mitochondrial membrane into the intermembrane space**



The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Components of the Respiratory Chain Are Contained in Four Large Protein Complexes Embedded in the Inner Mitochondrial Membrane

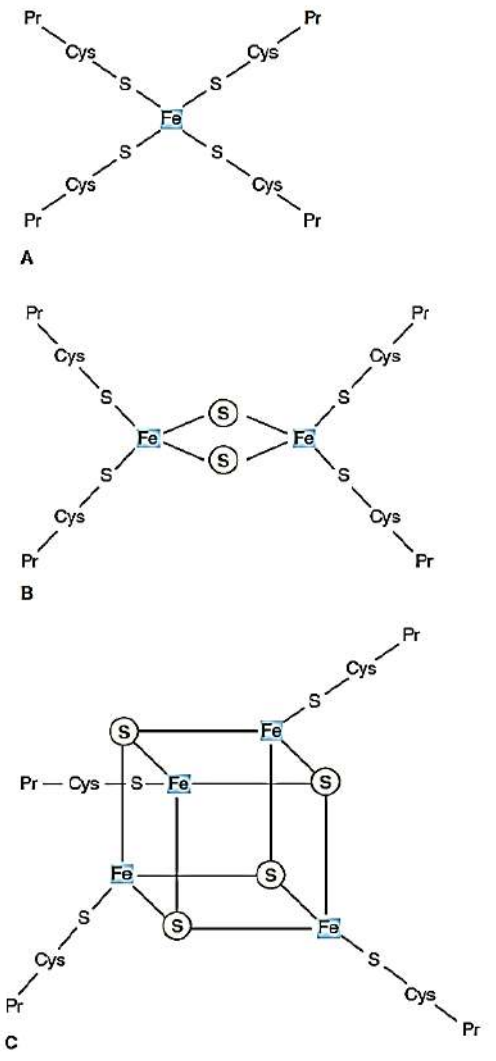


The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Flavoproteins & Iron-Sulfur Proteins (Fe-S) Are Components of the Respiratory Chain Complexes

- **Flavoproteins** (see Chapter 12) are important components of **Complexes I** and **II**.
- The **oxidized flavin nucleotide** (**FMN** or **FAD**) **can be reduced** in reactions involving the transfer of **two electrons** (to form **FMNH2** or **FADH2**), but they can also accept one electron to form the semiquinone (see Figure 12–2).
- **Ironsulfur proteins** (**nonheme iron proteins, Fe-S**) are found in **Complexes I**, **II**, and **III**.
- These may contain **one**, **two**, or **four Fe atoms** linked to **inorganic sulfur atoms** and/or via **cysteine- SH groups** **to the protein** (Figure 13–4).
- The **Fe-S** take part in **single electron transfer reactions** in which **one Fe** atom undergoes **oxidoreduction** between **Fe^{2+}** and **Fe^{3+}**.



The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Q Accepts Electrons via Complexes I & II

- **NADH-Q oxidoreductase** or **Complex I** is a large L-shaped multisubunit protein that catalyzes electron transfer from **NADH** to **Q**, coupled with the transfer of **four H^+** across the membrane:

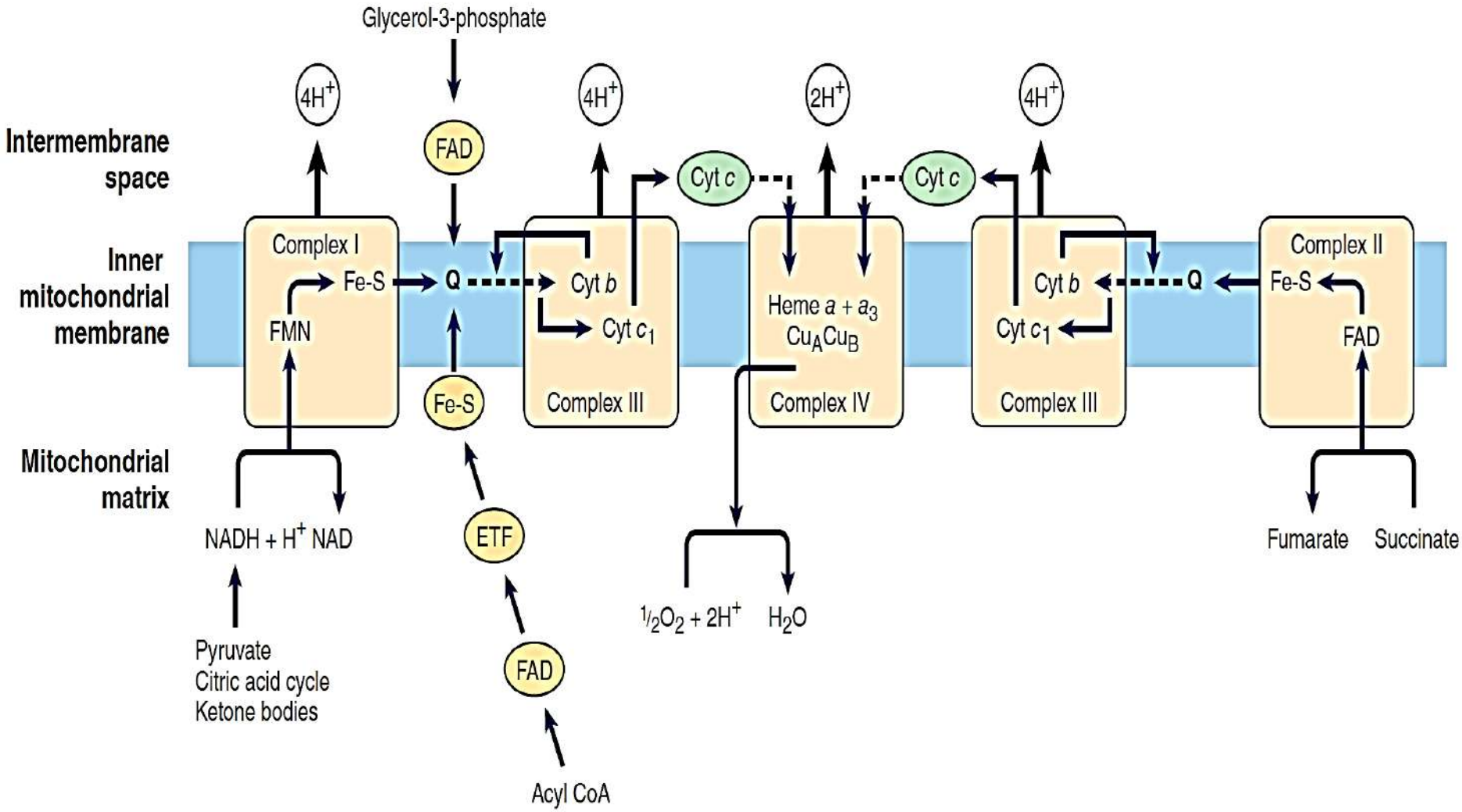
$$NADH + Q + 5H^+_{matrix} \rightarrow NAD + QH_2 + 4H^+_{intermembrane\ space}$$

- **Electrons** are transferred from **NADH** to **FMN** initially, then to a series of **Fe-S centers**, and **finally to Q** (Figure 13–5).
- **In Complex II** (**succinate-Q reductase**), **FADH2** is formed during the conversion of **succinate to fumarate** in the **citric acid cycle** (see Figure 16–3) and **electrons** are then passed via **several Fe-S centers** to **Q** (Figure 13–5).
- **Glycerol-3-phosphate** (generated in the breakdown of **triacylglycerols** or from **glycolysis**, Figure 17–2) and **acyl-CoA** also pass **electrons** to **Q** via different pathways involving **flavoproteins** (Figure 13–5).

The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Q Accepts Electrons via Complexes I & II



The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

The Q Cycle Couples Electron Transfer to Proton Transport in Complex III

- **Electrons** are passed from **QH2** to **cytochrome c** via **Complex III** (**Q-cytochrome c oxidoreductase**):

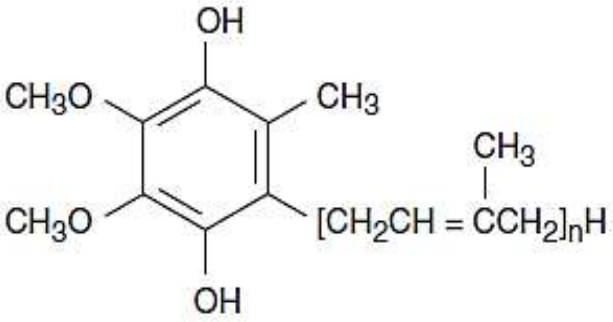
$$QH_2 + 2Cyt\,c_{oxidized} + 2H^+_{matrix} \rightarrow Q + 2Cyt\,c_{reduced} + 4H^+_{intermembrane\ space}$$

- The process is believed to involve **cytochromes** c1, **bL**, and **bH** and a **Rieske Fe-S** (**an unusual Fe-S in which one of the Fe atoms is linked to two histidine residues rather than two cysteine residues)** (Figure 13–5) and is known as the **Q cycle** (Figure 13–6).
- **Q** may exist in **three forms**: the **oxidized quinone**, **the reduced quinol**, or **the semiquinone** (Figure 13–6).
- The **semiquinone** is formed transiently during the cycle, one turn of which results in the **oxidation of 2QH2** to **Q**, releasing **4H+** into the **intermembrane space**, and the **reduction** of **one Q** to **QH2**, causing **2H+** to be taken up from the **matrix** (Figure 13–6).
- **Note that** while **Q carries two electrons**, the **cytochromes carry only one**, thus the **oxidation of one QH2** is coupled to the **reduction of two molecules of cytochrome c** via the **Q cycle**.

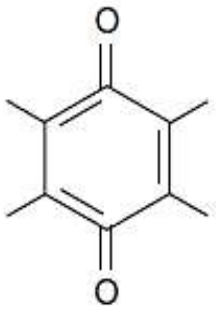
The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

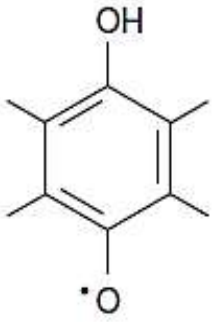
The Q Cycle Couples Electron Transfer to Proton Transport in Complex III



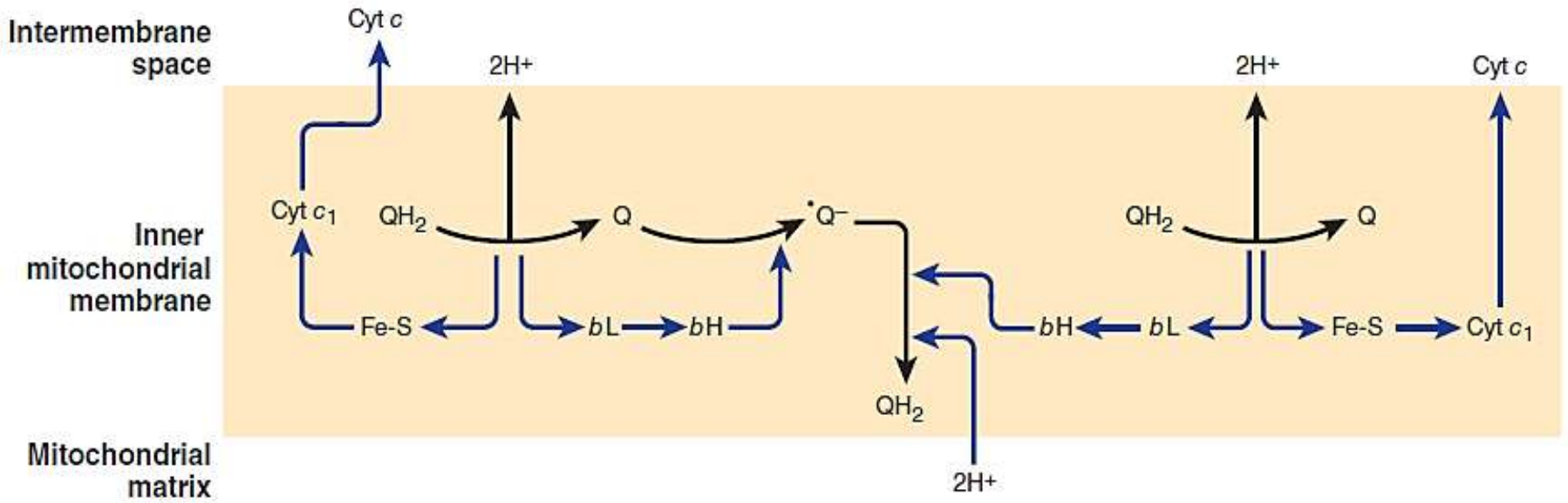
QH_2: Reduced (quinol) form (QH_2)



Q: Fully oxidized (quinone) form



$^{\bullet}Q^{-}$: Semiquinone (free radical) form



The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Molecular Oxygen Is Reduced to Water via Complex IV

- **Reduced cytochrome c is oxidized by Complex IV (cytochrome c oxidase)**, with the concomitant **reduction** of O_2 to two molecules of **water**:

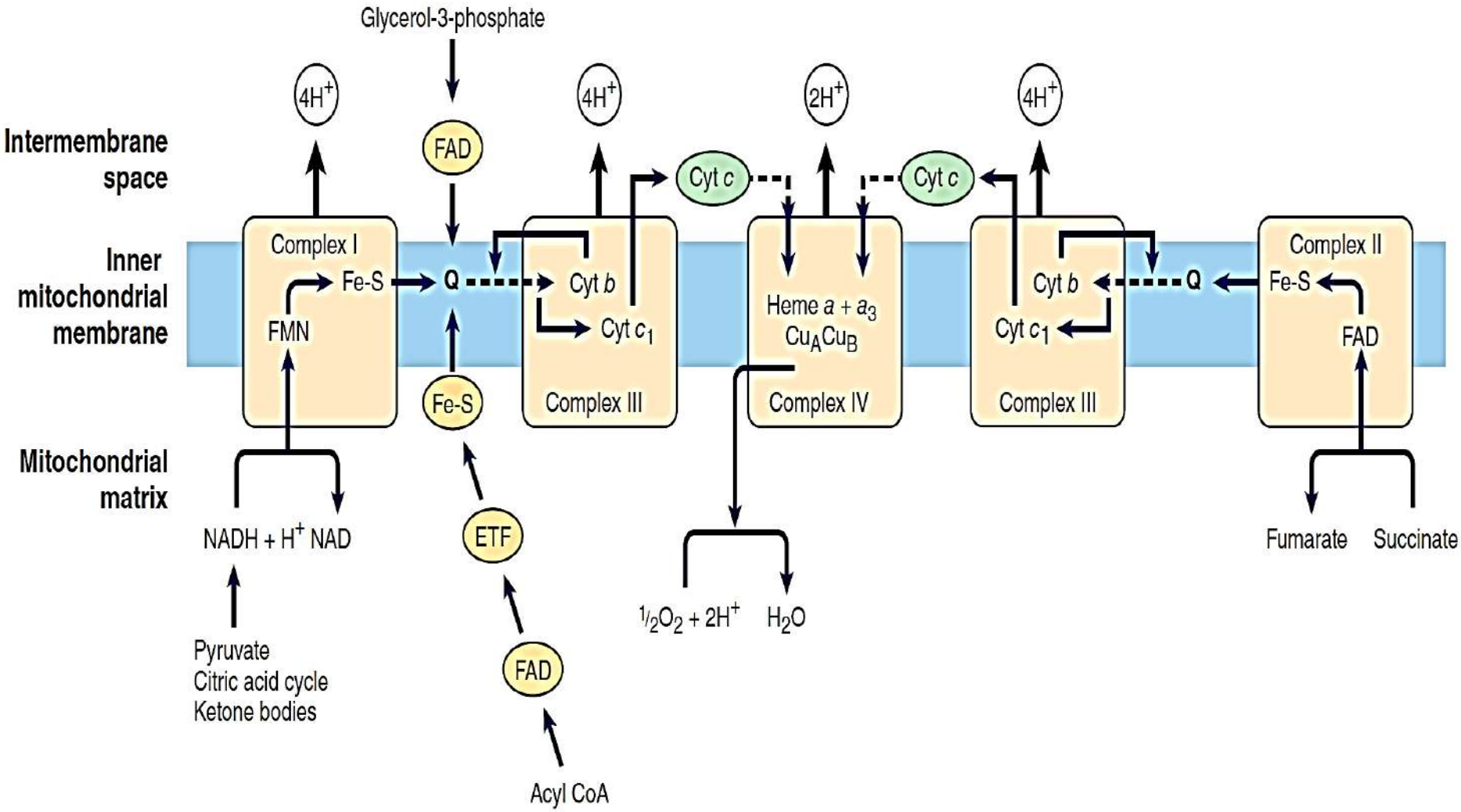
$$4\text{Cyt}\,c_{\text{reduced}} + \text{O}_2 + 8\text{H}^+_{\text{matrix}} \rightarrow 4\text{Cyt}\,c_{\text{oxidized}} + 2\text{H}_2\text{O} + 4\text{H}^+_{\text{intermembrane space}}$$

- This transfer of **four electrons** from **cytochrome c** to O_2 involves **two heme groups**, **a** and **a3**, and **Cu** (Figure 13–5).
- **Electrons** are **passed** initially to a **Cu** center (**CuA**), which contains **2Cu** atoms linked to two **protein cysteine-SH groups** (**resembling an Fe-S**), then in **sequence** to **heme a**, **heme a3**, a **second Cu** center, **CuB**, which is linked to **heme a3**, and **finally** to O_2.
- Of the **eight** H^+ removed from the matrix, **four** are used to form **two water** molecules and **four** are **pumped into the intermembrane space**.
- Thus, for every **pair** of **electrons** passing down the chain from **NADH** or **FADH2**, $2H^+$ are **pumped** across the membrane by **Complex IV**.
- The O_2 remains **tightly bound** to **Complex IV** until it is fully **reduced**, and this **minimizes** the **release of potentially damaging intermediates** such as **superoxide anions** or **peroxide** which are formed when O_2 accepts **one** or **two electrons**, **respectively**.

The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Oxidizes Reducing Equivalents & Acts As A Proton Pump

Molecular Oxygen Is Reduced to Water via Complex IV



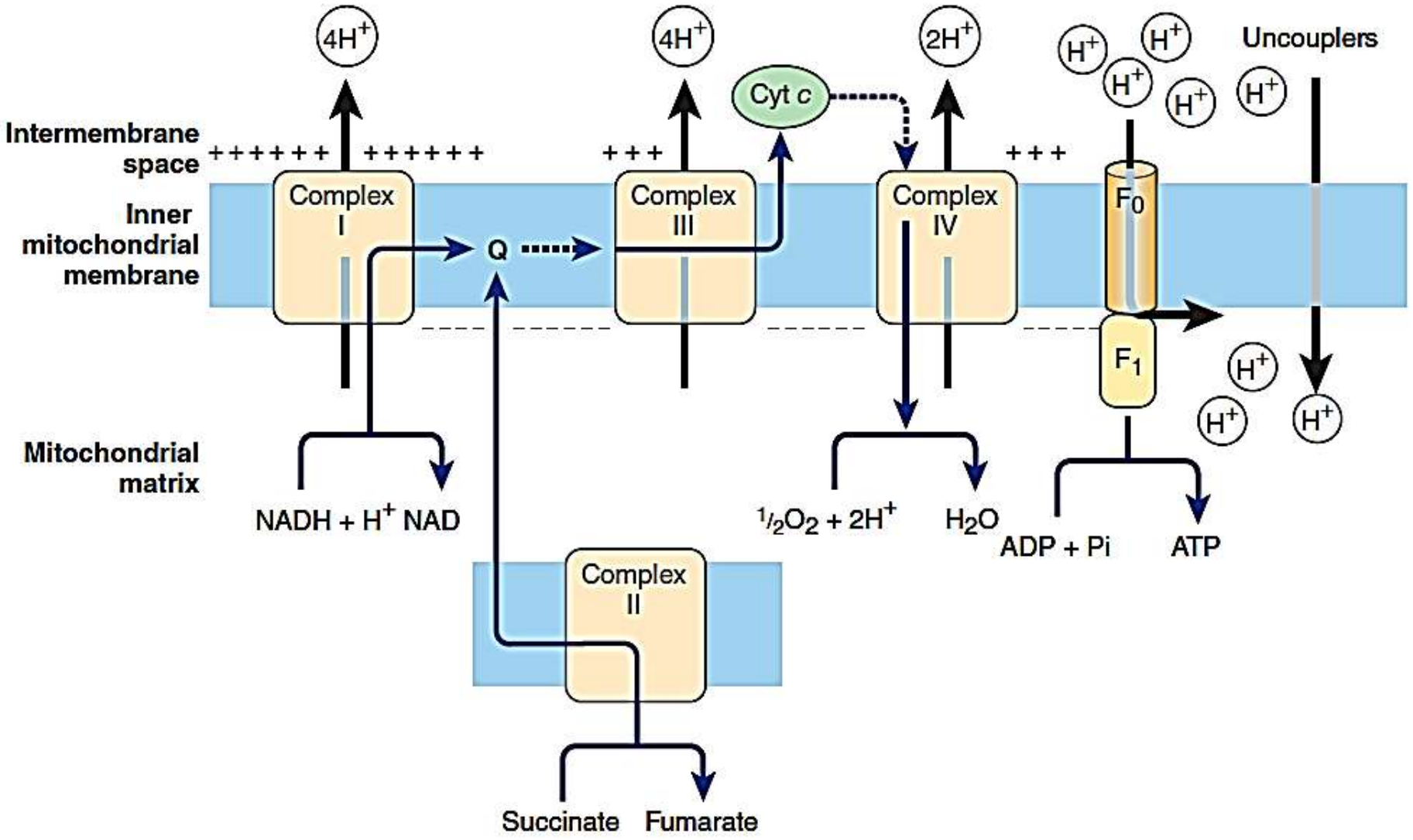
The Respiratory Chain & Oxidative Phosphorylation

Electron Transport Via The Respiratory Chain Creates A Proton Gradient Which Drives The Synthesis Of ATP

- The **flow** of **electrons** through the **respiratory chain generates ATP** by the process of **oxidative phosphorylation**.
- The **chemiosmotic theory**, proposed by Peter Mitchell in 1961, postulates that the two processes are coupled by a **proton gradient** across the **inner mitochondrial membrane** so that the **proton motive force** caused by the **electrochemical potential difference** (**negative on the matrix side**) drives the mechanism of **ATP synthesis**.
- As we have seen, **Complexes I**, **III**, and **IV** act as **proton pumps**.
- Since the **inner mitochondrial membrane** is **impermeable** to ions in general and particularly **to protons**, these **accumulate** in the **intermembrane space**, creating the **proton motive force** predicted by the **chemiosmotic theory**.

The Respiratory Chain & Oxidative Phosphorylation

Electron Transport Via The Respiratory Chain Creates A Proton Gradient Which Drives The Synthesis Of ATP



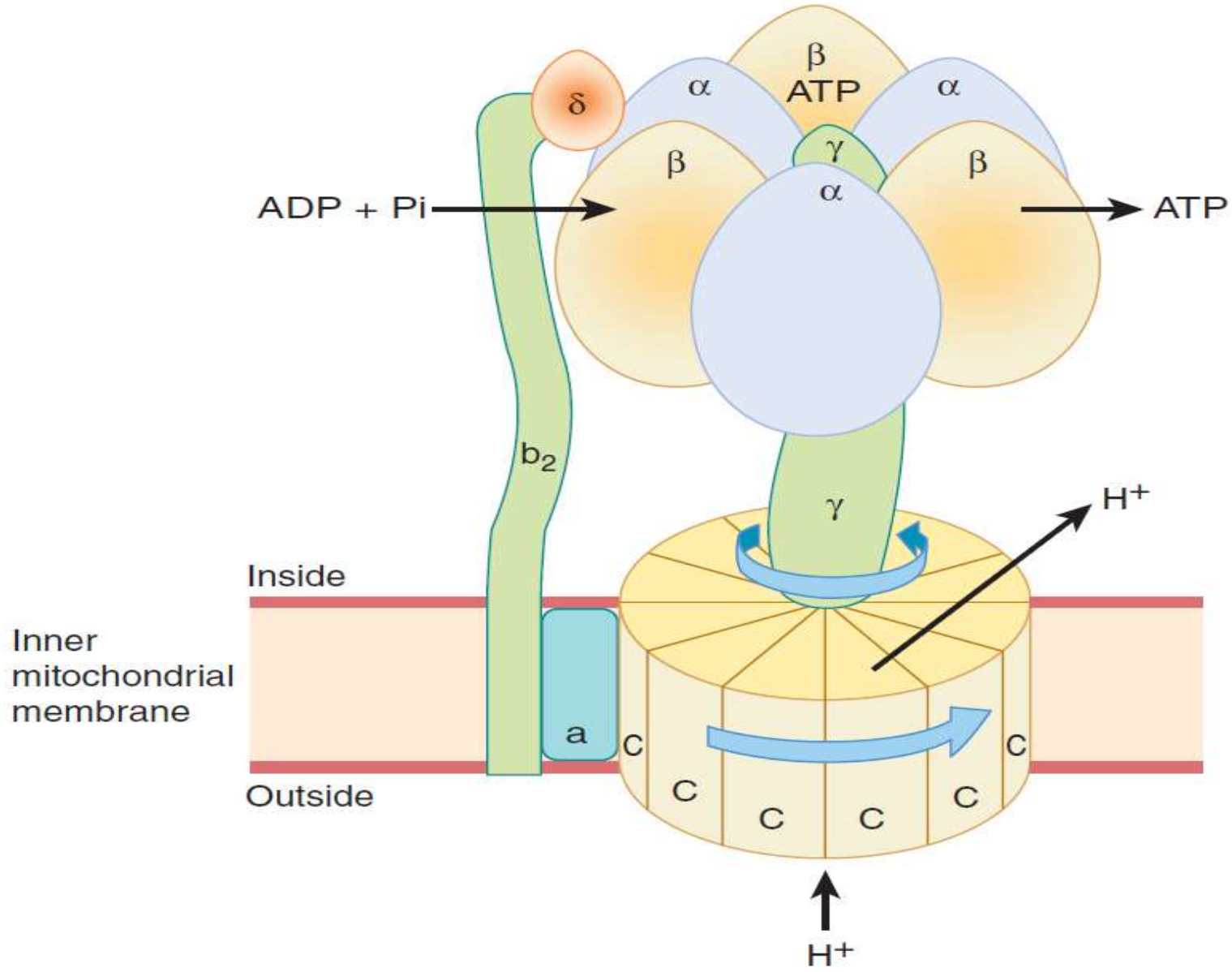
The Respiratory Chain & Oxidative Phosphorylation

A Membrane-Located ATP Synthase Functions as a Rotary Motor to Form ATP

- The **proton motive force** drives a membrane-located **ATP synthase** that forms **ATP** in the presence of **Pi + ADP**.
- **ATP synthase** is **embedded in the inner membrane**, **together** with the **respiratory chain complexes**.
- **Several subunits of the protein** form a ball-like shape arranged around an **axis** known as **F1**, which **projects into the matrix** and contains the **phosphorylation mechanism.**
- **F1** is attached to a **membrane protein complex** known as **F0**, which also consists of **several protein subunits**. **F0** spans the membrane and **forms a proton channel**. The **flow of protons** through **F0** causes it to **rotate**, driving the production of **ATP** in **the F1 complex**.
- This is thought to occur by a **binding change mechanism** in which the conformation of the **β-subunits** in **F1** is changed as the **axis rotates** from one that binds ATP tightly to one that releases ATP and binds ADP and Pi so that the next ATP can be formed.
- Estimates suggest that for each **NADH oxidized**, **Complexes I** and **III** translocate **four protons** each and **Complex IV** translocates **two**.

The Respiratory Chain & Oxidative Phosphorylation

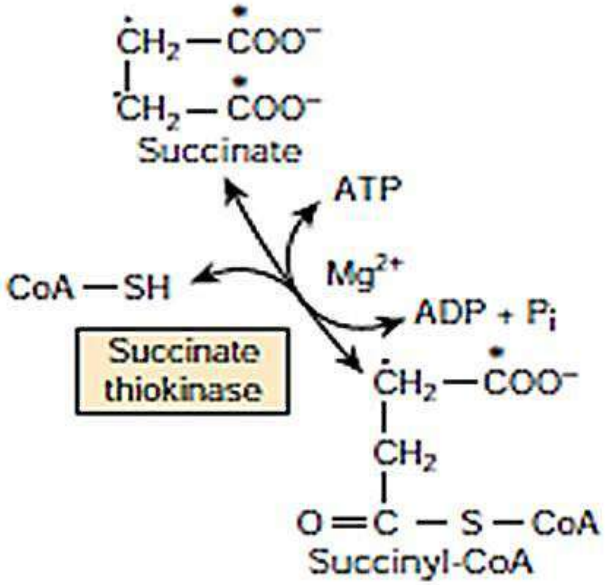
A Membrane-Located ATP Synthase Functions as a Rotary Motor to Form ATP



The Respiratory Chain & Oxidative Phosphorylation

The Respiratory Chain Provides Most Of The Energy Captured During Catabolism

- ADP captures, in the form of high-energy phosphate, a significant proportion of the free energy released by catabolic processes. The resulting ATP has been called the **energy** "**currency**" of the cell because it passes on this free energy to drive those processes requiring energy.
- There is a net direct capture of two high-energy phosphate groups in the glycolytic reactions. Two more high-energy phosphates per mole of glucose are captured in the citric acid cycle during the conversion of succinyl CoA to succinate. All of these **phosphorylations occur at the substrate level**.



- **For each mol of substrate oxidized via Complexes I, III, and IV in the respiratory chain (ie, via NADH), 2.5 mol of ATP are formed per 0.5 mol of O2 consumed; ie, the P:O ratio = 2.5.**
- **On the other hand, when 1 mol of substrate (eg, succinate ($FADH_2$) or 3-phophoglycerate) is oxidized via Complexes II, III, and IV, only 1.5 mol of ATP are formed; that is, P:O = 1.5.**
- These reactions are knownas **oxidative phosphorylation at the respiratory chain level**.
- Taking these values into account, it can be estimated that **nearly 90% of the high-energy phosphates produced from the complete oxidation of 1 mol glucose is obtained via oxidative phosphorylation coupled to the respiratory chain**.

The Respiratory Chain & Oxidative Phosphorylation

Respiratory Control Ensures a Constant Supply of ATP

- The **rate of respiration of mitochondria** can be **controlled** by the **availability** of **ADP**.
- **This is because oxidation and phosphorylation are tightly coupled; that is, oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP.**
- When work is performed, ATP is converted to ADP, allowing more respiration to occur, which in turn replenishes the store of ATP.
- Thus, the manner in which biologic oxidative processes allow the free energy resulting from the oxidation of foodstuffs to become available and to be captured is stepwise, efficient, and controlled—rather than explosive, inefficient, and uncontrolled, as in many nonbiologic processes.
- The remaining free energy that is not captured as high-energy phosphate is liberated as heat allowing continuous unidirectional flow and constant provision of ATP. It also contributes to maintenance of body temperature.
- Under certain conditions, the **concentration of inorganic phosphate** can also **affect the rate of functioning of the respiratory chain**

The Respiratory Chain & Oxidative Phosphorylation

Many Poisons Inhibit The Respiratory Chain

❑ **They may be classified as**:

A. inhibitors of oxidative phosphorylation:

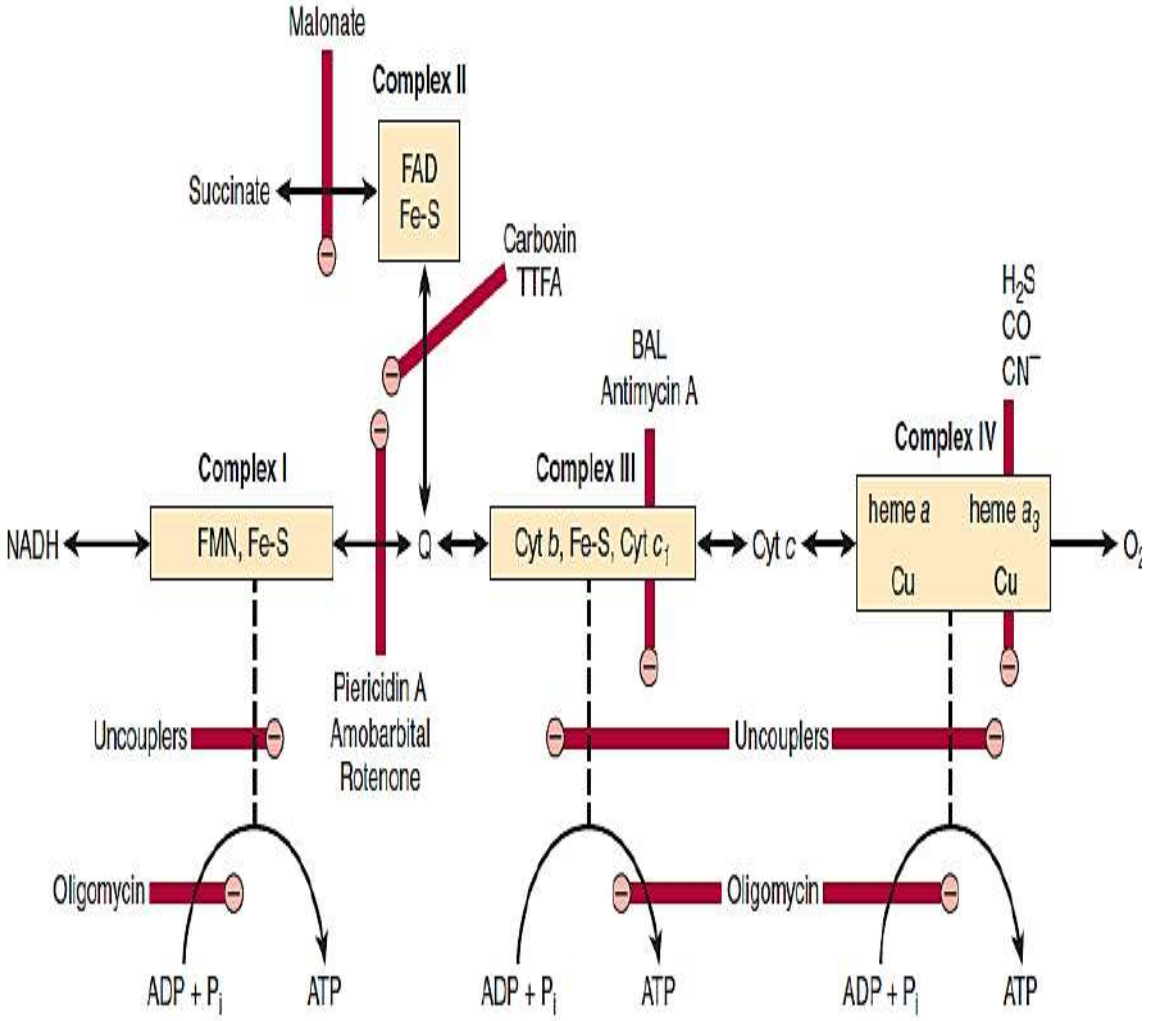
1) **Barbiturates** inhibit electron transport via **Complex I** by blocking the transfer from Fe-S to Q
2) **Antimycin A** and **dimercaprol** inhibit the respiratory chain at **Complex III**
3) The classic poisons **H_2S**, **carbon monoxide**, and **cyanide** inhibit **Complex IV**
4) **Malonate** is a competitive inhibitor of **Complex II**
5) **Atractyloside** inhibits the **transporter of ADP into and ATP** out of the mitochondrion
6) The antibiotic **oligomycin** completely **blocks the flow of protons through ATP synthase**

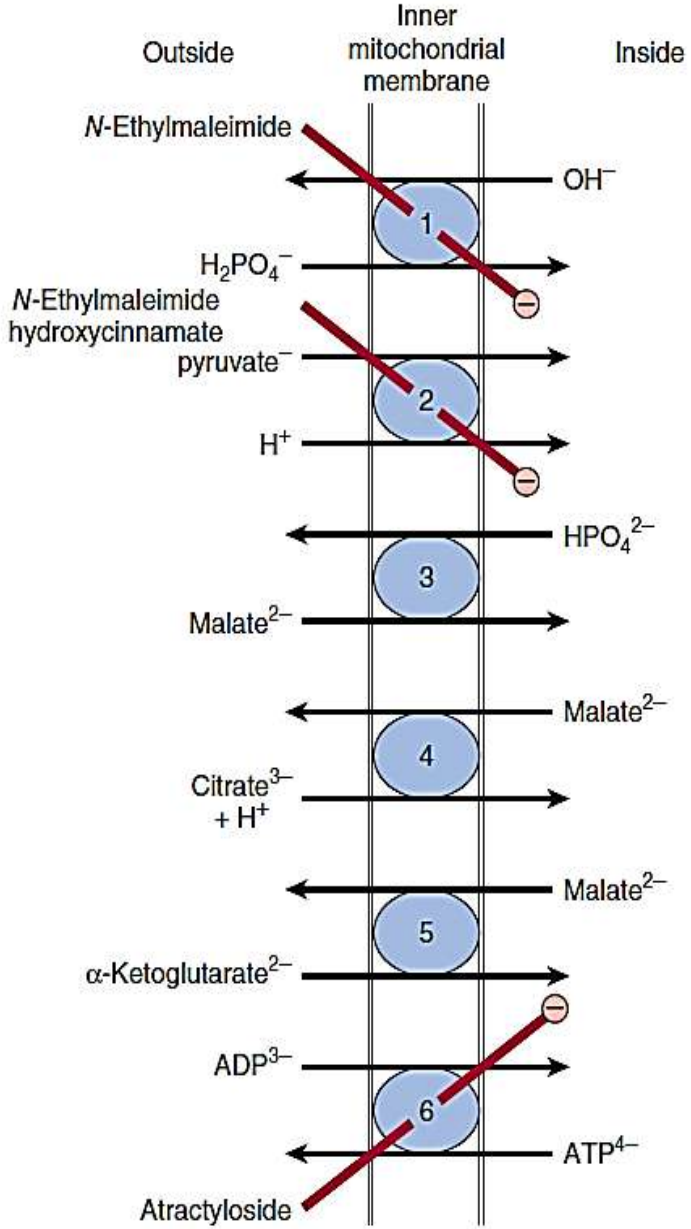
B. Uncouplers: **dissociate oxidation in the respiratory chain from phosphorylation**

1) **2,4-dinitrophenol** (These compounds are toxic in vivo, causing respiration to become uncontrolled).
2) **Thermogenin** (or the uncoupling protein) is a physiological uncoupler found in brown adipose tissue that functions to generate body heat, particularly for the newborn and during hibernation in animals

The Respiratory Chain & Oxidative Phosphorylation

Many Poisons Inhibit The Respiratory Chain





The Respiratory Chain & Oxidative Phosphorylation

The Selective Permeability Of The Inner Mitochondrial Membrane Necessitates Exchange Transporters

- Exchange diffusion systems involving transporter proteins that span the membrane are present in the membrane for exchange of anions against OH^- ions and cations against H^+ ions. Such systems are necessary for uptake and output of ionized metabolites while preserving electrical and osmotic equilibrium.
- The inner mitochondrial membrane is freely permeable to uncharged small molecules, such as oxygen, water, CO2, NH3, and to monocarboxylic acids, such as 3-hydroxybutyric, acetoacetic, and acetic, especially in their undissociated, more lipid soluble form.
- The adenine nucleotide transporter allows the exchange of ATP and ADP, but not AMP
- Ionophores are lipophilic molecules that complex specific cations and facilitate their transport through biologic membranes
- Energy-linked transhydrogenase, a protein in the inner mitochondrial membrane, couples the passage of protons down the electrochemical gradient from outside to inside the mitochondrion with the transfer of H from intramitochondrial NADH to NADPH for intramitochondrial enzymes such as glutamate dehydrogenase and hydroxylases involved in steroid synthesis

The Respiratory Chain & Oxidative Phosphorylation

Clinical Aspects

- **Fatal infantile mitochondrial myopathy (اعتلال عضلي) and renal dysfunction (فشل كلوي)** involves severe **diminution** or **absence** of most **oxidoreductases** of the respiratory chain.

- **MELAS (mitochondrial encephalopathy (اعتلال دماغي), lactic acidosis, and stroke (سكتة دماغية))** is **an inherited condition** due to **NADH-Q oxidoreductase** (**Complex I**) or **cytochrome oxidase** (**Complex IV**) **deficiency**. It is caused by a mutation in mitochondrial DNA and may be involved in **Alzheimer disease** and **diabetes mellitus.**

- **A number of drugs and poisons act by inhibition of oxidative phosphorylation**

The Respiratory Chain & Oxidative Phosphorylation

SUMMARY

1) **Virtually all energy released from the oxidation of carbohydrate, fat, and protein** is made available in mitochondria as **reducing equivalents (-H or e^-)**. These are funneled into the respiratory chain, where they are passed down a **redox gradient of carriers to their final reaction with oxygen to form water**.
2) **The redox carriers are grouped into four respiratory chain complexes in the inner mitochondrial membrane. Three of the four complexes are able to use the energy released in the redox gradient to pump protons to the outside of the membrane, creating an electrochemical potential between the matrix and the inner membrane space.**
3) **ATP synthase spans the membrane and acts like a rotary motor using the potential energy of the proton gradient or proton motive force to synthesize ATP from ADP and Pi. In this way, oxidation is closely coupled to phosphorylation to meet the energy needs of the cell.**
4) Since the inner mitochondrial membrane is impermeable to protons and other ions, special exchange transporters span the membrane to allow ions such as OH^-, ATP^{4-}, ADP^{3-}, and metabolites to pass through without discharging the electrochemical gradient across the membrane.
5) **Many well-known poisons such as cyanide arrest respiration by inhibition of the respiratory chain.**

Gluconeogenesis & the Control of Blood Glucose

Biomedical importance

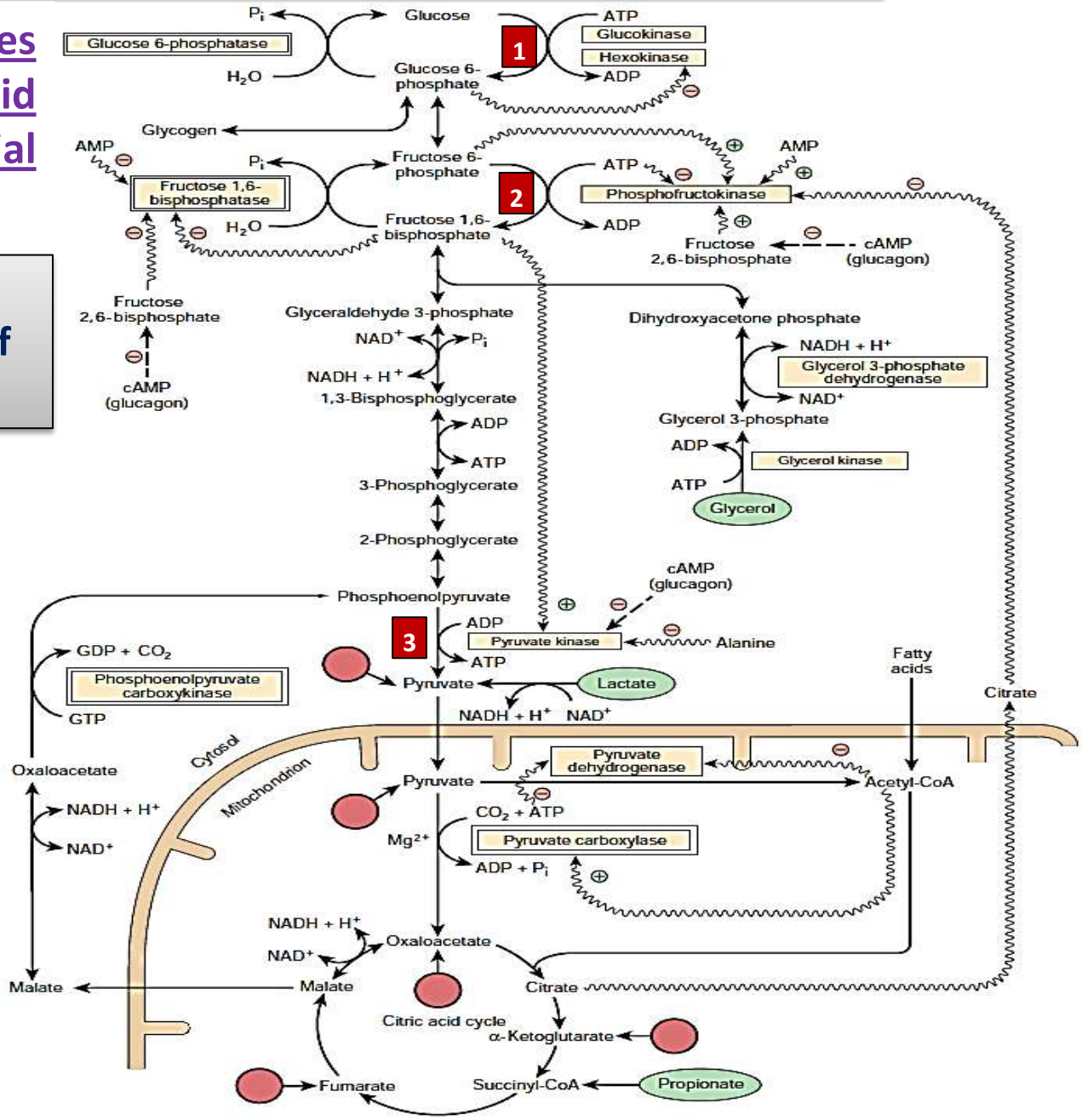
- **Gluconeogenesis** is the process of **synthesizing glucose** or **glycogen from noncarbohydrate precursors. Major substrates** are the **glucogenic amino acids**, **lactate**, **glycerol**, and **propionate**.
- **Liver and kidney** are the **major gluconeogenic tissues**, but the **small intestine** may also be a source of glucose in the **fasting state**.
- **Gluconeogenesis meets the needs** of the body for **glucose** when **insufficient carbohydrate** is available from the **diet** or **glycogen reserves**.
- A supply of **glucose is necessary** especially for the **nervous system** and **erythrocytes**.
- **Failure of gluconeogenesis** is usually **fatal**.
- **Hypoglycemia** causes **brain dysfunction**, which can lead to **coma** and **death**.
- **Glucose** is also important in **maintaining the level of intermediates of the citric acid cycle** even when **fatty acids** are the main **source of acetyl-CoA in the tissues**.
- **Gluconeogenesis clears lactate** produced by **muscle** and **erythrocytes** and **glycerol** produced by **adipose tissue**.
- In **ruminants**, **propionate** is a product of rumen metabolism of carbohydrates, and is a **major substrate for gluconeogenesis**.

Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis Involves Glycolysis, The Citric Acid Cycle, Plus Some Special Reactions

Thermodynamic Barriers Prevent a Simple Reversal of Glycolysis

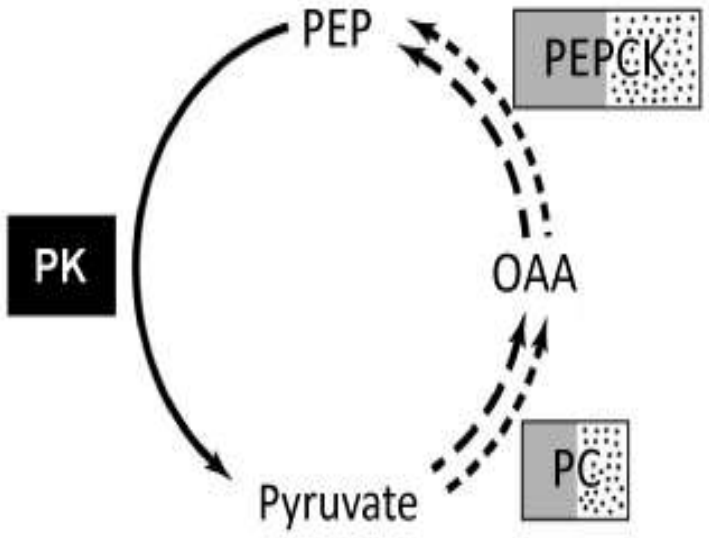
- Three nonequilibrium reactions in glycolysis catalyzed by :
  1. Hexokinase (1)
  2. Phosphofructokinase (3)
  3. pyruvate kinase (10)
- prevent simple reversal of glycolysis for glucose synthesis.



Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis Involves Glycolysis, The Citric Acid Cycle, Plus Some Special Reactions

1) Pyruvate & Phosphoenolpyruvate



- **Reversal** of the reaction catalyzed by **pyruvate kinase (PK)** in glycolysis involves **two endothermic reactions**:
  1. **Mitochondrial pyruvate carboxylase (PC)** catalyzes the **carboxylation of pyruvate to oxaloacetate**, an **ATP-requiring** reaction in which the **vitamin biotin is the coenzyme**. Biotin binds CO_2 from bicarbonate as carboxybiotin prior to the addition of the CO_2 to pyruvate.

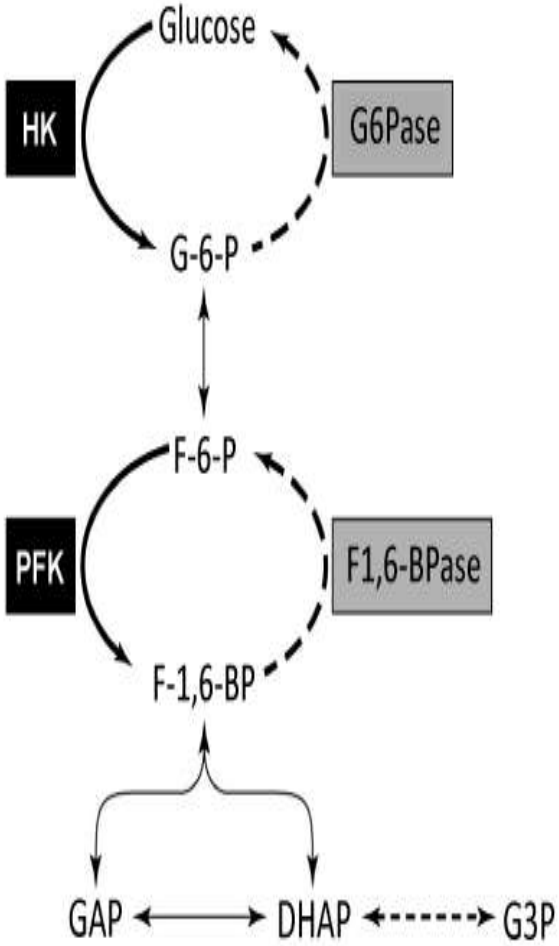
  2. **Phosphoenolpyruvate carboxykinase (PEPCK)**, catalyzes the **decarboxylation and phosphorylation of oxaloacetate to phosphoenolpyruvate** using **GTP as the phosphate donor**.

     In **liver** and **kidney**, the reaction of **succinate thiokinase (succinyl-CoA synthetase)** in the citric acid cycle (**reaction 5**) produces **GTP** (rather than ATP as in other tissues), and this GTP is used for the reaction of **phosphoenolpyruvate carboxykinase**, thus providing a link between citric acid cycle activity and gluconeogenesis, to prevent excessive removal of oxaloacetate for gluconeogenesis, which would impair citric acid cycle activity.

Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis involves glycolysis, the citric acid cycle, plus some special reactions

2) Fructose 1,6-Bisphosphate & Fructose 6-Phosphate



- The conversion of **fructose 1,6-bisphosphate** to **fructose 6-phosphate**, for the reversal of glycolysis, is catalyzed by:

  Fructose 1,6-bisphosphatase (F1,6BPase): Its presence determines whether a tissue is capable of synthesizing glucose (or glycogen) not only from pyruvate (from oxidation of lactate and some glucogenic amino acids catabolism), but also from triose phosphates (Glecero to glecerol 3-phosphate to dihydroxyacetone phosphate). It is **present in liver, kidney, and skeletal muscle**, but is **probably absent from heart and smooth muscle**.

3) Glucose 6-Phosphate & Glucose

- The conversion of **glucose 6-phosphate to glucose** is catalyzed by:

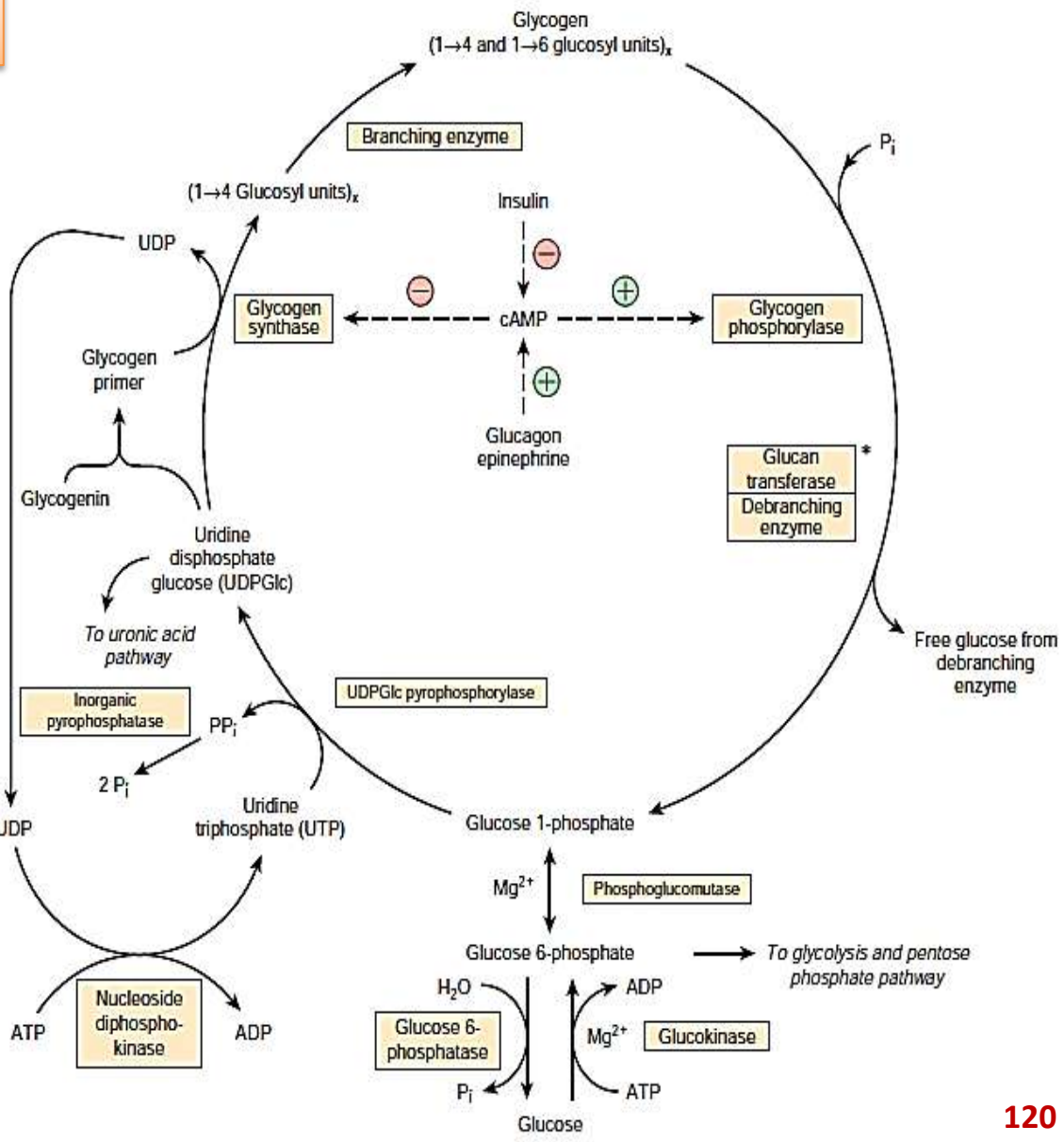
  Glucose 6-phosphatase (G6Pase): It is **present in liver and kidney** but **absent from muscle and adipose tissue**, which, therefore **cannot export glucose into the bloodstream**

Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis involves glycolysis, the citric acid cycle, plus some special reactions

Glucose 1-Phosphate & Glycogen

- The **breakdown** of **glycogen to glucose 1-phosphate** is catalyzed by **phosphorylase**.
- **Glycogen synthesis** involves a different pathway via **uridine diphosphate glucose** and **glycogen synthase**.

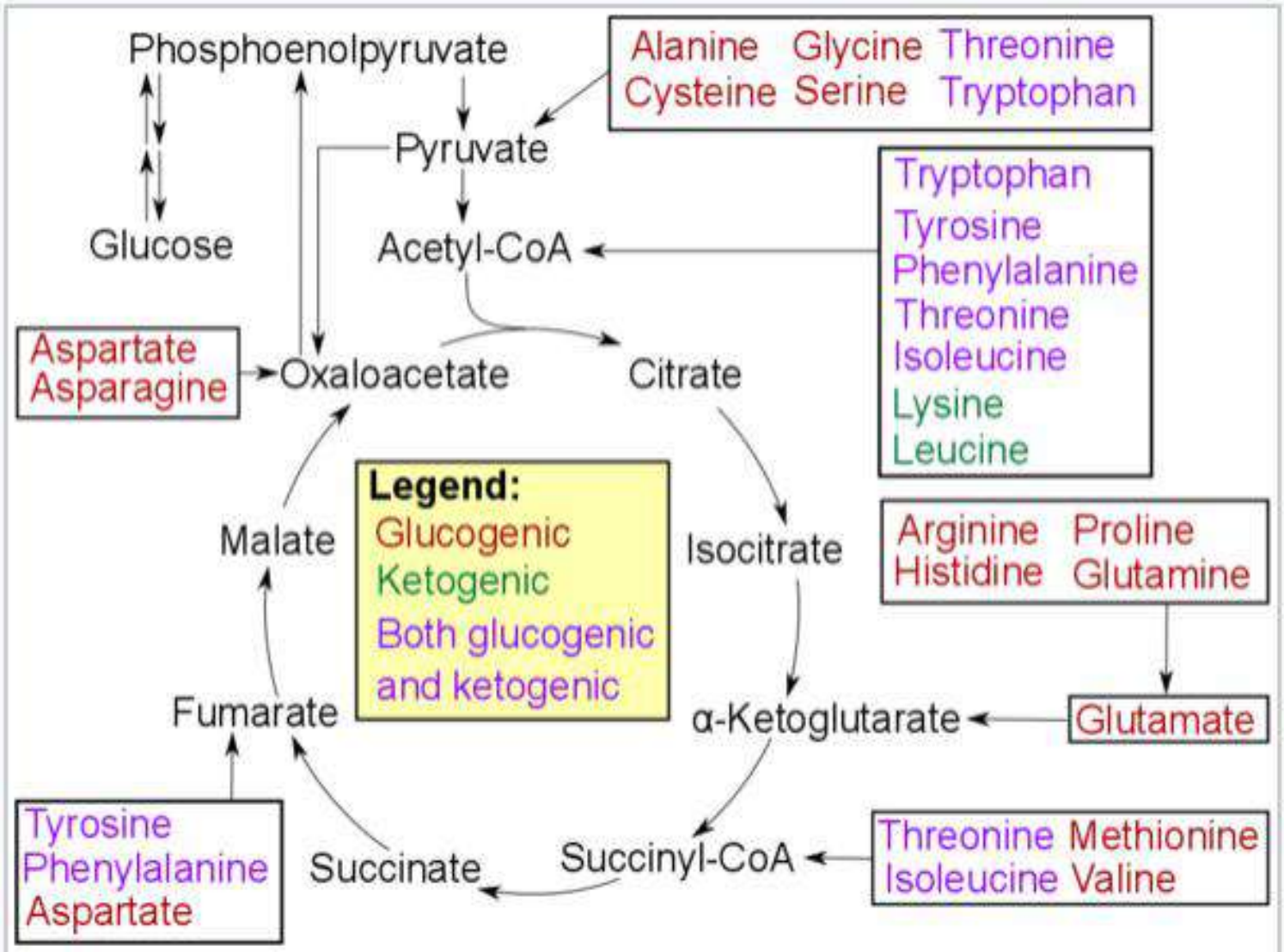


Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis involves glycolysis, the citric acid cycle, plus some special reactions

conversion of both lactate and glucogenic amino acids to glucose or glycogen

- **After transamination or deamination, glucogenic amino acids yield either pyruvate or intermediates of the citric acid cycle.**



Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis involves glycolysis, the citric acid cycle, plus some special reactions

conversion of both lactate and glucogenic amino acids

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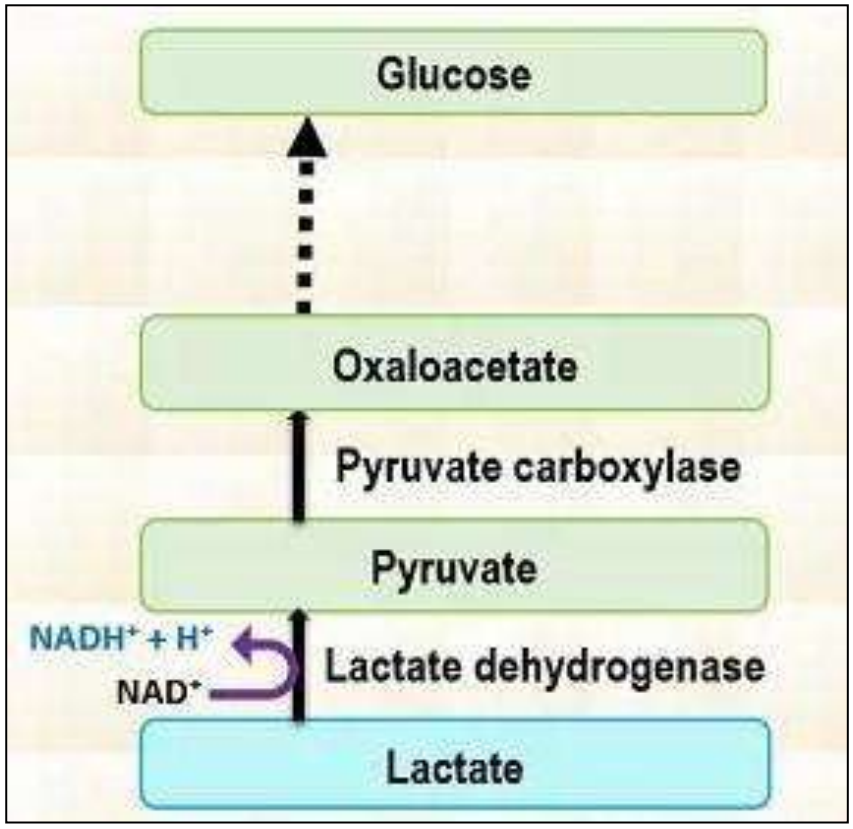
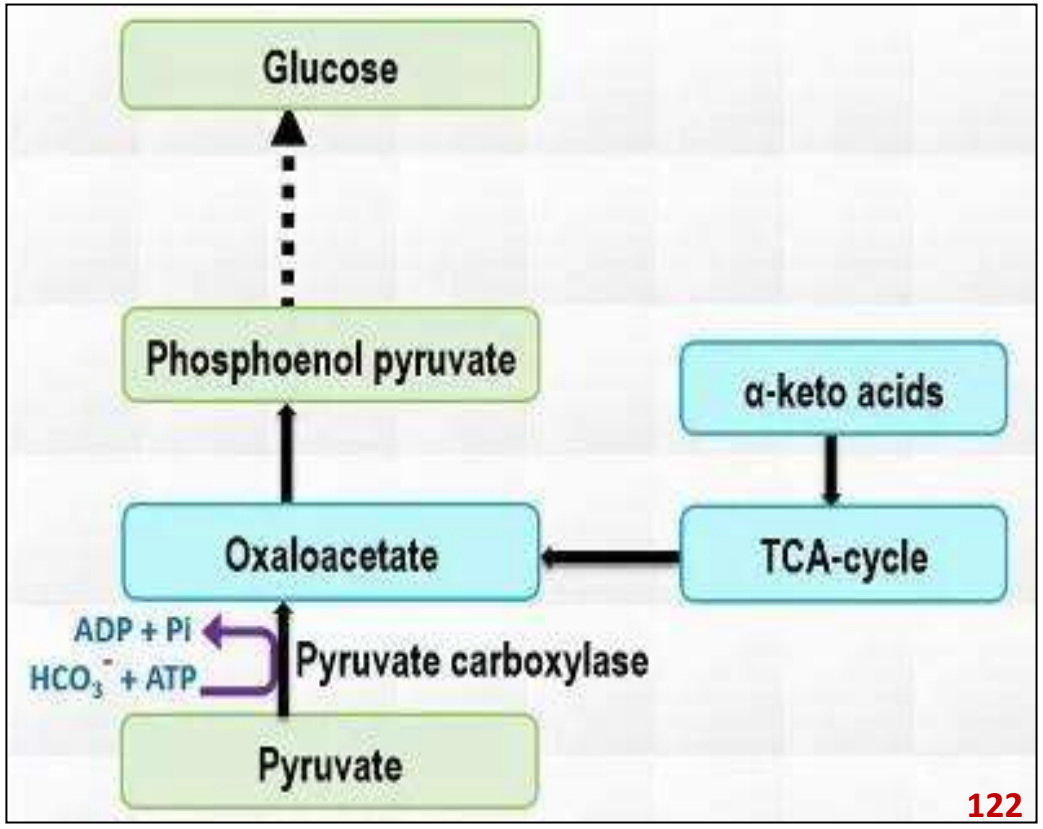


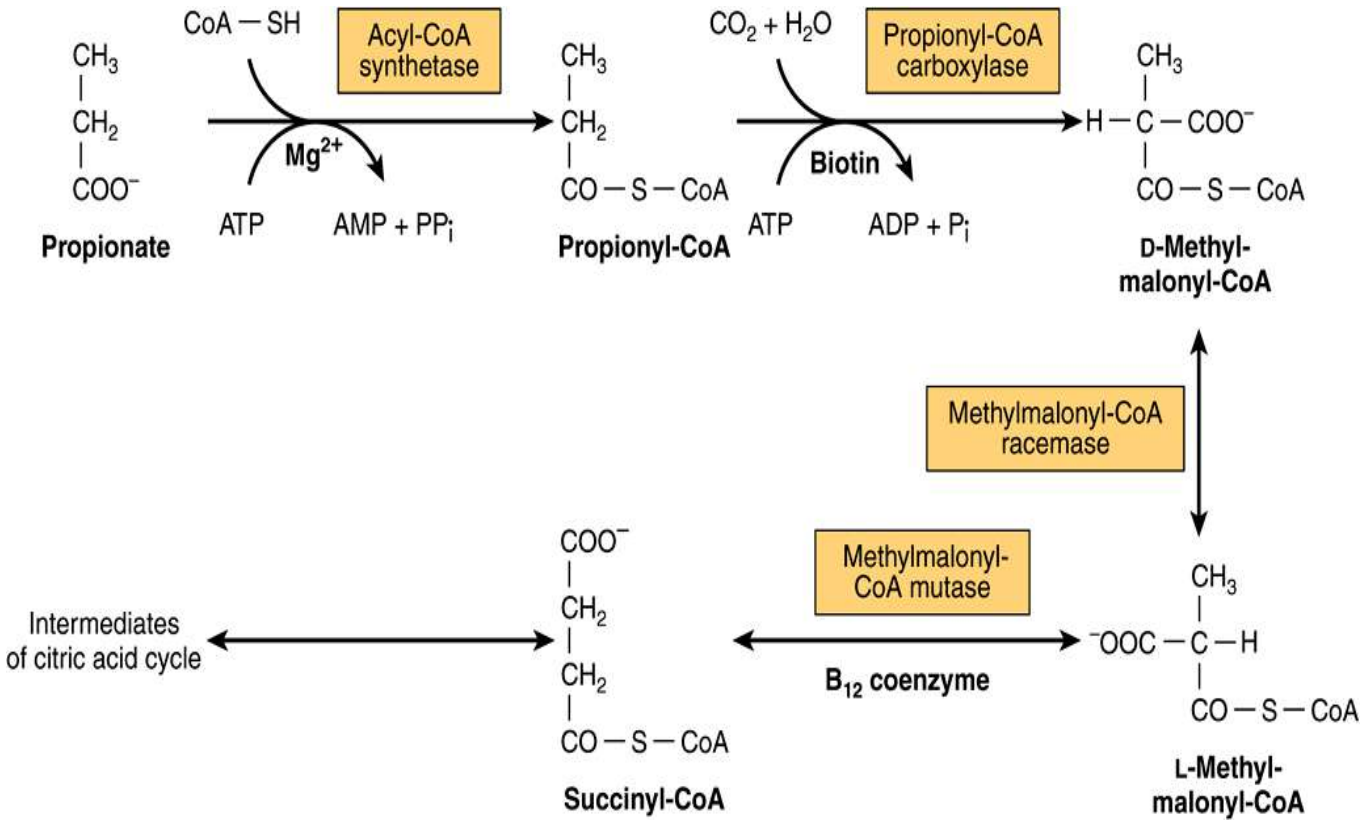


Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis involves glycolysis, the citric acid cycle, plus some special reactions

Propionate is a major precursor of glucose in ruminants

- **Propionate enters gluconeogenesis via the citric acid cycle.**
- After esterification with CoA, **propionyl-CoA is carboxylated to D-methylmalonyl-CoA**, catalyzed by **propionyl-CoA carboxylase**, a biotin-dependent.



- **Methylmalonyl-CoA racemase** catalyzes the **conversion of d-methylmalonyl-CoA to l-methylmalonyl-CoA**, which then undergoes **isomerization to succinyl-CoA** catalyzed by **methylmalonyl-CoA mutase**.

Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis involves glycolysis, the citric acid cycle, plus some special reactions

Propionate is a major precursor of glucose in ruminants

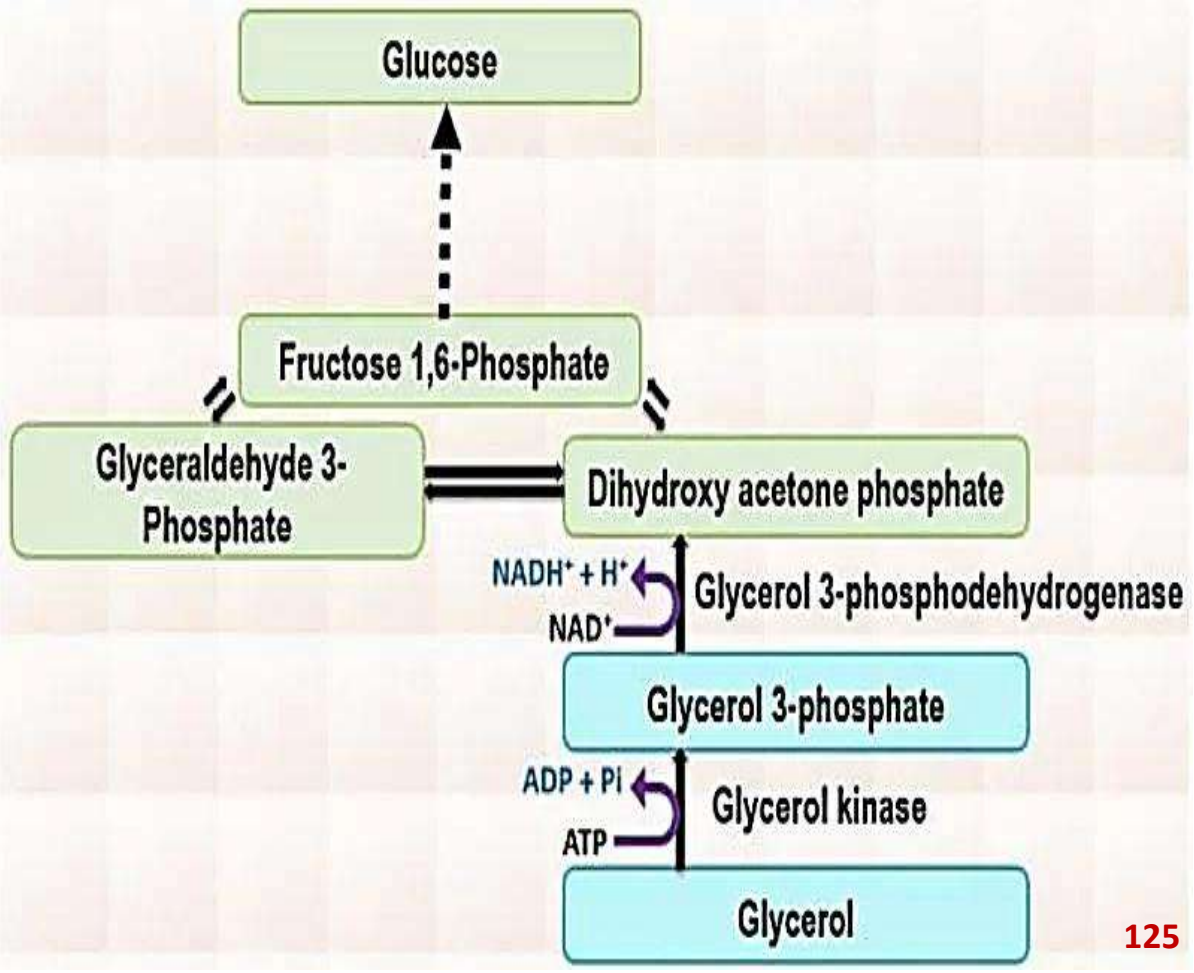
- In **non-ruminants**, including **humans**, **propionate arises from the β-oxidation of odd-chain fatty acids** that occur in ruminant lipids (Chapter 22), as well as **the oxidation of isoleucine** and **the side chain of cholesterol**, and is a (relatively minor) **substrate for gluconeogenesis**.
- **Methylmalonyl-CoA mutase** is a **vitamin B12-dependent enzyme**, and in **deficiency methylmalonic acid** is **excreted in the urine** (**methylmalonicaciduria**).

Gluconeogenesis & the Control of Blood Glucose

Gluconeogenesis Involves Glycolysis, The Citric Acid Cycle, Plus Some Special Reactions

Glycerol is a substrate for gluconeogenesis in the liver and kidneys

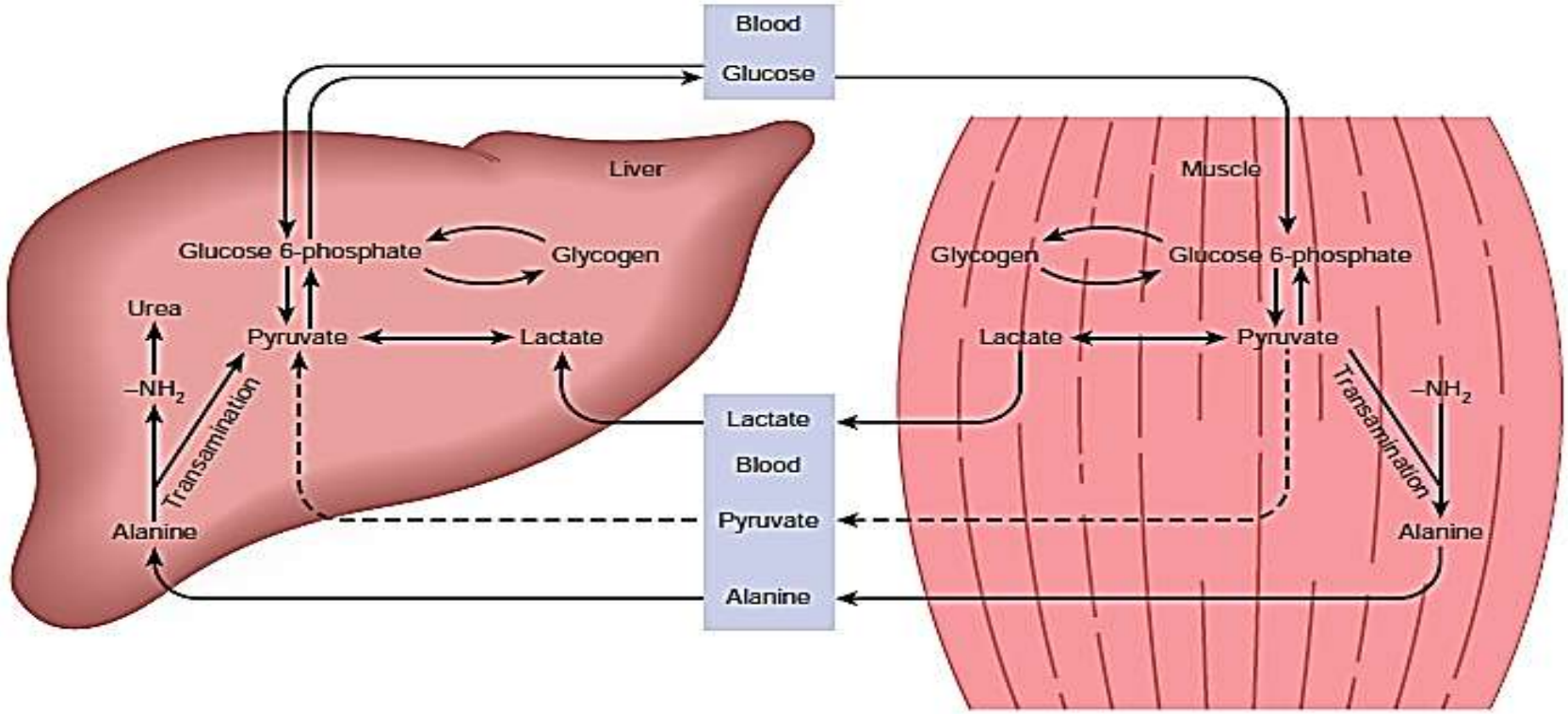
- **Glycerol** is released from **adipose tissue** as a result of **lipolysis of lipoprotein triacylglycerol**
- **in the fed state**; it may be used for re-esterification of free fatty acids to triacylglycerol in adipose tissue or liver, or **may be a substrate for gluconeogenesis in the liver**.
- **In the fasting state glycerol** released from **lipolysis of adipose tissue triacylglycerol is used solely** as a **substrate for gluconeogenesis in the liver and kidneys**.



Gluconeogenesis & the Control of Blood Glucose

Blood Glucose Is Derived From The Diet, Gluconeogenesis, & Glycogenolysis

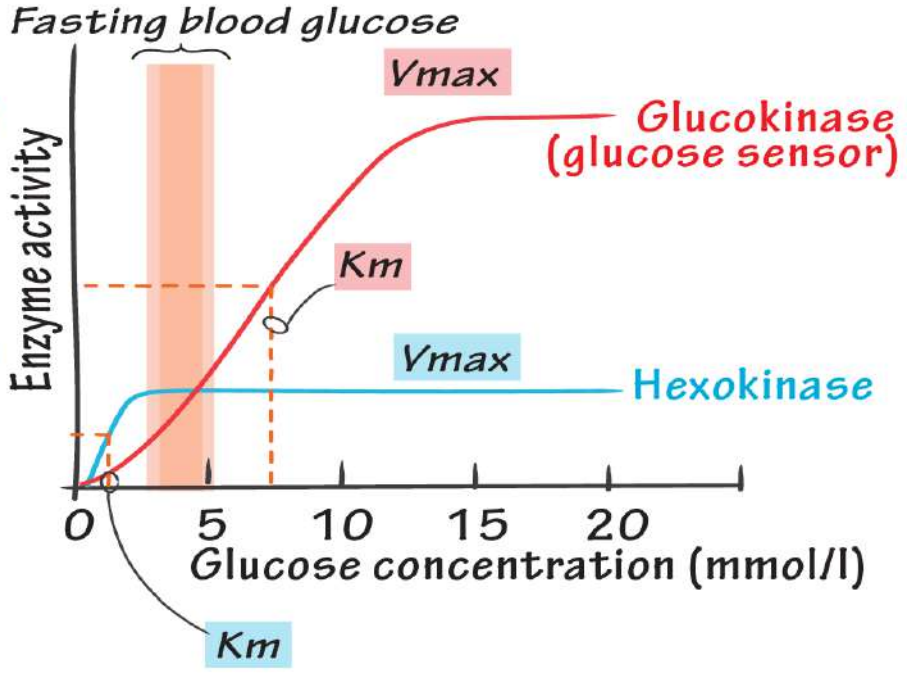
- **glucose-alanine cycle** (see Figure 20–4) provides an **indirect way of utilizing muscle glycogen to maintain blood glucose in the fasting state**.
- The **ATP required for the hepatic synthesis of glucose from pyruvate** is **derived from the oxidation of fatty acids**.
- Glucose is also formed from liver glycogen by glycogenolysis (Chapter 19).



Gluconeogenesis & the Control of Blood Glucose

Glucokinase Is Important in Regulating Blood Glucose After a Meal

- **Hexokinase** has a **low K_m for glucose**, and in the liver is saturated and acting at a constant rate under all normal conditions.
- **Glucokinase** has a considerably **higher K_m** (**lower affinity**) for glucose, so that its activity increases with increases in the concentration of glucose in the hepatic portal vein.
- It **promotes hepatic uptake of large amounts of glucose after a carbohydrate meal**.
- It is **absent from the liver of ruminants**, which have little glucose entering the portal circulation from the intestines.

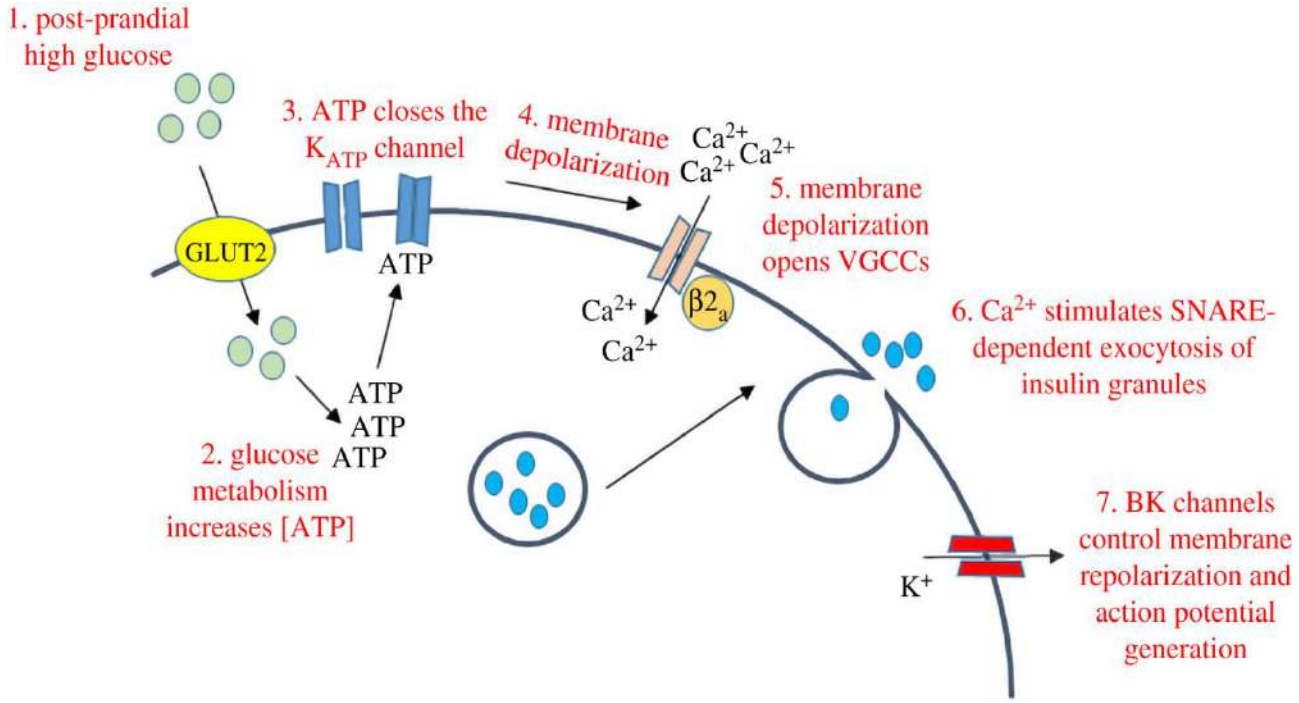


Glucokinase	Hexokinase	
liver and pancreas	All tissues	presnt
Glucose	All hexoses	substrate
High (lower affinity)	Low (Higheraffinity)	Km
High	low	Vmax

Gluconeogenesis & the Control of Blood Glucose

Insulin Plays a Central Role in Regulating Blood Glucose

- In addition to the direct effects of **hyperglycemia** in enhancing the uptake of glucose into the liver, the **hormone insulin** plays a central role in regulating blood glucose.
- It is produced by the **β cells of the islets of Langerhans in the pancreas** in response to hyperglycemia.



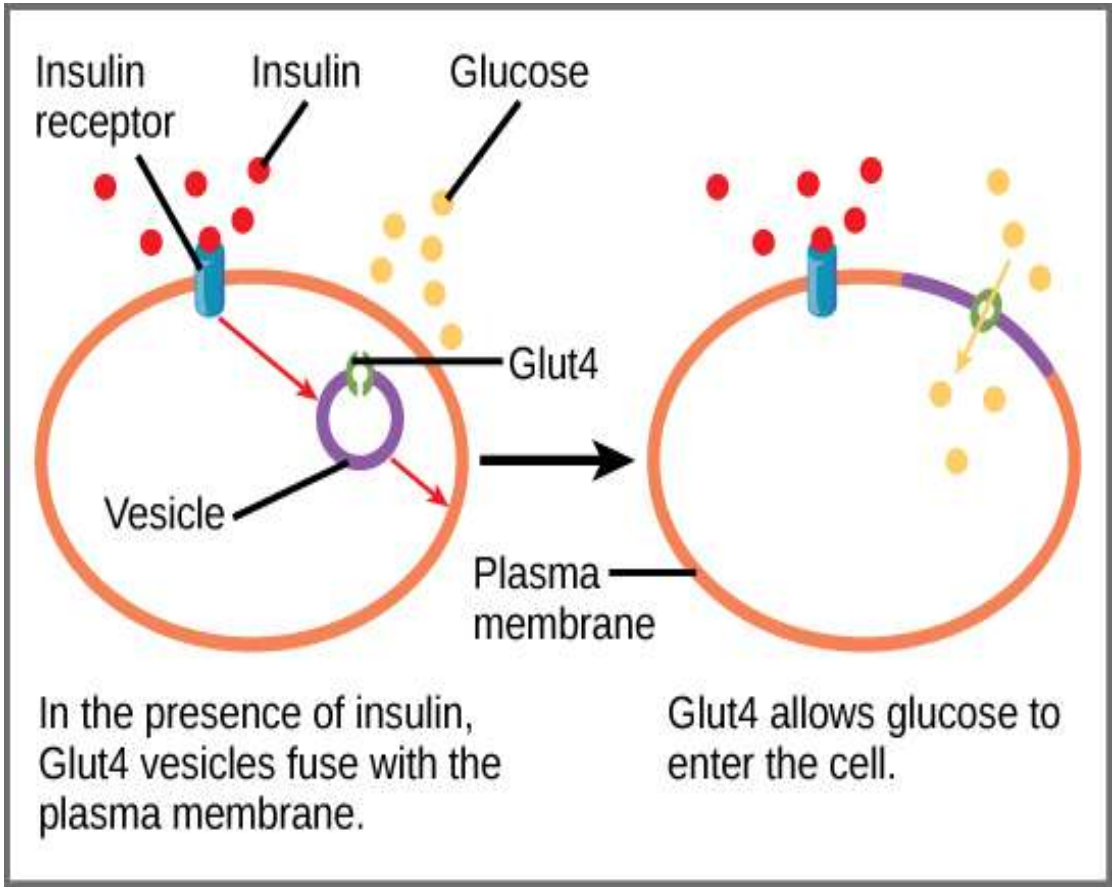
- **The β-islet cells** are **freely permeable to glucose via the GLUT 2 transporter**, and the glucose is phosphorylated by **glucokinase**.
- Therefore, **increasing blood glucose increases metabolic flux** through **glycolysis**, the **citric acid cycle**, and the **generation of ATP**.
- The **increase in [ATP] inhibits ATP-sensitive K^+ channels**, causing **depolarization of the cell membrane** which **increases Ca^{2+} influx via voltage-sensitive Ca^{2+} channels**, **stimulating exocytosis of insulin**.
- Thus, **the concentration of insulin** in the **blood parallels that of the blood glucose**.

Gluconeogenesis & the Control of Blood Glucose

Insulin Plays a Central Role in Regulating Blood Glucose

- **Other substances causing release of insulin** from the pancreas include **amino acids**, **free fatty acids**, **ketone bodies**, **glucagon**, **secretin**, and the **sulfonylurea drugs tolbutamide** and **glyburide**. These drugs are used to **stimulate insulin secretion in type 2 diabetes mellitus** (**NIDDM, noninsulin-dependent diabetes mellitus**); they act **by inhibiting the ATP-sensitive K^+ channels**
- **Epinephrine and norepinephrine block** the **release of insulin**.

- **Insulin lowers blood glucose immediately** by **enhancing glucose transport into adipose tissue** and **muscle** by **recruitment of glucose transporters (GLUT 4) from the interior of the cell to the plasma membrane**.
- **Although it does not affect glucose uptake into the liver directly**, **insulin does enhance long-term uptake as a result of its actions on the enzymes controlling glycolysis**, **glycogenesis**, and **gluconeogenesis**.



Gluconeogenesis & the Control of Blood Glucose

Glucagon Opposes the Actions of Insulin

- **Glucagon** is the **hormone produced by the α cells of the pancreatic islets**.
- Its secretion is **stimulated by hypoglycemia**.
- **In the liver** it **stimulates glycogenolysis by activating phosphorylase**.
- **Unlike epinephrine**, **glucagon does not have an effect on muscle phosphorylase**.
- **Glucagon** also **enhances gluconeogenesis from amino acids and lactate**.
- In all these actions, glucagon acts via generation of **cAMP** (see Table 20–1).
- Both **hepatic glycogenolysis** and **gluconeogenesis** contribute to the **hyperglycemic effect of glucagon**, whose actions **oppose those of insulin**.
- Most of the endogenous glucagon (and insulin) is cleared from the circulation by the liver (Table 20–3).

Gluconeogenesis & the Control of Blood Glucose

Other Hormones Affect Blood Glucose

- A number of **cytokines secreted by macrophages infiltrating adipose tissue** also **have insulin antagonistic actions**; together with **glucocorticoids secreted by adipose tissue**, **this explains the insulin resistance that commonly occurs in obese people.**
- **Epinephrine** is secreted by the **adrenal medulla** as a result of stressful stimuli (fear, excitement, hemorrhage, hypoxia, hypoglycemia, etc.) and leads to **glycogenolysis in liver and muscle** owing to **stimulation** of **phosphorylase** via **generation of cAMP**.
- **In muscle**, **glycogenolysis results in increased glycolysis**, whereas **in liver it results in the release of glucose into the bloodstream**.

Gluconeogenesis & the Control of Blood Glucose

SUMMARY

1) **Gluconeogenesis** is the **process of synthesizing glucose or glycogen from noncarbohydrate precursors**. It is of particular importance **when carbohydrate is not available from the diet**. **Significant substrates** are **amino acids**, **lactate**, **glycerol**, and **propionate**.
2) The **pathway of gluconeogenesis** in the **liver** and **kidney** utilizes those **reactions in glycolysis that are reversible plus four additional reactions that circumvent the irreversible nonequilibrium reactions.**
3) **Since glycolysis and gluconeogenesis share the same pathway but operate in opposite directions, their activities must be regulated reciprocally**.
4) **The liver regulates the blood glucose after a meal because it contains the high-Km glucokinase** that **promotes increased hepatic utilization of glucose**.
5) **Insulin** is secreted as a direct response to **hyperglycemia**; it **stimulates the liver to store glucose as glycogen** and **facilitates uptake of glucose into extrahepatic tissues**.
6) **Glucagon** is **secreted as a response to hypoglycemia** and **activates both glycogenolysis** and **gluconeogenesis** in the liver, causing **release of glucose into the blood**.

The pentose phosphate pathway & Other pathways of hexose Metabolism

Biomedical importance

- **The pentose phosphate pathway is an alternative route for the metabolism of glucose**.
- **It does not lead to formation of ATP** but **has two major functions:**

1. **the formation of NADPH for synthesis of fatty acids and steroids.**
2. **the synthesis of ribose for nucleotide and nucleic acid formation**.

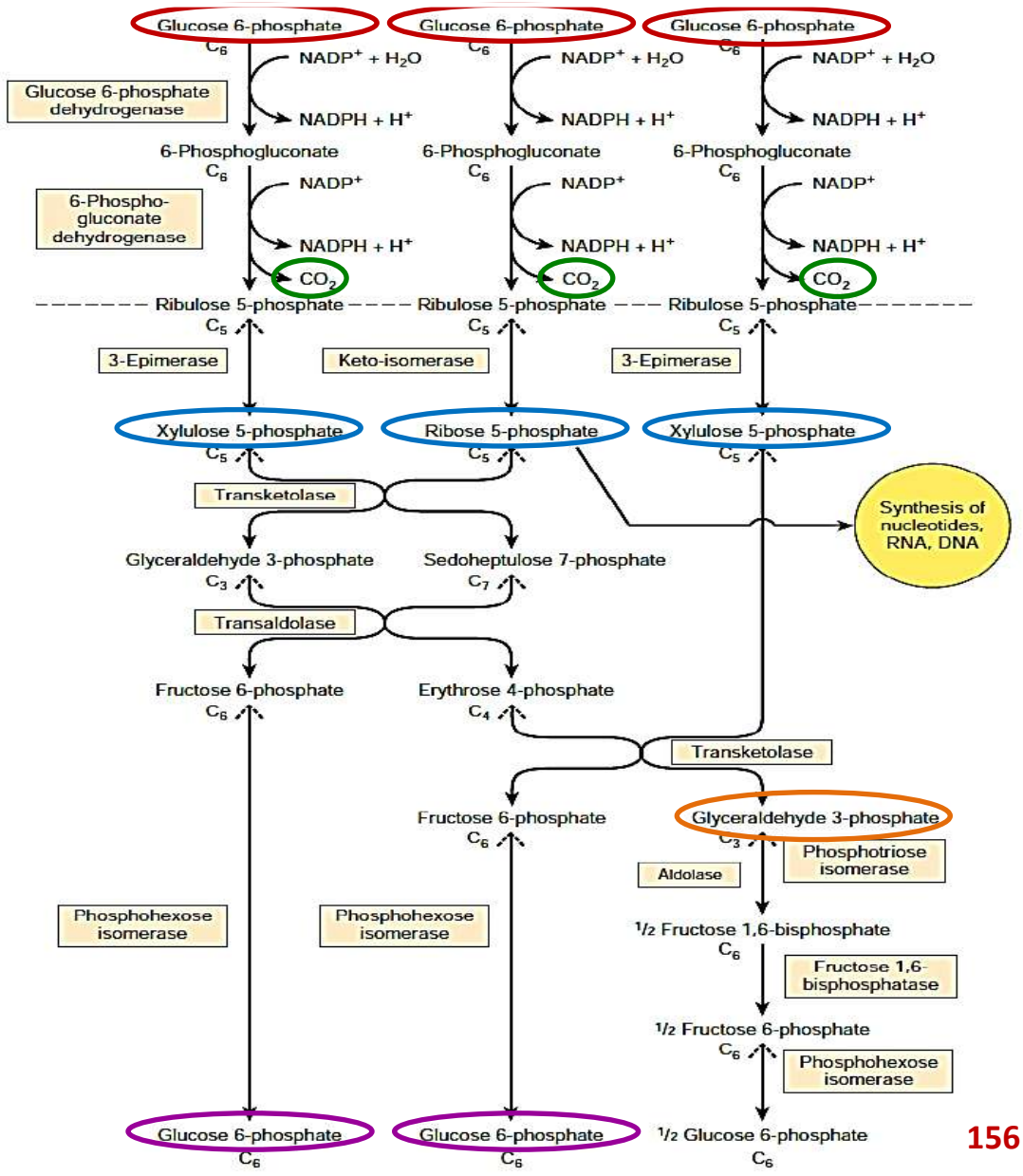
- **Glucose, fructose, and galactose are the main hexoses** absorbed from the gastrointestinal tract, derived from **dietary starch, sucrose, and lactose**, respectively.
- **Fructose** and **galactose** can be **converted to glucose**, mainly in the **liver**.

- **Genetic deficiency** of **glucose 6-phosphate dehydrogenase**, the **first enzyme of the pentose phosphate pathway**, is a major cause of hemolysis of red blood cells, resulting in **hemolytic anemia**.

The pentose phosphate pathway & Other pathways of hexose Metabolism

THE PENTOSE PHOSPHATE PATHWAY FORMS NADPH & RIBOSE PHOSPHATE

- The pentose phosphate pathway (**hexose monophosphate shunt**) is a more complex pathway than glycolysis.
- **Three molecules of glucose 6-phosphate** give rise to **three molecules of CO2** and **three 5-carbon sugars**.
- These are rearranged to regenerate **two molecules of glucose 6-phosphate** and one molecule of the glycolytic intermediate, **glyceraldehyde 3-phosphate**.
- **Since two molecules of glyceraldehyde 3-phosphate can regenerate glucose 6-phosphate, the pathway can account for the complete oxidation of glucose**.



REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY OCCUR IN THE CYTOSOL

- **Like glycolysis**, the **enzymes** of the pentose phosphate pathway are **cytosolic**.
- **Unlike glycolysis**, **oxidation** is achieved by **dehydrogenation** using **NADP+**, not NAD+, as the hydrogen acceptor.
- The sequence of reactions of **the pathway** may be divided into **two phases**:

oxidative nonreversible phase

glucose 6-phosphate undergoes dehydrogenation and decarboxylation to yield a pentose, **ribulose 5-phosphate**.

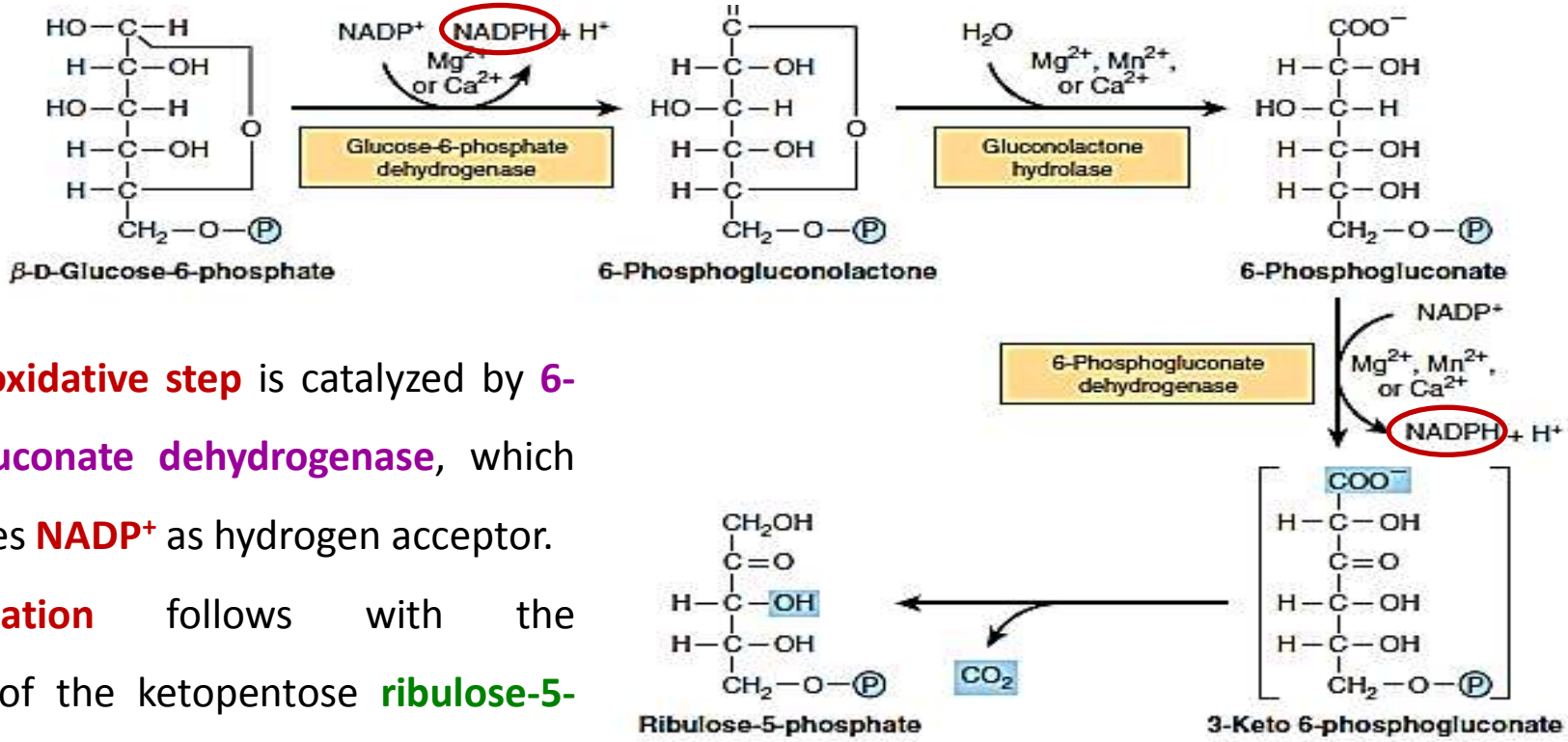
nonoxidative reversible phase

ribulose 5-phosphate is converted back to **glucose 6-phosphate** by a series of reactions involving mainly two enzymes: **transketolase** and **transaldolase**

REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY OCCUR IN THE CYTOSOL

The Oxidative Phase Generates NADPH

- **Dehydrogenation** of **glucose-6-phosphate** to **6-phosphogluconate** occurs via the formation of **6-phosphogluconolactone**, catalyzed by **glucose 6-phosphate dehydrogenase**, an **NADP dependent** enzyme. The **hydrolysis** of 6-phosphogluconolactone is accomplished by the enzyme **gluconolactone hydrolase**.



- A **second oxidative step** is catalyzed by **6-phosphogluconate dehydrogenase**, which also requires **NADP+** as hydrogen acceptor.
- **Decarboxylation** follows with the formation of the ketopentose **ribulose-5-phosphate.**

REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY OCCUR IN THE CYTOSOL

The Oxidative Phase Generates NADPH

- This phase of the pathway is particularly **important in the tissues** in which the pathway is **active use NADPH in reductive syntheses**, for example:
  1. In the **liver**, **lactating mammary glands**, and **adipose tissue**, which are active in the **NADPH-dependent biosynthesis of fatty acids**.
  2. In the **testes**, **ovaries**, and **adrenal cortex**, which are active in the **NADPH-dependent biosynthesis of steroid hormones.**
  3. **In red blood cells, which require NADPH to keep glutathione reduced**.

- The **synthesis of glucose-6-phosphate dehydrogenase** and **6-phosphogluconate dehydrogenase** may also be **induced** by **insulin** in the **fed state**, when **lipogenesis increases.**

The pentose phosphate pathway & Other pathways of hexose Metabolism

REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY OCCUR IN THE CYTOSOL

The Nonoxidative Phase Generates Ribose Precursors

Ribulose-5-phosphate is the substrate for **two enzymes**:

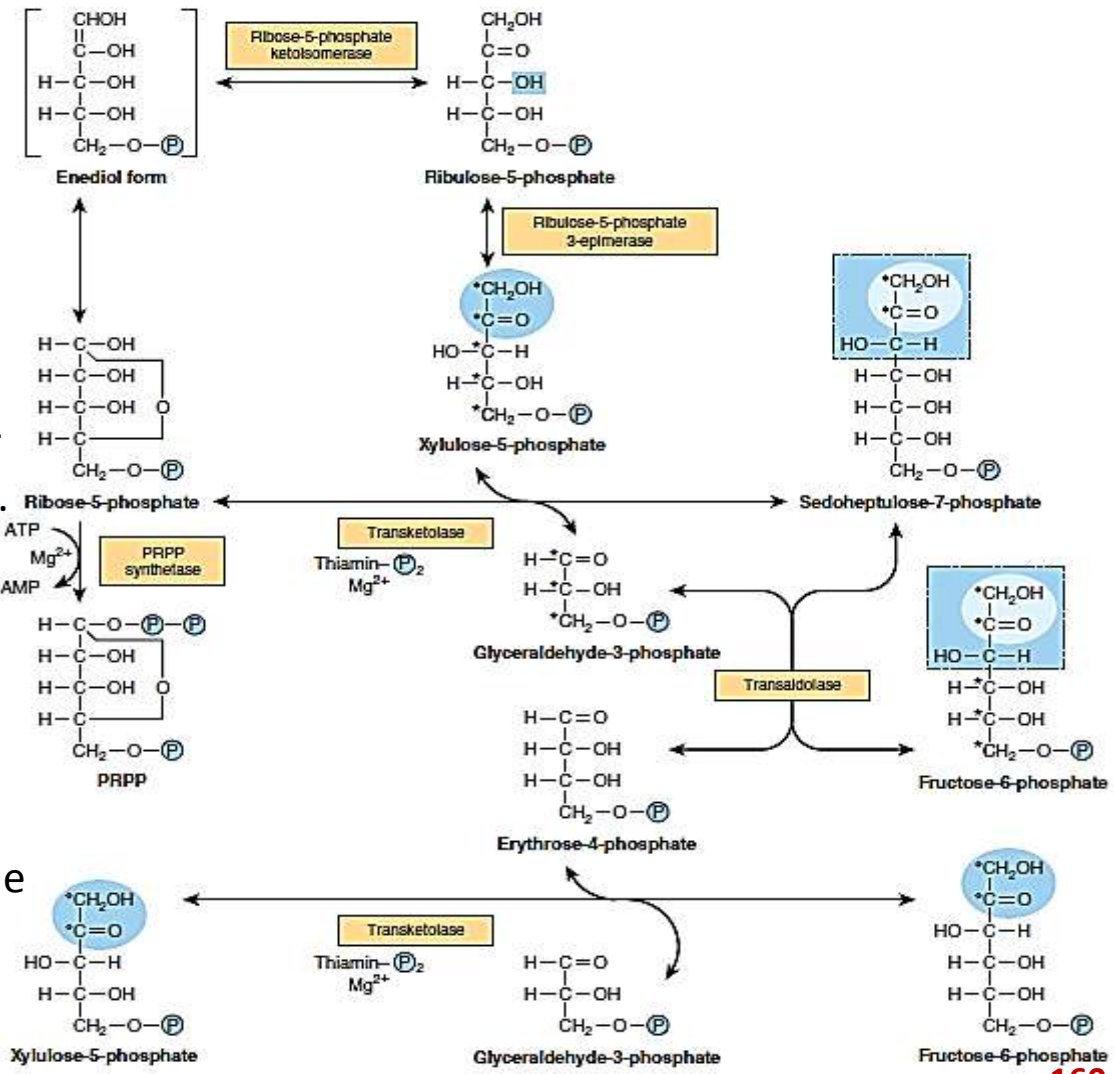
1. **Ribulose-5-phosphate 3-epimerase** alters the configuration to **xylulose 5-phosphate**.

2. **Ribose-5-phosphate ketoisomerase** converts ribulose 5-phosphate to the, **ribose-5-phosphate**, which is used for **nucleotide and nucleic acid synthesis**.

Next: these two products are converted back to Glucose 6-phosphates by a series of reactions involving especially two enzymes

3. **Transketolase: (Mg2+ and thiamin diphosphate (vitamin B1)** catalyzes the transfer of the **two-carbon** (2C)

4. **Transaldolase: (no cofactor)**catalyzes the transfer of a **three carbon** (3C)



The pentose phosphate pathway & Other pathways of hexose Metabolism

REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY OCCUR IN THE CYTOSOL

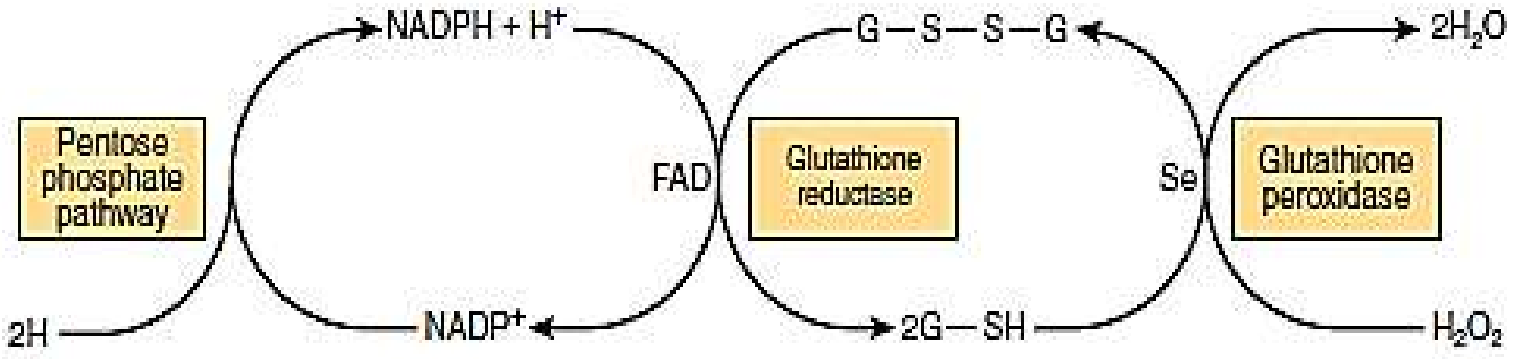
The Two Major Pathways for the Catabolism of Glucose Have Little in Common

	pentose phosphate pathway	glycolysis
site	cytosol	cytosol
glucose-6-phosphate	Yes	Yes
Oxidation utilizes	$NADP^+$	NAD^+
CO_2 production	Yes	No
ATP generation	No	Yes

The pentose phosphate pathway & Other pathways of hexose Metabolism

THE PENTOSE PHOSPHATE PATHWAY & GLUTATHIONE PEROXIDASE PROTECT ERYTHROCYTES AGAINST HEMOLYSIS

- The radical anion superoxide, $O_2^{\cdot -}$, is generated in **red blood cells** by the **autoxidation of hemoglobin to methemoglobin**, and that leads to **formation of H_2O_2**.
- Accumulation of **H_2O_2** may decrease the life span of the erythrocyte by causing oxidative damage to the cell membrane, leading to **hemolysis**.
- **Reduced glutathione removes H_2O_2** in a reaction catalyzed by **glutathione peroxidase**.
- In red blood cells, **the pentose phosphate pathway is the sole source of NADPH** for the **reduction of oxidized glutathione** catalyzed by **glutathione reductase**.
- In other tissues, NADPH can also be generated by the converting of **malate** to **pyruvate** by the **malic enzyme** (**NADP malate dehydrogenase**).



The pentose phosphate pathway & Other pathways of hexose Metabolism

SUMMARY

- The **pentose phosphate pathway**, present in the **cytosol**, can account for the **complete oxidation of glucose**, **producing NADPH and CO_2** but not ATP.

- The pathway has
  1) an **oxidative phase**, which is **irreversible** and **generates NADPH**, and a
  2) **nonoxidative phase**, which is **reversible** and **provides ribose precursors for nucleotide synthesis**.

- The complete pathway is present mainly in those **tissues having** a **requirement for NADPH for reductive syntheses**, eg, **lipogenesis** or **steroidogenesis**, whereas the **nonoxidative phase is present in all cells requiring ribose**.

- In **erythrocytes**, the **pathway has a major function in preventing hemolysis** by **providing NADPH to maintain glutathione in the reduced state as the substrate for glutathione peroxidase.**

Metabolism of Glycogen

Biomedical importance

- **Glycogen** is the major **storage carbohydrate** in animals, corresponding to **starch in plants**; it is **a branched polymer of α-D glucose**.
- It occurs mainly in **liver and muscle**.
- Although the **liver content of glycogen is greater than that of muscle**, because the muscle mass of the body is considerably greater than that of the liver, about **three-quarters of total body glycogen is in muscle**.
- **Muscle glycogen provides** a readily available source of **glucose (Glucose-6 phosphate)** for **glycolysis** within the muscle itself.
- **Liver glycogen functions to store and export glucose to maintain blood glucose between meals**.

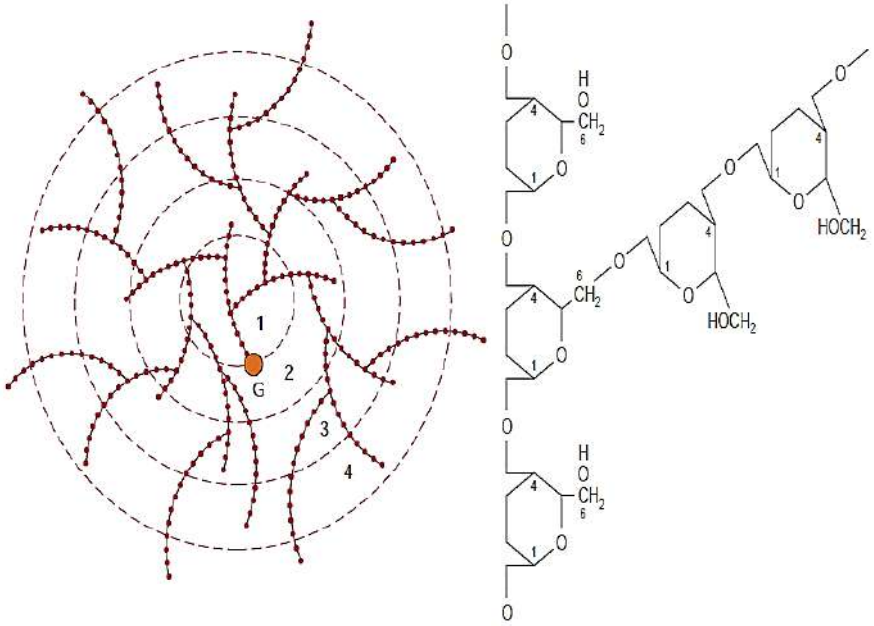


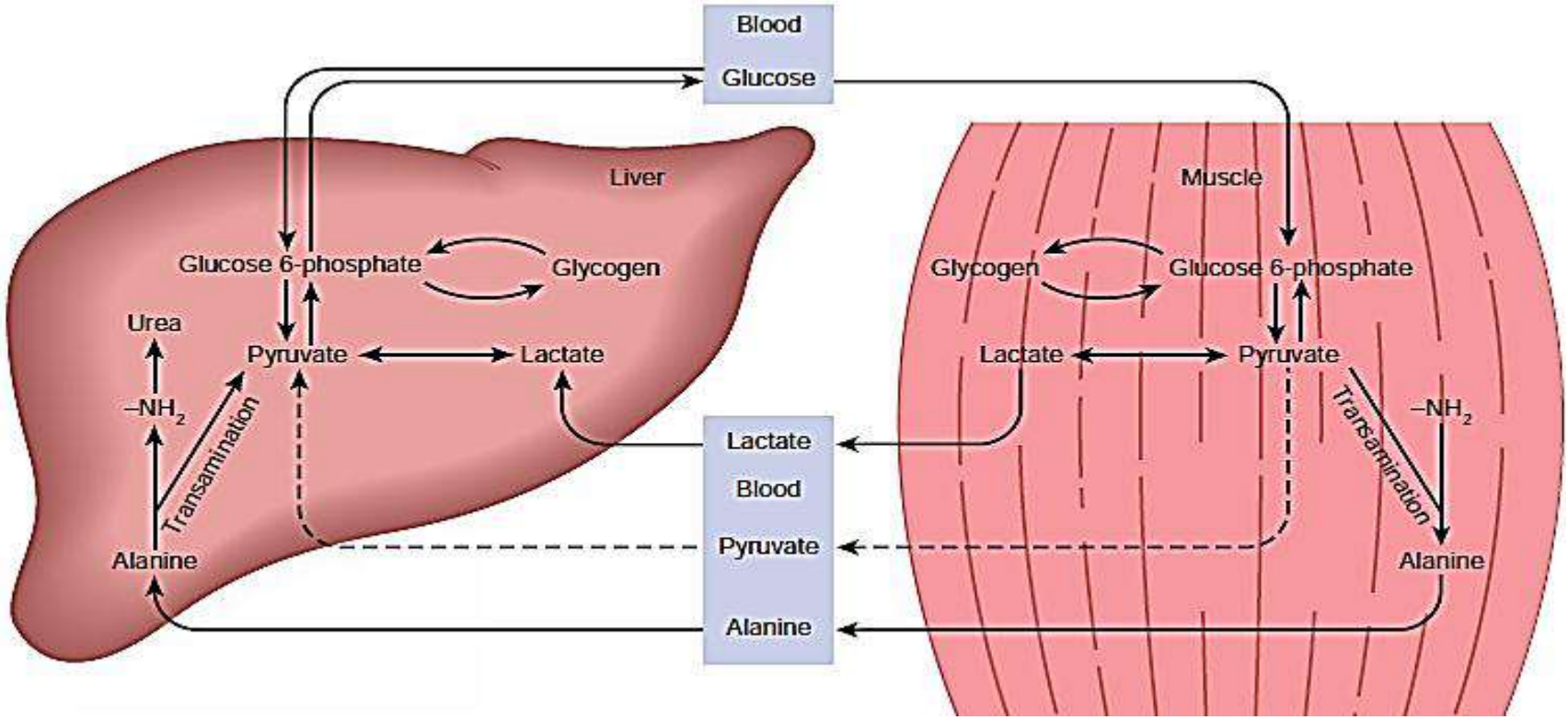
TABLE 19–1: Storage of Carbohydrate in a 70-kg Human Being

	Percentage of Tissue Weight	Tissue Weight	Body Content
Liver glycogen	5.0	1.8 kg	90 g
Muscle glycogen	0.7	35 kg	245 g
Extracellular glucose	0.1	10 L	10 g

Metabolism of Glycogen

Biomedical importance

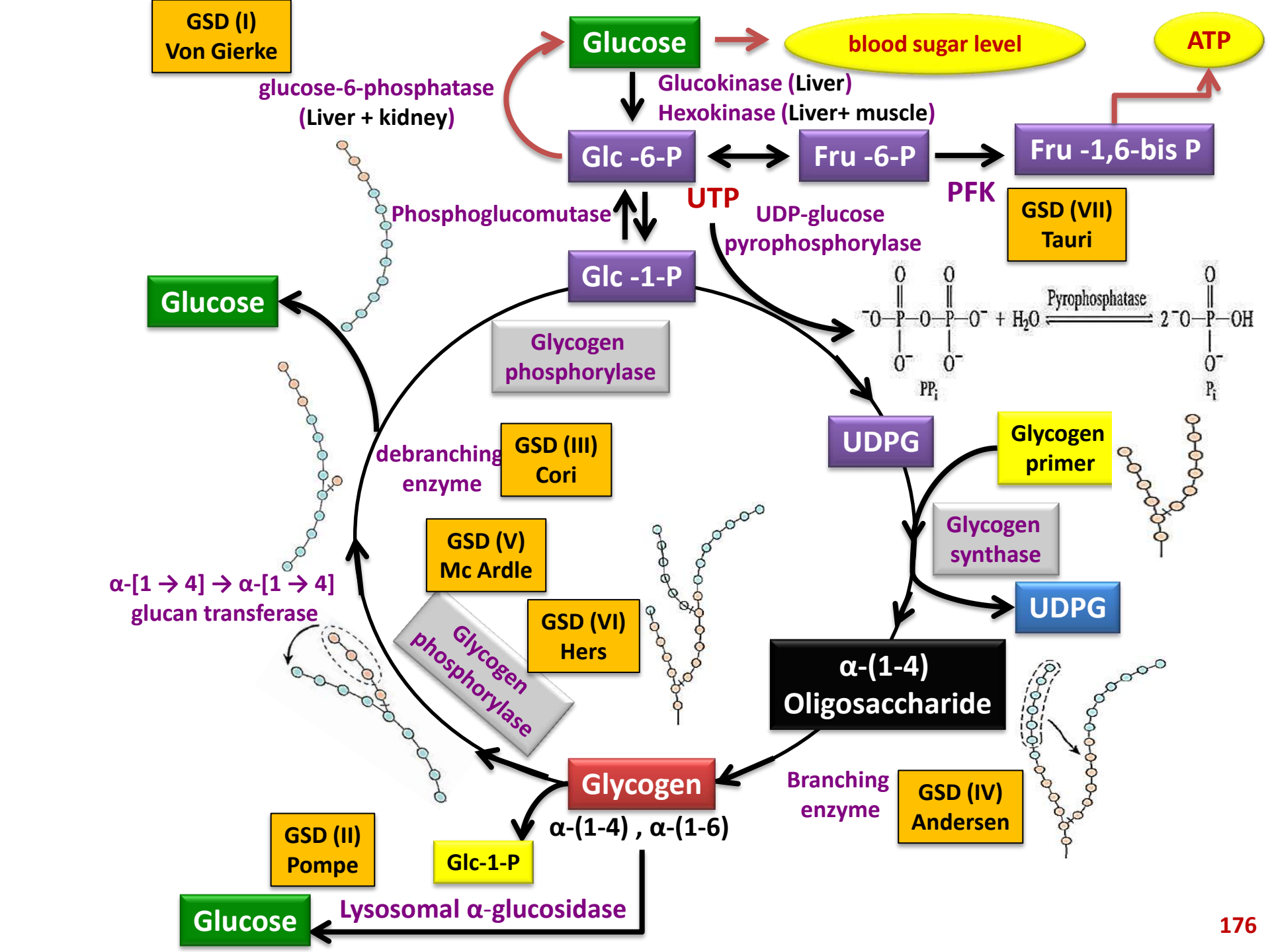
- The **liver concentration of glycogen** is about **450 mM after a meal**, **falling to about 200 mM after an overnight fast**; **after 12–18 h of fasting, liver glycogen is almost totally depleted**.
- Although **muscle glycogen does not directly yield free glucose** (**because muscle lacks glucose 6-phosphatase**), **pyruvate formed by glycolysis in muscle** can undergo **transamination to alanine,** which is **exported from muscle** and used for **gluconeogenesis in the liver.**



Metabolism of Glycogen

Biomedical importance

- **Glycogen storage diseases** are a group of **inherited disorders** characterized by **deficient mobilization of glycogen** or **deposition of abnormal forms of glycogen**, leading to **muscle weakness**; some glycogen storage diseases result in **early death**.
- The **highly branched structure of glycogen** provides a **large number of sites** for **glycogenolysis**, permitting **rapid release of glucose 1-phosphate for muscle activity**.
- **Endurance athletes require a slower, more sustained release of glucose 1-phosphate**.
- The formation of **branch points in glycogen is slower than the addition of glucose units to a linear chain**, and **some endurance athletes practice carbohydrate loading —exercise to exhaustion** (when muscle glycogen in largely depleted) **followed by a high-carbohydrate meal, which results in rapid glycogen synthesis**, with **fewer branch points than normal**.

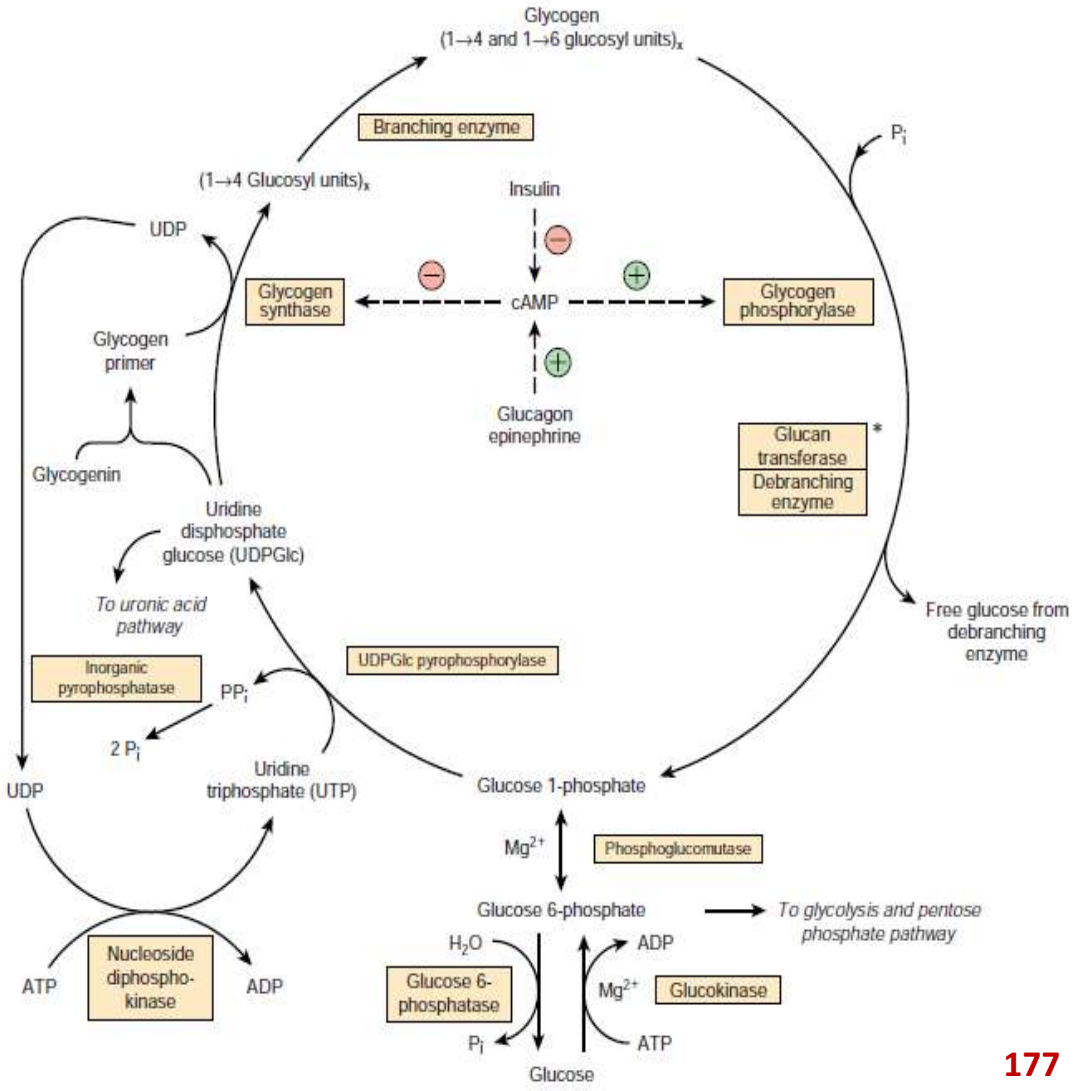

GSD (I)
Von Gierke
Glucose
blood sugar level
ATP
glucose-6-phosphatase
(Liver + kidney)
Glucokinase (Liver)
Hexokinase (Liver+ muscle)
Glc -6-P
Fru -6-P
Fru -1,6-bis P
PFK
GSD (VII)
Tauri
UTP
UDP-glucose
pyrophosphorylase
Phosphoglucomutase
Glc -1-P
Glucose
Glycogen
phosphorylase
Pyrophosphatase
PPi
Pi
UDPG
Glycogen
primer
debranching
enzyme
GSD (III)
Cori
Glycogen
synthase
GSD (V)
Mc Ardle
GSD (VI)
Hers
α-[1 → 4] → α-[1 → 4]
glucan transferase
Glycogen
phosphorylase
UDPG
α-(1-4)
Oligosaccharide
Glycogen
Branching
enzyme
GSD (IV)
Andersen
α-(1-4) , α-(1-6)
GSD (II)
Pompe
Glc-1-P
Glucose
Lysosomal α-glucosidase

Metabolism of Glycogen

Glycogenesis occurs mainly in muscle & liver

The pathway of glycogen biosynthesis involves a special nucleotide of glucose

- As in **glycolysis, glucose is phosphorylated to glucose 6-phosphate**, catalyzed by **hexokinase** in muscle and **glucokinase** in liver.
- **Glucose 6-phosphate** is **isomerized** to **glucose 1-phosphate** by **phosphoglucomutase**.
- The enzyme **itself is phosphorylated**, and the phospho-group takes part in a **reversible reaction** in which **glucose 1,6-bisphosphate is an intermediate**.

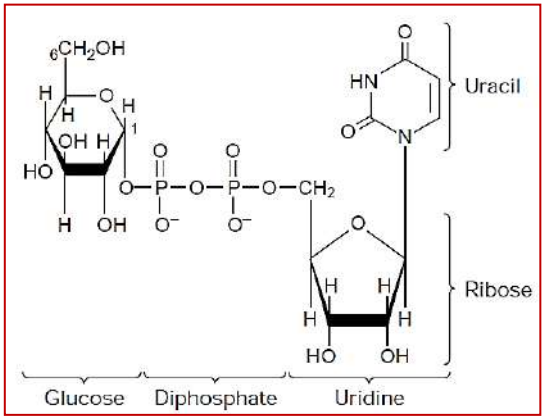


Metabolism of Glycogen

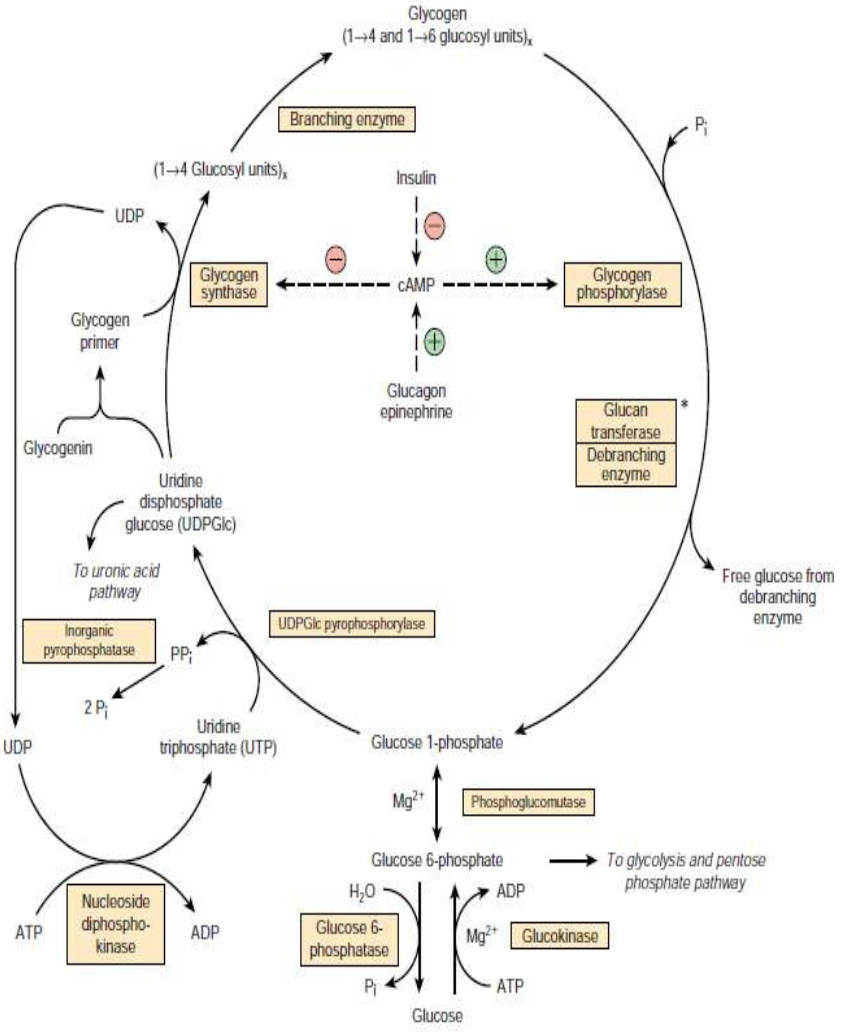
Glycogenesis occurs mainly in muscle & liver

The pathway of glycogen biosynthesis involves a special nucleotide of glucose

- Next, **glucose 1-phosphate reacts** with **uridine triphosphate (UTP)** to form the **active nucleotide uridine diphosphate glucose (UDPGlc)** and **pyrophosphate** catalyzed by **UDPGlc pyrophosphorylase**.



- **The reaction proceeds in the direction of UDPGlc formation because pyrophosphatase catalyzes hydrolysis of pyrophosphate to 2 × phosphate**, so **removing one of the reaction products.**

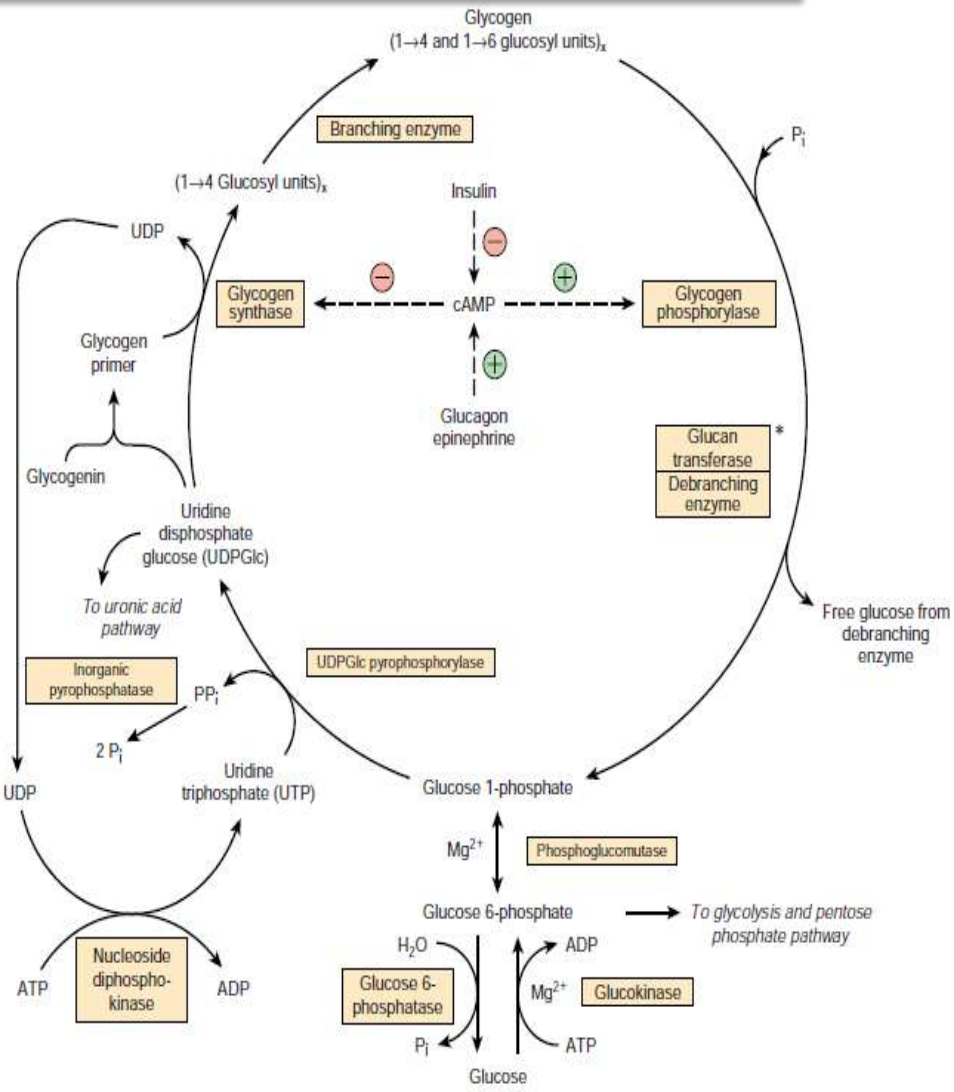


Metabolism of Glycogen

Glycogenesis occurs mainly in muscle & liver

The pathway of glycogen biosynthesis involves a special nucleotide of glucose

- **Glycogen synthase catalyzes the formation of a glycoside bond between C-1 of the glucose of UDPGlc and C-4 of a terminal glucose residue of glycogen, liberating uridine diphosphate (UDP).**
- A **preexisting glycogen molecule**, or "**glycogen primer**," must be present to initiate this reaction. The **glycogen primer in turn is formed on a protein primer** known as **glycogenin**. Glycogenin is a **37 kDa protein** that is **glucosylated on a specific tyrosine residue by UDPGlc**.

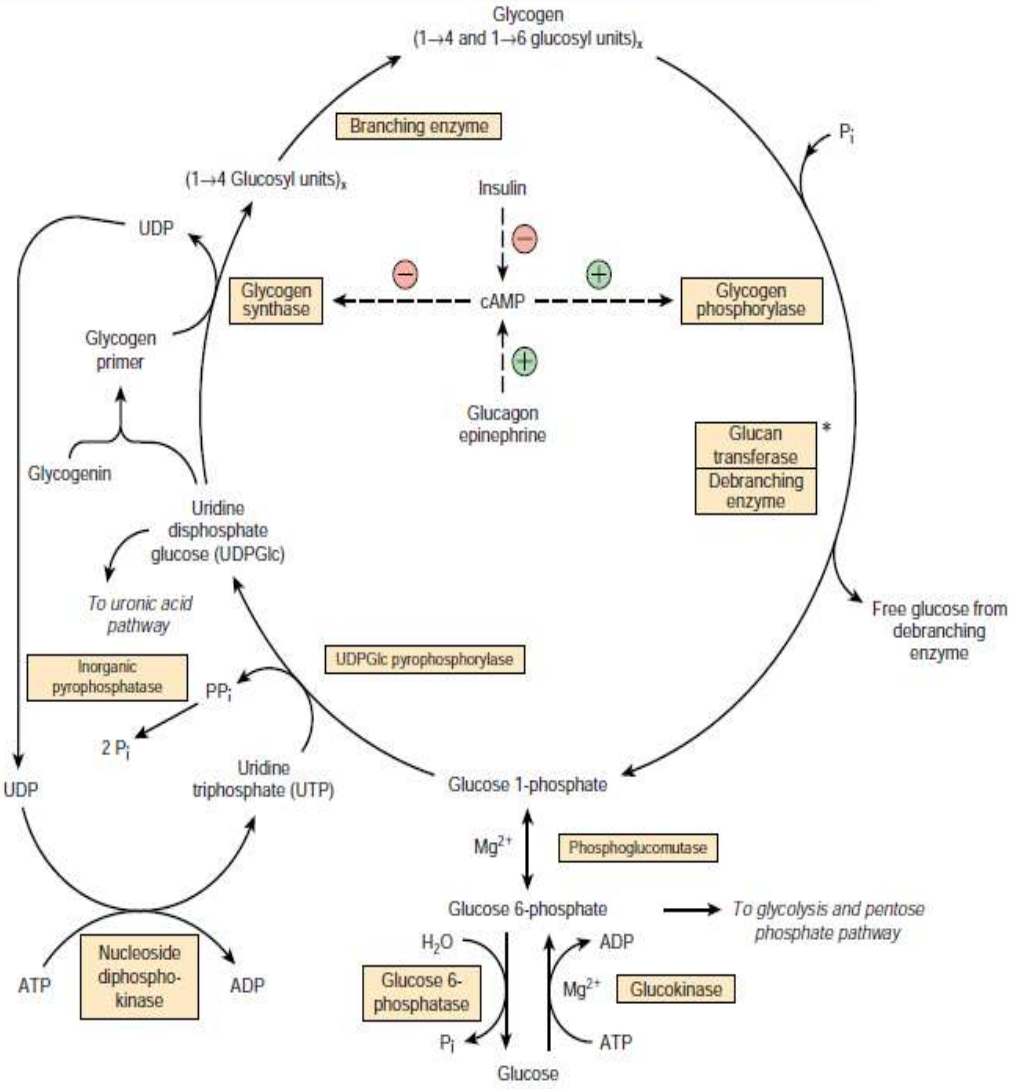


Metabolism of Glycogen

Glycogenesis occurs mainly in muscle & liver

The pathway of glycogen biosynthesis involves a special nucleotide of glucose

- Further glucose residues are attached in the 1 → 4 position (**catalyzed by glycogenin it self**) to form a short chain that is a substrate for **glycogen synthase.**
- **In skeletal muscle**, **glycogenin** remains **attached** in the **center** of the **glycogen** molecule.
- **in liver** the number of **glycogen** molecules is **greater than** the number of **glycogenin** molecules.

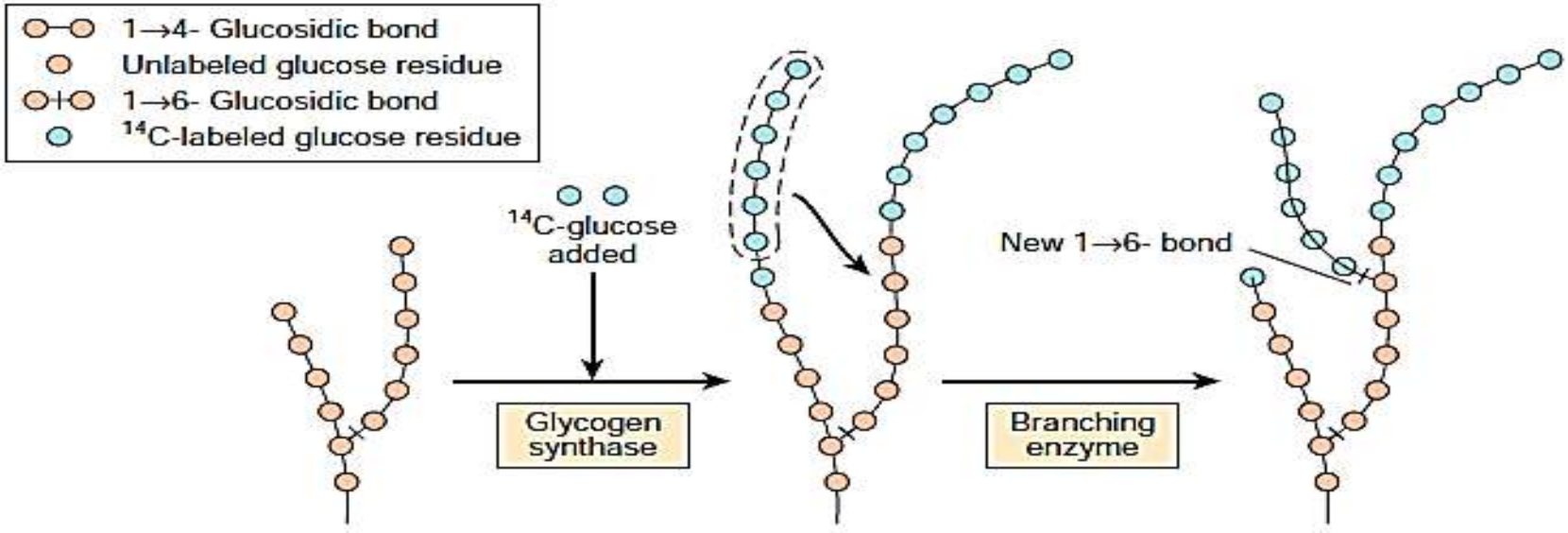


Metabolism of Glycogen

Glycogenesis occurs mainly in muscle & liver

Branching Involves Detachment of Existing Glycogen Chains

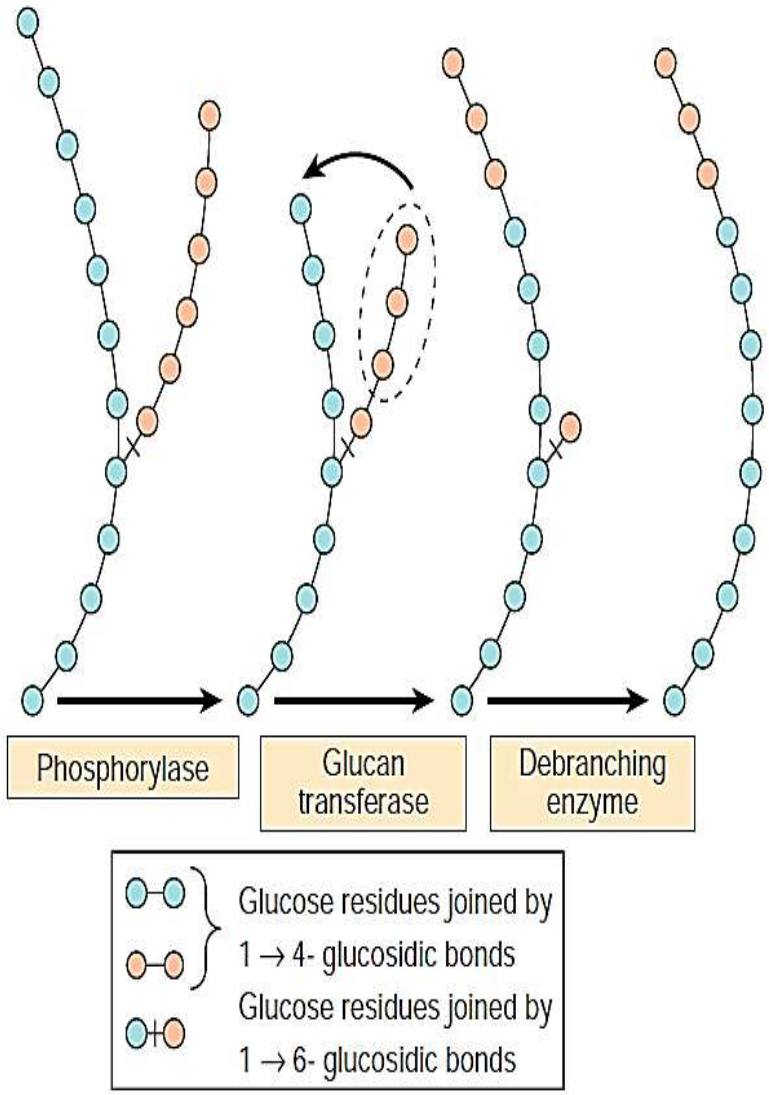
- The **addition of a glucose residue to a preexisting glycogen chain**, or "primer," occurs at the **nonreducing**, outer end of the molecule, so that the branches of the glycogen molecule become **elongated as successive 1 → 4 linkages are formed.**
- When **the chain is at least 11 glucose residues long**, **branching enzyme** transfers a part of the 1 → 4-chain (**at least six glucose residues**) to a neighboring chain to form a 1 → 6 linkage, establishing a branch point.
- The branches grow by further additions of 1 → 4-glucosyl units and further branching.



Metabolism of Glycogen

Glycogenolysis is not the reverse of glycogenesis, but is a separate pathway

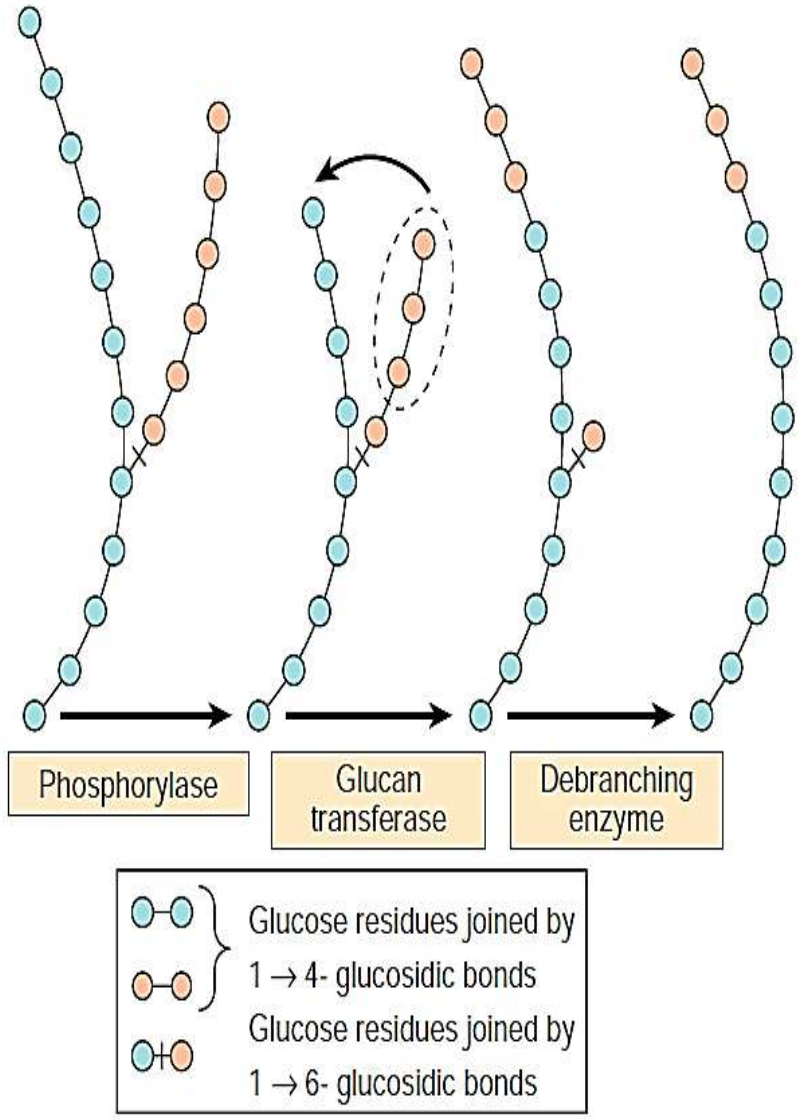
- **Glycogen phosphorylase** catalyzes the rate-limiting step in **glycogenolysis** by catalyzing the phosphoroylytic **cleavage (phosphorolysis; of hydrolysis) of the 1 → 4 linkages of glycogen to yield glucose 1-phosphate**.
- **Glycogen phosphorylase** requires pyridoxal phosphate (PLP) as its coenzyme.
- Unlike the reactions of amino acid metabolism (Chapter 29), in which the aldehyde is **the reactive group, in phosphorylase it is the phosphate group that it catalytically active.**
- **The terminal glucosyl residues from the outermost chains of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a 1 → 6 branch**.



Metabolism of Glycogen

Glycogenolysis is not the reverse of glycogenesis, but is a separate pathway

- Another enzyme (**α-[1 → 4] → α-[1 → 4] glucan transferase**) transfers a **trisaccharide** unit from one branch to the other, **exposing the 1 → 6 branch point**.
- **Hydrolysis of the 1 → 6 linkages** requires the **debranching enzyme**; **glucan transferase and the debranching enzyme are separate activities of a single protein with two catalytic sites**.
- Further phosphorylase action can then proceed.
- The **combined action** of **phosphorylase** and these **other enzymes** leads to the **complete breakdown of glycogen**.



Metabolism of Glycogen

Glycogenolysis is not the reverse of glycogenesis, but is a separate pathway

- The **reaction catalyzed** by **phosphoglucomutase is reversible**, so that **glucose 6-phosphate can be formed from glucose 1-phosphate**.
- **In liver (and kidney),** but **not in muscle**, **glucose 6-phosphatase hydrolyzes glucose 6-phosphate, yielding glucose** that is **exported**, **leading to an increase in the blood glucose concentration**.
- **Glucose 6-phosphatase** is in the lumen of the smooth endoplasmic reticulum, and **genetic defects of the glucose 6-phosphate transporter** can cause a variant of **type I glycogen storage disease** (see Table 19–2).

Metabolism of Glycogen

the regulation of glycogenolysis & glycogenesis

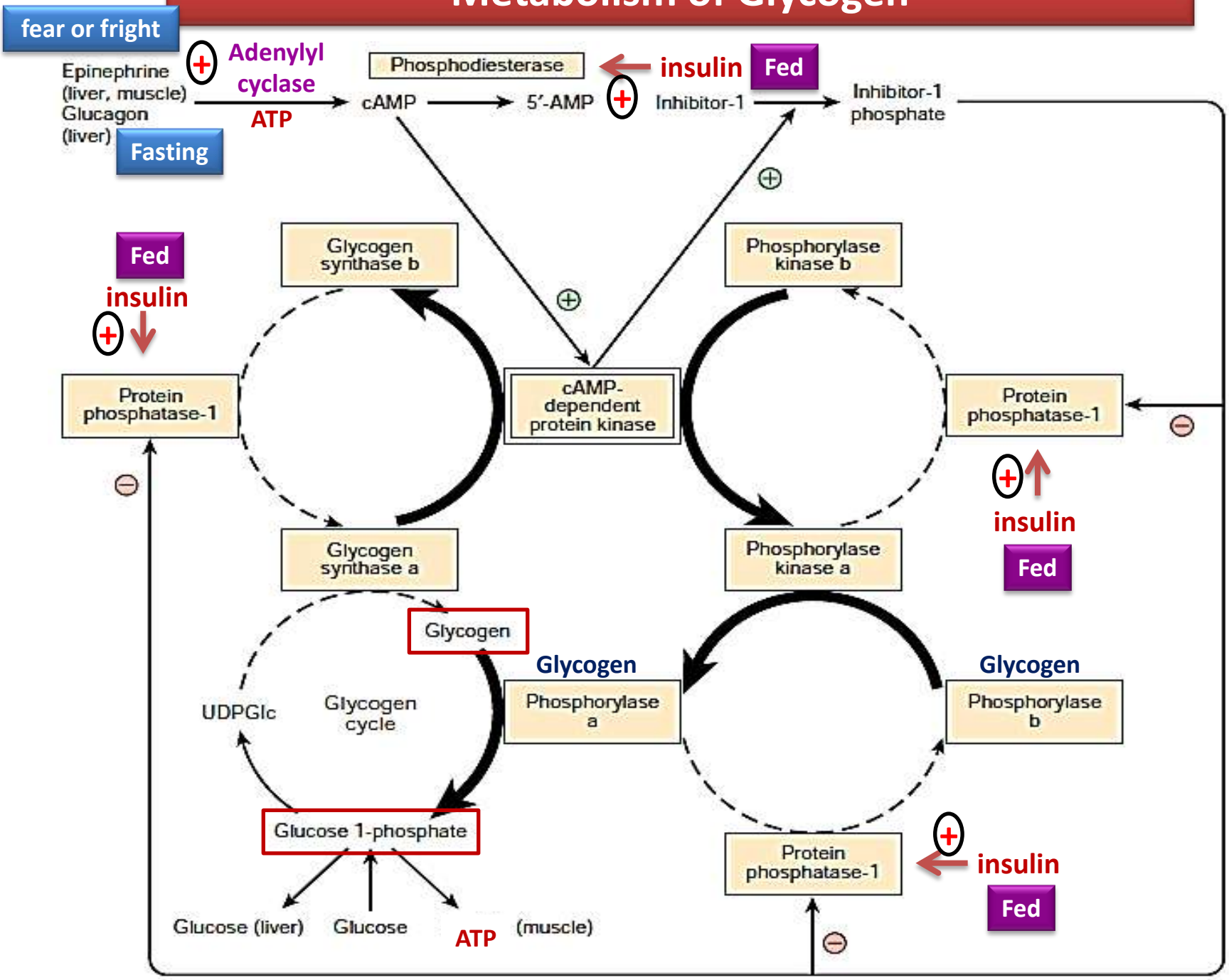
glycogen phosphorylase / glycogen synthase

Hormons
covalent modification
phosphorylation /dephosphorylation

allosteric mechanisms

glycogen phosphorylase

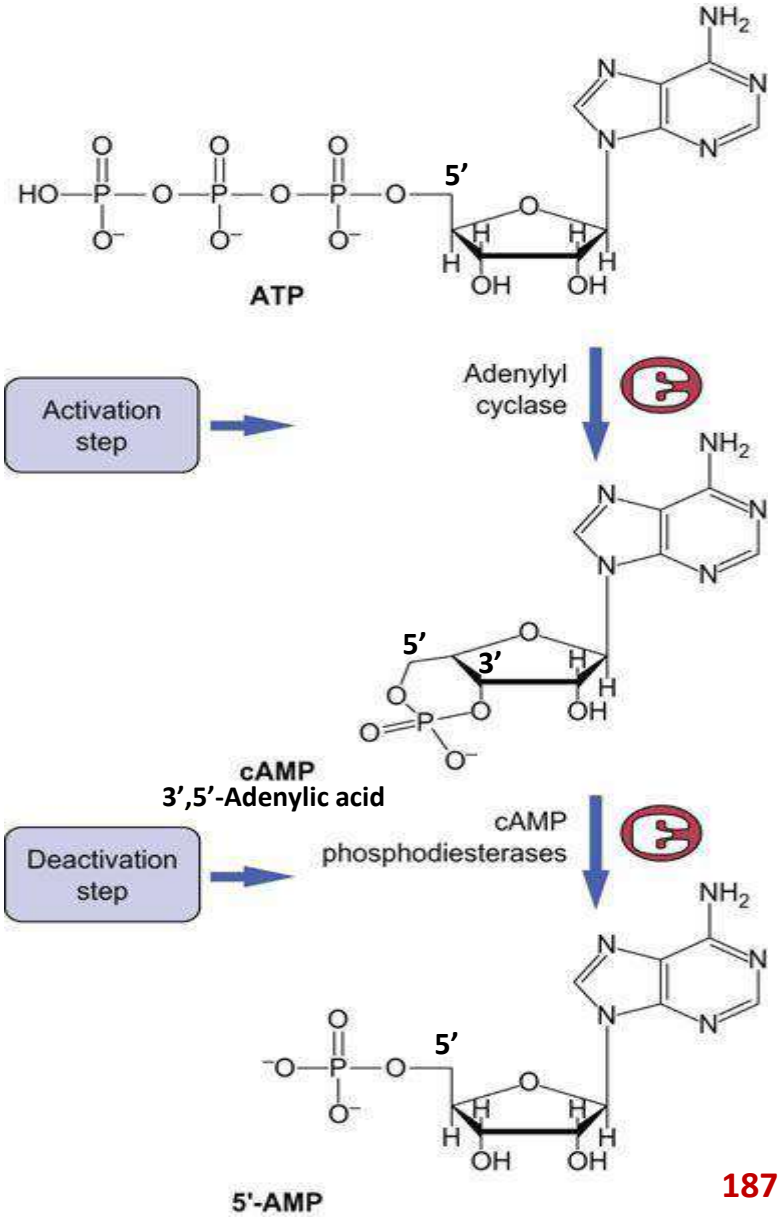
Metabolism of Glycogen



Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

- The principal enzymes controlling **glycogen metabolism**—**glycogen phosphorylase** and **glycogen synthase**—are **regulated by allosteric mechanisms** and **covalent modification** by **reversible phosphorylation and dephosphorylation of enzyme protein in response to hormone action** .
- **Phosphorylation is increased** in response to cyclic adenosine monophosphate (**cyclic AMP; cAMP)** formed from **ATP** by **adenylyl cyclase** at the inner surface of cell membranes **in response to hormones** such as **epinephrine, norepinephrine, and glucagon**.
- **cAMP** is **hydrolyzed** by **phosphodiesterase**, so terminating hormone action; in liver **insulin increases** the **activity** of **phosphodiesterase**.



Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

The Control of Phosphorylase Differs Between Liver & Muscle

- **In the liver** the **role of glycogen** is to provide free **glucose** for export to **maintain the blood concentration of glucose**.
- **In muscle** the **role of glycogen** is to provide a **source of glucose 6-phosphate for glycolysis** in response to the **need for ATP for muscle contraction**.
- **In both tissues**, the **enzyme is activated** by **phosphorylation** catalyzed by **phosphorylase kinase** (to yield **phosphorylase a**) and **inactivated** by **dephosphorylation** catalyzed by **phosphoprotein phosphatase** (to yield **phosphorylase b**), in response to hormonal and other signals.
- There is instantaneous overriding of this hormonal control.
- **In both tissues Active phosphorylase a** is **allosterically inhibited by ATP and glucose 6-phosphate**
- **In liver, but not muscle**, **free glucose** is also an **inhibitor**.

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

The Control of Phosphorylase Differs Between Liver & Muscle

- **Muscle phosphorylase differs from** the **liver isoenzyme in having a binding site for 5'AMP**, which acts as an **allosteric activator** of the (**inactive**) **dephosphorylated b-form of the enzyme.**

- **5'AMP** acts as a **potent signal of the energy state** of the **muscle cell**; it is formed as the concentration of **ADP** begins to **increase** (indicating the **need for increased substrate metabolism** to **permit ATP formation**), **as a result of the reaction of adenylate kinase**:

$$2 \times ADP \leftrightarrow ATP + 5'AMP$$

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

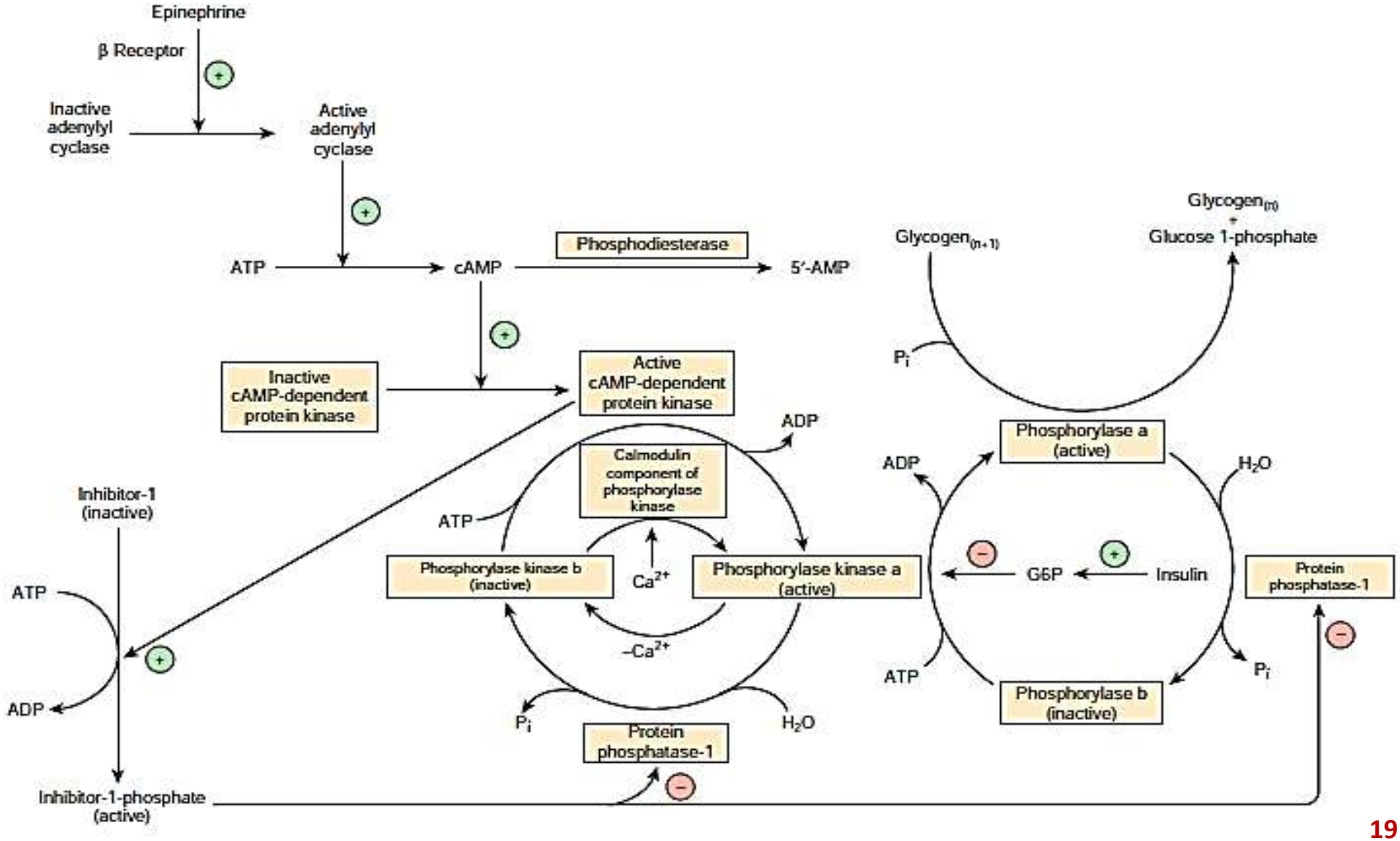
cAMP Activates Phosphorylase

- **Phosphorylase kinase** is **activated** in response to **cAMP**.
- **Increasing** the concentration of **cAMP activates cAMP-dependent protein kinase**, which **catalyzes** the **phosphorylation** by **ATP** of **inactive phosphorylase kinase b** to **active phosphorylase kinase a**, which in turn, **phosphorylates phosphorylase b** to **phosphorylase a**.
- **In the liver**, **cAMP** is formed in response to **glucagon**, which is **secreted** in response to **falling blood glucose**.
- **Muscle** is **insensitive** to **glucagon**; in muscle, the signal for increased **cAMP** formation is the action of **norepinephrine**, which is secreted in response to **fear or fright**, when there is a **need** for **increased glycogenolysis** to **permit rapid muscle activity**.

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

cAMP Activates Phosphorylase



Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

Ca^{2+} Synchronizes the Activation of Phosphorylase With Muscle Contraction

- Glycogenolysis in muscle increases several 100-fold at the onset of contraction; the same signal (increased cytosolic Ca^{2+} ion concentration) is responsible for initiation of both contraction and glycogenolysis.
- **Muscle phosphorylase kinase**, which **activates glycogen phosphorylase**, is a tetramer of four different subunits, α, β, γ, and δ.
- **The α and β subunits** contain serine residues that are **phosphorylated by cAMP-dependent protein kinase**.
- **The δ subunit** is identical to the Ca^{2+}-binding protein calmodulin (Chapter 42), and **binds four Ca^{2+}**.
- **The binding of Ca^{2+} activates** the **catalytic site of the γ subunit even while the enzyme is in the dephosphorylated b state**; the **phosphorylated a form** is **only fully activated in the presence of high concentrations of Ca^{2+}**.

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

Glycogenolysis in Liver Can Be cAMP-Independent

- In the **liver**, there is **cAMP-independent activation** of **glycogenolysis** in response to **stimulation** of **α1 adrenergic receptors** by **epinephrine** and **norepinephrine**.
- This involves **mobilization of Ca^{2+} into the cytosol**, followed by the **stimulation** of a **Ca^{2+}/calmodulin-sensitive phosphorylase kinase**.
- **cAMP-independent glycogenolysis** is **also activated** by **vasopressin, oxytocin, and angiotensin II** acting either through **calcium** or the **phosphatidylinositol bisphosphate pathway** .

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

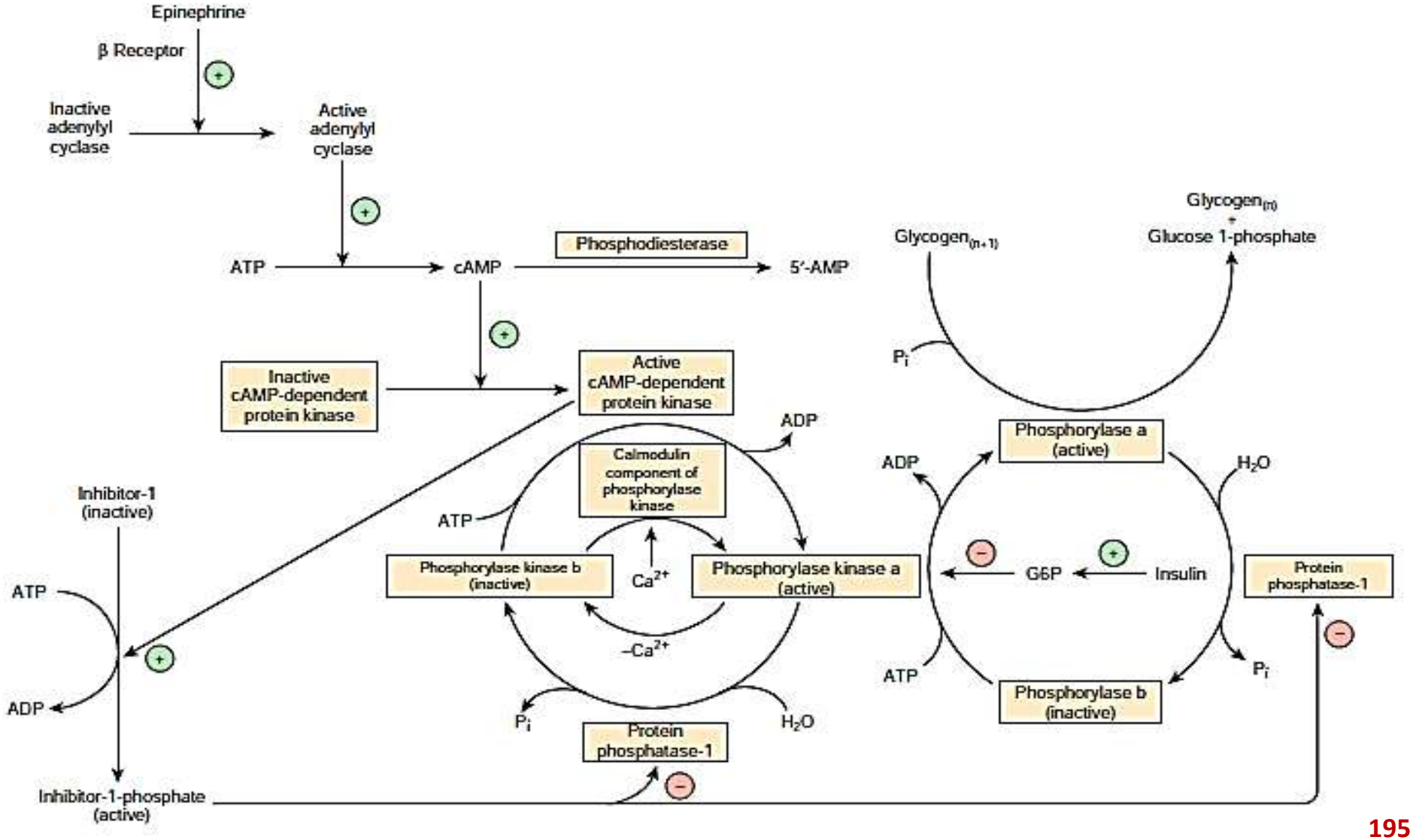
Protein Phosphatase-1 Inactivates Phosphorylase

- Both **phosphorylase a** and **phosphorylase kinase a** are **dephosphorylated** and **inactivated** by **protein phosphatase-1**.
- **Protein phosphatase-1** is **inhibited** by a **protein, inhibitor-1**, which is **active only** after it has been **phosphorylated** by **cAMP-dependent protein kinase**.
- Thus, **cAMP controls both** the **activation** and **inactivation** of **phosphorylase** (Figure 19–6).
- **Insulin reinforces** this effect by **inhibiting** the **activation of phosphorylase b** to **a**
- It does this **indirectly** by **increasing uptake of glucose**, leading to **increased formation** of **glucose 6-phosphate**, which is an **inhibitor of phosphorylase kinase**.

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

Protein Phosphatase-1 Inactivates Phosphorylase



Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

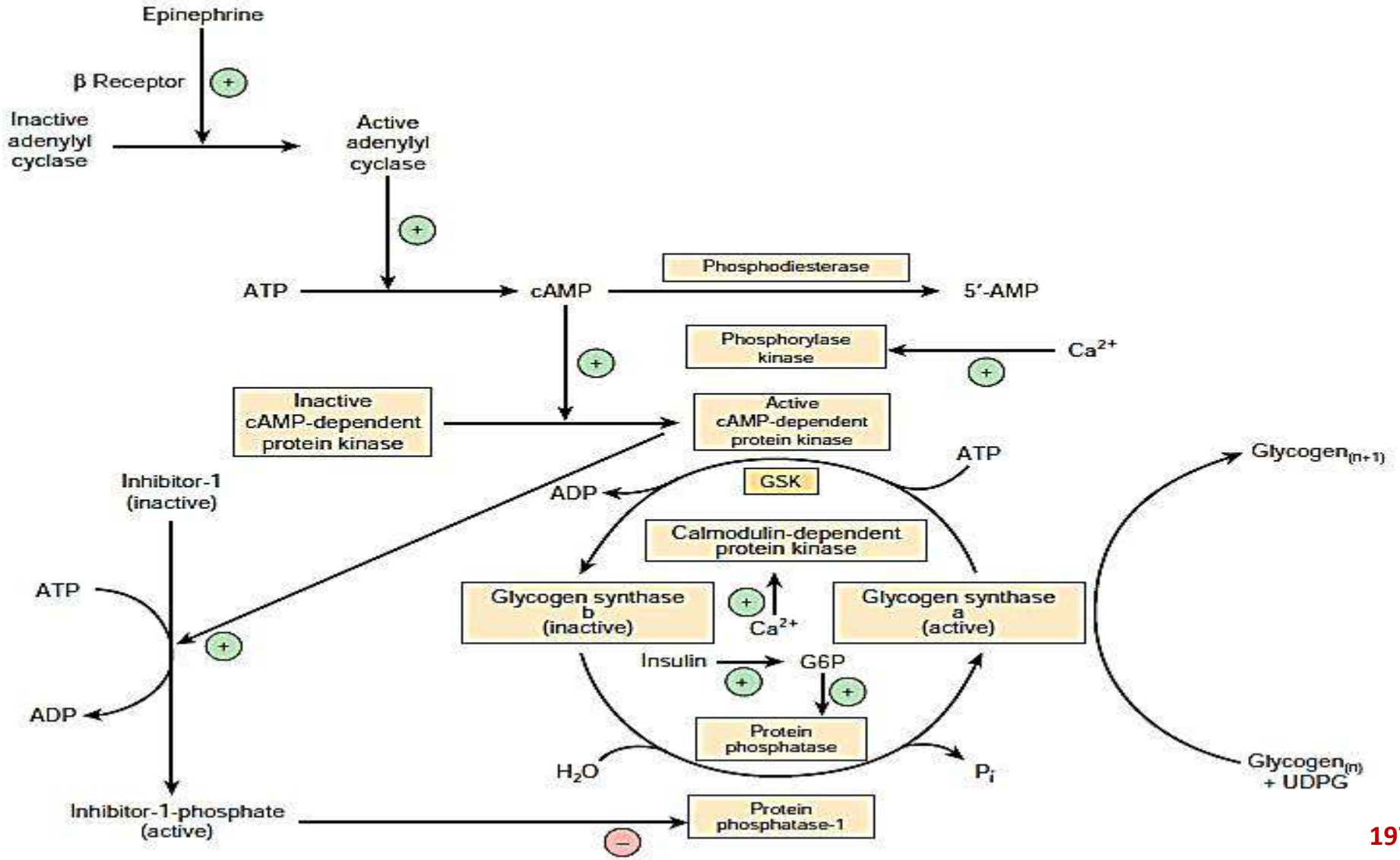
Glycogen Synthase & Phosphorylase Activity Are Reciprocally Regulated

- Like **phosphorylase**, **glycogen synthase** exists in both **phosphorylated** and **nonphosphorylated** states, and the **effect of phosphorylation is the reverse of that seen in phosphorylase** (Figure 19–7).
- **Active glycogen synthase a** is **dephosphorylated** and **inactive glycogen synthase b** is **phosphorylated**.
- **Six different protein kinases act** on **glycogen synthase**. **Two** are **Ca2+/calmodulin-dependent** (one of these is **phosphorylase kinase**).
- **Another kinase** is **cAMP-dependent protein kinase**, which **allows cAMP-mediated hormonal action** to **inhibit glycogen synthesis** synchronously with the **activation of glycogenolysis**.
- **Insulin** also **promotes glycogenesis in muscle** at the same time as **inhibiting glycogenolysis** by **raising glucose 6-phosphate concentrations**, which **stimulates** the **dephosphorylation** and **activation** of **glycogen synthase**.
- **Dephosphorylation** of **glycogen synthase b** is carried out by **protein phosphatase-1**, which is under the control of **cAMP-dependent protein kinase**.

Metabolism of Glycogen

Cyclic amp integrates the regulation of glycogenolysis & glycogenesis

Glycogen Synthase & Phosphorylase Activity Are Reciprocally Regulated

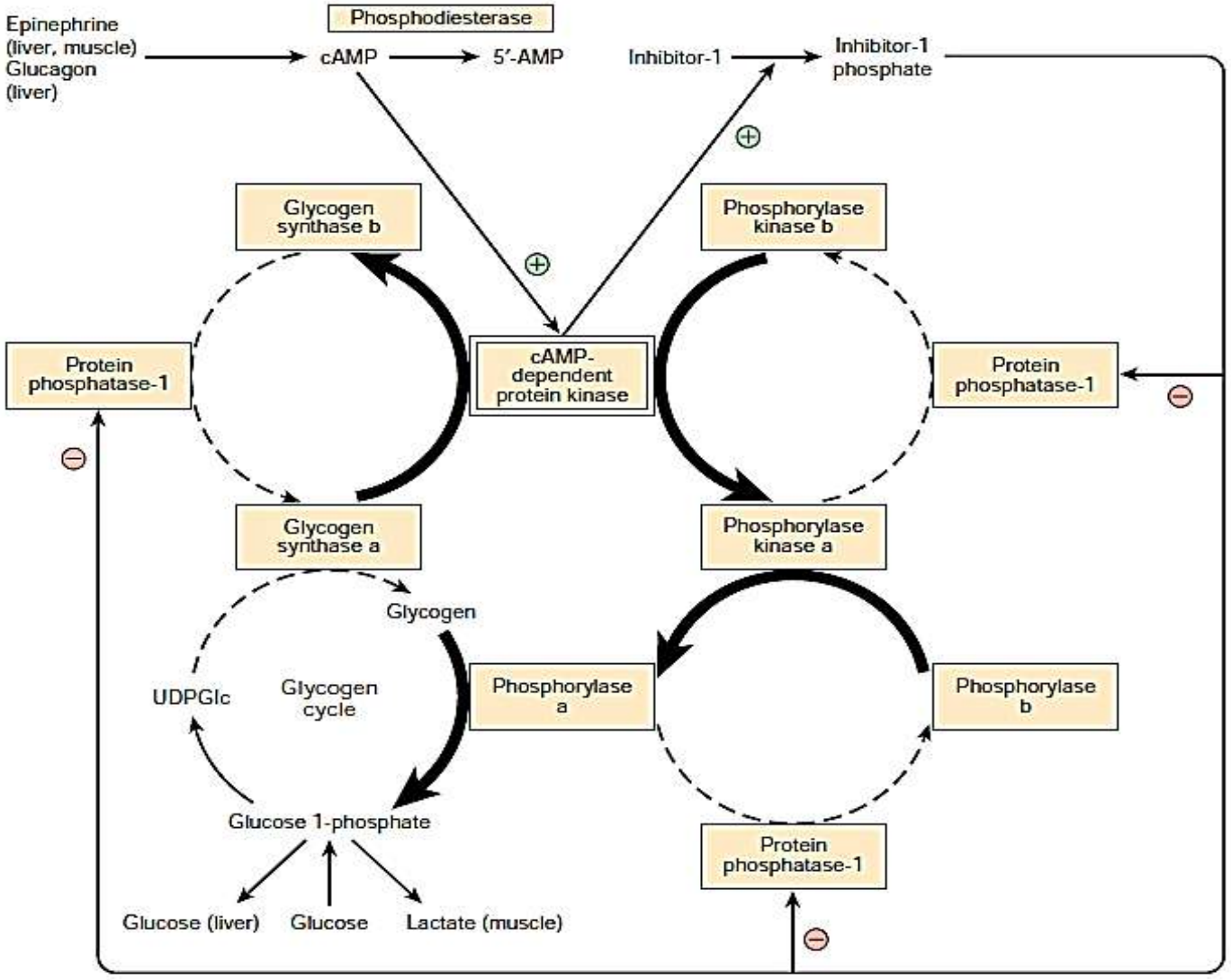


Metabolism of Glycogen

Regulation of glycogen metabolism is effected by a balance in activities Between glycogen synthase & phosphorylase

- At the **same time as phosphorylase** is **activated** by a **rise in concentration of cAMP** (via **phosphorylase kinase**),

glycogen synthase is **converted to the inactive form**; **both effects are mediated** via **cAMP-dependent protein kinase**



Metabolism of Glycogen

Regulation of glycogen metabolism is effected by a balance in activities Between glycogen synthase & phosphorylase

- Thus, **inhibition of glycogenolysis enhances net glycogenesis**, and **inhibition** of **glycogenesis enhances net glycogenolysis**.
- Also, the **dephosphorylation** of **phosphorylase** a, **phosphorylase kinase**, and **glycogen synthase b** is **catalyzed by a single enzyme** with **broad specificity—protein phosphatase-1**.
- In turn, **protein phosphatase-1** is **inhibited by cAMP-dependent protein kinase via inhibitor-1**.
- Thus, **glycogenolysis can be terminated** and **glycogenesis** can be **stimulated**, or **vice versa, synchronously**, **because both processes are dependent on the activity of cAMP-dependent protein kinase**.
- **Both phosphorylase kinase** and **glycogen synthase may be reversibly phosphorylated** at more than one site by **separate kinases and phosphatases**.
- These **secondary phosphorylations modify the sensitivity** of the **primary sites to phosphorylation and dephosphorylation** (**multisite phosphorylation**).
- Also, **they allow insulin**, **by way of increased glucose 6-phosphate**, **to have effects that act reciprocally to those of cAMP** (see Figures 19–6 & 19–7).

Metabolism of Glycogen

CLINICAL ASPECTS glycogen storage diseases are inherited

- "**Glycogen storage disease**" is a generic term to describe a group of inherited disorders characterized by **deposition of an abnormal type or quantity** of **glycogen in tissues**, or **failure** to **mobilize glycogen**.

Type	Name	Enzyme Deficiency	Clinical Features
0	—	Glycogen synthase	Hypoglycemia; hyperketonemia; early death
Ia	Von Gierke's disease	Glucose 6-phosphatase	Glycogen accumulation in liver and renal tubule cells; hypoglycemia; lactic acidemia; ketosis; hyperlipemia
Ib	—	Endoplasmic reticulum glucose 6-phosphate transporter	As type Ia; neutropenia and impaired neutrophil function leading to recurrent infections
II	Pompe's disease	Lysosomal α1→4 and α1→6 glucosidase (acid maltase)	Accumulation of glycogen in lysosomes: juvenile onset variant, muscle hypotonia, death from heart failure by age 2; adult onset variant, muscle dystrophy
IIIa	Limit dextrinosis, Forbe's or Cori's disease	Liver and muscle debranching enzyme	Fasting hypoglycemia; hepatomegaly in infancy; accumulation of characteristic branched polysaccharide (limit dextrin); muscle weakness
IIIb	Limit dextrinosis	Liver debranching enzyme	As type IIIa, but no muscle weakness
IV	Amylopectinosis, Andersen's disease	Branching enzyme	Hepatosplenomegaly; accumulation of polysaccharide with few branch points; death from heart or liver failure before age 5
V	Myophosphorylase deficiency, McArdle's syndrome	Muscle phosphorylase	Poor exercise tolerance; muscle glycogen abnormally high (2.5–4%); blood lactate very low after exercise
VI	Hers' disease	Liver phosphorylase	Hepatomegaly; accumulation of glycogen in liver; mild hypoglycemia; generally good prognosis
VII	Tarui's disease	Muscle and erythrocyte phosphofructokinase 1	Poor exercise tolerance; muscle glycogen abnormally high (2.5–4%); blood lactate very low after exercise; also hemolytic anemia
VIII		Liver phosphorylase kinase	Hepatomegaly; accumulation of glycogen in liver; mild hypoglycemia; generally good prognosis
IX		Liver and muscle phosphorylase kinase	Hepatomegaly; accumulation of glycogen in liver and muscle; mild hypoglycemia; generally good prognosis
X		cAMP-dependent protein kinase A	Hepatomegaly; accumulation of glycogen in liver

Metabolism of Glycogen

SUMMARY

1) Glycogen represents the principal storage carbohydrate in the body, mainly in the liver and muscle.
2) In the liver, its major function is to provide glucose for extrahepatic tissues. In muscle, it serves mainly as a ready source of metabolic fuel for use in muscle. Muscle lacks glucose 6-phosphatase and cannot release free glucose from glycogen.
3) Glycogen is synthesized from glucose by the pathway of glycogenesis. It is broken down by a separate pathway, glycogenolysis.
4) Cyclic AMP integrates the regulation of glycogenolysis and glycogenesis by promoting the simultaneous activation of phosphorylase and inhibition of glycogen synthase. Insulin acts reciprocally by inhibiting glycogenolysis and stimulating glycogenesis.
5) Inherited deficiencies of enzymes of glycogen metabolism in both liver and muscle cause glycogen storage diseases.

Oxidation of Fatty Acids: Ketogenesis

Biomedical Importance

- Although **fatty acids** are **broken down by oxidation to acetyl- CoA** and also **synthesized from acetyl-CoA**, fatty acid oxidation is **not the simple reverse** of fatty acid biosynthesis but an entirely different process **taking place in a separate compartment of the cell**.
- The separation of **fatty acid oxidation in mitochondria** from **biosynthesis in the cytosol** allows each process to be individually controlled and integrated with tissue requirements.
- **Each step in fatty acid oxidation involves acyl-CoA derivatives**, is **catalyzed by separate enzymes**, **utilizes NAD^+ and FAD as coenzymes**, and **generates ATP**.
- It is an **aerobic process**, requiring the presence of **oxygen**.

Oxidation of Fatty Acids: Ketogenesis

Biomedical Importance

- **Increased fatty acid oxidation** is a characteristic of **starvation and of diabetes mellitus**, and leads to **increased ketone body production by the liver** (**ketosis**).
- **Ketone bodies are acidic** and when produced in **excess over long periods**, as in **diabetes**, cause **ketoacidosis**, which is ultimately **fatal**.
- Because **gluconeogenesis is dependent upon fatty acid oxidation**, any **impairment in fatty acid oxidation** leads to **hypoglycemia**.
- This occurs in various states of **carnitine deficiency** or **deficiency of essential enzymes** in fatty acid oxidation, for example, **carnitine palmitoyltransferase**, or **inhibition of fatty acid oxidation by poisons**, for example, **hypoglycin**.

Oxidation of Fatty Acids: Ketogenesis

Oxidation of fatty acids occurs in mitochondria

Fatty acids are transported in the blood as free fatty acids

- **Free fatty acids** (**FFAs**)—also called **unesterified** (**UFA**) or **nonesterified** (**NEFA**) fatty acids—are fatty acids that are in the **unesterified state**.
- In **plasma**, **longer chain FFA** are combined with **albumin**, and **in the cell** they are attached to a **fatty acid binding protein**, so that in fact they are **never really** "**free**."
- **Shorter chain fatty acids** are **more water-soluble** and **exist as the unionized acid or as a fatty acid anion**.

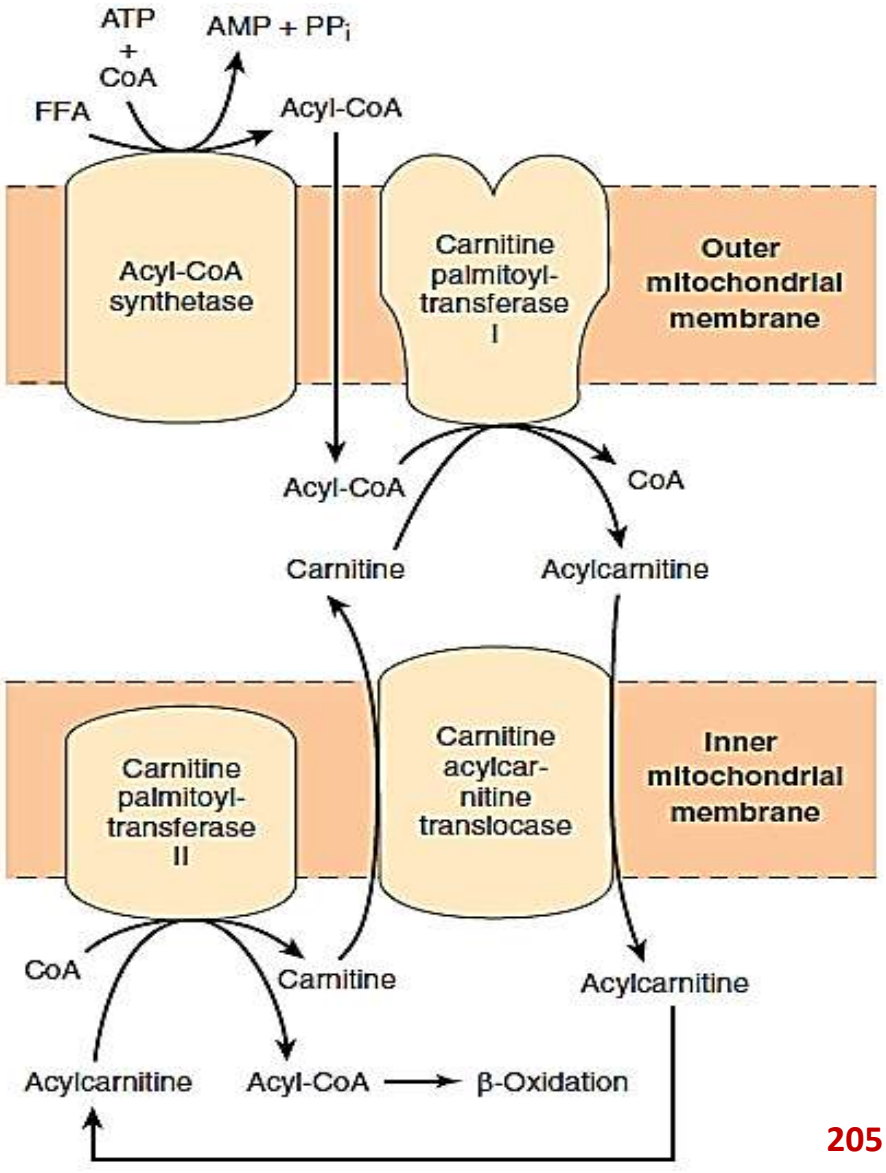
Oxidation of Fatty Acids: Ketogenesis

Oxidation Of Fatty Acids Occurs In Mitochondria

Fatty Acids Are Activated Before Being Catabolized

1) **Fatty acids** must first be **converted to an active intermediate** before they can be catabolized.

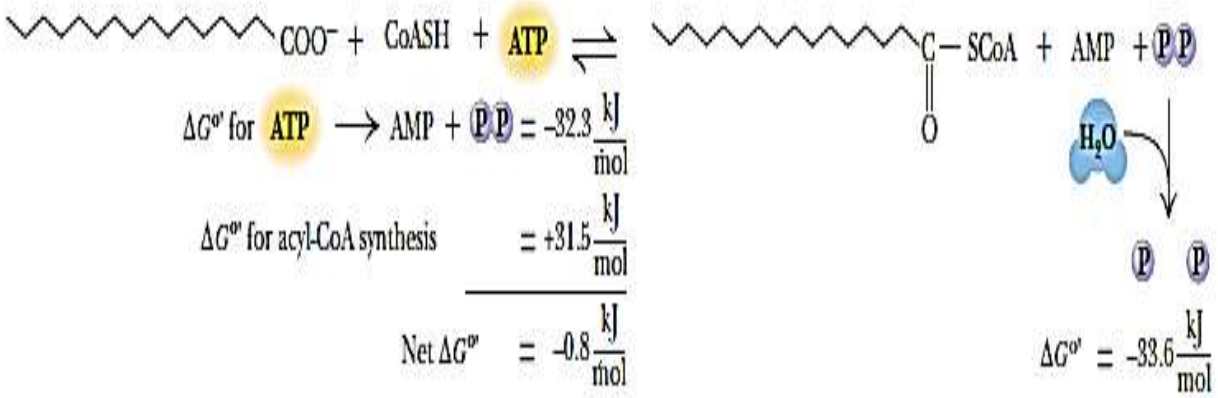
- This is the **only step** in the complete degradation of a fatty acid that **requires energy from ATP**.
- In the **presence** of **ATP** and **coenzyme A**, the enzyme **acyl-CoA synthetase** (**fatty acid thiokinase**) catalyzes the conversion of a **fatty acid** (or **FFA**) to an "**active fatty acid**" or **acyl-CoA**, using one high-energy phosphate and forming **AMP** and **PPi**.
- The **PPi** is **hydrolyzed** by **inorganic pyrophosphatase** with the loss of a further high energy phosphate, **ensuring** that the **overall reaction goes to completion**.
- **Acyl-CoA synthetases** are found in the **endoplasmic reticulum**, **peroxisomes**, and **inside and on the outer membrane of mitochondria**.

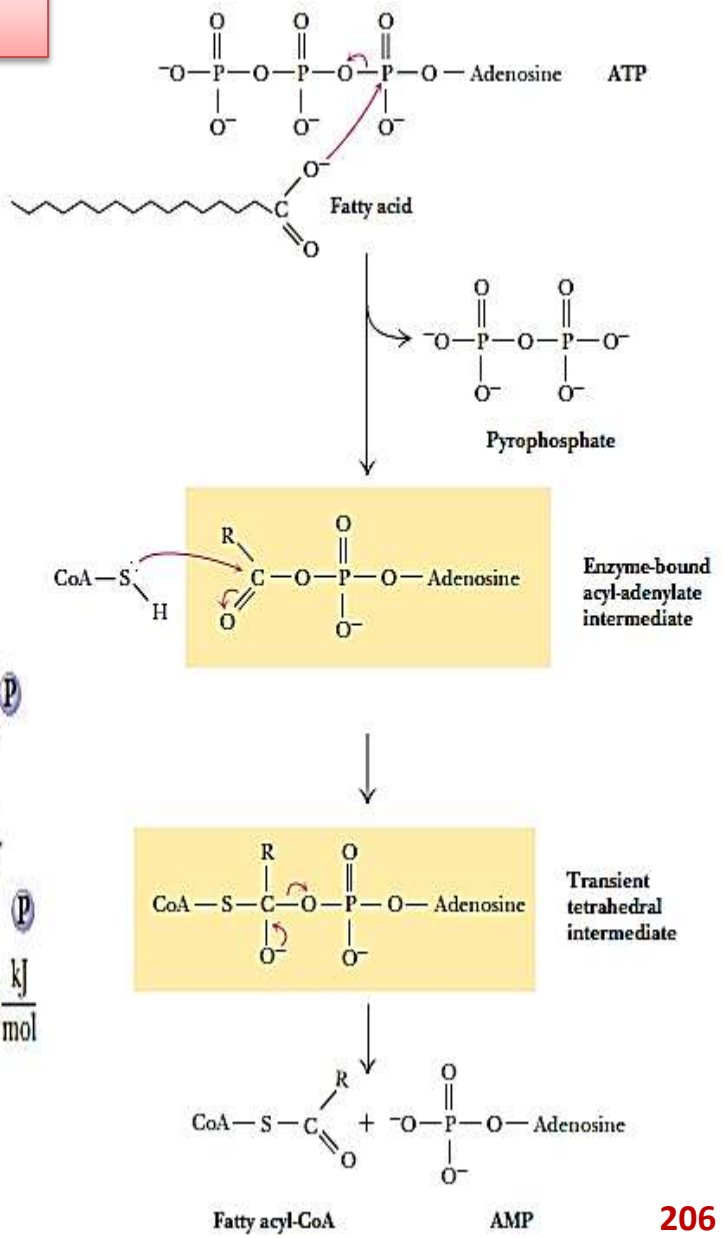


Oxidation of Fatty Acids: Ketogenesis

Oxidation Of Fatty Acids Occurs In Mitochondria

Fatty Acids Are Activated Before Being Catabolized



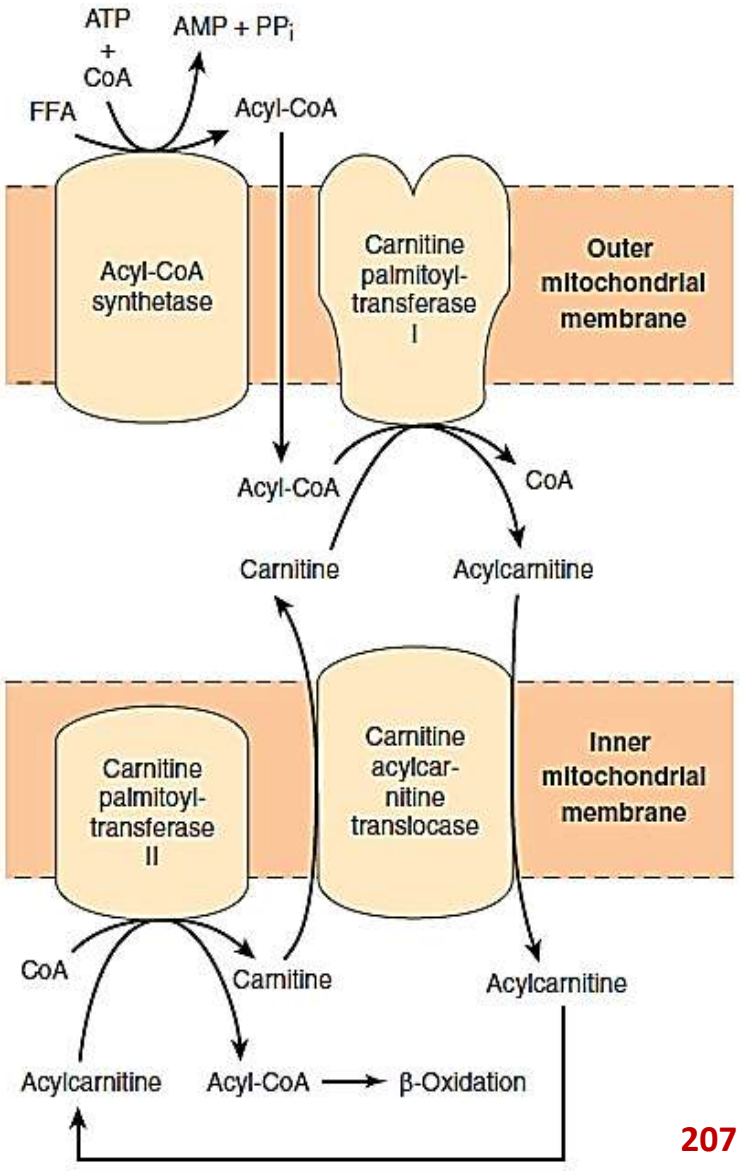


Oxidation of Fatty Acids: Ketogenesis

Oxidation Of Fatty Acids Occurs In Mitochondria

Long-Chain Fatty Acids Penetrate the Inner Mitochondrial Membrane as Carnitine Derivatives

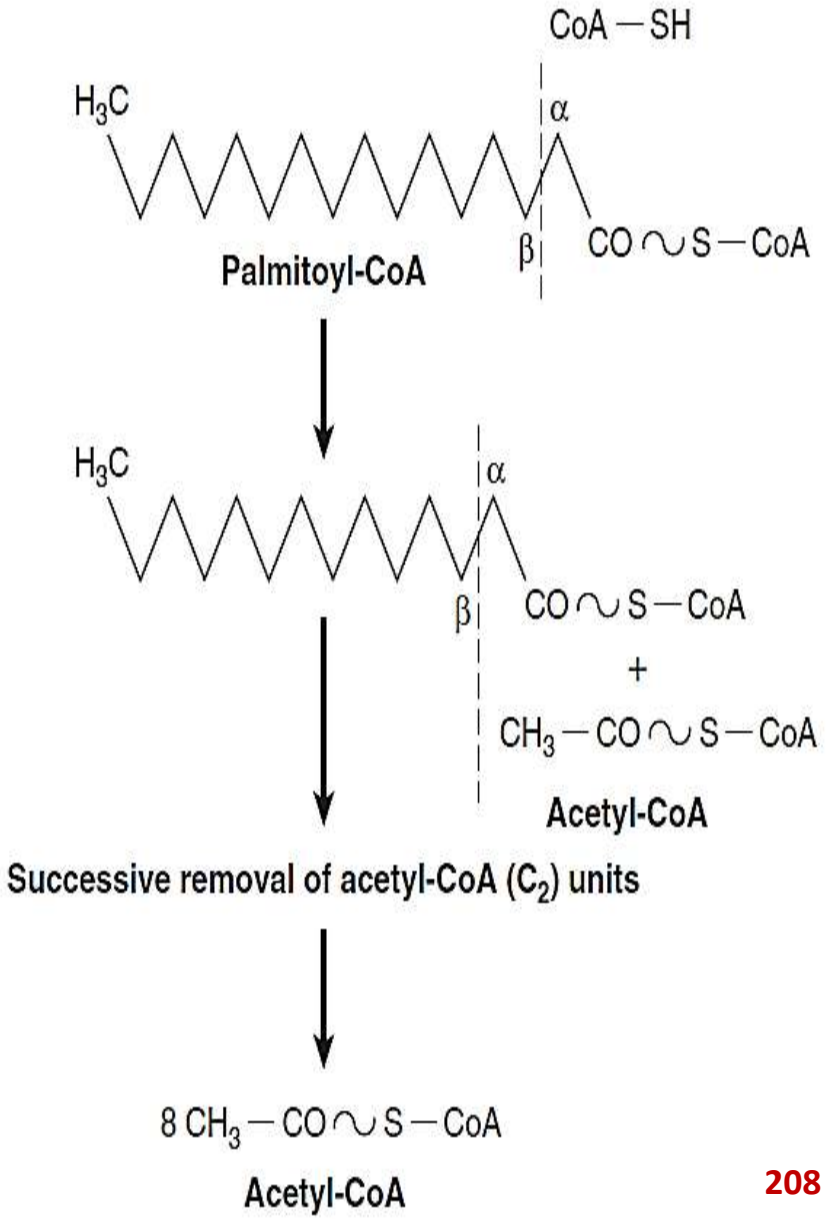
- **Carnitine** (**β-hydroxy-γ-trimethylammonium butyrate**), **$(CH3)_3N^+$-CH2-CH(OH)-CH2-COO^-**, is **widely distributed** and is particularly abundant in **muscle**.
- **Long-chain acyl-CoA** (or FFA) **cannot penetrate the inner membrane of mitochondria**.
- In the presence of carnitine, however, **carnitine palmitoyltransferase-I**, located in the **outer mitochondrial membrane**, **transfers long-chain acyl group from CoA to carnitine**, **forming acylcarnitine** and **releasing CoA**.
- **Acylcarnitine** is able to **penetrate the inner membrane** and gain **access to the β-oxidation system of enzymes** via the **inner membrane exchange transporter carnitine-acylcarnitine translocase**.
- **The transporter binds acylcarnitine** and **transports it across the membrane in exchange for carnitine**.
- **The acyl group** is then **transferred to CoA** so that **acyl-CoA is reformed** and **carnitine is liberated** by **carnitine palmitoyltransferase-II**, which is located on **the inside of the inner membrane** .



Oxidation of Fatty Acids: Ketogenesis

β-oxidation Of Fatty Acids Involves Successive Cleavage With Release Of Acetyl-CoA

- In the **β-oxidation pathway**, **two carbons at a time are cleaved from acyl-CoA molecules**, **starting at the carboxyl end**.
- The chain is broken between the **α(2)- and β(3)- carbon atoms**—hence the **name β-oxidation.**
- The **two-carbon units** formed are **acetyl-CoA**; thus, **palmitoyl-CoA forms eight acetyl-CoA molecules**.
- **Several enzymes, known collectively** as "**fatty acid oxidase**," are found in the **mitochondrial matrix** or **inner membrane adjacent to the respiratory chain**.
- These **catalyze the oxidation of acyl-CoA** to **acetyl-CoA via the β-oxidation pathway**.
- The system proceeds in **cyclic fashion** which results in the **degradation of long fatty acids to acetyl CoA**.

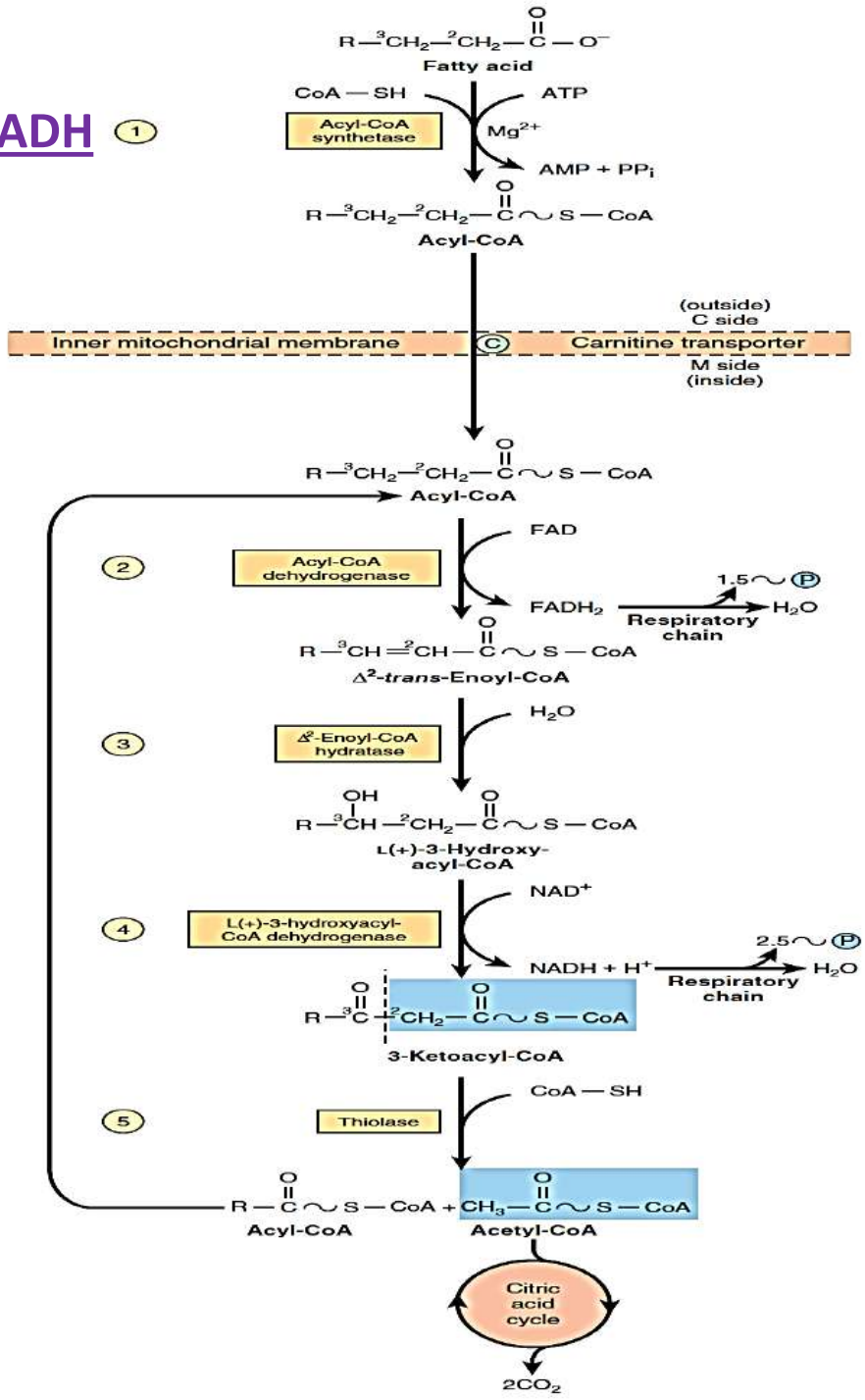


Oxidation of Fatty A

The β-Oxidation Cycle Generates FADH2 & NADH

- In the **β-Oxidation process**, **large quantities of** the reducing equivalents **$FADH_2$** and **NADH** are **generated** and are used to **form ATP** by **oxidative phosphorylation.**

  2) **The first step** is the **removal of two hydrogen atoms** from the 2(α)- and 3(β)-carbon atoms, catalyzed by **acyl-CoA dehydrogenase** and requiring **FAD**. This results in the formation of **Δ^2-trans-enoyl-CoA** and **$FADH_2$** . The **reoxidation** of **$FADH_2$** by the **respiratory chain** requires the **mediation of another flavoprotein**, termed **electron-transferring flavoprotein**.



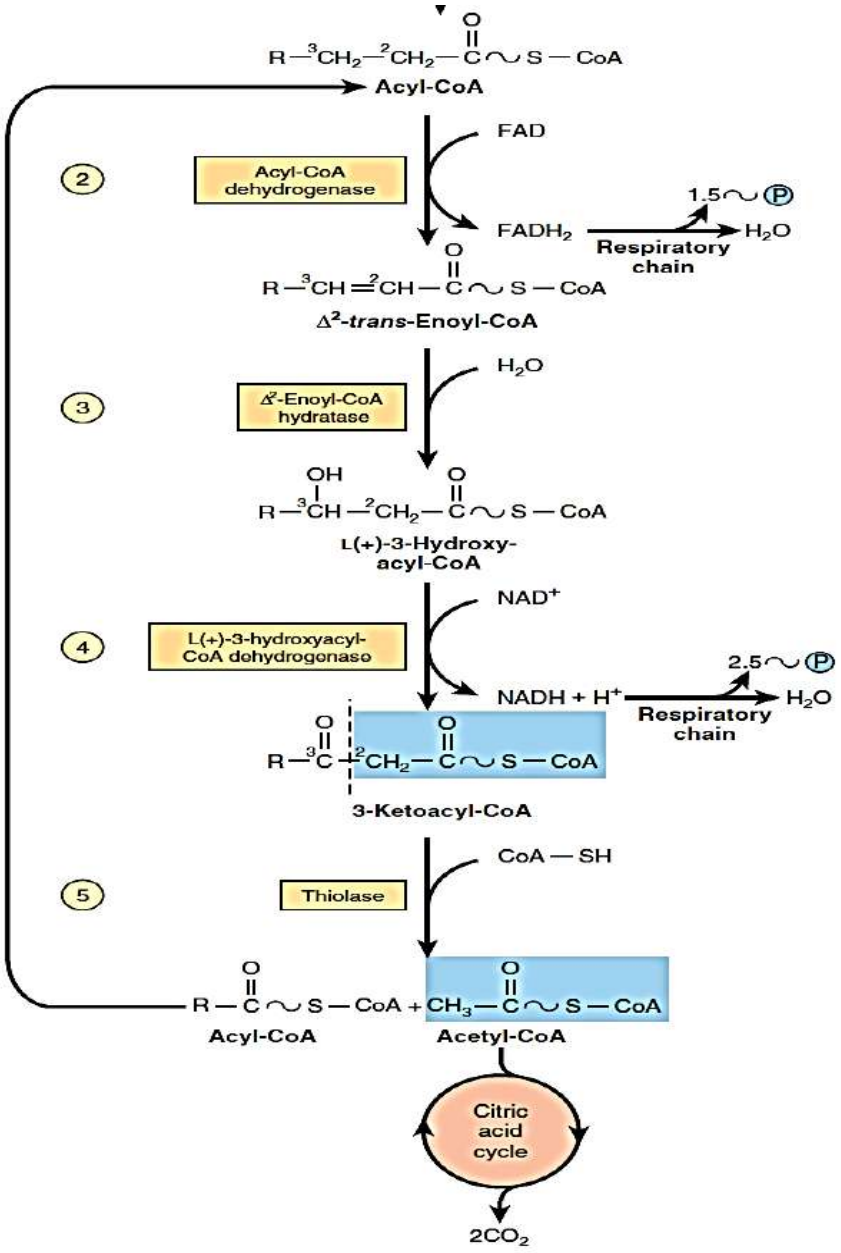
Oxidation of Fatty Acids: Ketogenesis

The β-Oxidation Cycle Generates FADH2 & NADH

3) **Water is added to saturate the double bond** and **form 3-hydroxyacyl-CoA**, catalyzed by **Δ^2-enoyl-CoA hydratase**.

4) The **3-hydroxy derivative** undergoes further **dehydrogenation** on the 3-carbon catalyzed by **L(+)-3-hydroxyacyl-CoA dehydrogenase** to form the corresponding **3-ketoacyl-CoA** compound. In this case, **NAD^+** is the **coenzyme** involved.

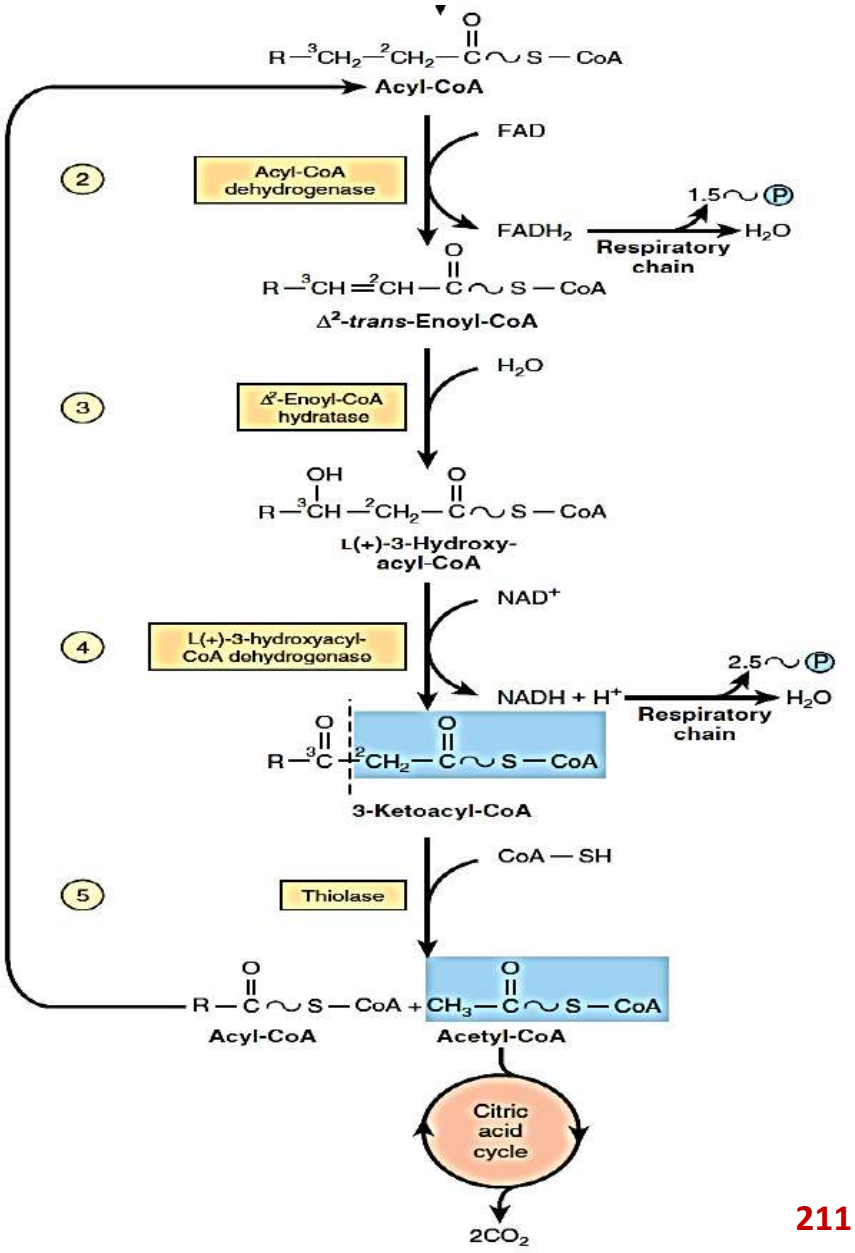
5) **Finally**, **3-ketoacyl-CoA** is split at the 2,3-position by **thiolase** (**3-ketoacyl-CoA-thiolase**), **forming acetyl-CoA** and a **new acyl-CoA two carbons shorter than the original acyl-CoA molecule**.



Oxidation of Fatty Acids: Ketogenesis

The β-Oxidation Cycle Generates FADH2 & NADH

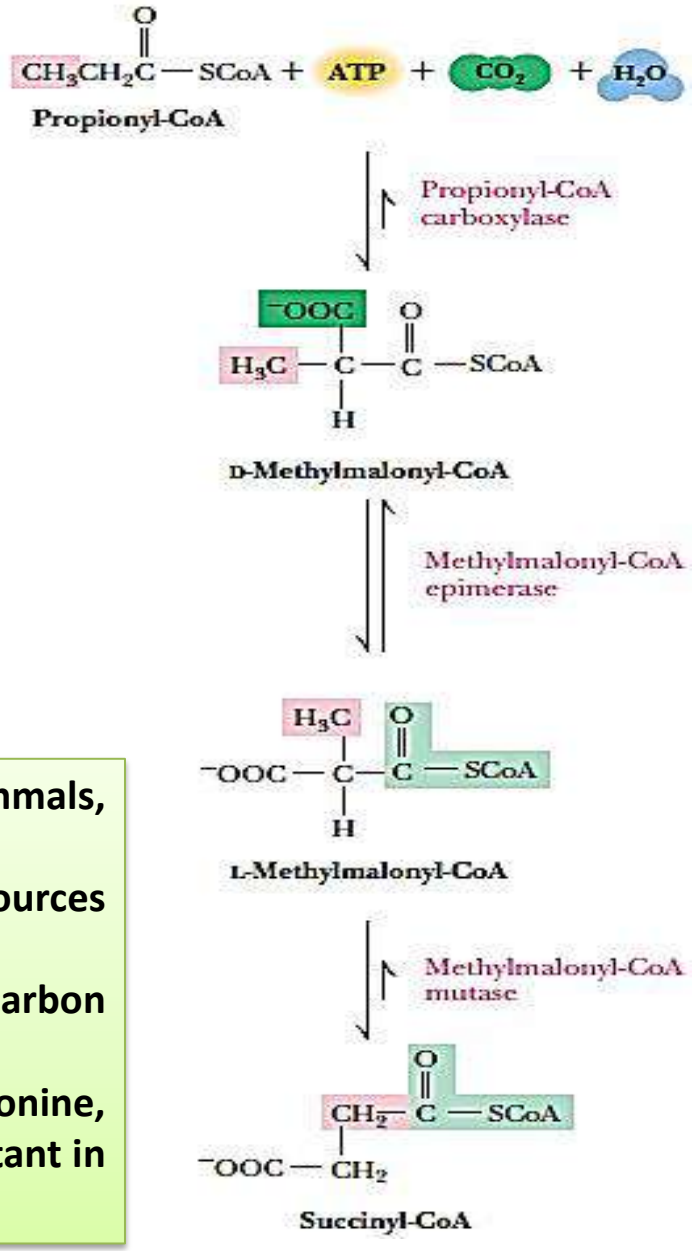
- The shorter acyl-CoA formed in the cleavage reaction reenters the oxidative pathway at reaction 2.
- In this way, a long-chain fatty acid with an even number of carbons may be degraded completely to acetyl-CoA (C2 units).
- For example, after seven cycles, the C16 fatty acid, palmitate, would be converted to eight acetyl CoA molecules.
- Since acetyl-CoA can be oxidized to CO_2 and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.



Oxidation of Fatty Acids: Ketogenesis

Oxidation of a Fatty Acid With an Odd Number of Carbon Atoms Yields Acetyl-Co A Plus a Molecule of Propionyl-CoA

- **Fatty acids** with an **odd number** of carbon atoms are oxidized by the pathway of β-oxidation described above producing acetyl CoA until a three-carbon (**propionyl-CoA**) residue remains.
- This compound is converted to **succinyl-CoA**, a constituent of the citric acid cycle.
- Hence, the **propionyl residue** from an odd-chain fatty acid is the only part of a fatty acid that is **glucogenic**.



1. **Fatty acids with odd numbers of carbon atoms are rare in mammals, but fairly common in plants and marine organisms.**
2. **Humans and animals whose diets include these food sources metabolize odd-carbon fatty acids via the β-oxidation pathway**
3. **The final product of β-oxidation in this case is the 3-carbon propionyl- CoA instead of acetyl-CoA**
4. **Because propionyl-CoA is a degradation product of methionine, valine, and isoleucine, this sequence of reactions is also important in amino acid catabolism**

Oxidation of Fatty Acids: Ketogenesis

Oxidation of Fatty Acids Produces a Large Quantity of ATP

- Transport of electrons from **$FADH_2$** and **NADH** via the respiratory chain leads to the synthesis of four high-energy phosphates (**1.5 +2.5 = 4 ATP**) for each of the **seven cycles** needed for the breakdown of the C16 fatty acid, palmitate, to acetyl-CoA (**7 × 4 = 28**).
- A total of **8 mol of acetyl-CoA** is formed, and each gives rise to **10 mol of ATP** on oxidation in the **citric acid cycle**, making **8 × 10 = 80 mol**.
- **Two must be subtracted** for the **initial activation** of the fatty acid, yielding a net **gain of 106 mol** of ATP per mole of palmitate, or **106 × 30.5* = 3233 kJ**. This represents **33%** of the free energy of combustion of palmitic acid.

$$^{*}\mathrm{ATP} \rightarrow \mathrm{ADP} + \mathrm{P_i}\ (\Delta G^{0'} = -30.5\ \mathrm{kJ/mol})$$

Step	Product	Amount Product Formed (mol)/mol Palmitate C16:0	ATP Formed (mol)/mol Product	Total ATP Formed (mol)/mol Palmitate	ATP Used (mol)/mol Palmitate
Activation		-			2
β-Oxidation	$FADH_2$	7	1.5	10.5	-
β-Oxidation	NADH	7	2.5	17.5	-
Citric acid cycle	Acetyl CoA	8	10	80	-
SUM				108	2
net gain of ATP = 108 - 2 = 106 ATP					

Oxidation of Fatty Acids Produces a Large Quantity of ATP

- Transport of electrons from **$FADH_2$** and **NADH** via the respiratory chain leads to the synthesis of four high-energy phosphates (**1.5 +2.5 = 4 ATP**) for each of the **seven cycles** needed for the breakdown of the C16 fatty acid, palmitate, to acetyl-CoA (**7 × 4 = 28**).
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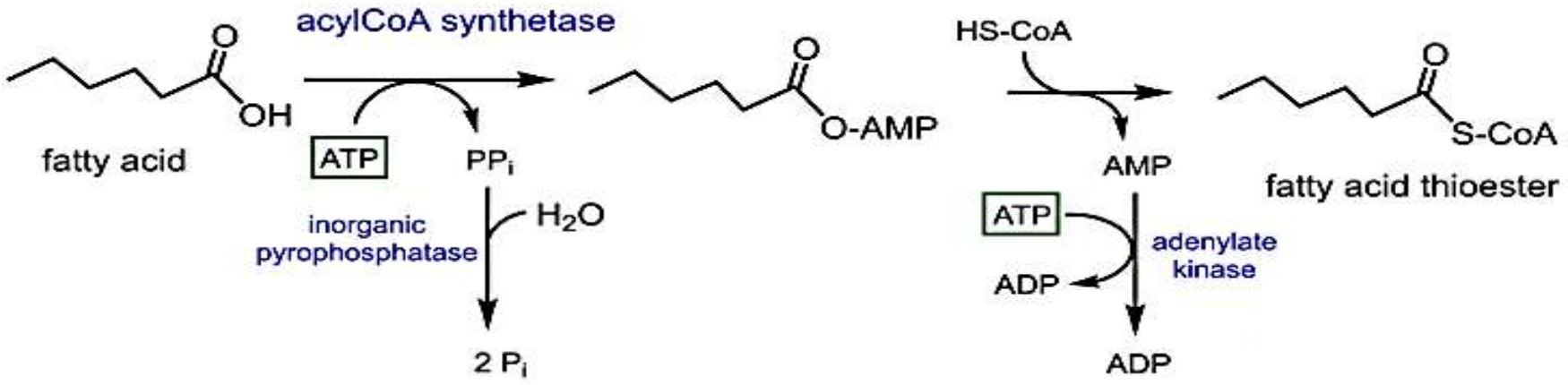
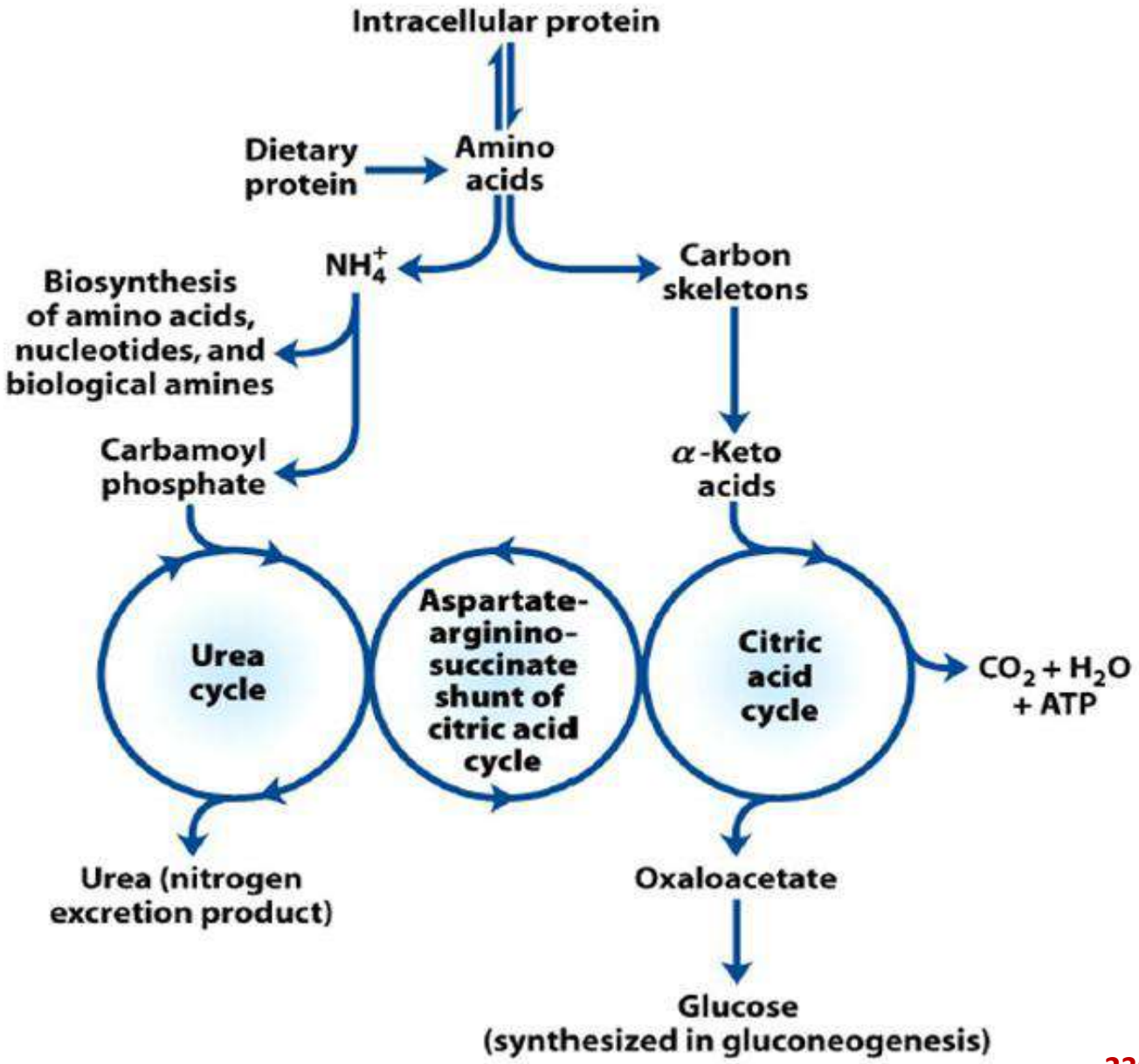
- **Two must be subtracted** for the **initial activation** of the fatty acid, yielding a net **gain of 106 mol**

To recycle the AMP, a second equivalent of ATP is needed to make ADP, which is the substrate used by the complex V of mitochondria to regenerate ATP. Hence, the overall reaction of fatty acid activation requires 2 equivalents of ATP



Biomedical Importance

- In **normal adults**, **nitrogen intake matches nitrogen excreted**.
- **Positive nitrogen balance**, an **excess of ingested over excreted nitrogen**, **accompanies growth** and **pregnancy**.
- **Negative nitrogen balance**, where **output exceeds intake**, **may follow surgery**, **advanced cancer**, and the **nutritional disorders kwashiorkor** and **marasmus**.

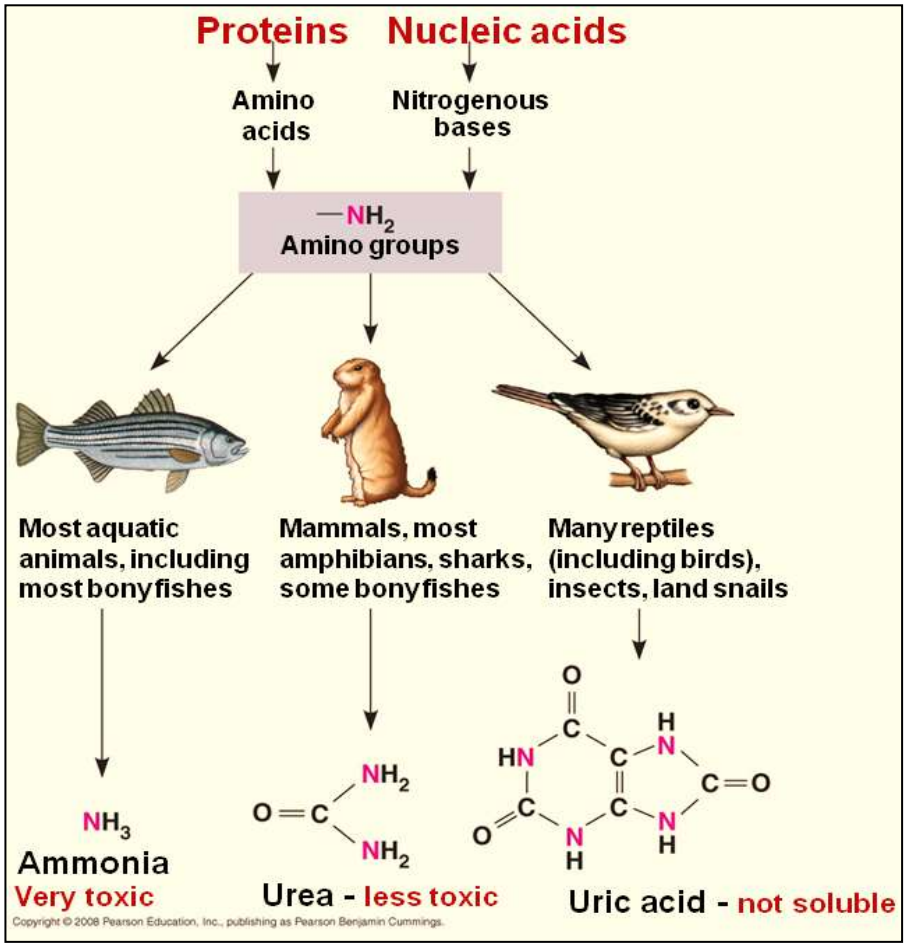


Biomedical Importance

- This chapter describes how the nitrogen of amino acids is converted to urea, and the metabolic disorders that accompany defects in this process.
- Ammonia, which is highly toxic, arises in humans primarily from the α-amino nitrogen of amino acids.
- Tissues therefore convert ammonia to the amide nitrogen of the nontoxic amino acid glutamine.
- Subsequent deamination of glutamine in the liver releases ammonia, which is efficiently converted to urea, which is not toxic.
- However, if liver function is compromised, as in cirrhosis or hepatitis, elevated blood ammonia levels generate clinical signs and symptoms.
- Each enzyme of the urea cycle provides examples of metabolic defects and their physiologic consequences.
- In addition, the urea cycle provides a useful molecular model for the study of other human metabolic defects.

Animals convert α-amino nitrogen to varied end products

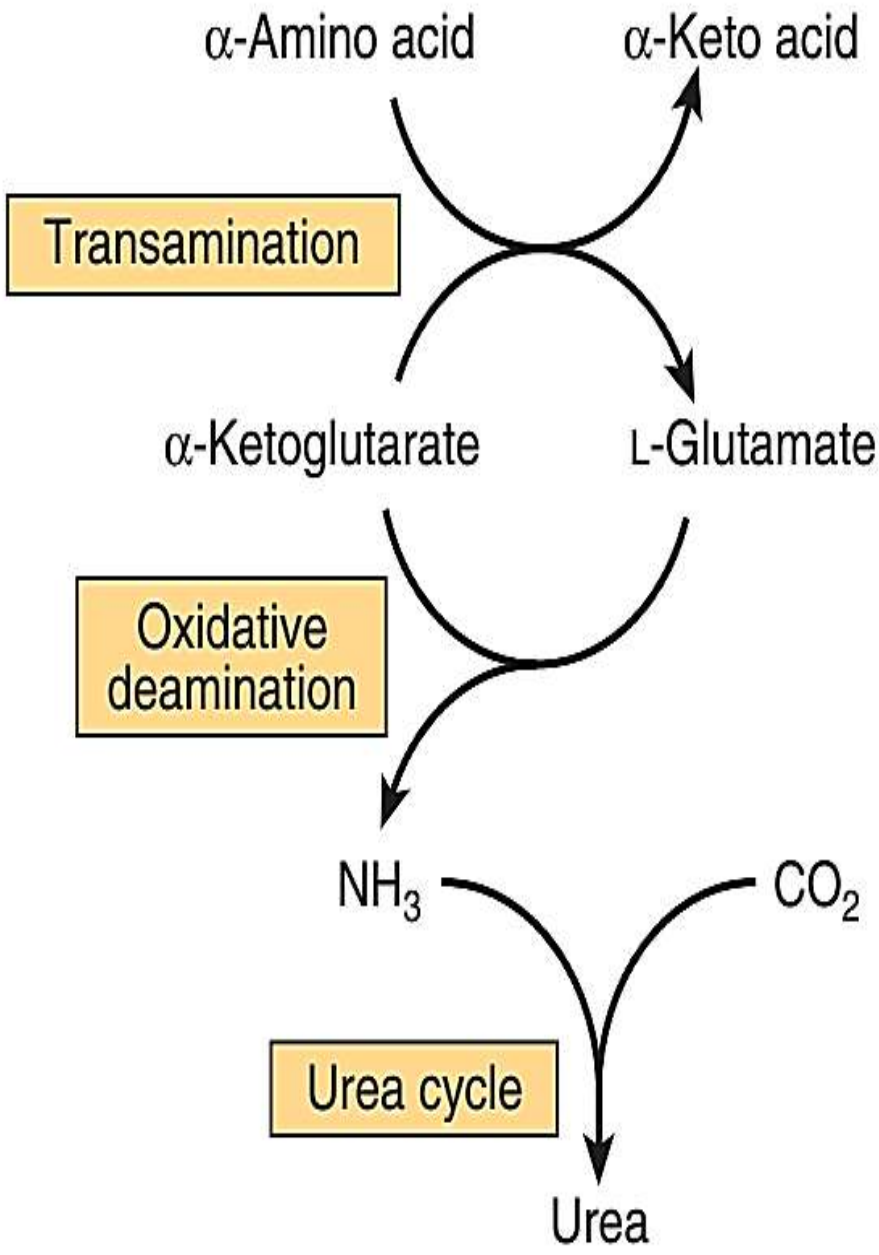
- Depending on their ecological niche and physiology, different animals excrete excess nitrogen as **ammonia**, as **uric acid**, or as **urea**.
- The aqueous environment of teleostean **fish**, which are **ammonotelic** (**excrete ammonia**), permits them to excrete water continuously to facilitate excretion of **ammonia**, which is **highly toxic**.
- **Birds** must both **conserve water** and **maintain low weight**. **Birds**, which are **uricotelic**, address both problems by excreting nitrogen-rich **uric acid** as **semisolid guano**.



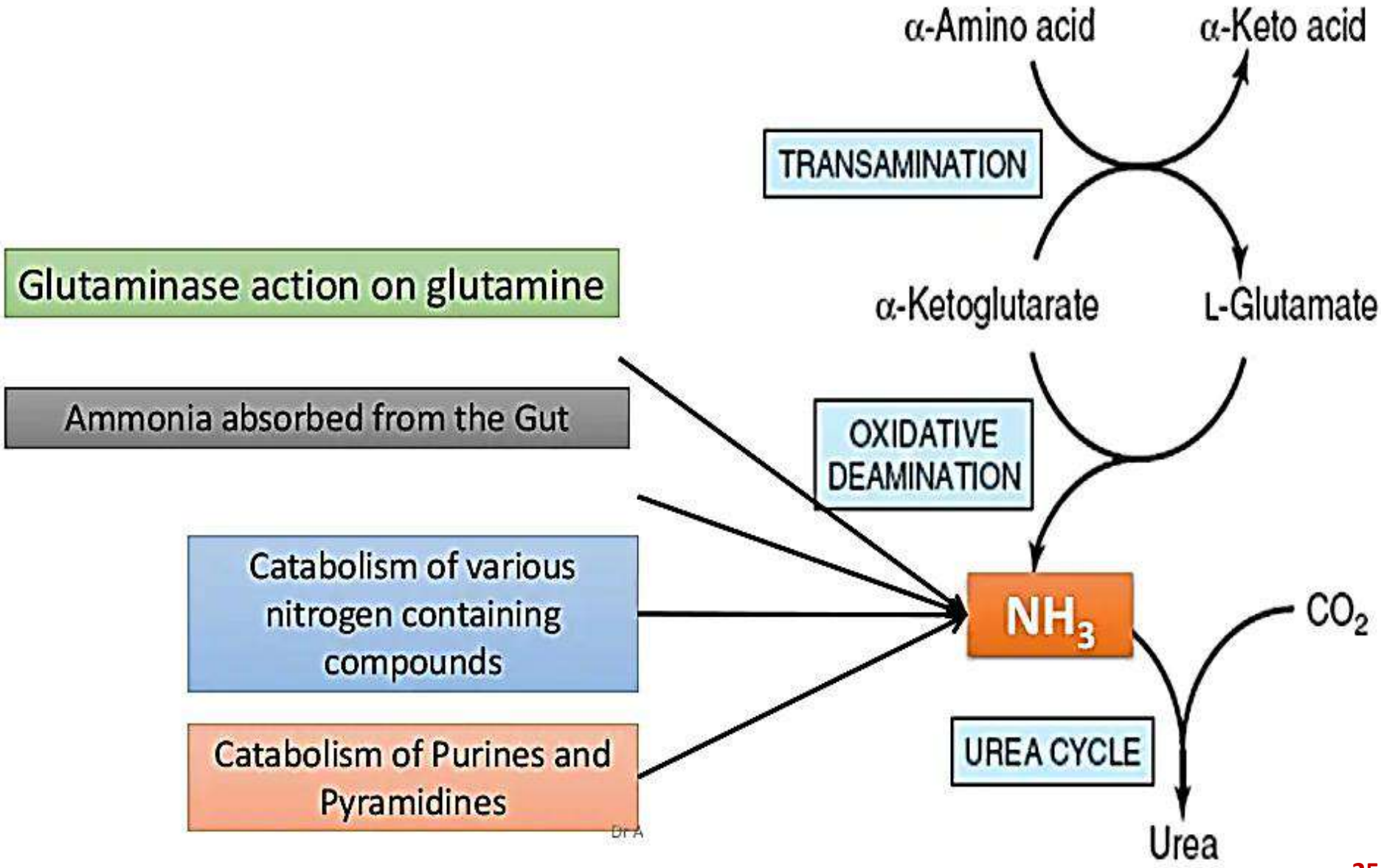
- Many **land animals**, including **humans**, are **ureotelic** and excrete nontoxic, highly water-soluble **urea**.
- Since **urea** is **nontoxic to humans**, **high blood levels in renal disease are a consequence, not a cause, of impaired renal function.**

Biosynthesis of urea

- **Urea biosynthesis occurs in four stages** (Figure 28–8) :
  1) **transamination**,
  2) **oxidative deamination of glutamate,**
  3) **ammonia transport, and**
  4) **reactions of the urea cycle**
- **The expression in liver of the RNAs for all the enzymes of the urea cycle increases several fold in starvation, probably secondary to enhanced protein degradation to provide energy**

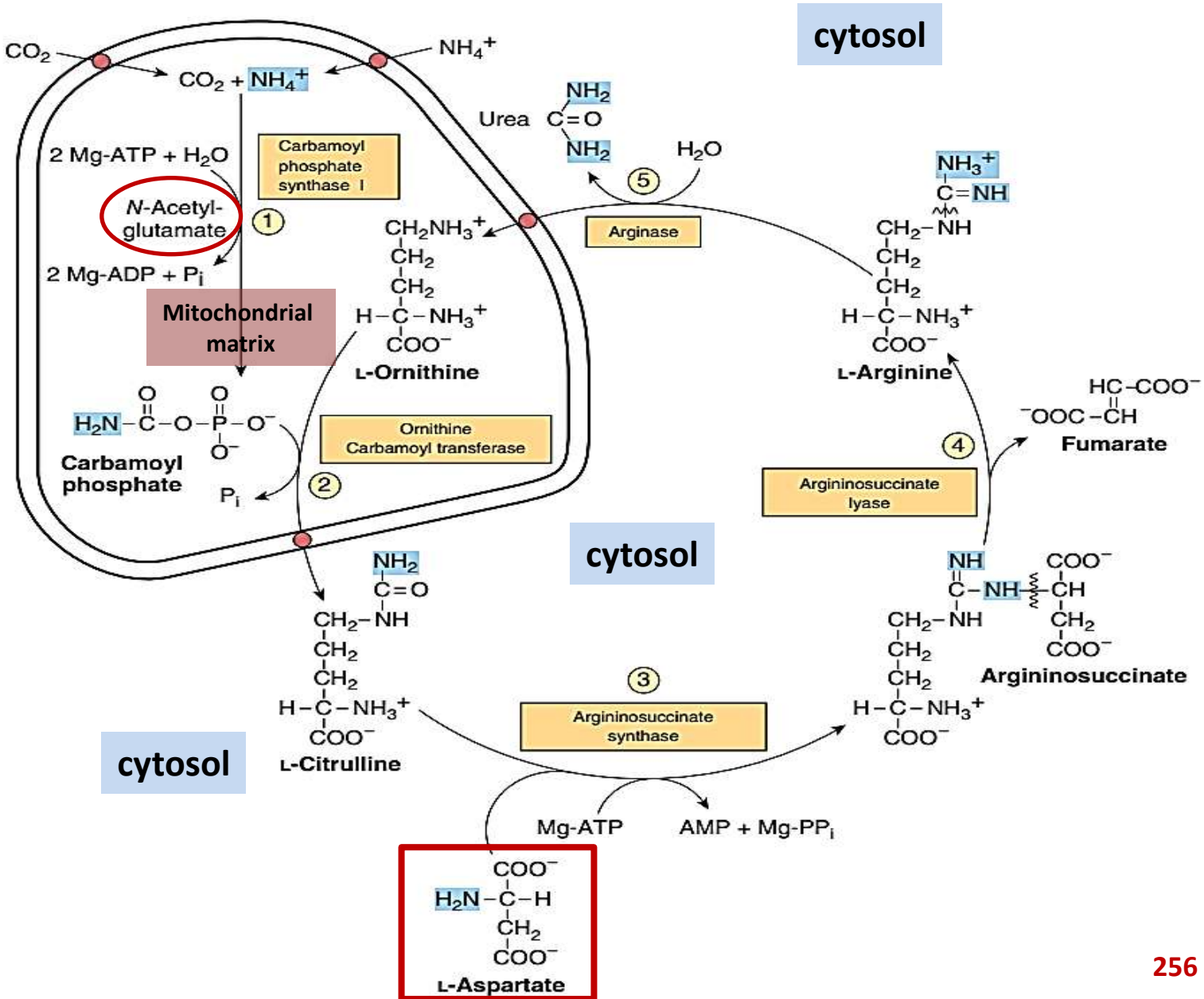


Ammonia sources



Urea is the major end product of nitrogen catabolism in humans

- **Synthesis** of **1 mol** of **urea** requires
- **3 mol** of **ATP**,
- **1 mol** each of **ammonium ion** and of
- **1 mol** of **aspartate**,
- **employs five enzymes**



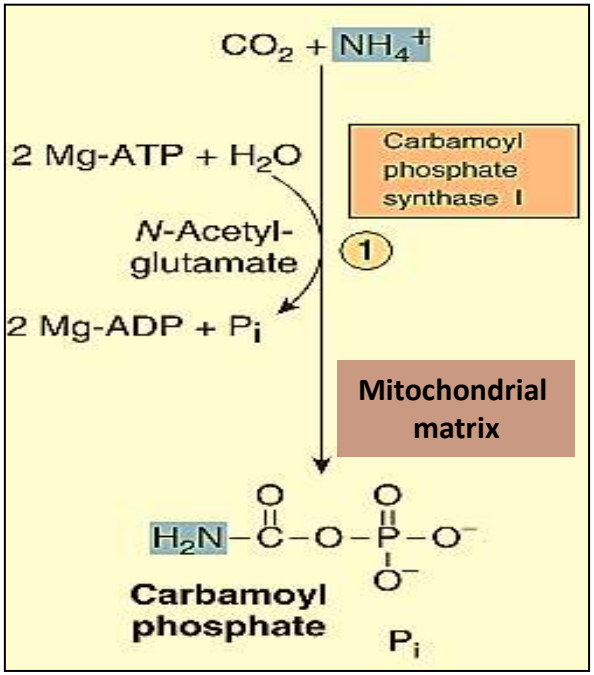
Urea is the major end product of nitrogen catabolism in humans

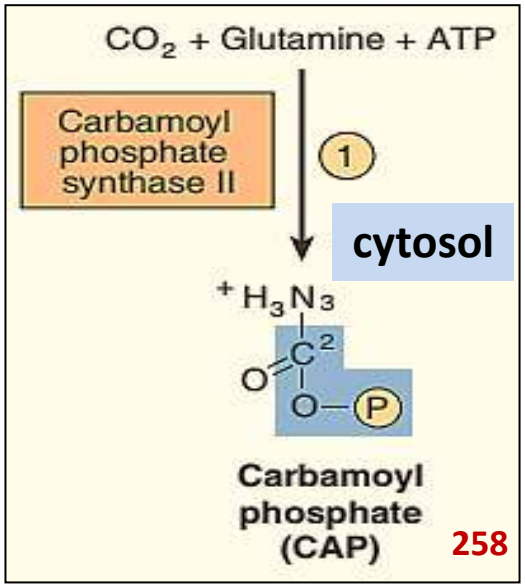
- Of the **six participating amino acids**, **N-acetylglutamate** functions solely as an **enzyme activator.**
- The others serve as **carriers** of the atoms that ultimately become **urea**.
- The major metabolic role of **ornithine**, **citrulline**, and **argininosuccinate** in mammals is **urea synthesis.**
- **Urea synthesis is a cyclic process**.
- While **ammonium ion**, **CO2**, **ATP**, and **aspartate** are **consumed**, the **ornithine consumed** in **reaction 2** is **regenerated** in **reaction 5**.
- There thus is **no net loss** or **gain** of **ornithine**, **citrulline**, **argininosuccinate**, or **arginine (only loss of Aspartate)**.
- As indicated in Figure 28–16, some reactions of **urea synthesis** occur in the **matrix** of the **mitochondrion (reaction 1+2)**, and other reactions in the **cytosol (reaction 3+4+5)**.

Carbamoyl phosphate synthase I initiates urea biosynthesis

Reaction 1 (mitachondrial)

- **Condensation** of **CO_2**, **ammonia**, and **ATP** to **form** **carbamoyl phosphate** is catalyzed by **mitochondrial carbamoyl phosphate synthase I** (**EC 6.3.4.16**).
- A **cytosolic form** of this enzyme, **carbamoyl phosphate synthase II**, uses **glutamine** **rather than** **ammonia** as the **nitrogen donor** and functions in **pyrimidine biosynthesis**
- The concerted action of **glutamate dehydrogenase** and **carbamoyl phosphate synthase 1** thus **shuttles amino nitrogen into carbamoyl phosphate**, a compound with high group transfer potential.
- **Carbamoyl phosphate synthase I**, the rate-limiting enzyme of the urea cycle, is **active only** in the presence **of** **N-acetylglutamate**, an **allosteric activator** that **enhances the affinity** of the **synthase** for **ATP**.
- Synthesis of **1 mol** of **carbamoyl phosphate** requires **2 mol** of **ATP**.

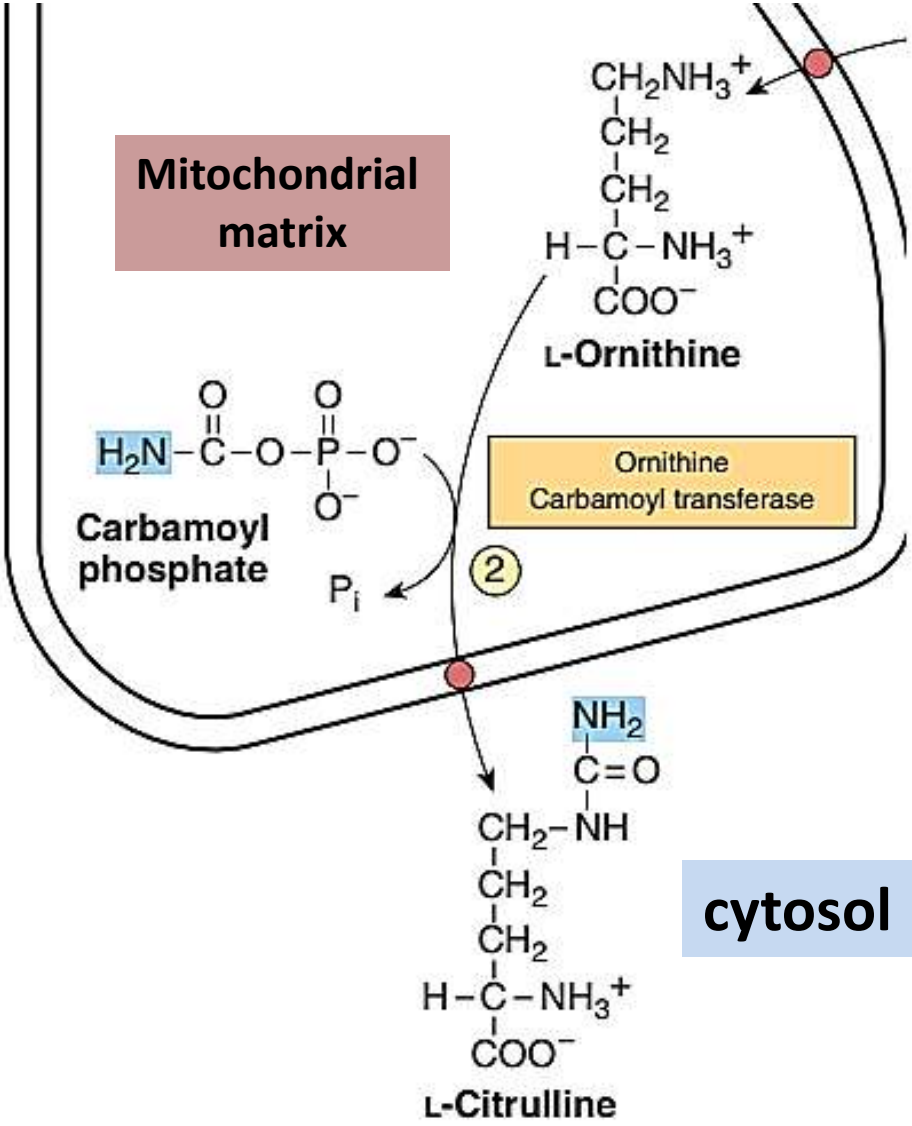




Carbamoyl Phosphate Plus Ornithine Forms Citrulline

Reaction 2 (mitachondrial)

- **l-Ornithine transcarbamoylase** (EC 2.1.3.3) catalyzes **transfer** of the **carbamoyl group** of **carbamoyl phosphate** **to** **ornithine**, **forming** **citrulline** and **orthophosphate**.
- While the **reaction occurs in the mitochondrial matrix**, both the **formation of ornithine** and the subsequent **metabolism of citrulline** take place in the **cytosol**.
- **Entry of ornithine into mitochondria** and **exodus of citrulline from mitochondria** therefore involve **mitochondrial inner membrane permeases.**



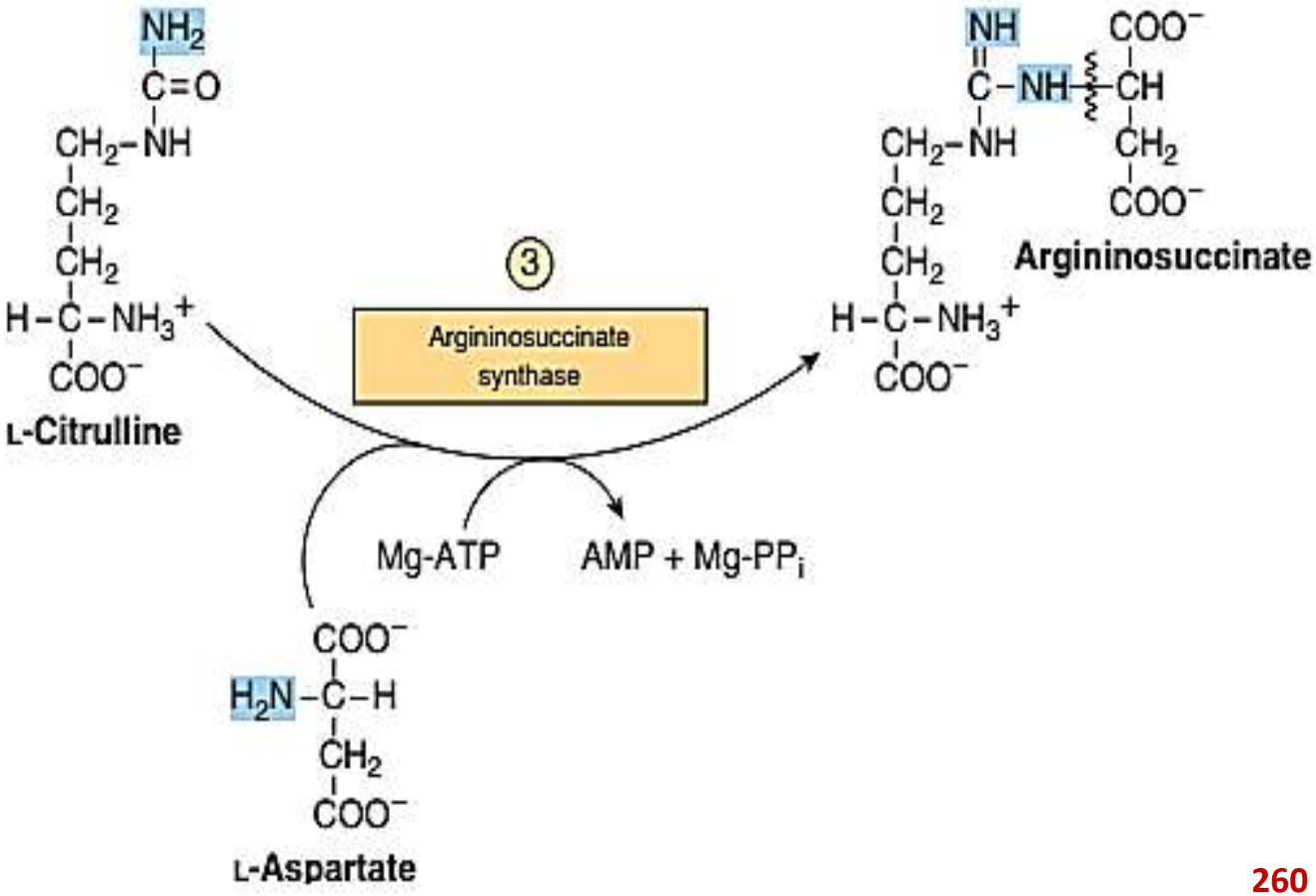
Citrulline plus aspartate forms argininosuccinate

Reaction 3 (Cytosolic)

- **Argininosuccinate synthase (synthetase) (EC 6.3.4.5) links aspartate** and **citrulline** via the **amino group of aspartate** and **provides the second nitrogen of urea**.

- The **reaction requires ATP** and involves intermediate formation of citrullyl-AMP.

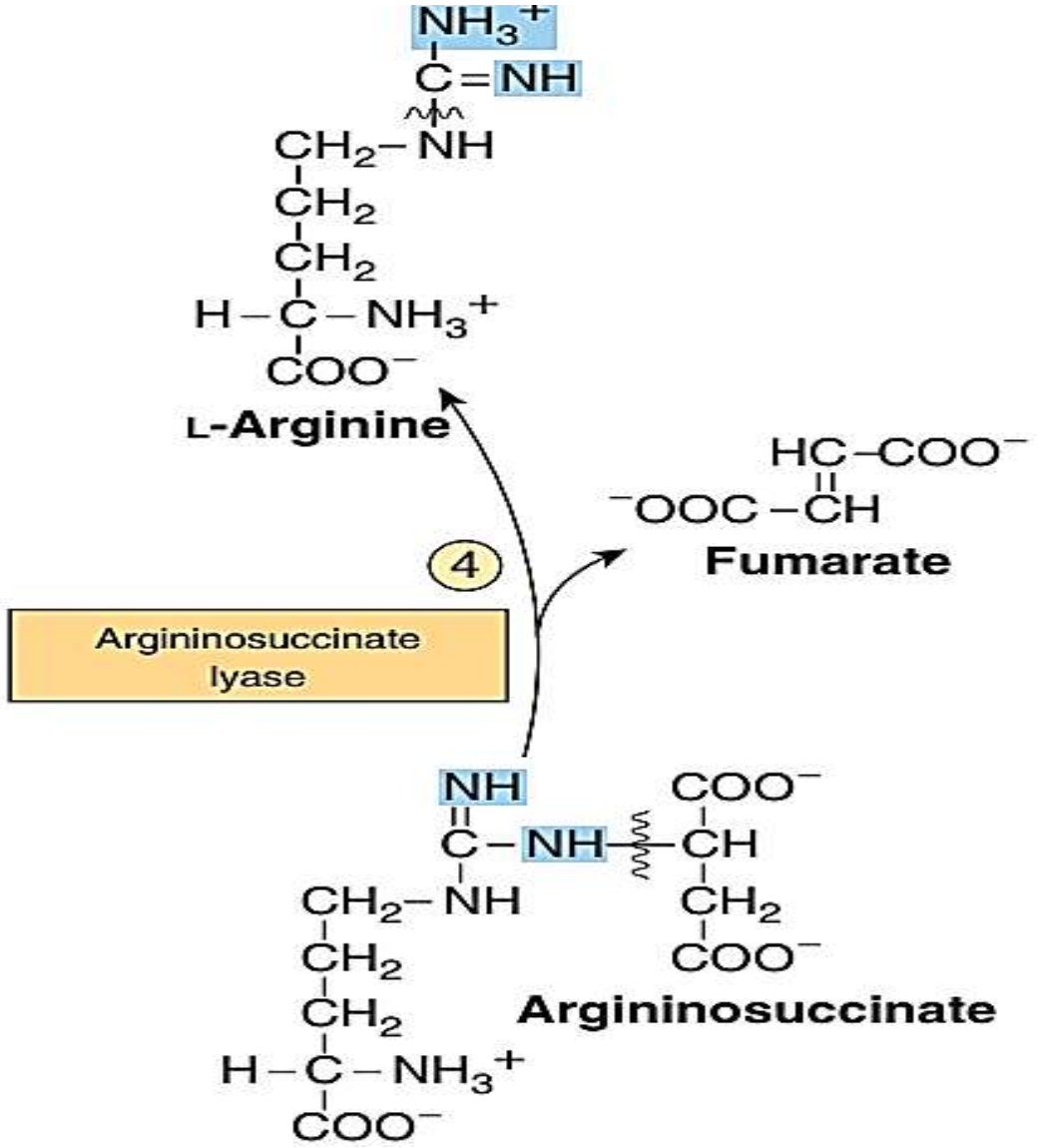
- Subsequent displacement of AMP by aspartate then forms **argininosuccinate**



Cleavage of argininosuccinate forms arginine & fumarate

Reaction 4 (Cytosolic)

- **Cleavage of argininosuccinate** is catalyzed by **argininosuccinate lyase** (**EC 4.3.2.1**).
- The reaction proceeds with **retention of all three nitrogens** in **arginine** and **release** of the **aspartate skeleton** as **fumarate**.
- The **carbon skeleton** of **aspartate-fumarate** thus acts as a **carrier of the nitrogen** of **glutamate** into a **precursor of urea**.

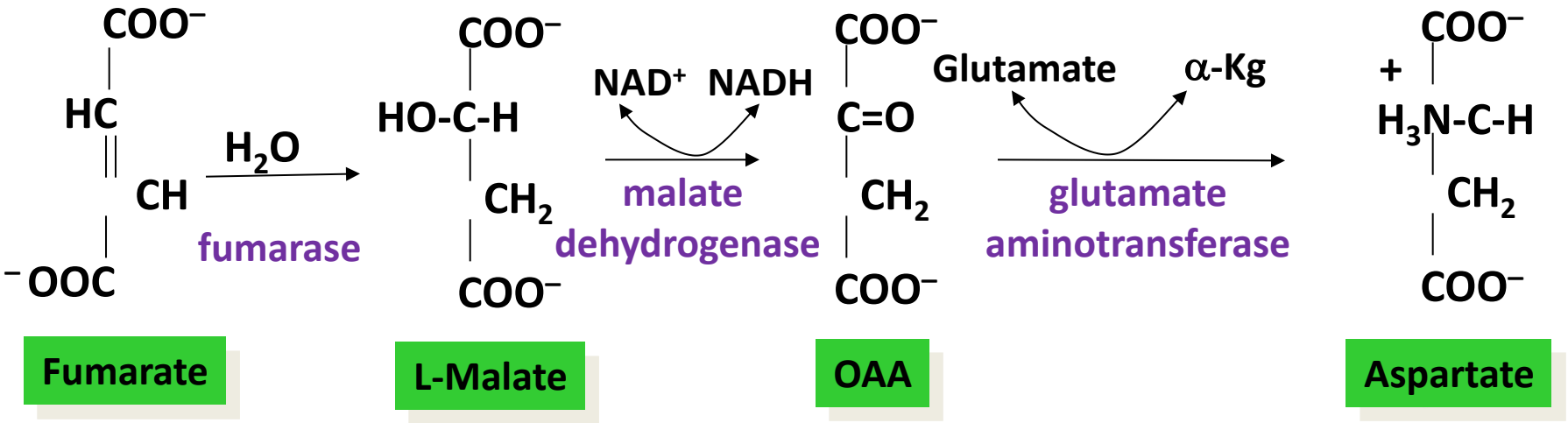


Cleavage of argininosuccinate forms arginine & fumarate

Reaction 4 (Cytosolic)

- Subsequent **addition of water** to **fumarate** forms **l-malate**, whose subsequent **NAD$^+$-dependent oxidation** forms **oxaloacetate**. These two reactions are analogous to reactions of the citric acid cycle (**reaction 7+8**), but are catalyzed by **cytosolic fumarase** and **malate dehydrogenase**.

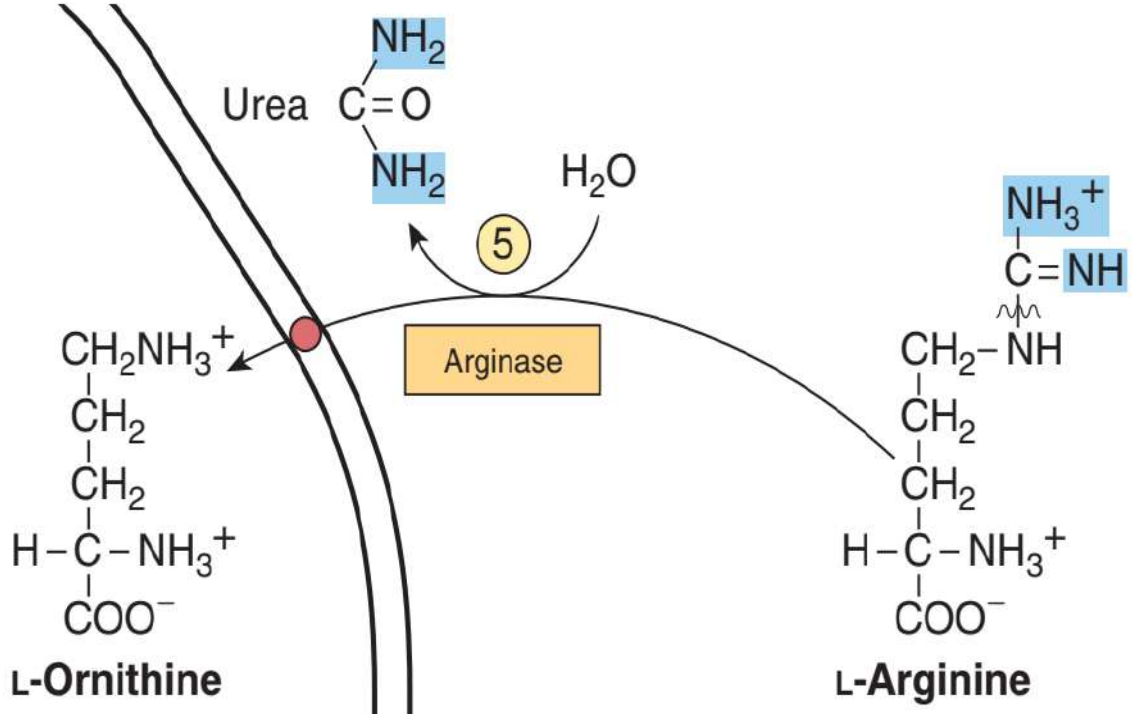
- **Transamination** of **oxaloacetate** by **glutamate aminotransferase** then r**e-forms aspartate**.



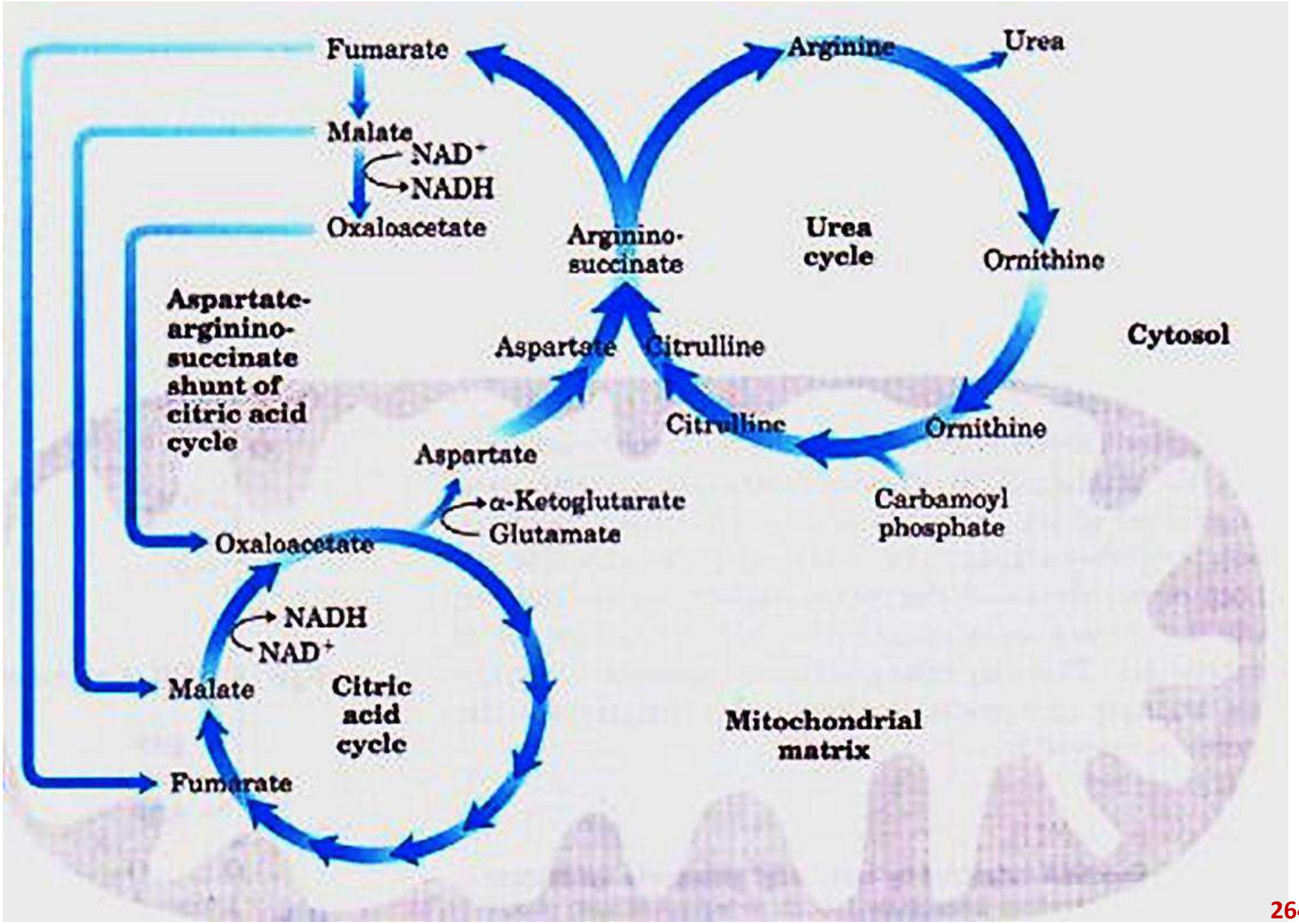
Cleavage of arginine releases urea & re-forms ornithine

Reaction 5 (Cytosolic)

- **Hydrolytic cleavage** of the **guanidino group** of **arginine**, catalyzed by liver **arginase** (**EC 3.5.3.1**), **releases urea**.
- The other product, **ornithine**, **reenters liver mitochondria** and participates in **additional rounds of urea synthesis.**
- **Ornithine** and **lysine** are **potent inhibitors** of **arginase**, and **compete** with **arginine**.
- **Arginine** also serves as the **precursor** of the **potent muscle relaxant nitric oxide** (**NO**) in a **Ca^{2+}-dependent reaction catalyzed** by **NO synthase**.



Catabolism of Proteins & of Amino Acid Nitrogen



Biomedical Importance

- **Amino acid deficiency** states can result if **nutritionally essential amino acids** are **absent from the diet**, or are **present in inadequate amounts**.
- **Examples** in certain regions of West Africa include **kwashiorkor**, which results when a **child is weaned (فطام) onto a starchy diet poor in protein**, and **marasmus**, in which **both caloric intake and specific amino acids are deficient.**
- **Patients** with **short bowel syndrome (متلازمة الأمعاء القصيرة) unable to absorb sufficient quantities of calories and nutrients** suffer from **significant nutritional and metabolic abnormalities** (اضطراب تغذية وتمثيل غذائي).
- Both the **nutritional disorder scurvy**, **a dietary deficiency of vitamin C**, and **specific genetic disorders** are associated with an **impaired ability (ضعف قدرة)** of **connective tissue (نسيج ضام)** to **form hydroxyproline** and **hydroxylysine**. **The resulting conformational instability of collagen** (التهايؤ غير المستقر للكولاجين) **results in bleeding gums (نزيف لثة), swelling joints (ورم مفاصل), poor wound healing (ضعف التئام الجروح)**, and **ultimately in death (في النهاية الموت)**.

Nutritionally essential & nutritionally nonessential Amino acids

- While often employed with reference to amino acids, the terms "**essential**" and "**nonessential**" are **misleading** since **all 20 common amino acids are essential to ensure health**.
- Of these 20 amino acids, **10 must be present in the human diet**, and thus are best termed "**nutritionally essential**." **The other (10+2=12) amino acids are "nutritionally nonessential" since they need not be present in the diet**.
- **Amino acid deficiency disorders** are endemic (مستوطنة) in certain regions of West Africa where diets rely heavily on grains that are **poor sources of tryptophan and lysine.**
- These nutritional disorders include :

Nutritionally Essential	Nutritionally Nonessential
Arginine[a]	Alanine
Histidine	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Hydroxyproline[b]
Tryptophan	Hydroxylysine[b]
Valine	Proline
	Serine
	Tyrosine

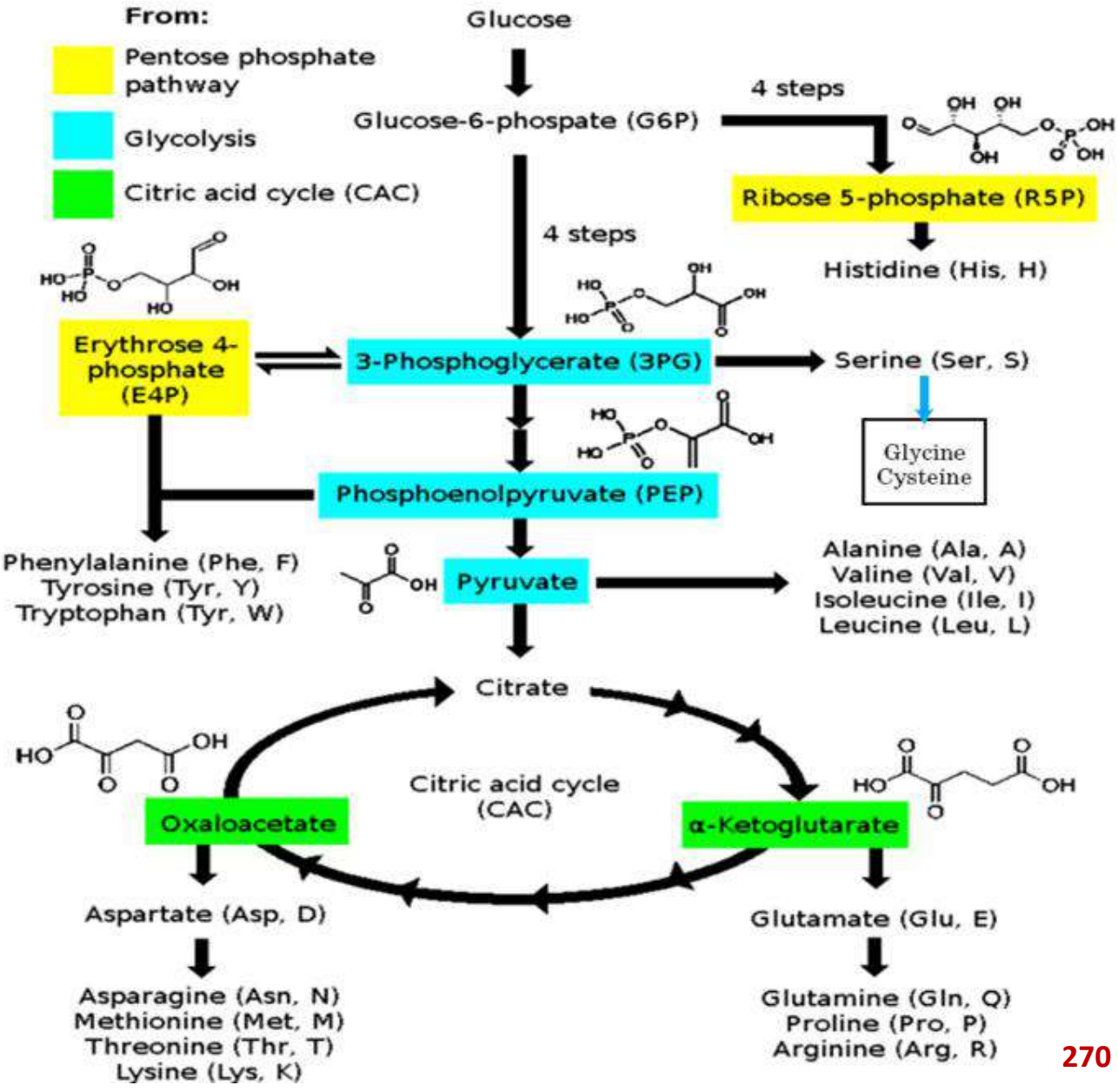
[a]Nutritionally "semiessential." Synthesized at rates inadequate to support growth of children.
[b]Not necessary for protein synthesis, but is formed during post-translational processing of collagen.

1. **Kwashiorkor (enfant (*kwashi*) rouge (*orkor*))**, which results when a **child is weaned onto a starchy diet poor in protein**,
2. **Marasmus (withering/ذبول)**, in which **both caloric intake and specific amino acids are deficient.**

Biosynthesis of all amino acids

Three Pathways of Amino Acid Biosynthesis:

1) glycolysis pathway:
   - ❑ 3-phosphoglycerate
   - ❑ pyruvate
2) pentose phosphate pathway:
   - ❑ E4P
   - ❑ R5P
3) Citric acid pathway:
   - ❑ α-ketoglutarate
   - ❑ Oxaloacetate

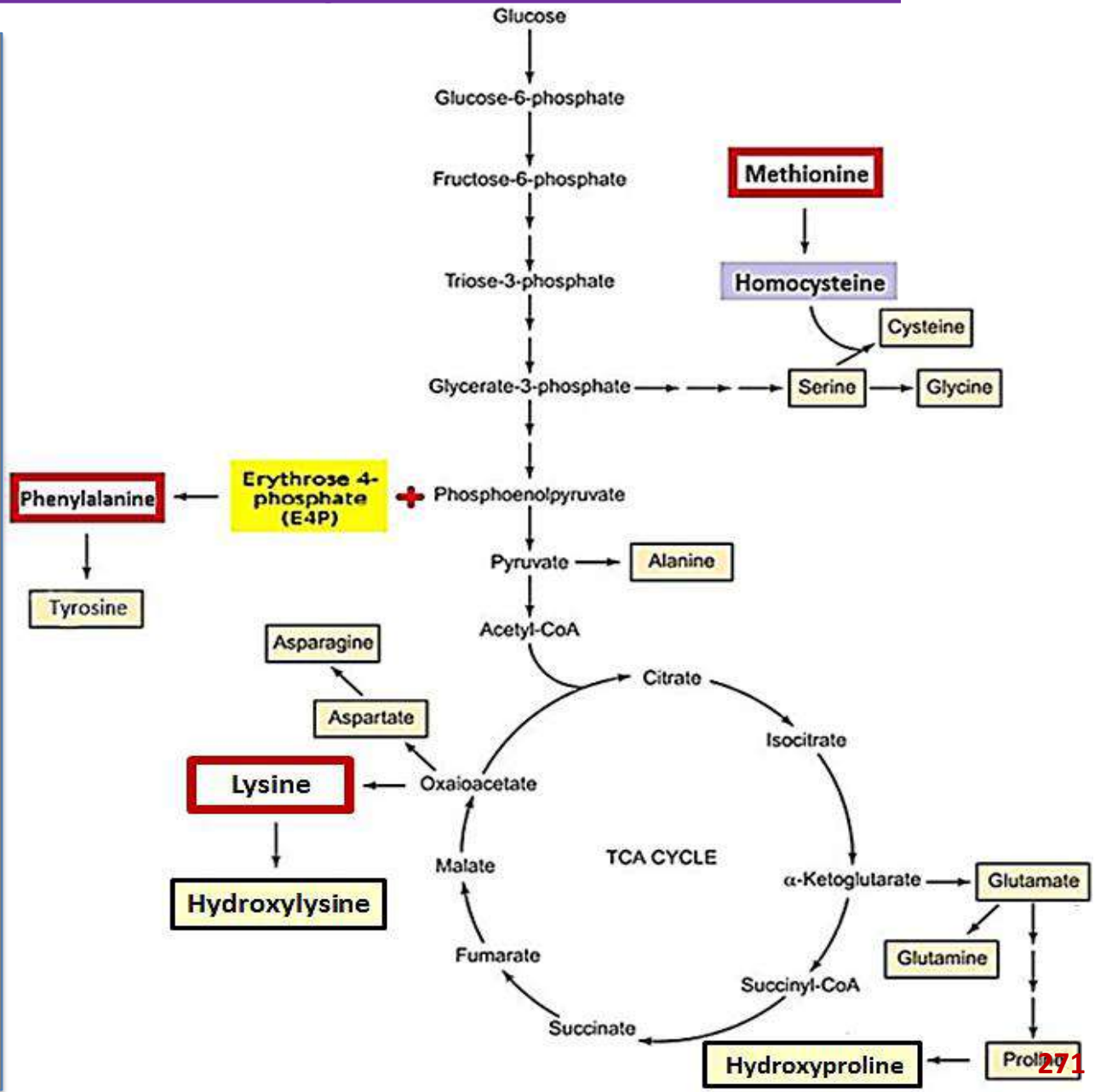


Biosynthesis of the Nutritionally Nonessential Amino Acids

Biosynthesis of the human nutritionally nonessential amino acids

Three pathways of nutritionally nonessential amino acid biosynthesis:

1) glycolysis pathway:
   - ❑ 3-phosphoglycerate
   - ❑ pyruvate
2) Dietary amino acids
3) Citric acid pathway:
   - ❑ α-ketoglutarate
   - ❑ Oxaloacetate



Metabolism of Purine & Pyrimidine Nucleotides

Biomedical Importance

- Despite a diet that may be rich in **nucleoproteins**, **dietary purines** and **pyrimidines** are **not incorporated** directly into **tissue nucleic acids**.
- **Humans synthesize the nucleic acids** and **their derivatives ATP, NAD^+, coenzyme A**, etc, **from amphibolic intermediates**.
- However, **injected purine or pyrimidine analogs**, **including potential anticancer drugs**, may nevertheless be **incorporated into DNA**.
- The biosyntheses of purine and pyrimidine ribonucleotide triphosphates (NTPs) and dNTPs are precisely regulated events.
- Coordinated feedback mechanisms ensure their production in appropriate quantities and at times that match varying physiologic demand (eg, cell division).
- **Human diseases** that involve **abnormalities in purine metabolism** include gout, Lesch-Nyhan syndrome, adenosine deaminase deficiency, and purine nucleoside phosphorylase deficiency.
- **Diseases** of **pyrimidine biosynthesis** are rarer, but include orotic acidurias.

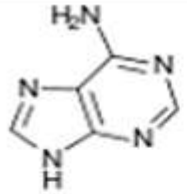
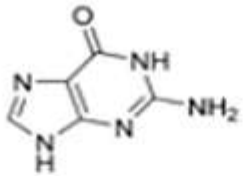
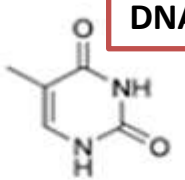
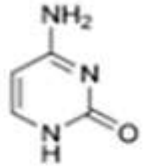
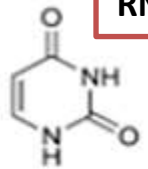
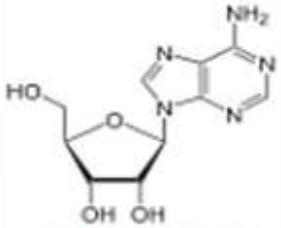
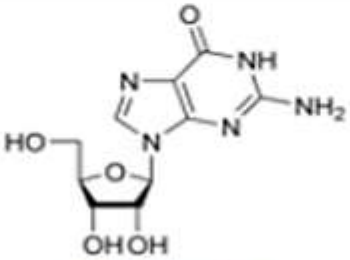
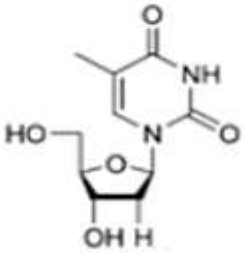
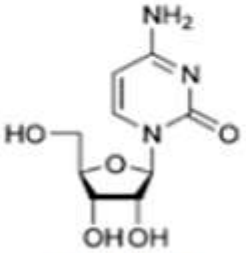
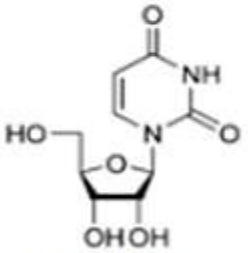
Metabolism of Purine & Pyrimidine Nucleotides

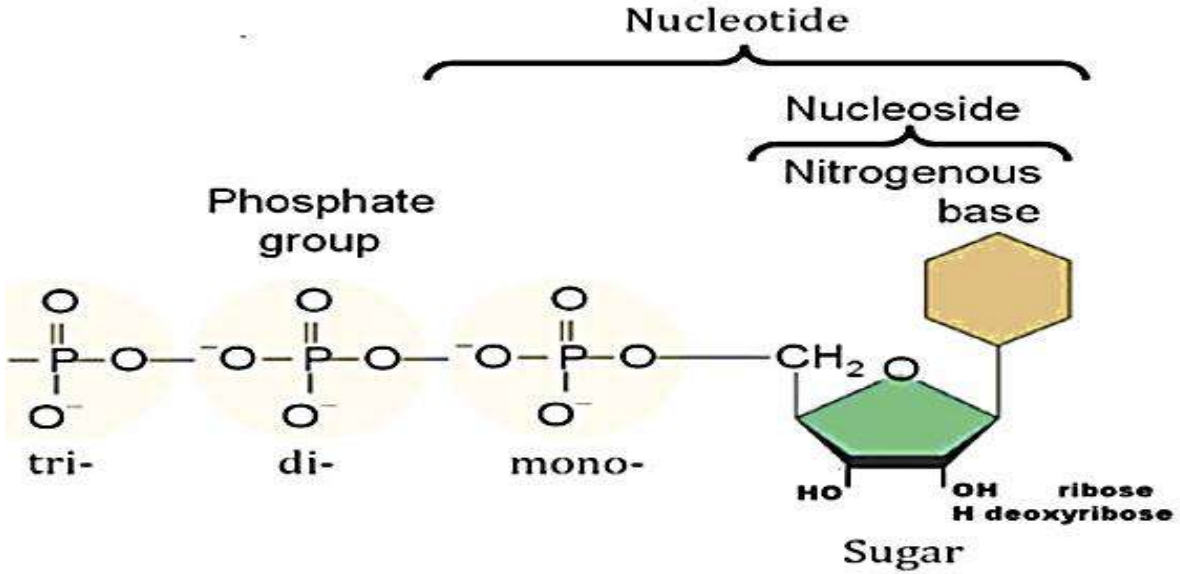
Biomedical Importance

- Unlike the **low solubility** of **uric acid** formed by **catabolism of purines**, the end products of **pyrimidine catabolism** (**carbon dioxide, ammonia, β-alanine, and γ-aminoisobutyrate**) are **highly water soluble**.
- One **genetic disorder of pyrimidine catabolism**, **β-hydroxybutyric aciduria**, is due to total or partial **deficiency of the enzyme dihydropyrimidine dehydrogenase**.
- This **disorder of pyrimidine catabolism**, also known as **combined uraciluria-thyminuria**, is also a **disorder of β-amino acid biosynthesis**, since the formation of **β-alanine** and of **β-aminoisobutyrate** is **impaired**.
- A **nongenetic form** can be triggered by administration of **5-fluorouracil** to patients with low levels of **dihydropyrimidine dehydrogenase**.

Metabolism of Purine & Pyrimidine Nucleotides

Biomedical Importance

Nucleobase	 Adenine	 Guanine	DNA  Thymine	 Cytosine	RNA  Uracil
Nucleoside	 Adenosine A	 Guanosine G	 Thymidine T	 Cytidine C	 Uridine U

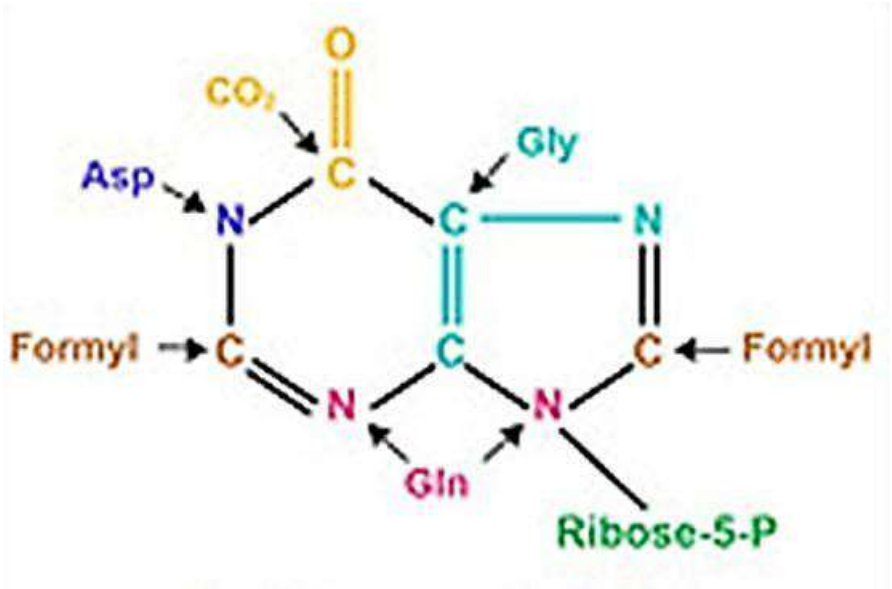


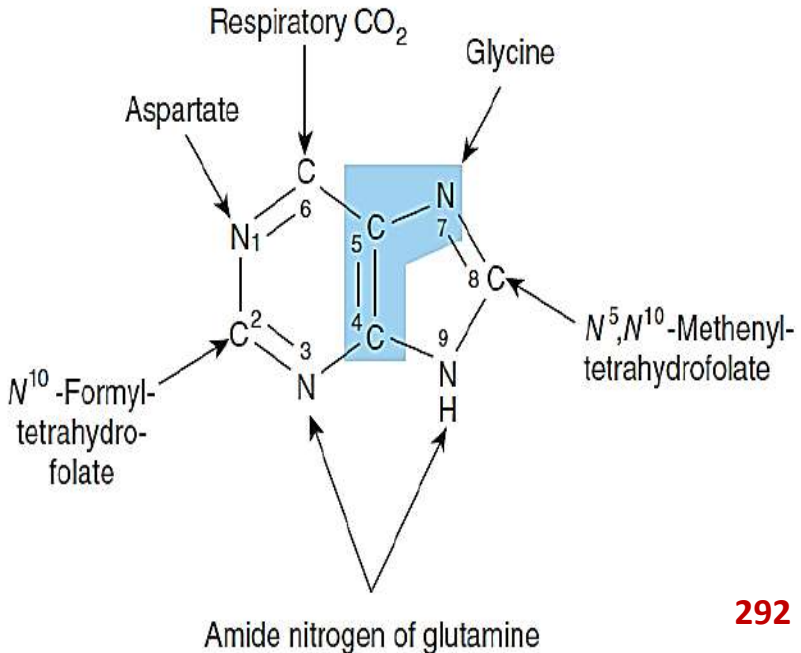
PURINES & PYRIMIDINES ARE DIETARILY NONESSENTIAL

- Normal **human** tissues can synthesize **purines and pyrimidines** from **amphibolic intermediates** in quantities and at times appropriate to meet variable physiologic demand.
- Ingested nucleic acids and nucleotides therefore are **dietarily nonessential**.
- Following their degradation in the intestinal tract, the resulting mononucleotides may be **absorbed or converted to purine and pyrimidine bases**.
- The **purine** bases are then **oxidized to uric acid**, which may be absorbed and excreted **in the urine**.
- While **little or no dietary purine or pyrimidine** is **incorporated into tissue nucleic acids**, **injected compounds are incorporated**.
- **The incorporation of injected [3H]thymidine into newly synthesized DNA thus can be used to measure the rate of DNA synthesis**

BIOSYNTHESIS OF PURINE NUCLEOTIDES

- **Purine and pyrimidine nucleotides** are synthesized in vivo at **rates consistent** with **physiologic need**.
- Early **investigations of nucleotide biosynthesis** first employed **birds**, and later **Escherichia coli**.
- Isotopic precursors of **uric acid fed to pigeons** established the **source of each atom** of a **purine** and initiated study of the **intermediates of purine biosynthesis**. (Figure 33–1)
- **Avian tissues also** served as a source of **cloned genes that encode enzymes of purine biosynthesis** and the **regulatory proteins** that control the rate of purine biosynthesis.

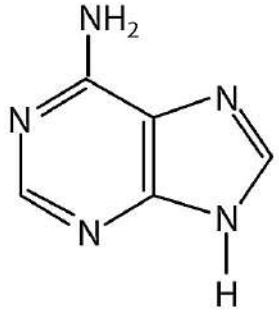




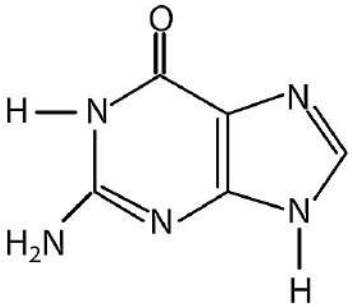
BIOSYNTHESIS OF PURINE NUCLEOTIDES

- **The three processes that contribute to purine nucleotide biosynthesis** are, in order of decreasing importance.
  1) **Synthesis from amphibolic intermediates (synthesis de novo).**
  2) **Phosphoribosylation of purines.**
  3) **Phosphorylation of purine nucleosides.**

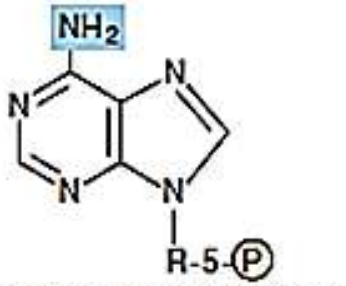
Purine



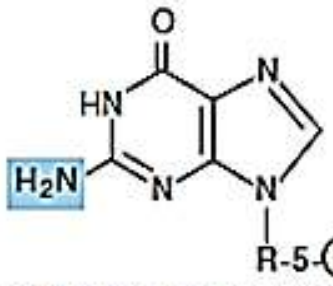
Adenine (A)



Guanine (G)



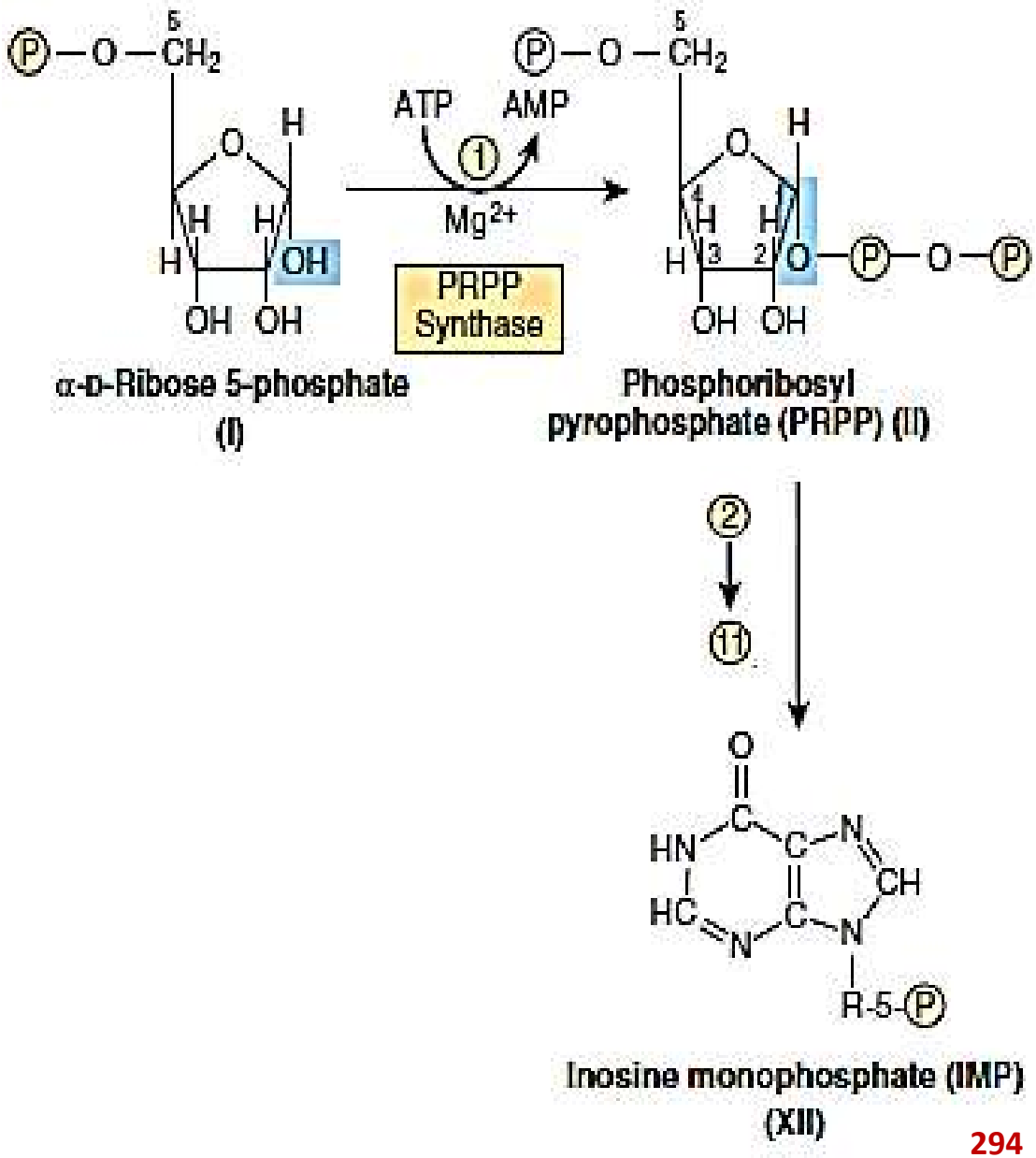
Adenosine monophosphate (AMP)



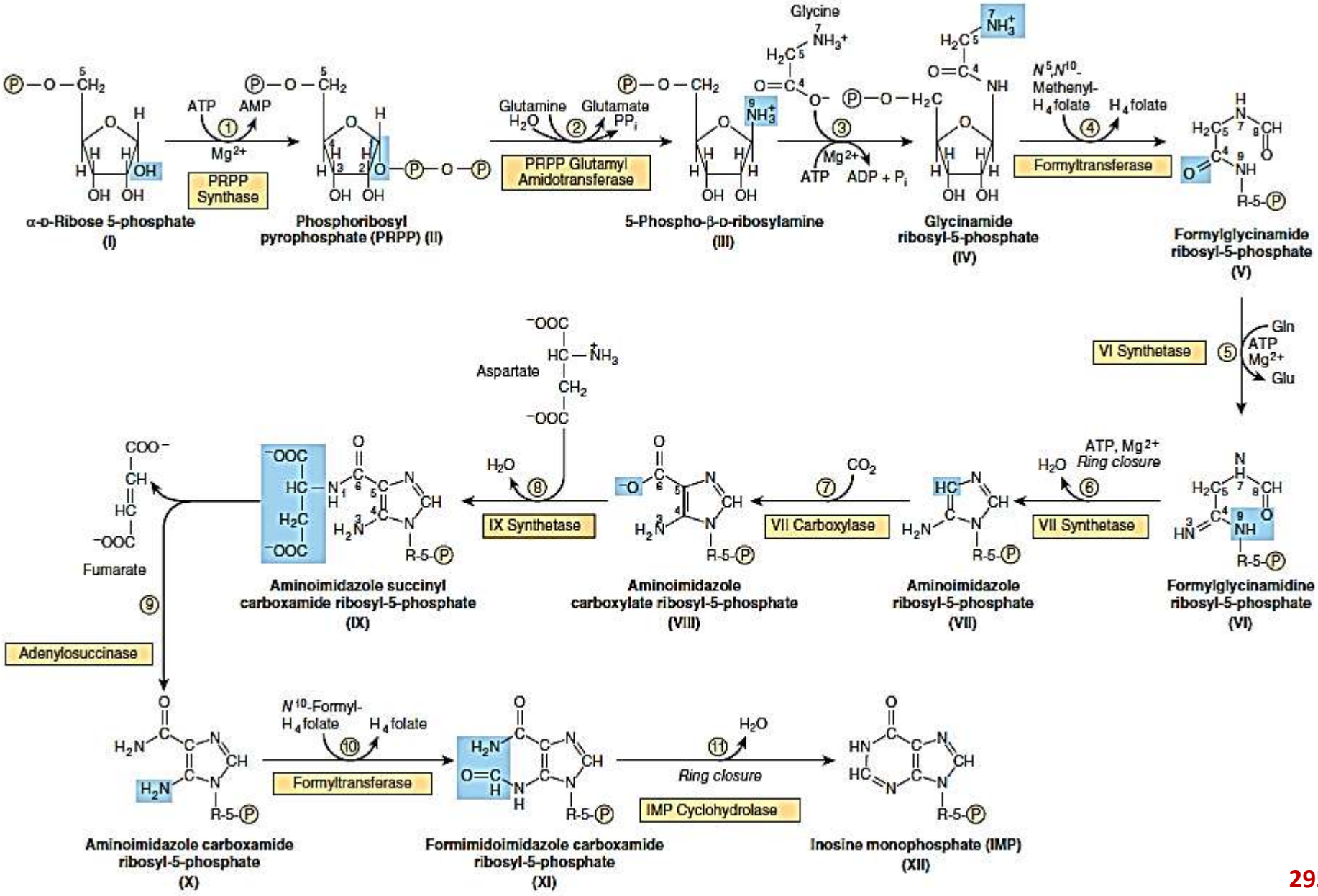
Guanosine monophosphate (GMP)

Inosine Monophosphate (IMP) Is Synthesized From Amphibolic Intermediates

- Figure 33–2 depicts the **intermediates** and the **11 enzyme catalyzed reactions** that convert **α-D-ribose 5-phosphate** to **inosine monophosphate** (**IMP**).
- The first intermediate formed in the **de novo pathway** for **purine biosynthesis** is **5-phosphoribosyl 1-pyrophosphate** (**PRPP**)
- **PRPP** is also an intermediate in the **pyrimidine nucleotides biosynthesis**, **NAD⁺**, and **NADP⁺**.
- Stepwise assembly of the **9-membered purine ring** then takes place on **PRPP** as a scaffold.

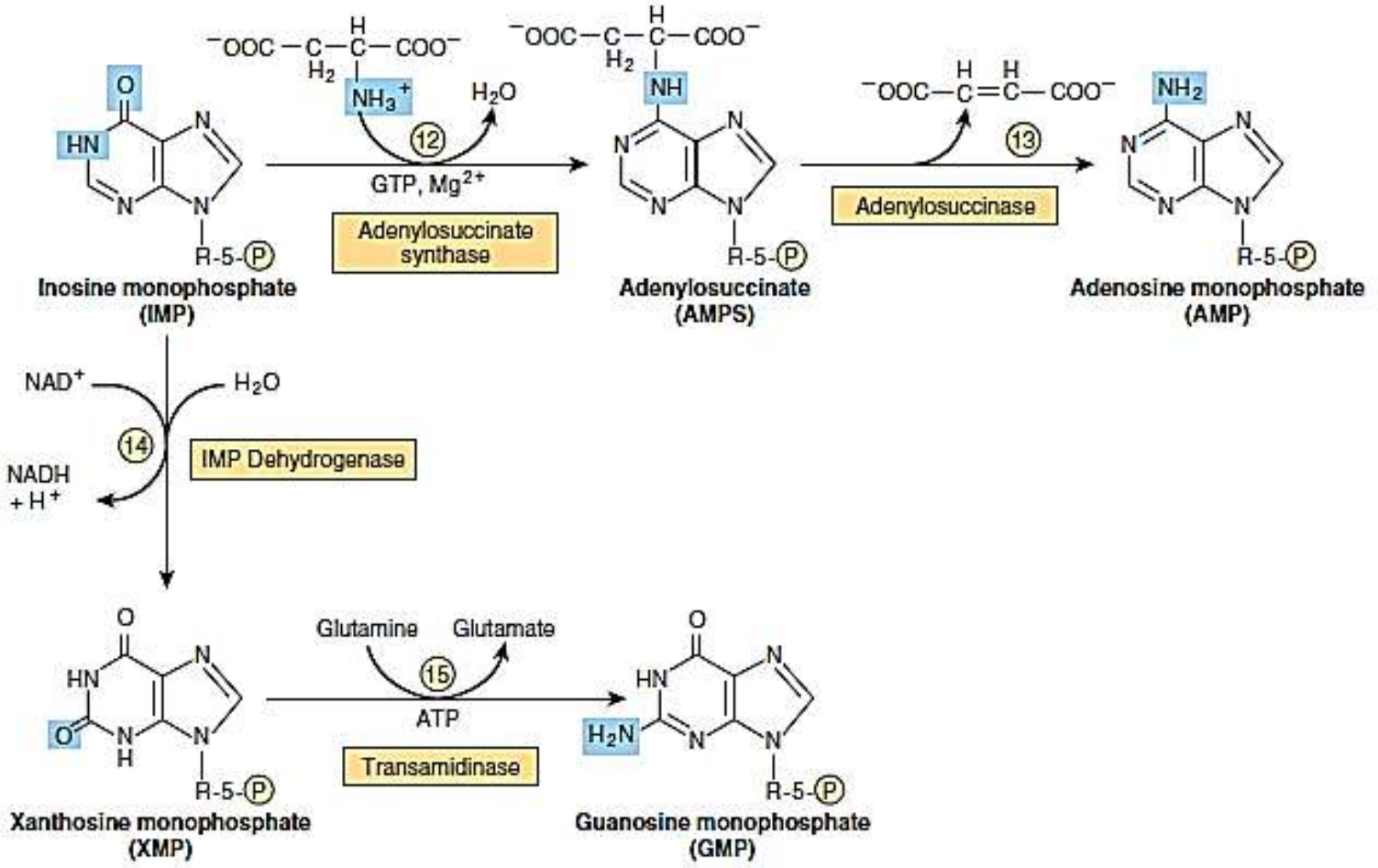


Inosine Monophosphate (IMP) Is Synthesized From Amphibolic Intermediates



Inosine Monophosphate (IMP) Is Synthesized From Amphibolic Intermediates

- Following synthesis of **IMP**, separate branches lead to **AMP** and **GMP**.



- Subsequent **phosphoryl transfer from ATP** converts **AMP** to **ADP** and **GMP** to **GDP**.

- Conversion of **GDP** to **GTP** involves a **second phosphoryl transfer from ATP**, whereas conversion of **ADP** to **ATP** is achieved primarily by **oxidative phosphorylation.**

Inosine Monophosphate (IMP) Is Synthesized From Amphibolic Intermediates

Multifunctional Catalysts Participate in Purine Nucleotide Biosynthesis

In prokaryotes, each reaction of **de novo pathway** is catalyzed by a **different polypeptide**.

By contrast, the **enzymes** of **eukaryotes** are **polypeptides** that possess **multiple catalytic activities** (**multifunctional enzymes**) whose adjacent catalytic sites **facilitate channeling of intermediates between sites**.

Three distinct **multifunctional enzymes** **catalyze reactions ③, ④, and ⑥**; **reactions ⑦ and ⑧**; and **reactions ⑩ and ⑪**

Antifolate Drugs & Glutamine Analogs Block Purine Nucleotide Biosynthesis

The carbons added in reactions ④ and ⑩ of **de novo pathway** are contributed by **derivatives of tetrahydrofolate**.

Purine deficiency states, while rare in humans, generally **reflect a deficiency of folic acid**.

Compounds that **inhibit formation of tetrahydrofolates** and therefore **block purine synthesis have been used in cancer chemotherapy**.

Inhibitory compounds and the reactions they inhibit include **azaserine** (reaction ⑤), **diazanorleucine** (reaction ②), **6-mercaptopurine** (reactions ⑬ and ⑭), and **mycophenolic acid** (reaction ⑭).

Metabolism of Purine & Pyrimidine Nucleotides

"Salvage Reactions" Convert Purines & Their Nucleosides To Mononucleotides

- **Conversion** of **purines**, their **ribonucleosides**, and their **deoxyribonucleosides to mononucleotides** involves "**salvage reactions**" that require far **less energy** than **de novo synthesis**.

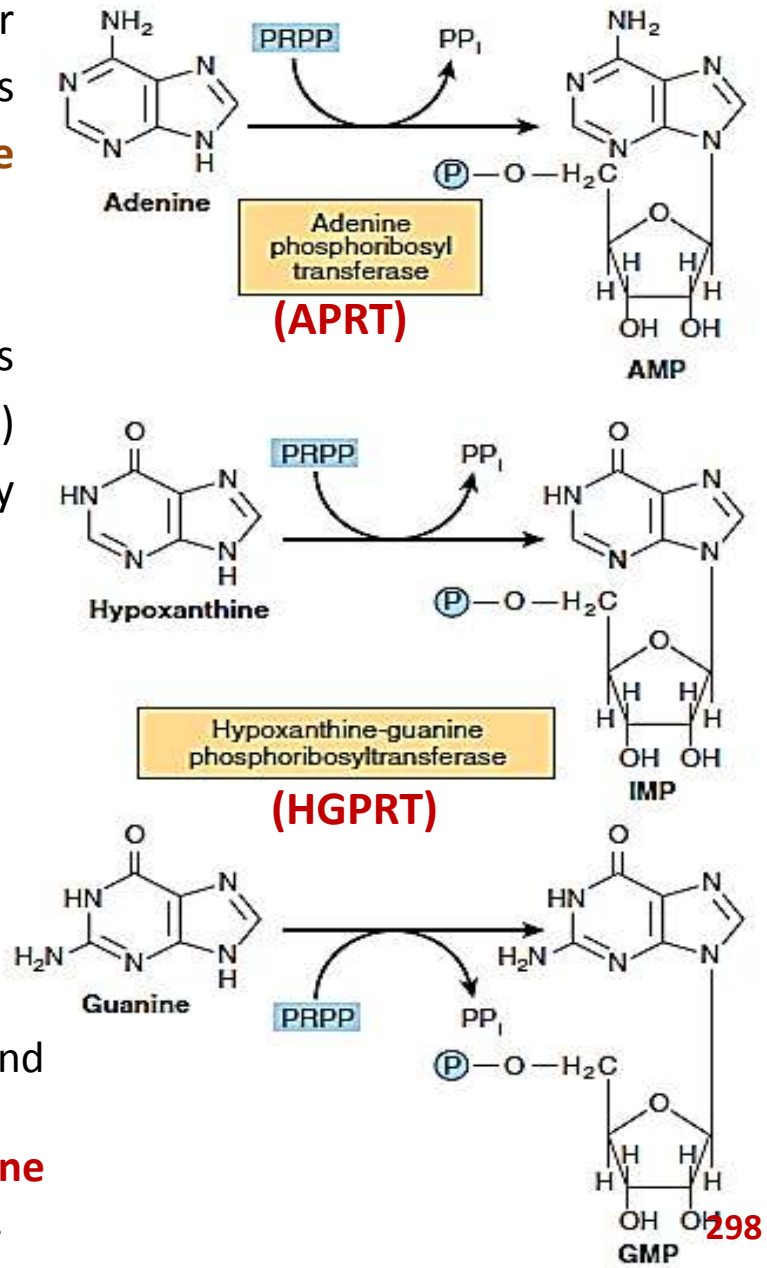
1) **The more important mechanism** involves **phosphoribosylation** by **PRPP** of a **free purine** (**Pu**) to form a **purine 5'-mononucleotide** (**Pu-RP**) by **phosphoribosyltransferase (APRT, HGPRT)**

Pu + PR-PP → Pu-RP + PPi

2) **A second salvage mechanism** involves **phosphoryl transfer** from **ATP** to a **purine ribonucleoside** (**Pu-R**) (EX: **adenosine kinase**).

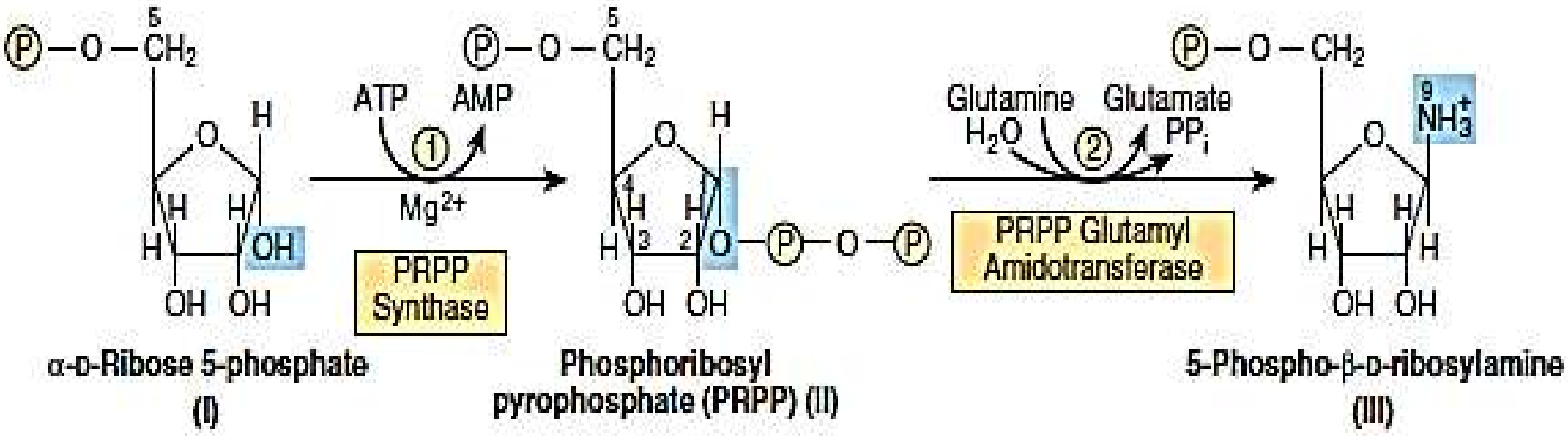
Pu-R + ATP → PuR-P + ADP

1. **Adenosine Kinase** converts **adenosine** and **deoxyadenosine** to **AMP** and **dAMP**.
2. **Deoxycytidine kinase** phosphorylates **deoxycytidine** and **2'-deoxyguanosine**, forming **dCMP** and **dGMP**.



"Salvage Reactions" Convert Purines & Their Nucleosides To Mononucleotides

- **Liver**, the major **site** of **purine nucleotide biosynthesis**, **provides purines and purine nucleosides for salvage** and **for utilization by tissues incapable of their biosynthesis**.
- **Human brain** tissue has a **low level** of **PRPP glutamyl amidotransferase**, EC 2.4.2.14 (**reaction** ②) and hence **depends** in part on **exogenous purines**.
- **Erythrocytes** and polymorphonuclear leukocytes **cannot synthesize 5-phosphoribosylamine** (**structure III**) and, therefore, als0 **utilize exogenous purines** to form **nucleotides**



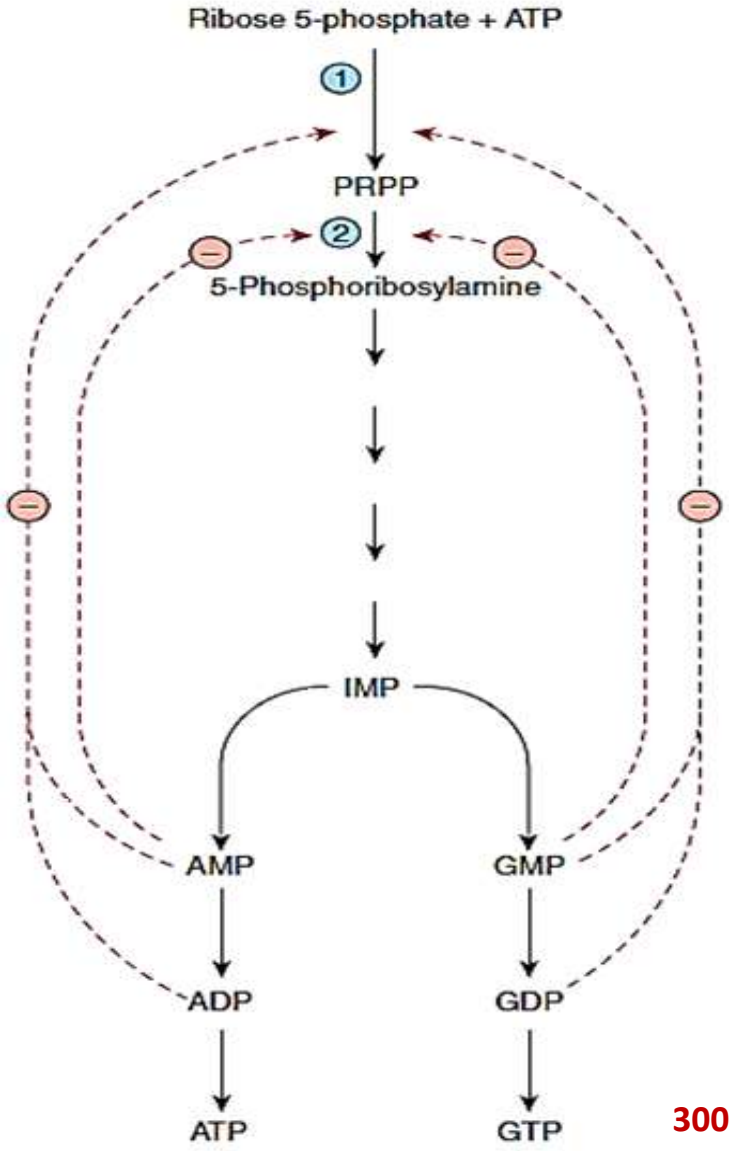
Metabolism of Purine & Pyrimidine Nucleotides

Hepatic Purine Biosynthesis Is Stringently Regulated

AMP & GMP Feedback Regulate PRPP Glutamyl Amidotransferase

1) regulation at the level of PRPP biosynthesis

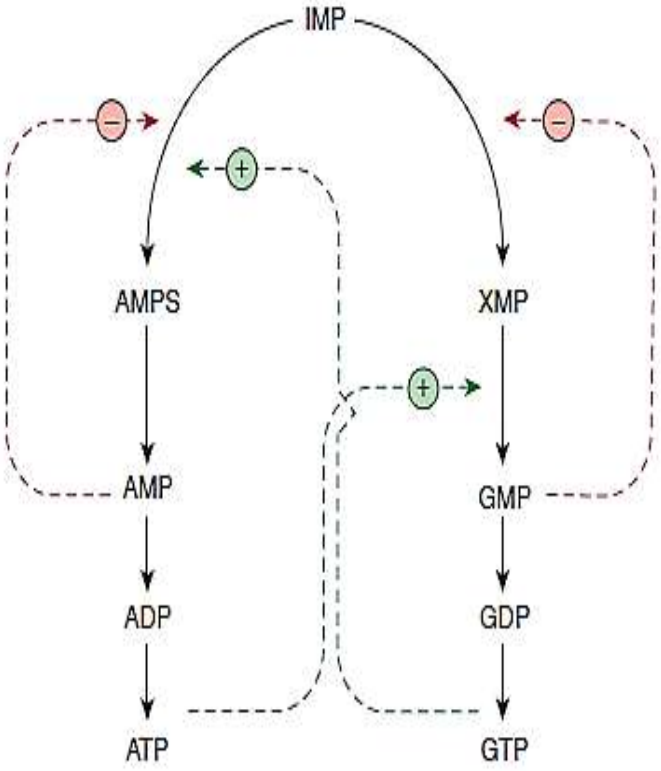
- **PRPP synthetase**, (**reaction 1**), an enzyme **feedback inhibited by AMP, ADP, GMP, and GDP**. **Elevated levels of these nucleoside phosphates** thus signal a physiologically appropriate overall **decrease in their biosynthesis.**
- **PRPP Glutamyl Amidotransferase** (**reaction 2**) an enzyme **feedback inhibited by AMP & GMP.**



Hepatic Purine Biosynthesis Is Stringently Regulated

AMP & GMP Feedback Regulate Their Formation From IMP

2) regulate conversion of IMP to ATP and GTP

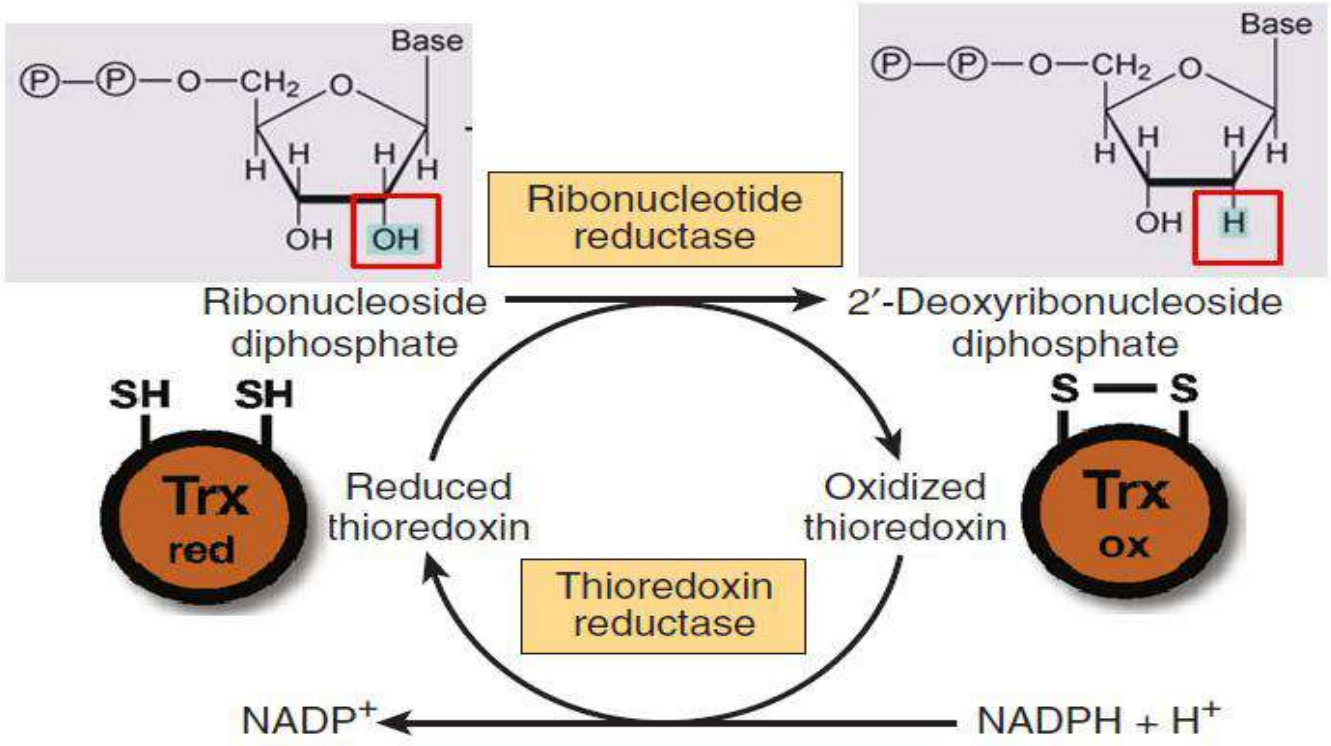


- **AMP inhibits adenylosuccinate synthase**, (**reaction** ⑫)
- **GMP inhibits IMP dehydrogenase**, (**reaction** ⑭).
- Furthermore, conversion of IMP to adenylosuccinate en route to AMP (reaction ⑫) requires GTP, and conversion of xanthinylate (XMP) to GMP requires ATP.

- This cross-regulation between the pathways of IMP metabolism thus serves to balance the biosynthesis of purine nucleoside triphosphates by decreasing the synthesis of one purine nucleotide when there is a deficiency of the other nucleotide.
- AMP and GMP also inhibit hypoxanthine-guanine phosphoribosyltransferase, which converts hypoxanthine and guanine to IMP and GMP (Figure 33–4), and GMP feedback inhibits PRPP glutamyl amidotransferase (reaction ②, Figure 33–2).

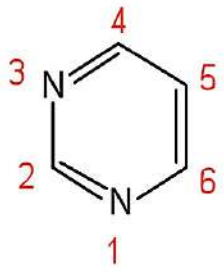
Reduction Of Ribonucleoside Diphosphates Forms Deoxyribonucleoside Diphosphates

- The reduction of ribonucleoside diphosphates (NDPs) to deoxyribonucleoside diphosphates (dNDPs) catalyzed by the ribonucleotide reductase complex IS needed for both the synthesis and repair of DNA.
- Reduction requires reduced thioredoxin, thioredoxin reductase and NADPH.

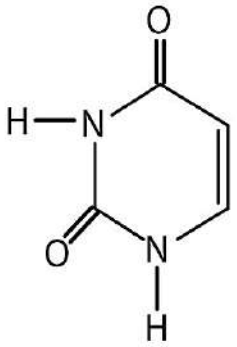


Biosynthesis Of Pyrimidine Nucleotides

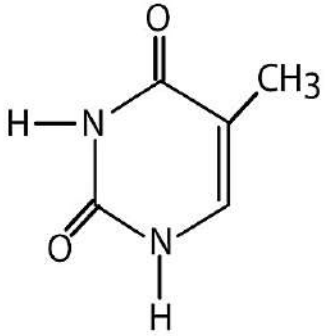
- **Figure 33–9** illustrates the intermediates and enzymes of **pyrimidine nucleotide biosynthesis**.
- **The catalyst for the initial reaction is cytosolic carbamoyl phosphate synthase II** (EC 6.3.5.5), a **different enzyme from** the **mitochondrial carbamoyl phosphate synthase I of urea synthesis**.
- **Compartmentation** thus provides an **independent pool** of **carbamoyl phosphate** for each process.
- **Unlike** in **purine biosynthesis** where **PRPP** serves as a **scaffold for assembly** of the **purine ring**, **PRPP** participates in **pyrimidine biosynthesis** only **subsequent** to **assembly** of the **pyrimidine ring**.
- **biosynthesis of pyrimidines,** the **biosynthesis of the purine nucleosides** is **energetically costly**.



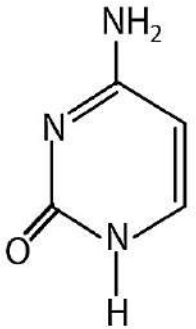
Pyrimidine



Uracil (U)
RNA only



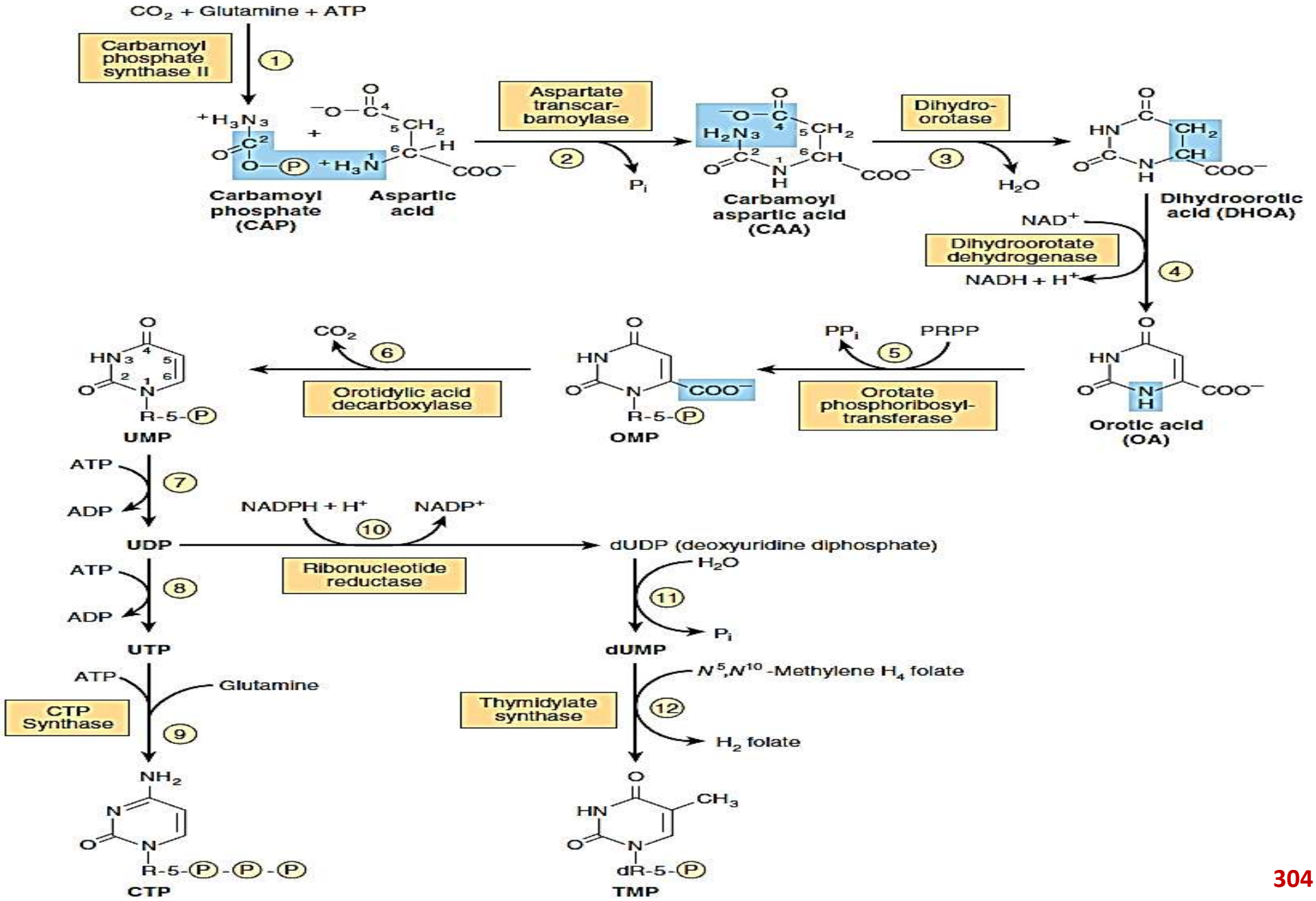
Thymine (T)
DNA only



Cytosine (C)
both DNA and RNA

Metabolism of Purine & Pyrimidine Nucleotides

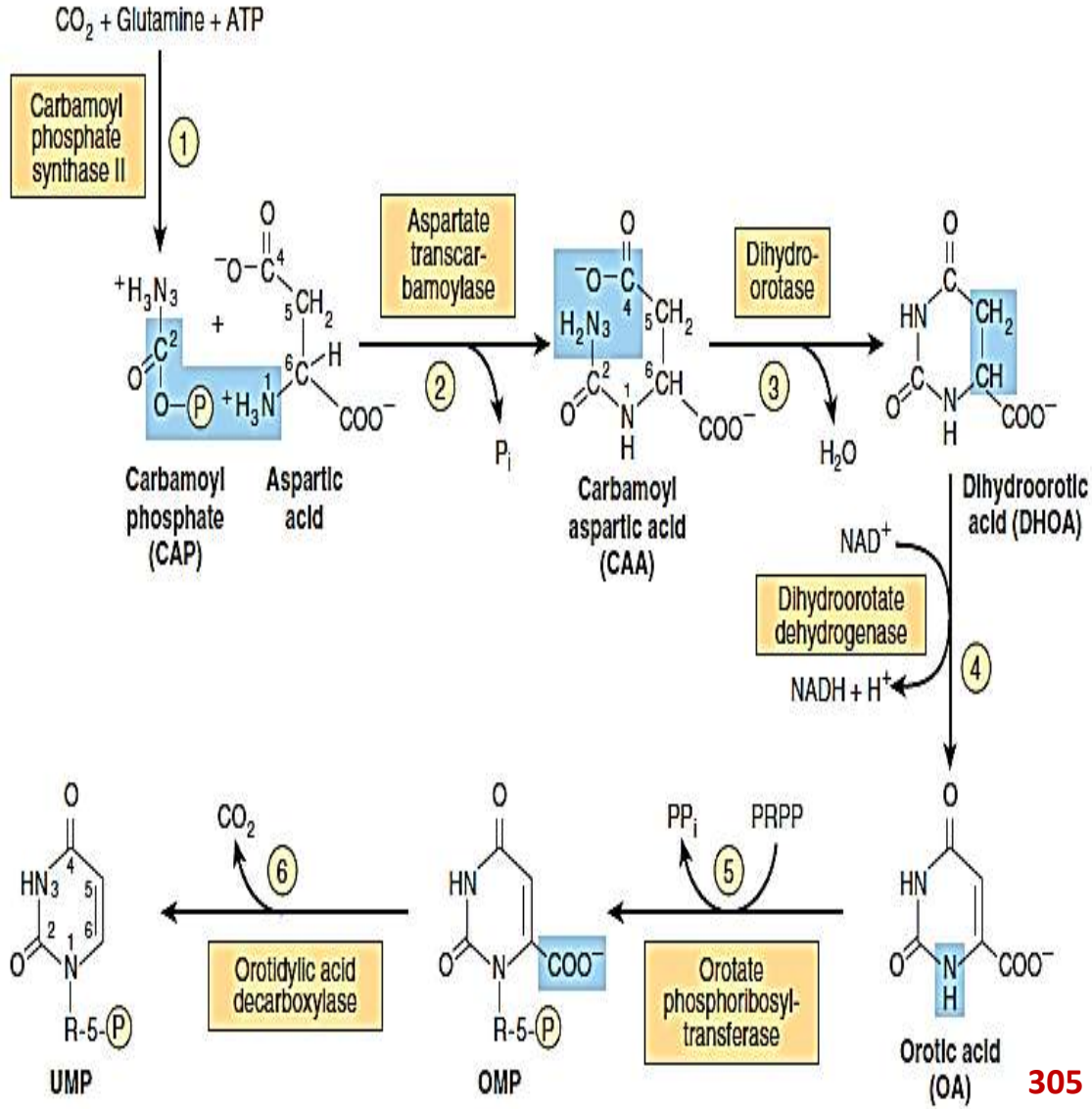
Biosynthesis Of Pyrimidine Nucleotides



Biosynthesis Of Pyrimidine Nucleotides

Multifunctional Proteins Catalyze The Early Reactions Of Pyrimidine Biosynthesis

- **Five** of the first six **enzyme activities** of **pyrimidine biosynthesis** reside on **multifunctional polypeptides**.
- **One polypeptide catalyzes the first three reactions** of Figure 33–9.
- **A second bifunctional enzyme catalyzes reactions ⑤ and ⑥** of Figure 33–9.
- The close proximity of multiple active sites on a **multifunctional polypeptide facilitates efficient channeling of the intermediates** of **pyrimidine biosynthesis**.



The Deoxyribonucleosides Of Uracil & Cytosine Are Salvaged

- **Adenine**, **guanine**, and **hypoxanthine** released during the turnover of nucleic acids, notably messenger RNAs, are **reconverted** to **nucleoside triphosphates** via so-called **salvage pathways**.
- While mammalian cells reutilize **few free pyrimidines**, "**salvage reactions**" **convert** the **pyrimidine ribonucleosides uridine** and **cytidine** and the **pyrimidine deoxyribonucleosides thymidine** and **deoxycytidine** to their respective **nucleotides**.

Adenine+ PRPP → AMP+ PPi

Guanine + PRPP → GMP + PPi

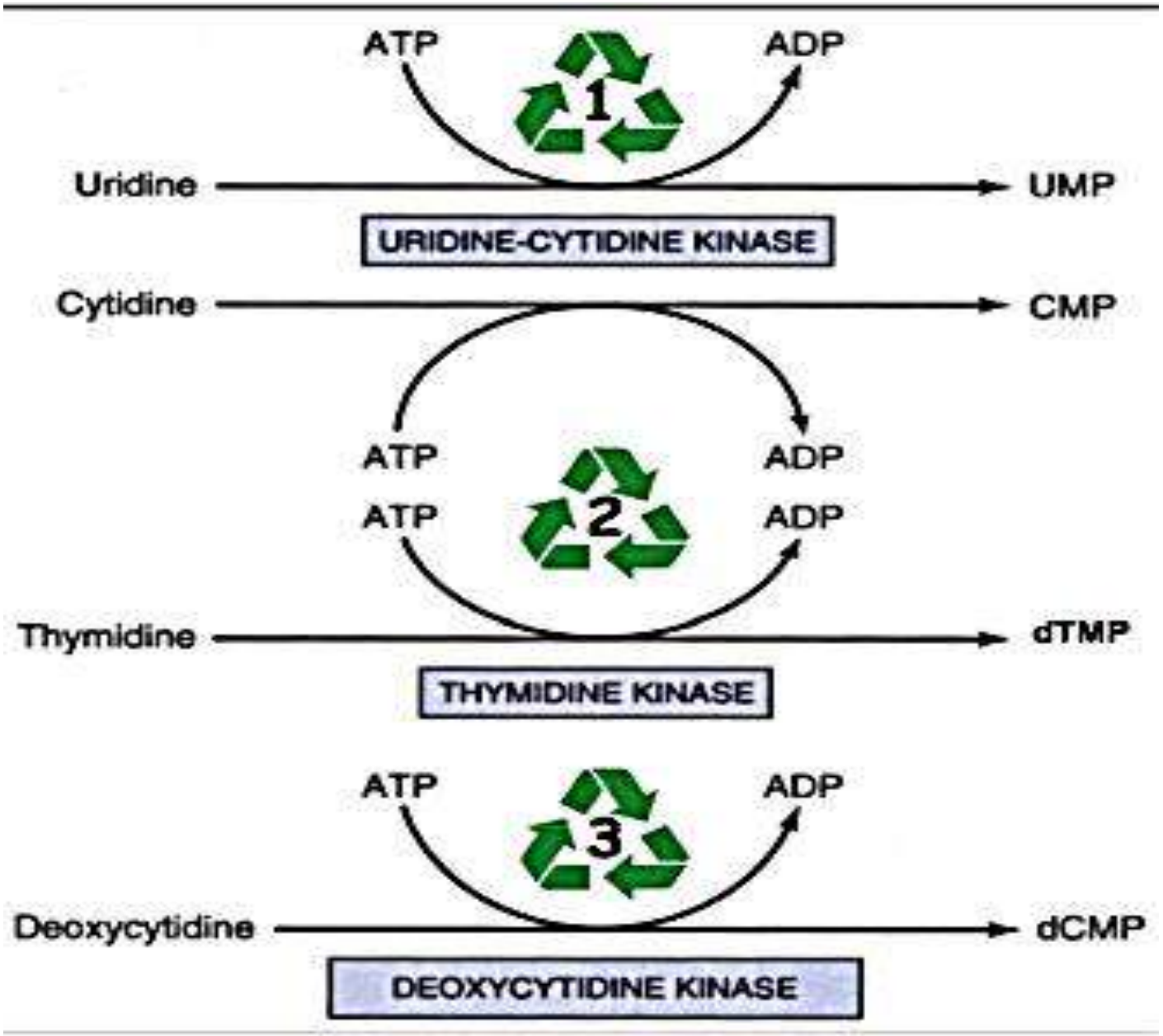
Hypoxanthine + PRPP → IMP + PPi

- **Phosphoryltransferases** (**kinases**) catalyze transfer of the **γ-phosphoryl group** of **ATP to the diphosphates** of the **dNDPs 2′-deoxycytidine**, **2′-deoxyguanosine**, and **2′-deoxyadenosine**, **converting them to** the corresponding **nucleoside triphosphates**.

NDP + ATP → NTP + ADP

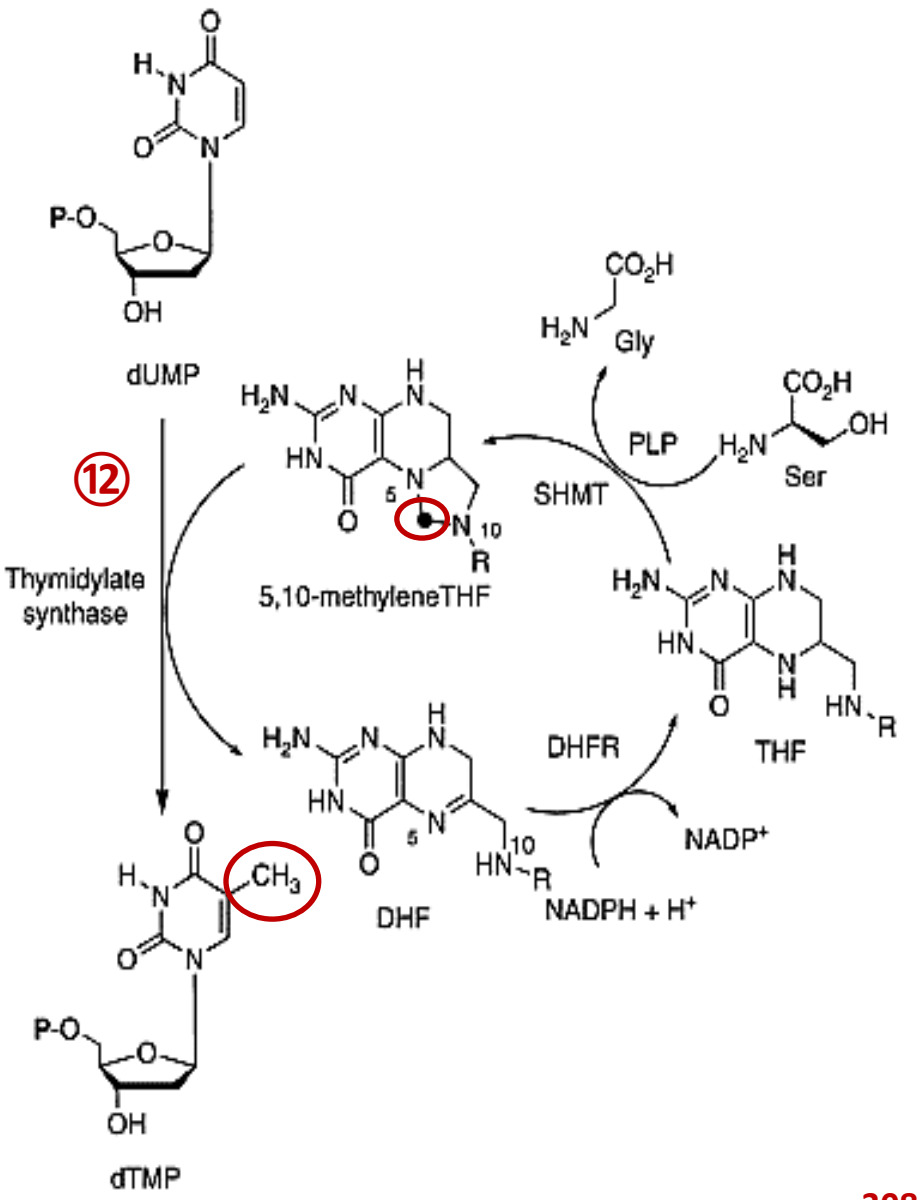
dNDP + ATP → dNTP + ADP

The Deoxyribonucleosides Of Uracil & Cytosine Are Salvaged



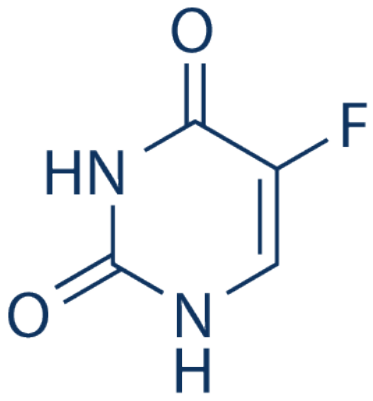
Methotrexate Blocks Reduction of Dihydrofolate

- The reaction catalyzed by **thymidylate synthase**, EC 2.1.1.45 (**reaction ⑫** of Figure 33–9) is the only reaction of **pyrimidine nucleotide biosynthesis** that **requires a tetrahydrofolate derivative**.
- During this reaction the **methylene group** of ***N*5,*N*10-methylene-tetrahydrofolate** is **reduced** to the **methyl group** that is transferred to the 5-position of the pyrimidine ring, and **tetrahydrofolate** is **oxidized** to **dihydrofolate**.
- **For further pyrimidine synthesis** to occur, **dihydrofolate** must be **reduced back** to **tetrahydrofolate**.
- This reduction, catalyzed by **dihydrofolate reductase** (EC 1.5.1.3), is **inhibited** by **methotrexate.**
- **Dividing cells**, which must generate TMP and dihydrofolate, thus are **especially sensitive** to **inhibitors of dihydrofolate reductase** such as the **anticancer drug methotrexate.**

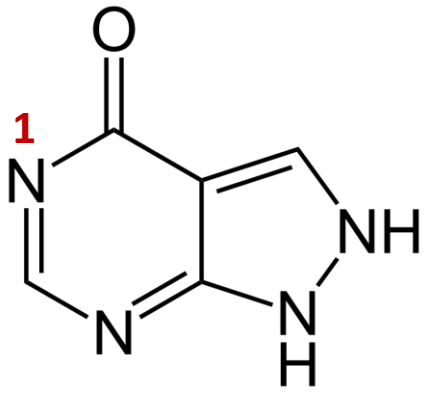


Certain Pyrimidine Analogs Are Substrates for Enzymes of Pyrimidine Nucleotide Biosynthesis

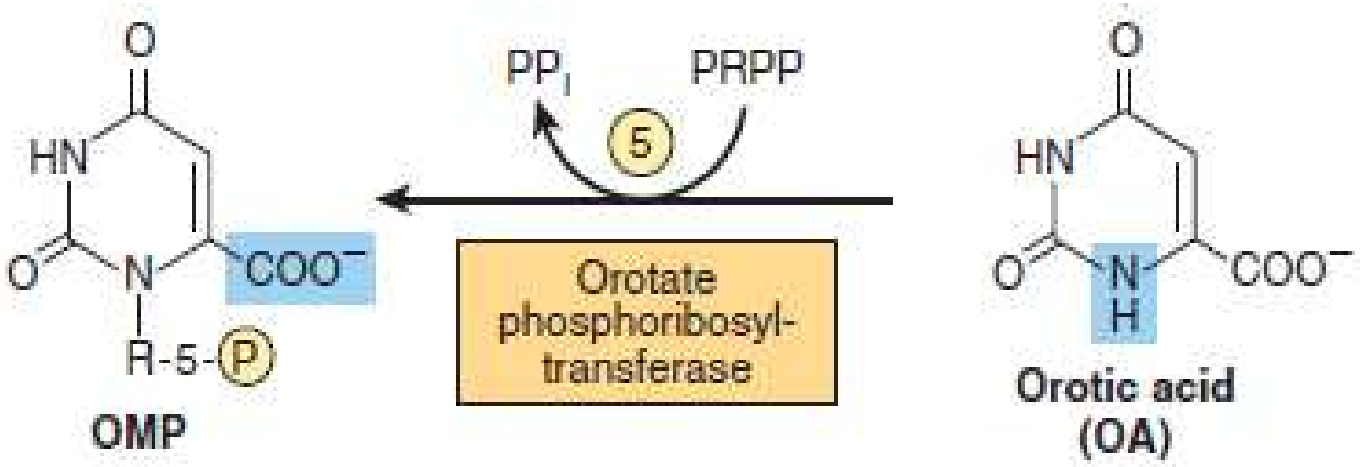
- **Allopurinol** and the **anticancer** drug **5-fluorouracil** are **alternate substrates** for **orotate phosphoribosyltransferase**, (**reaction ⑤**).
- Both drugs are **phosphoribosylated**, and **allopurinol** is converted to a **nucleotide** in which the ribosyl phosphate is attached to N1 of the pyrimidine ring.



5-fluorouracil



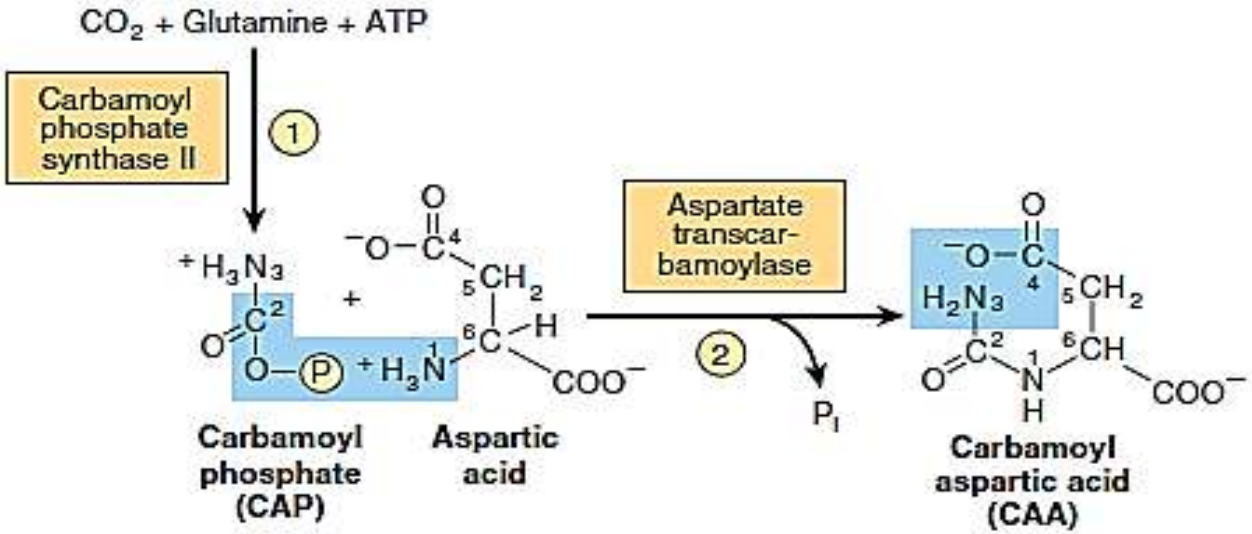
Allopurinol

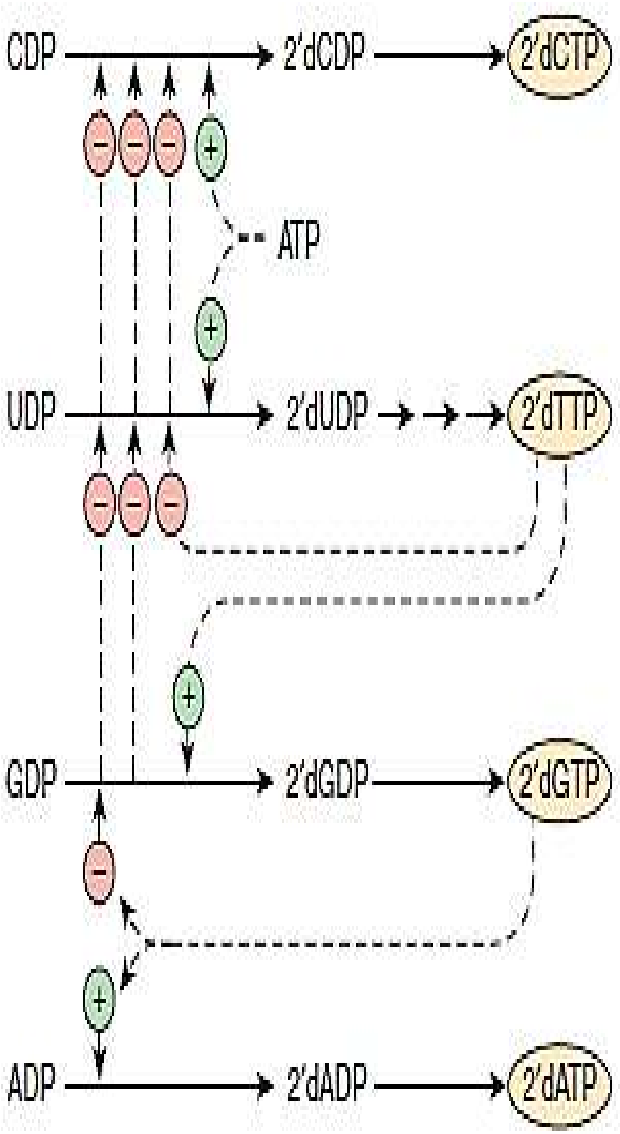


Regulation Of Pyrimidine Nucleotide Biosynthesis

Gene Expression & Enzyme Activity Both Are Regulated

- The activities of the first and second enzymes of pyrimidine nucleotide biosynthesis are controlled by allosteric regulation.
- **Carbamoyl phosphate synthase II** (**reaction ①**) is **inhibited** by **UTP** and purine nucleotides but **activated** by **PRPP**.
- **Aspartate transcarbamoylase**, EC 2.1.3.2 (**reaction ②**) is **inhibited** by **CTP** but is **activated** by **ATP** (Figure 33–10). In addition, the first three and the last two enzymes of the pathway are regulated by coordinate repression and derepression.

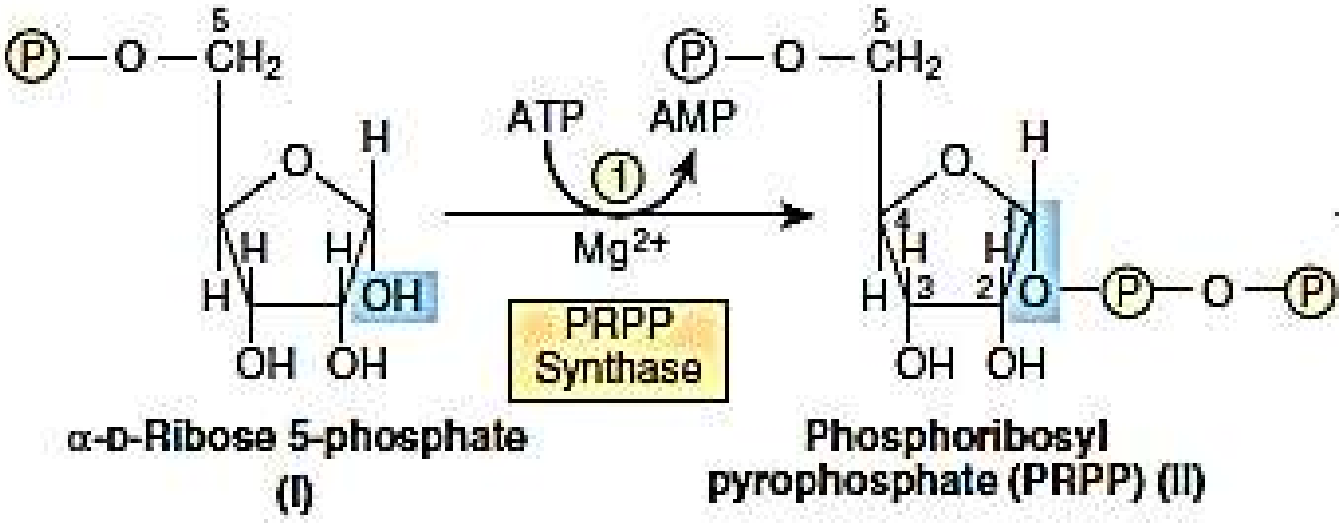




Regulation Of Pyrimidine Nucleotide Biosynthesis

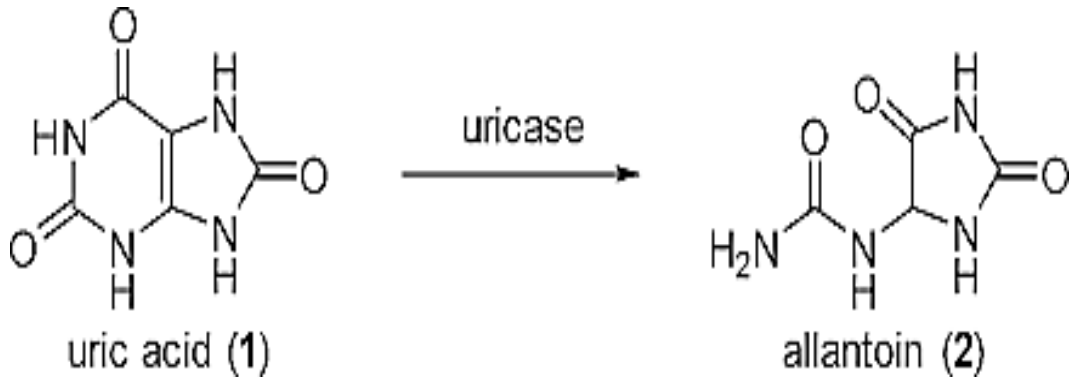
Purine & Pyrimidine Nucleotide Biosynthesis Are Coordinately Regulated

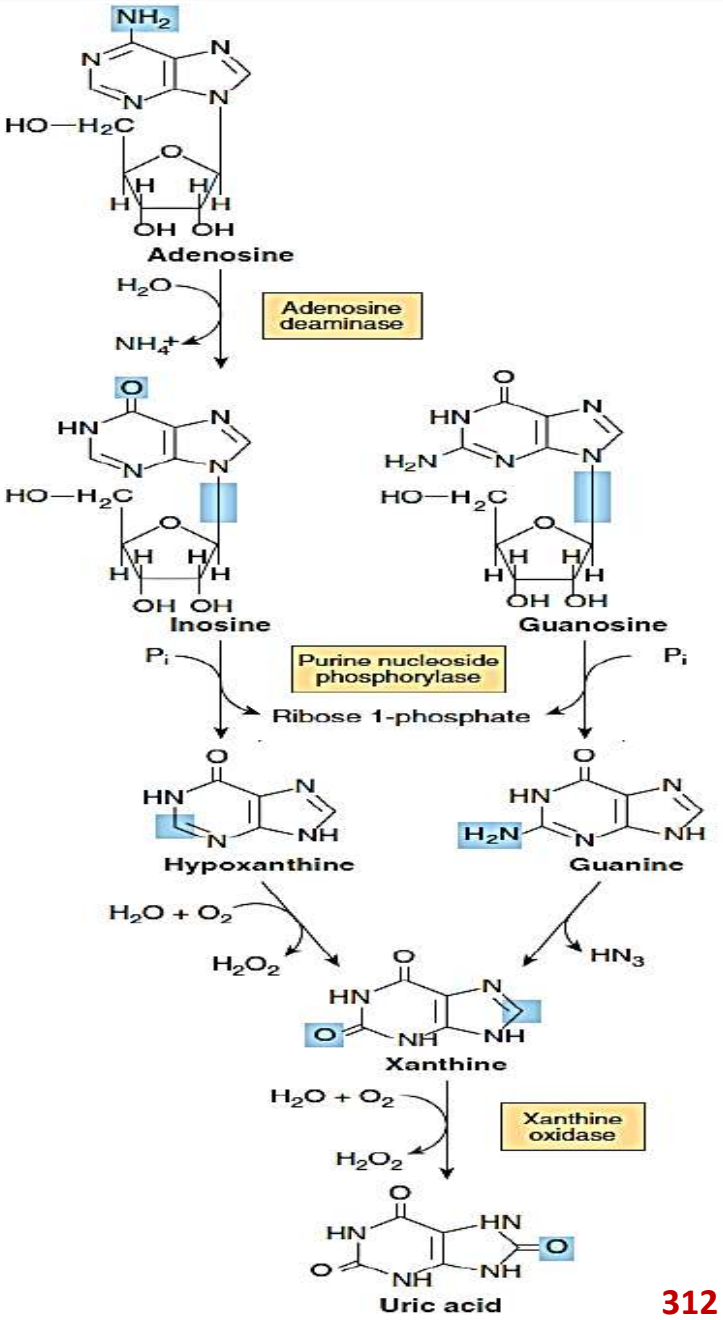
- **Purine** and **pyrimidine** **biosynthesis parallel one another quantitatively**, that is, mole for mole, **suggesting coordinated control of their biosynthesis**.
- Several sites of cross-regulation characterize the pathways that lead to the biosynthesis of purine and pyrimidine nucleotides.
- **PRPP synthase** (**reaction ①**), which forms a **precursor** essential for **both processes**, is **feedback inhibited** by both **purine** and **pyrimidine nucleotides**.



Humans Catabolize Purines To Uric Acid

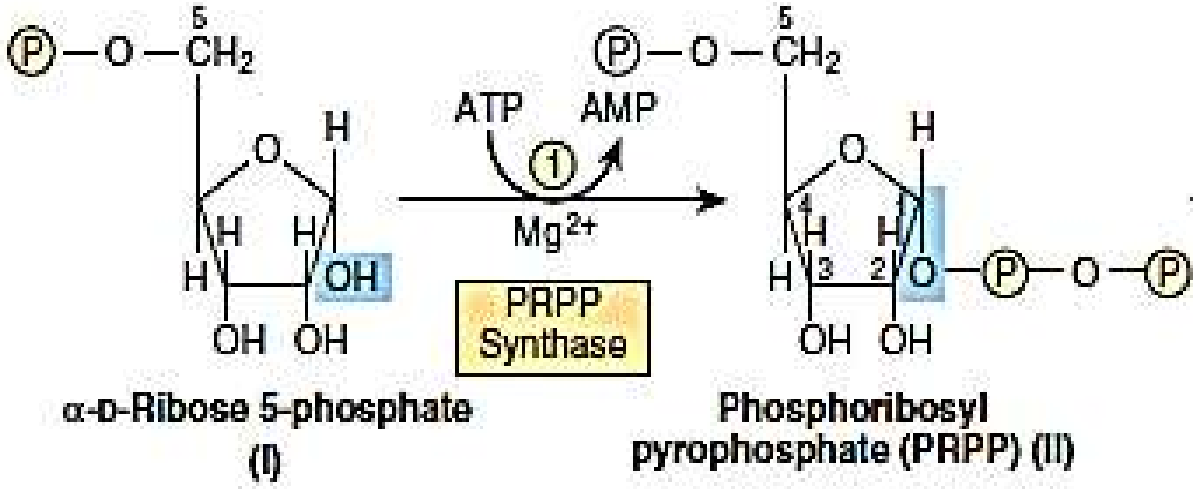
- Humans convert **adenosine and guanosine** to **uric acid** (Figure 33–11).
- **Adenosine** is first converted to **inosine** by **adenosine deaminase**, EC 3.5.4.4.
- **In mammals other than higher primates**, **uricase**, (EC 1.7.3.3) converts **uric acid** to the **watersoluble product allantoin**.
- However, since **humans lack uricase**, **the end product of purine catabolism in humans** is **uric acid**.





Gout Is A Metabolic Disorder Of Purine Catabolism

- Various **genetic defects** in **PRPP synthase** (**reaction ①**) present clinically as **gout**.
- Each defect—for example, an elevated Vmax, increased affinity for ribose 5-phosphate, or resistance to feedback inhibition—results in **overproduction** and **overexcretion** of **purine catabolites**.
- When **serum urate** levels **exceed the solubility limit**, **sodium urate crystalizes** in **soft tissues** and **joints** and causes an inflammatory reaction, **gouty arthritis**.
- However, **most cases of gout** reflect **abnormalities** in **renal handling** of **uric acid**.



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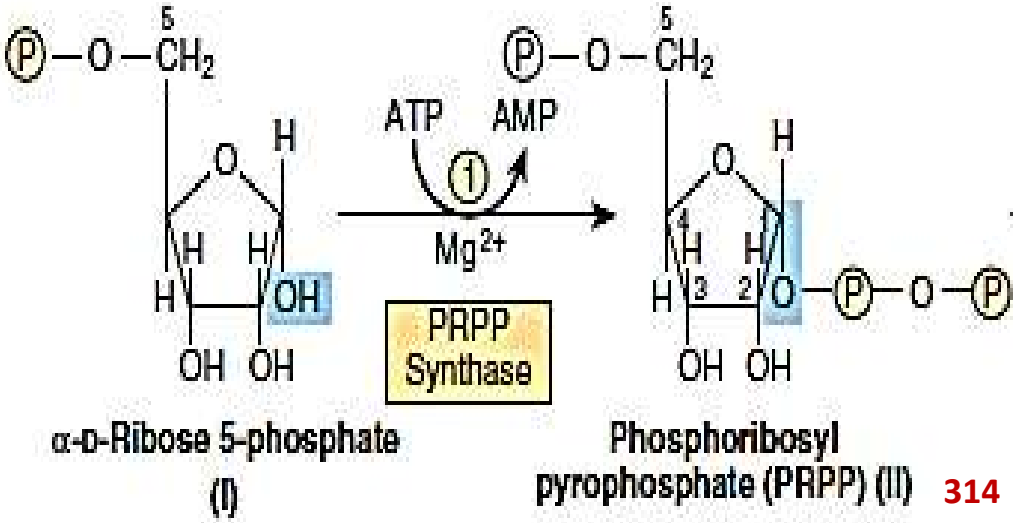
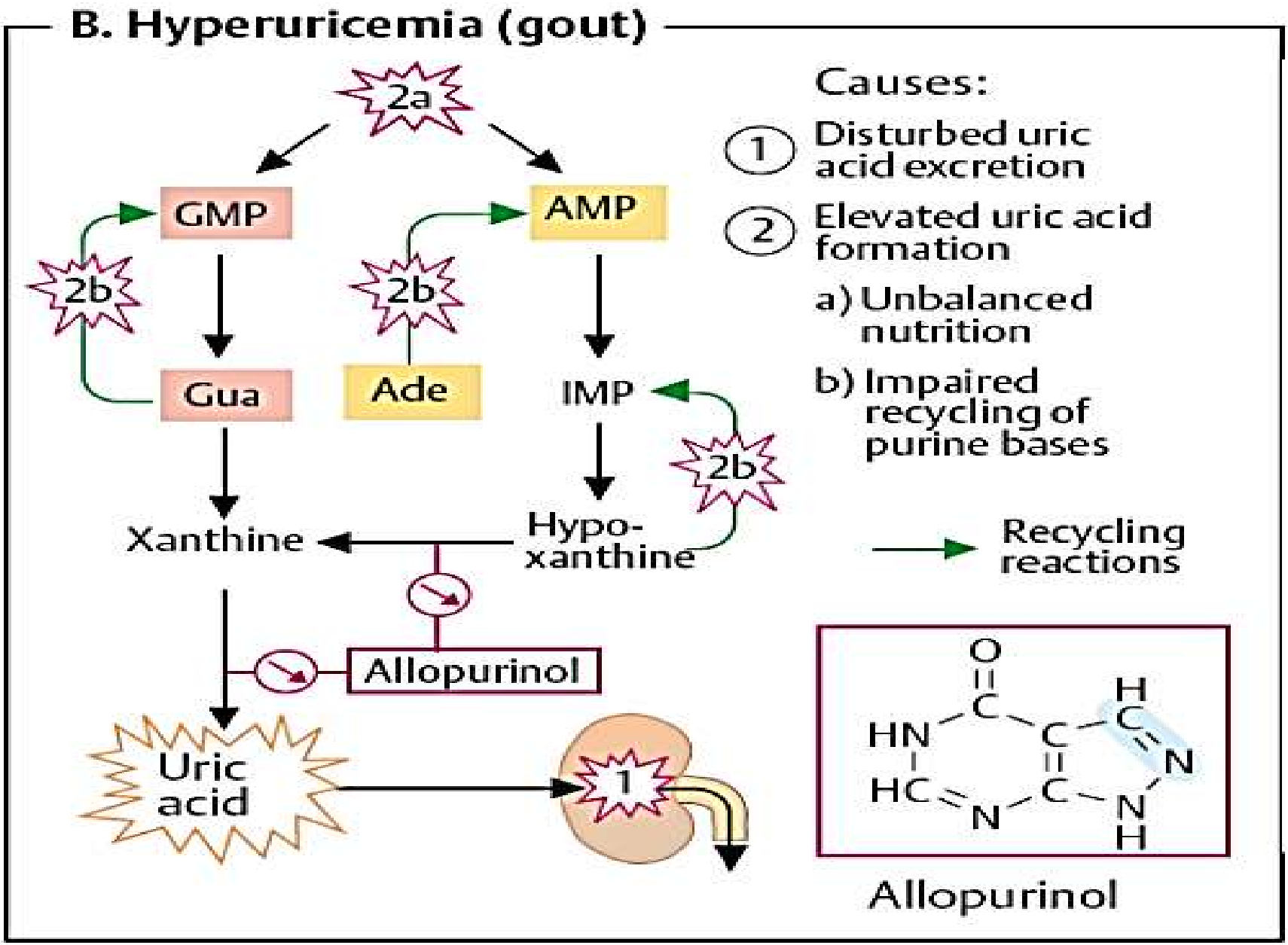
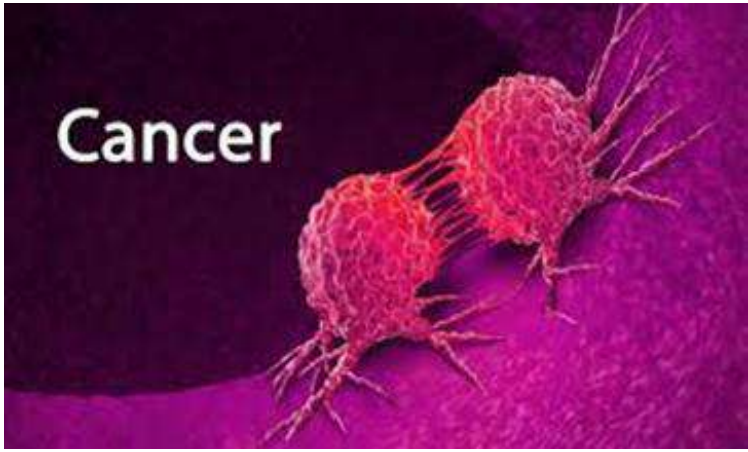


Gout Is A Metabolic Disorder Of Purine Catabolism



Other Disorders Of Purine Catabolism

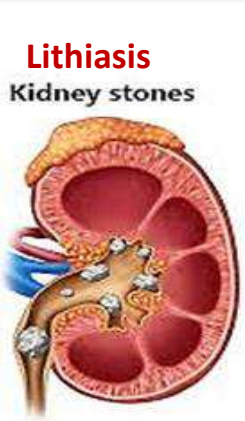
- While **purine deficiency states are rare in human** subjects, there are **numerous genetic disorders of purine catabolism**.
- **Hyperuricemias** may be **differentiated** based on whether **patients excrete normal** or **excessive** quantities of **total urates**.
- **Some hyperuricemias** reflect **specific enzyme defects**. **Others** are **secondary** to **diseases** such as **cancer** or **psoriasis** that **enhance tissue turnover**.

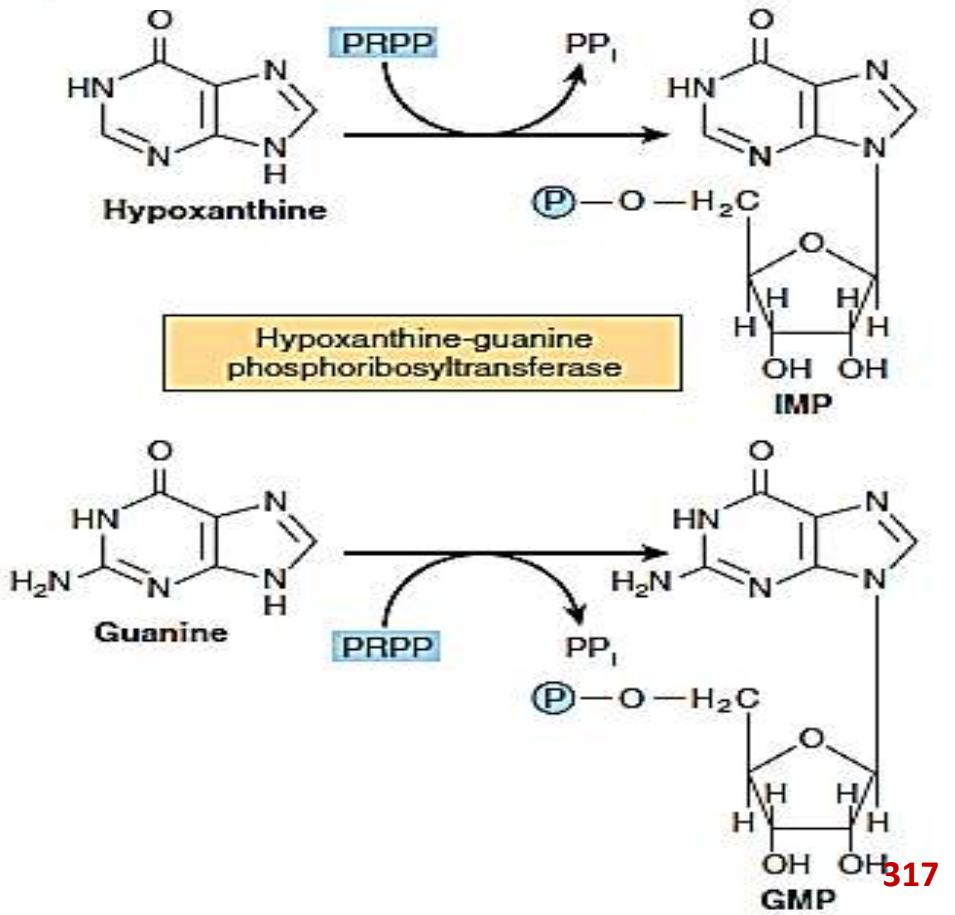


Other Disorders Of Purine Catabolism

Lesch-Nyhan Syndrome

- The **Lesch-Nyhan syndrome**, an **overproduction hyperuricemia** characterized by frequent episodes of uric acid **lithiasis** and a bizarre syndrome of **self-mutilation**, reflects a defect in **hypoxanthine-guanine phosphoribosyl transferase**, an enzyme of **purine salvage** .
- The accompanying rise in intracellular **PRPP** results in **purine overproduction**.
- Mutations that **decrease** or **abolish** **hypoxanthine-guanine phosphoribosyltransferase** activity include deletions, frameshift mutations, base substitutions, and aberrant mRNA splicing.





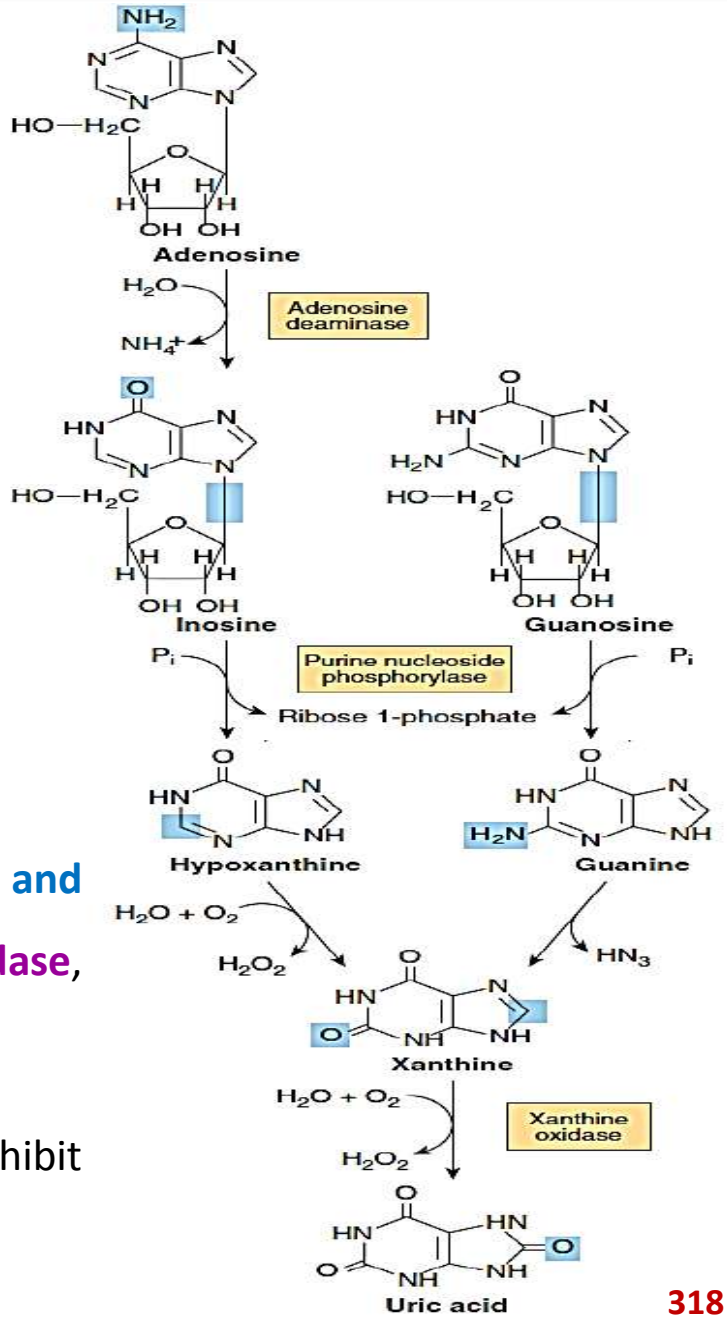
Other Disorders Of Purine Catabolism

von Gierke Disease

- **Purine overproduction and hyperuricemia** in **von Gierke disease** (**glucose-6-phosphatase deficiency**) **occurs secondary** to enhanced generation of the **PRPP** precursor **ribose 5-phosphate**.
- An associated **lactic acidosis elevates the renal threshold for urate**, **elevating total body urates**.

Hypouricemia

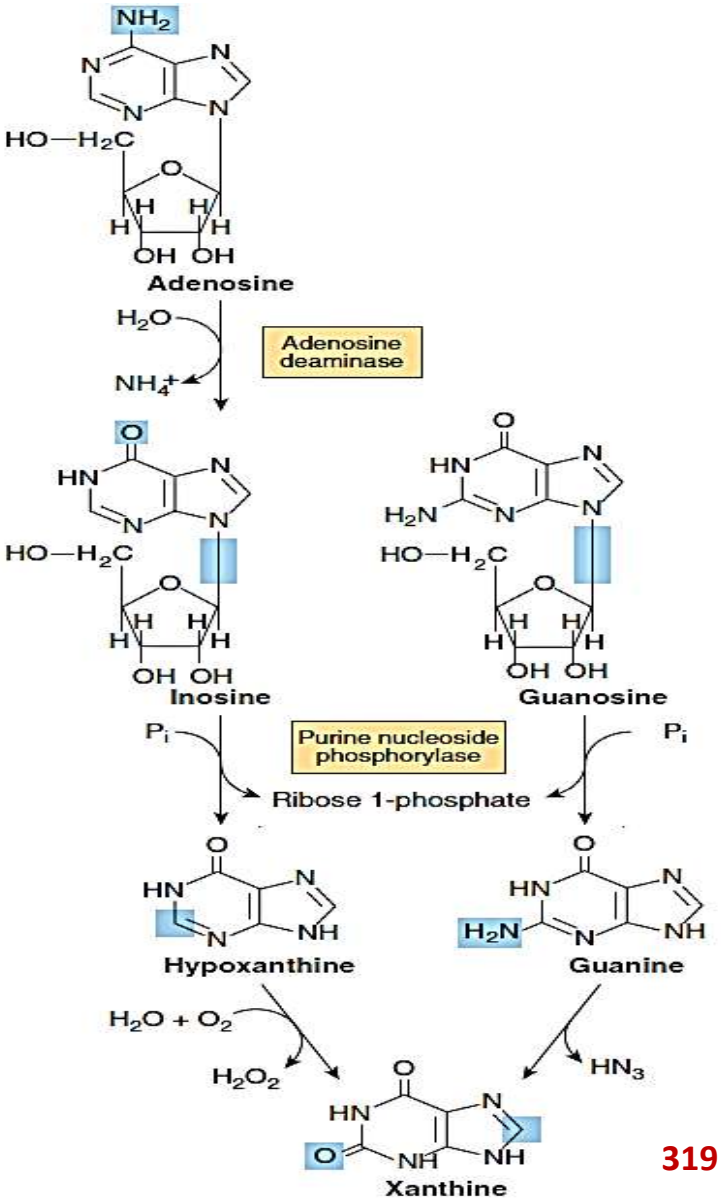
- **Hypouricemia** and **increased excretion of hypoxanthine and xanthine** are associated with a **deficiency in xanthine oxidase**, due to a **genetic defect** or to **severe liver damage**.
- Patients with a severe enzyme deficiency may exhibit **xanthinuria** and **xanthine lithiasis**..



Other Disorders Of Purine Catabolism

Adenosine Deaminase & Purine Nucleoside Phosphorylase Deficiency

- **Adenosine deaminase deficiency** is associated with an **immunodeficiency disease** in which both **thymus derived lymphocytes (T cells)** and **bone marrow-derived lymphocytes (B cells)** are sparse and dysfunctional.
- Patients suffer from severe **immunodeficiency**.
- In the absence of enzyme replacement or bone marrow transplantation, infants often succumb to fatal infections.
- Defective activity of **purine nucleoside phosphorylase** (EC 2.4.2.1) is associated with a **severe deficiency of T cells**, but **apparently normal B-cell function**.
- **Immune dysfunctions** appear to result from **accumulation** of **dGTP and dATP**, which **inhibit ribonucleotide reductase** and thereby deplete cells of DNA precursors.
- Table 33–1 summarizes known disorders of purine metabolism.



Other Disorders Of Purine Catabolism

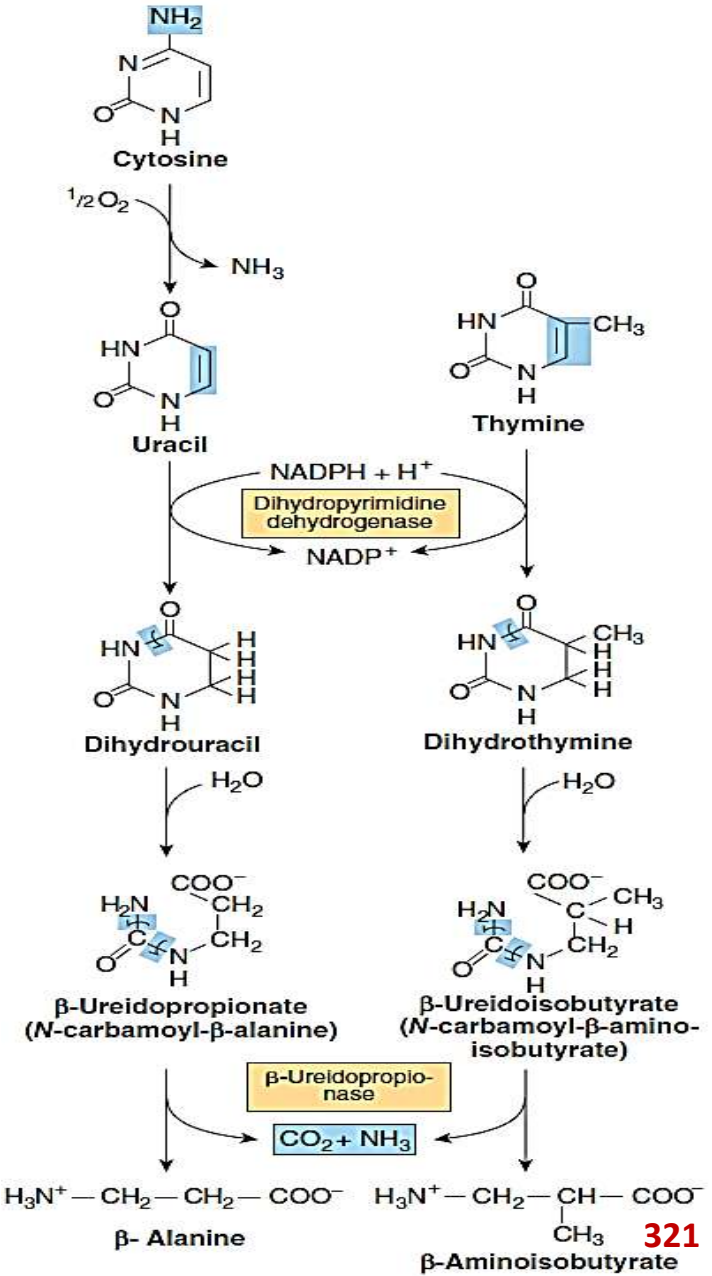
Adenosine Deaminase & Purine Nucleoside Phosphorylase Deficiency

- Table 33–1 summarizes known disorders of purine metabolism.

Defective Enzyme	Enzyme Catalog Number	OMIM Reference	Major Signs and Symptoms	Figure and Reaction
Purine Metabolism				
Hypoxanthine-guanine phosphoribosyl transferase	2.4.2.8	308000	Lesch-Nyhan syndrome. Uricemia, self-mutilation	33–4 ②
PRPP synthase	2.7.6.1	311860	Gout; gouty arthritis	33–2 ①
Adenosine deaminase	3.5.4.6	102700	Severely compromised immune system	33–1 ①
Purine nucleoside phosphorylase	2.4.2.1	164050	Autoimmune disorders; benign and opportunistic infections	33–11 ②
Pyrimidine Metabolism				
Dihydropyrimidine dehydrogenase	1.3.1.2	274270	Can develop toxicity to 5-fluorouracil, also a substrate for this dehydrogenase	33–12 ②
Orotate phosphoribosyl transferase and orotidylic acid decarboxylase	2.4.2.10 and 4.1.1.23	258900	Orotic acid aciduria type 1; megaloblastic anemia	33–9 ⑤ and ⑥
Orotidylic acid decarboxylase	4.1.1.23	258920	Orotic acid aciduria type 2	33—9 ⑥

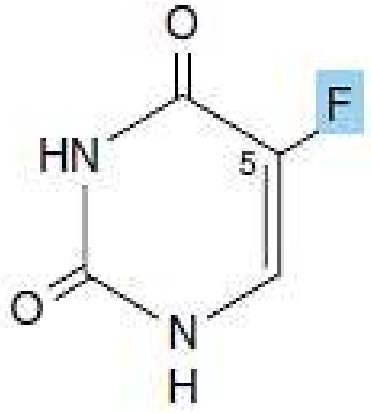
Catabolism Of Pyrimidines Produces Water-soluble Metabolites

- Unlike the **low solubility products of purine catabolism**, **catabolism of the pyrimidines forms highly water-soluble products**— **CO2**, **NH3**, **β-alanine**, and **β-aminoisobutyrate** .
- Humans transaminate **β-aminoisobutyrate** to **methylmalonate semialdehyde**, which then forms **succinyl-CoA.**
- **Excretion** of **β-aminoisobutyrate increases** in **leukemia** and **severe x-ray radiation exposure** due to **increased destruction of DNA**.
- However, many persons of Chinese or Japanese ancestry routinely excrete **β-aminoisobutyrate**.
- **Disorders** of **β-alanine** and **β-aminoisobutryrate** metabolism arise from **defects** in **enzymes of pyrimidine catabolism**.
- These include **a-hydroxybutyric aciduria**, a disorder due to total or partial **deficiency** of the enzyme **dihydropyrimidine dehydrogenase**, EC 1.3.1.2.
- The **genetic disease** reflects an **absence** of the **enzyme**.
- A disorder of pyrimidine catabolism, known also as combined uraciluria-thyminuria, is also a disorder of β-amino acid metabolism, since the formation of β-alanine



Catabolism Of Pyrimidines Produces Water-soluble Metabolites

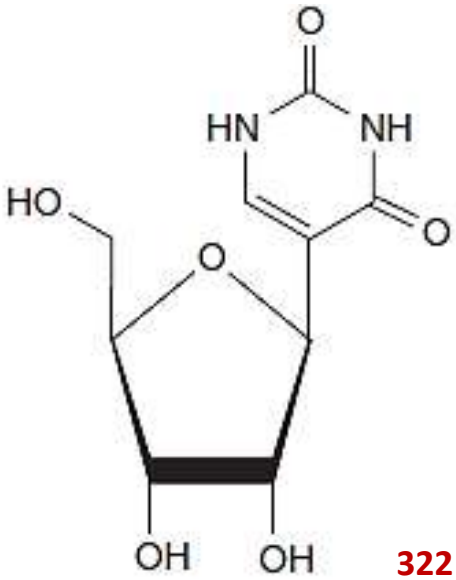
- **A disorder of pyrimidine catabolism**, known also as combined **uraciluria-thyminuria**, is also a disorder of **β-amino acid metabolism**, since the formation of **β-alanine and of β-aminoisobutyrate** is **impaired**.
- When due to an **inborn error**, there are serious **neurologicalcomplications**.
- A **nongenetic** form is **triggered** by the administration of the **anticancer drug 5-fluorouracil** to patients with **low levels** of **dihydropyrimidine dehydrogenase**.



5-Fluorouracil

Pseudouridine Is Excreted Unchanged

- **No human enzyme catalyzes hydrolysis** or **phosphorolysis** of the **pseudouridine** (ψ) derived from the **degradation of RNA molecules**.
- This **unusual nucleotide** therefore is **excreted unchanged** in the **urine** of normal subjects.
- **Pseudouridine** was indeed **first isolated from human urine**



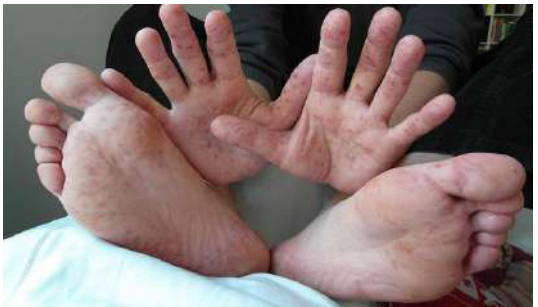
Overproduction Of Pyrimidine Catabolites Is Only Rarely Associated With Clinically Significant Abnormalities

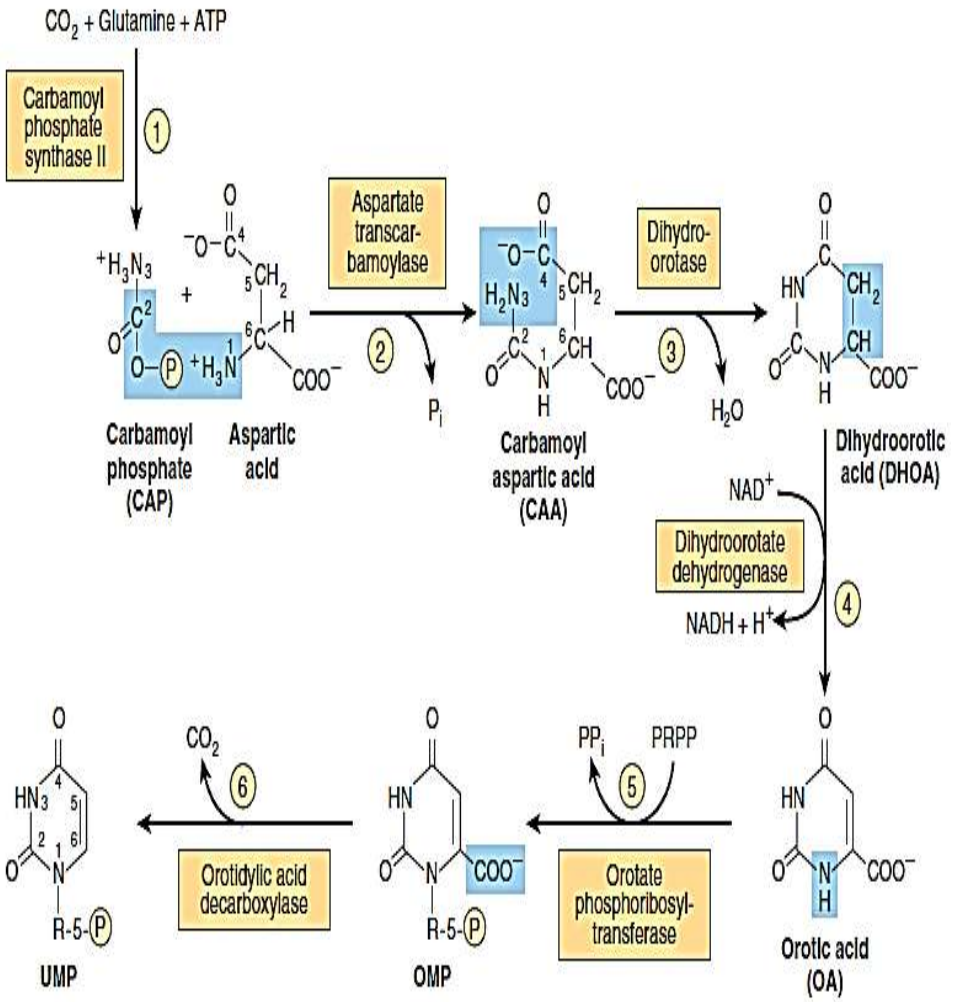
- Since the end products of pyrimidine catabolism are highly water-soluble, pyrimidine overproduction results in few clinical signs or symptoms. Table 33–1 lists exceptions.
- In hyperuricemia associated with severe overproduction of PRPP, there is overproduction of pyrimidine nucleotides and increased excretion of β-alanine.
- Since N5,N10-methylenetetrahydrofolate is required for thymidylate synthesis, disorders of folate and vitamin B12 metabolism result in deficiencies of TMP.

Overproduction Of Pyrimidine Catabolites Is Only Rarely Associated With Clinically Significant Abnormalities

Orotic Aciduria

- The **orotic aciduria** that accompanies the **Reye syndrome** probably is a consequence of the inability of **severely damaged mitochondria to utilize carbamoyl phosphate**, which then becomes **available for cytosolic overproduction of orotic acid**.
- **Type-I orotic aciduria** reflects a deficiency of both **orotate phosphoribosyltransferase** (EC 2.1.3.3) and **orotidylate decarboxylase**, EC 4.1.1.23 (**reactions ⑤** and **⑥**). The
- **rarer Type-II orotic aciduria** is due to a deficiency only of **orotidylate decarboxylase** (**reaction ⑥**).





Overproduction Of Pyrimidine Catabolites Is Only Rarely Associated With Clinically Significant Abnormalities

Deficiency of a Urea Cycle Enzyme Results in Excretion of Pyrimidine Precursors

- Increased excretion of orotic acid, uracil, and uridine accompanies
- a deficiency in liver mitochondrial ornithine transcarbamoylase
- (see reaction ②, Figure 28–16). Excess carbamoyl
- phosphate exits to the cytosol, where it stimulates pyrimidine
- nucleotide biosynthesis. The resulting mild orotic aciduria is
- increased by high-nitrogen foods.

Overproduction Of Pyrimidine Catabolites Is Only Rarely Associated With Clinically Significant Abnormalities

Drugs May Precipitate Orotic Aciduria

- Allopurinol (see Figure 32–13), an alternative substrate for
- orotate phosphoribosyltransferase (reaction ⑤, Figure 33–9), competes with orotic acid. The resulting nucleotide product also inhibits orotidylate decarboxylase (reaction ⑥, Figure 33–9), resulting in orotic aciduria and orotidinuria.
- 6-Azauridine, following conversion to 6-azauridylate, also competitively
- inhibits orotidylate decarboxylase (reaction ⑥, Figure 33–9),
- enhancing excretion of orotic acid and orotidine. Four genes
- that encode urate transporters have been identified. Two of the
- encoded proteins are localized to the apical membrane of proximal
- tubular cells.

Metabolism of Purine & Pyrimidine Nucleotides

SUMMARY

1) Ingested nucleic acids are degraded to purines and pyrimidines. Purines and pyrimidines are formed from amphibolic intermediates and thus are dietarily nonessential.
2) Several reactions of IMP biosynthesis require folate derivatives and glutamine. Consequently, antifolate drugs and glutamine analogs inhibit purine biosynthesis.
3) IMP is a precursor both of AMP and of GMP. Glutamine provides the 2-amino group of GMP, and aspartate the 6-amino group of AMP.
4) Phosphoryl transfer from ATP converts AMP and GMP to ADP and GDP. A second phosphoryl transfer from ATP forms GTP, but ADP is converted to ATP primarily by oxidative phosphorylation.
5) Hepatic purine nucleotide biosynthesis is stringently regulated by the pool size of PRPP and by feedback inhibition of PRPP glutamyl amidotransferase by AMP and GMP.
6) Coordinated regulation of purine and pyrimidine nucleotide biosynthesis ensures their presence in proportions appropriate for nucleic acid biosynthesis and other metabolic needs.
7) Humans catabolize purines to uric acid (pKa 5.8), present as the relatively insoluble acid at acidic pH or as its more soluble sodium urate salt at a pH near neutrality. Urate crystals are diagnostic of gout. Other disorders of purine catabolism include Lesch-Nyhan syndrome, von Gierke disease, and hypouricemias.
8) Since pyrimidine catabolites are water-soluble, their overproduction does not result in clinical abnormalities.
9) Excretion of pyrimidine precursors can, however, result from a deficiency of ornithine transcarbamoylase because excess carbamoyl phosphate is available for pyrimidine biosynthesis.

The Diversity of the Endocrine System

Abbreviations

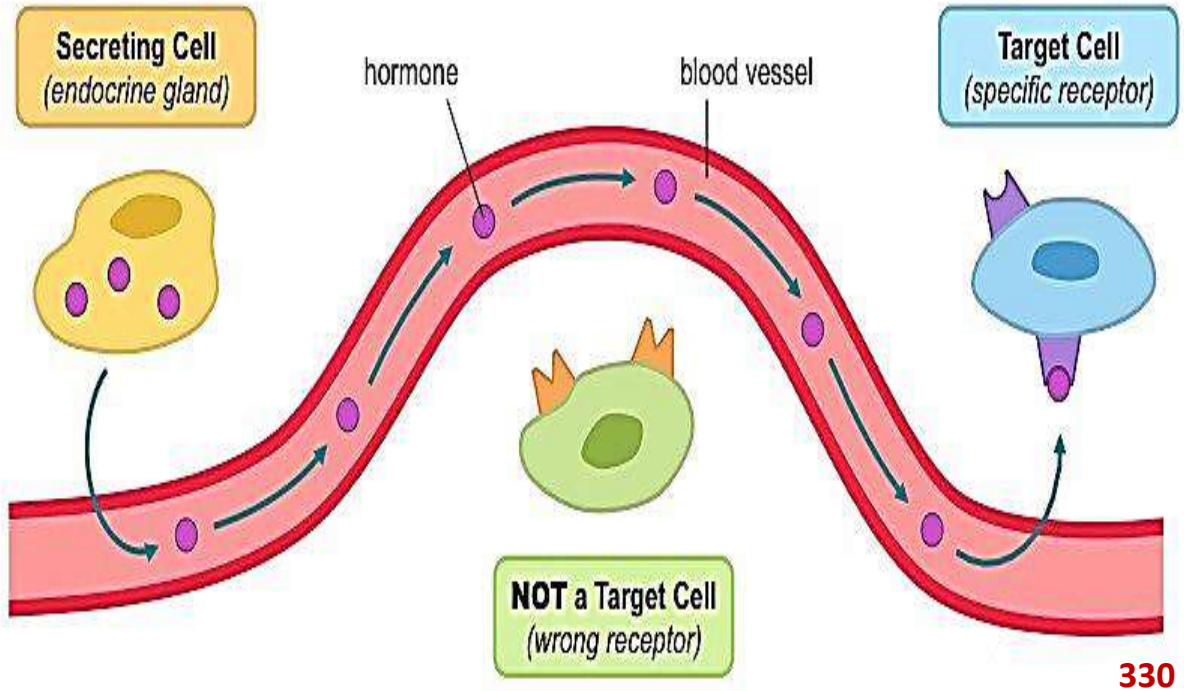
ACTH	Adrenocorticotropic hormone
ANF	Atrial natriuretic factor
cAMP	Cyclic adenosine monophosphate
CBG	Corticosteroid-binding globulin
CG	Chorionic gonadotropin
cGMP	Cyclic guanosine monophosphate
CLIP	Corticotropin-like intermediate lobe peptide
DBH	Dopamine β-hydroxylase
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DIT	Diiodotyrosine
DOC	Deoxycorticosterone
EGF	Epidermal growth factor
FSH	Follicle-stimulating hormone
GH	Growth hormone
IGF-I	Insulin-like growth factor-I
LH	Luteotropic hormone
LPH	Lipotropin
MIT	Monoiodotyrosine
MSH	Melanocyte-stimulating hormone
OHSD	Hydroxysteroid dehydrogenase
PNMT	Phenylethanolamine-*N*-methyltransferase
POMC	Pro-opiomelanocortin
SHBG	Sex hormone-binding globulin
StAR	Steroidogenic acute regulatory (protein)
TBG	Thyroxine-binding globulin
TEBG	Testosterone-estrogen-binding globulin
TRH	Thyrotropin-releasing hormone
TSH	Thyrotropin-stimulating hormone

Biomedical Importance

- The survival of multicellular organisms depends on their ability to adapt to a constantly changing environment.
- Intercellular communication mechanisms are necessary requirements for this adaptation.
- The **nervous system** and the **endocrine system** **provide this intercellular, organism-wide communication.**
- The nervous system was originally viewed as providing a fixed communication system, whereas the endocrine system supplied hormones, which are mobile messages.
- In fact, there is a remarkable convergence of these regulatory systems.
- For example, neural regulation of the endocrine system is important in the production and secretion of some hormones; many neurotransmitters resemble hormones in their synthesis, transport, and mechanism of action; and many hormones are synthesized in the nervous system.

Biomedical Importance

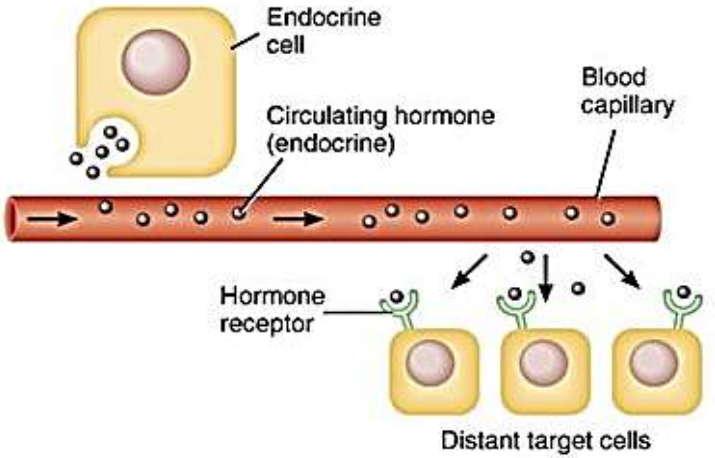
- The word "**hormone**" is derived from a Greek term that means to arouse to activity.
- As classically defined, a hormone is a substance that is synthesized in one organ and transported by the circulatory system to act on another tissue.
- However, this original description is too restrictive because hormones can act on adjacent cells (paracrine action) and on the cell in which they were synthesized (autocrine action) without entering the systemic circulation.
- A diverse array of hormones—each with distinctive mechanisms of action and properties of biosynthesis, storage, secretion, transport, and metabolism—has evolved to provide homeostatic responses.
- This biochemical diversity is the topic of this chapter.



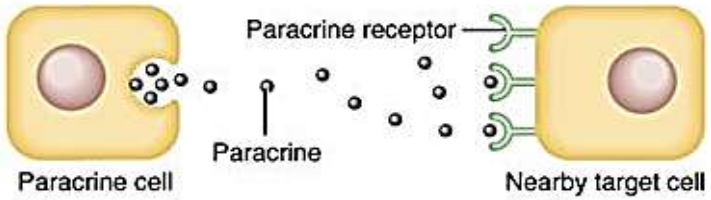
Hormones: Mechanisms of Signaling

hormone producing cell = **endocrine cell**
- e.g. thyroid, pituitary

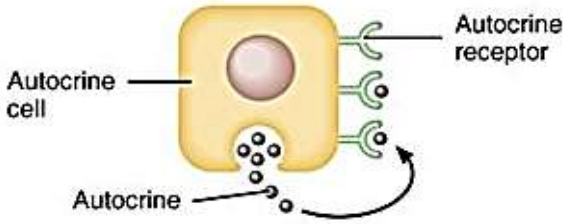
- **Endocrine signaling**
  - circulating hormones (**endocrine hormones**)
  - act on distant targets
  - travel in blood



- **Paracrine signaling**
  - local action
  - local hormone (**paracrine hormones**) act on neighboring cells
  - autocrines act on same cell that secreted them



- **Autocrine signaling**
  - cell responds to the hormone it produces



Classifications ofhormones

Hormones are classified according to:

1- *Chemical composition* (protein or others)

Type of Hormone		
Type of Compound	**Formed From**	**Examples**
Amines	Amino acids	Norepinephrine, epinephrine
Peptides	Amino acids	Antidiuretic hormone, oxytocin, thyrotropin-releasing hormone
Proteins	Amino acids	Parathyroid hormone, growth hormone, prolactin
Glycoproteins	Protein and carbohydrate	Follicle-stimulating hormone, luteinizing hormone, thyroid-stimulating hormone
Steroids	Cholesterol	Estrogen, testosterone, aldosterone, cortisol

2- *Solubility* (hydrophilic or lipophilic)

3- *Location* of receptors (intra- or extra-cellular)

4- Nature of *signal* used to mediate hormone action within cells

According to their classifications, hormones are classified into

Group I & Group II (according to mechanism of action)

Hormone: Classification

- **Category # 1. According to Chemical Nature:**
- **(a) Steroid Hormones:**
- These are made up of lipids, which basically derived from cholesterol, e.g. Testosterone, Estrogen, Progesterone etc.
- **(b) Amine Hormones:**
- These hormones are made up of amines. Amine hormone is derivative of the amino acid tyrosine.
- e.g. T_3, T_4, epinephrine, norepinephrine.
- **(c) Peptide Hormones:**
- These hormones are made up of few amino acid residues only and present as simple linear chain.
- e.g. Oxytocin and vasopressin both consist of only 9-amino acid residues only.
- **(d) Protein Hormones:**
- These hormones are also made amino acid residues which are much more in numbers. They represent primary, secondary and tertiary configuration.
- e.g. Insulin, glucagon, STH etc.
- **(e) Glycoprotein Hormones:**
- These hormones are glycoprotein in nature. They are conjugated protein where carbohydrate groups are mannose, galactose, fucose etc.
- e.g. LH, FSH, TSH etc.

Classification of Hormones by Mechanism of Action

I. *Hormones that bind to intracellular receptors*

- Androgens
- Calcitriol ($1,25[OH]_2$-D_3)
- Estrogens
- Glucocorticoids
- Mineralocorticoids
- Progestins
- Retinoic acid
- Thyroid hormones (T_3 and T_4)

II. *Hormones that bind to cell surface receptors*

A. The second messenger is cAMP

- α_2-Adrenergic catecholamines
- β-Adrenergic catecholamines
- Adrenocorticotropic hormone (ACTH)
- Antidiuretic hormone (vasopressin)
- Calcitonin
- Chorionic gonadotropin, human (CG)
- Corticotropin-releasing hormone
- Follicle-stimulating hormone (FSH)
- Glucagon
- Lipotropin (LPH)
- Luteinizing hormone (LH)
- Melanocyte-stimulating hormone (MSH)
- Parathyroid hormone (PTH)
- Somatostatin
- Thyroid-stimulating hormone (TSH)

B. The second messenger is cGMP

- Atrial natriuretic factor
- Nitric oxide

C. The second messenger is calcium or phosphatidylinositols (or both)

- Acetylcholine (muscarinic)
- α_1-Adrenergic catecholamines
- Angiotensin II
- Antidiuretic hormone (vasopressin)
- Cholecystokinin
- Gastrin
- Gonadotropin-releasing hormone
- Oxytocin
- Platelet-derived growth factor (PDGF)
- Substance P
- Thyrotropin-releasing hormone (TRH)

D. The second messenger is a kinase or phosphatase cascade

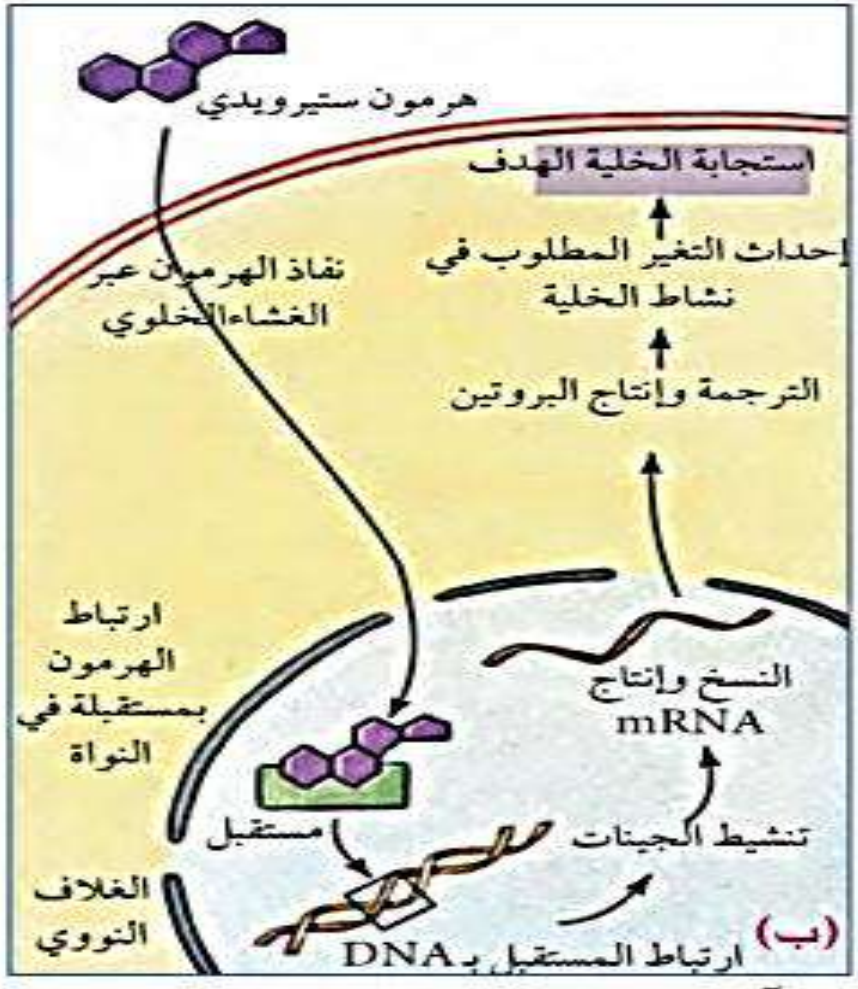
- Adiponectin
- Chorionic somatomammotropin
- Epidermal growth factor (EGF)
- Erythropoietin (EPO)
- Fibroblast growth factor (FGF)
- Growth hormone (GH)
- Insulin
- Insulin-like growth factors I and II
- Leptin
- Nerve growth factor (NGF)
- Platelet-derived growth factor
- Prolactin

General Features of Hormone Classes

	Group I	Group II
Types	Steroids, iodothyronines, calcitriol, retinoids	Polypeptides, proteins, glycoproteins, catecholamines
Solubility	Lipophilic	Hydrophilic
Transport proteins	Yes	No
Plasma half-life	Long (hours to days)	Short (minutes)
Receptor	Intracellular	Plasma membrane
Mediator	Receptor-hormone complex	cAMP, cGMP, Ca^{2+}, metabolites of complex phosphinositols, kinase cascades

Lipophilic hormones

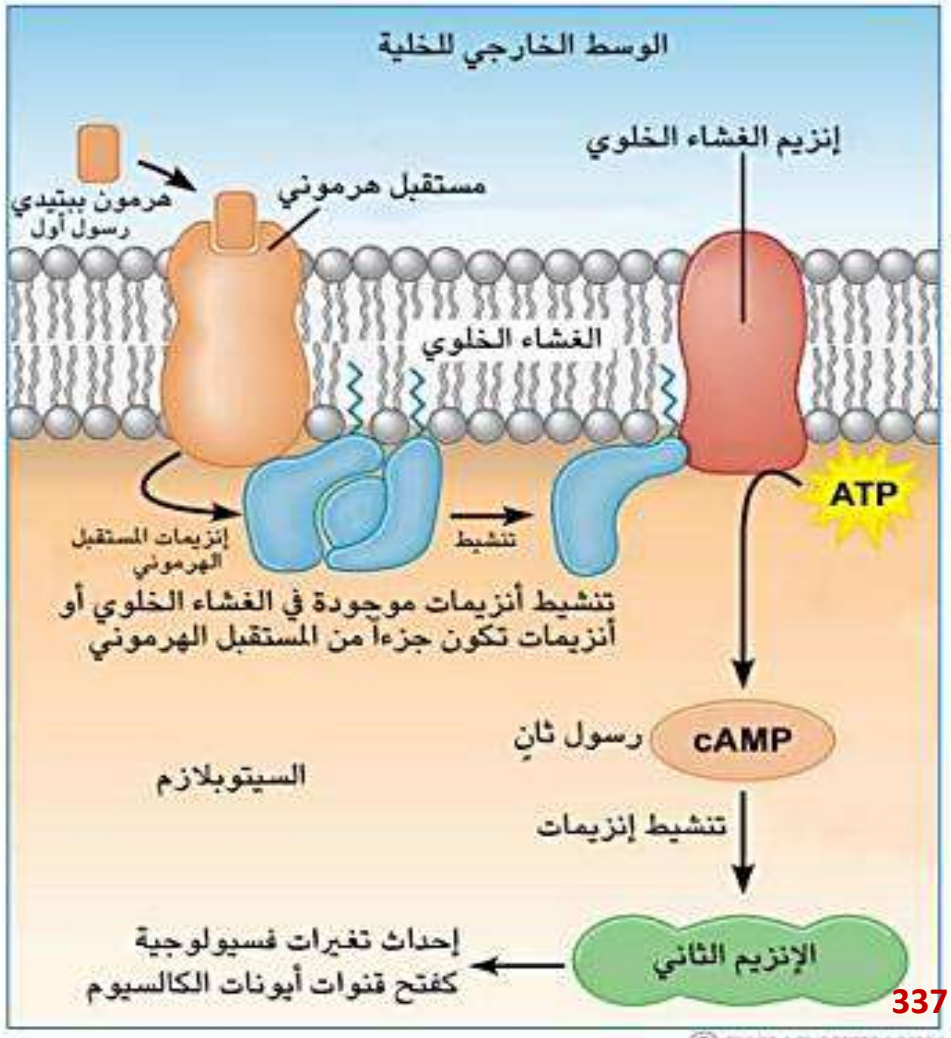
- After secretion, these hormones associate with plasma transport or carrier proteins, a process that circumvents the problem of solubility while prolonging the plasma half-life of the hormone.
- The relative percentages of bound and free hormone are determined by the amount, binding affinity, and binding capacity of the transport protein.
- The free hormone, which is the biologically active form, readily traverses the lipophilic plasma membrane of all cells and encounters receptors in either the cytosol or nucleus of target cells.
- The ligand-receptor complex is the intracellular messenger in this group.



آلية عمل الهرمونات الستيرويدية

water-soluble hormones

- The second major group consists of water-soluble hormones that bind to specific receptors spanning the plasma membrane of the target cell.
- Hormones that bind to these surface receptors of cells communicate with intracellular metabolic processes through intermediary molecules called second messengers (the hormone itself is the first messenger), which are generated as a consequence of the ligand-receptor interaction.
- The second messenger concept arose from an observation that epinephrine binds to the plasma membrane of certain cells and increases intracellular cAMP.
- This was followed by a series of experiments in which cAMP was found to mediate the effects of many hormones.



water-soluble hormones

- Hormones that employ this mechanism are shown in group II.A of Table 41–3. Atrial natriuretic factor (ANF) uses cGMP as its second messenger (group II.B).
- Several hormones, many of which were previously thought to affect cAMP, appear to use ionic calcium (Ca^{2+}) or metabolites of complex phosphoinositides (or both) as the intracellular second messenger signal.
- These are shown in group II.C of the table.
- The intracellular messenger for group II.D is a protein kinase-phosphatase cascades; several have been identified, and a given hormone may use more than one kinase cascade.
- A few hormones fit into more than one category, and assignments change as new information is discovered.

Endocrine Glands and Their Hormones

Pineal gland : Melatonin

Hypothalamus : ADH, Oxytocin

Pituitary : LH, FSH, Prolactin,GH, TSH, Oxytocin, ADH, ACTH

Thyroid gland : T3,T4, Calcitonin

Parathyroid gland : Parathyroid hormone

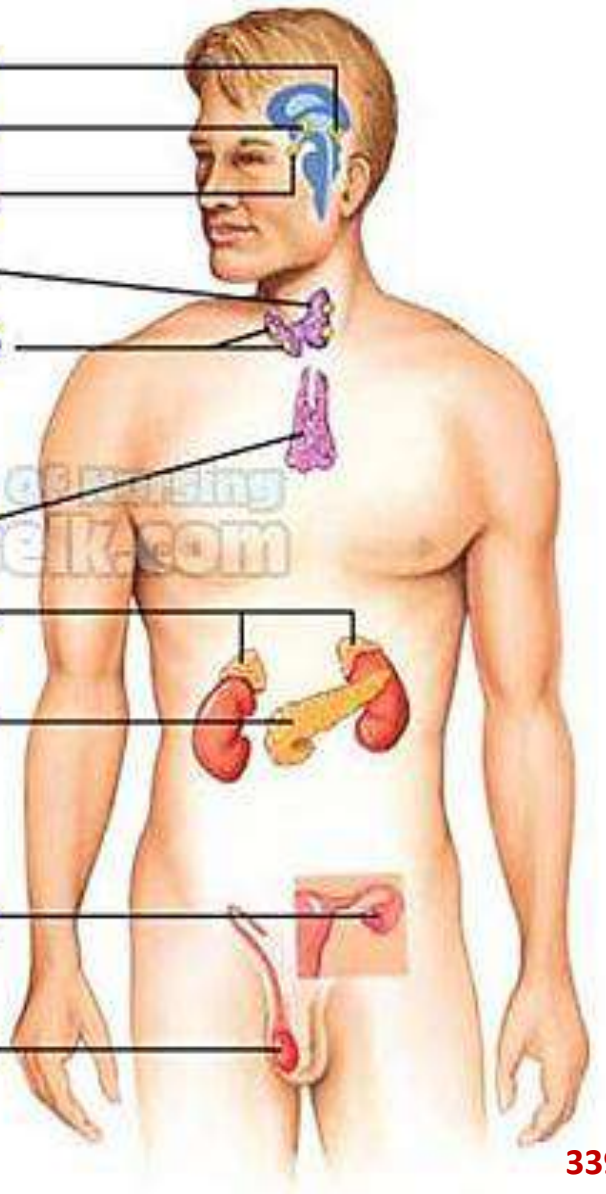
Thymus : Thymosin

Adrenal glands : Adrenaline, Noradrenaline, Cortisol, Aldosteron

Pancreas : Insulin, Glucagon, Somatostatin

Ovaries (females) : Oestrogen, Progesterone

Testes (males only) : Testosterone



The Diversity of the Endocrine System

Endocrine Gland	Hormone	Hormonal Function
Posterior Pituitary Lobe	Antidiuretic hormone (ADH)	Increases water reabsorption by the kidneys
	Oxytocin (OT)	Stimulates uterine contractions
Anterior Pituitary Lobe	Growth hormone (GH)	Stimulates cell division and growth
	Prolactin (PRL)	Initiates milk synthesis
	Follicle Stimulating hormone (FSH)	Stimulates gamete production and sex hormone regulation
	Luteinizing hormone (LH)	Stimulates secretion of sex hormones and ovulation
	Thyroid Stimulating hormone (TSH)	Stimulates secretions of the thyroid gland
	Adrenocorticotropic hormone (ACTH)	Stimulates secretions of the adrenal cortex
Pineal Gland	Melatonin	Involved in the sleep/wake cycles
Thymus Gland	Thymosin	Stimulates increased production of T-cells
Thyroid Gland	Triiodothyronine (T3)	Increases metabolic rate
	Thyroxine (T4)	Increases metabolic rate
	Calcitonin	Stimulates osteoblast to remove calcium from the blood; lowering blood calcium levels
Parathyroid Gland	Parathyroid hormone (PTH)	Stimulates osteoclast to release stored calcium into the blood; raising blood calcium levels
Adrenal Cortex	Cortisol	Maintains blood sugar levels between meals
	Aldosterone	Increases sodium and water reabsorption by the kidneys
Adrenal Medulla	Epinephrine/Norepinephrine	Initiates response similar to the sympathetic nervous system
Pancreas	Glucagon	Stimulates an increase in blood sugar levels
	Insulin	Stimulates a decrease in blood sugar levels
Ovaries	Estrogen	Stimulates the development of the female reproductive system
	Progesterone	Stimulates the development of the female reproductive system
Testes	Testosterone	Stimulates the development of the male reproductive system

Biomedical Importance

- In addition to **water**, the **diet** must provide metabolic fuels (mainly **carbohydrates** and **lipids**), **protein** (for growth and turnover of tissue proteins, as well as a source of metabolic fuel), **fiber** (for bulk in the intestinal lumen), **minerals** (containing elements with specific metabolic functions), and **vitamins** and **essential fatty acids** (organic compounds needed in smaller amounts for other metabolic and physiologic functions).

- The **polysaccharides**, **triacylglycerols**, and **proteins** that make up the bulk of the **diet must be hydrolyzed to their constituent monosaccharides**, **fatty acids**, and **amino acids**, respectively, **before absorption and utilization**.

- **Minerals** and **vitamins must be released from the complex matrix of food before they can be absorbed and utilized**

- Globally, **undernutrition** is widespread, leading to **impaired growth**, **defective immune system**, and **reduced work capacity**.

- By contrast, **in developed countries, and increasingly in developing countries**, there is **excessive food consumption** (**especially of fat**), leading to **obesity**, and the **development of diabetes**, **cardiovascular disease**, and **some cancers**.

Biomedical Importance

- **Worldwide, there are more overweight and obese people than undernourished people.**
- Deficiencies of vitamin A, iron, and iodine pose major health concerns in many countries, and deficiencies of other vitamins and minerals are a major cause of ill health.
- In **developed countries nutrient deficiency** is **rare**, although there are vulnerable sections of the population at risk.
- **Intakes of minerals and vitamins** that are **adequate to prevent deficiency may be inadequate to promote optimum health and longevity**.
- **Excessive secretion of gastric acid**, associated with **Helicobacter pylori infection**, can result in the development of **gastric and duodenal ulcers / قرحة معدية**; small changes in the composition of bile can result in crystallization of cholesterol as **gallstones / حصى المرارة**; failure of exocrine pancreatic secretion (as in **cystic fibrosis / تليف كيسي**) leads to **undernutrition** and **steatorrhea (اسهال/ دهني)**.
- **Lactose intolerance** is the result of **lactase deficiency**, leading to **diarrhea / اسهال** and intestinal discomfort when lactose is consumed.
- **Absorption of intact peptides** that **stimulate antibody responses** causes **allergic reactions**; **celiac disease / (اضطرابات هضمية)** is an allergic reaction to **wheat gluten**.

Digestion & Absorption Of Carbohydrates

- The **digestion of carbohydrates** is by **hydrolysis to liberate oligosaccharides**, **then free mono- and disaccharides**.
- The **increase in blood glucose** after a **test dose of a carbohydrate compared with that after an equivalent amount of glucose** (as glucose or from a reference starchy food) is **known as the glycemic index**.
- **Glucose** and **galactose** have an **index of 1** (or **100%**), as do **lactose**, **maltose**, **isomaltose**, and **trehalose**, which **give rise to these monosaccharides on hydrolysis**.
- **Fructose** and the **sugar alcohols** are **absorbed less rapidly** and **have a lower glycemic index**, as does **sucrose**.
- The **glycemic index of starch** varies between near 1 (or **100%**) and near **0** as a result of variable rates of hydrolysis, and that of **nonstarch polysaccharides** is 0.
- **Foods** that have a **low glycemic index** are considered to be more beneficial since they cause **less fluctuation in insulin secretion**.
- Resistant starch and nonstarch polysaccharides provide substrates for bacterial fermentation in the large intestine, and the resultant butyrate and other short chain fatty

SUMMARY

1) Ingested nucleic acids are degraded to purines and pyrimidines. Purines and pyrimidines are formed from amphibolic intermediates and thus are dietarily nonessential.
2) Several reactions of IMP biosynthesis require folate derivatives and glutamine. Consequently, antifolate drugs and glutamine analogs inhibit purine biosynthesis.
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