SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEEING
DEPARTMENT OF BIOTECHNOLOGY

UNIT I –INTERMEDIARY METABOLISM - SBB 1202

Introduction

Thermodynamics can be defined as the study of energy, energy transformations and its relation to matter. The analysis of thermal systems is achieved through the application of the governing equations. The branch of physical chemistry known as thermodynamics is concerned with the study of the transformations of energy. That concern might seem remote from chemistry, let alone biology; indeed, thermodynamics was originally formulated by physicists and engineers interested in the efficiency of steam engines.

However, thermodynamics has proved to be of immense importance in both chemistry and biology. Not only does it deal with the energy output of chemical reactions but it also helps to answer questions that lie right at the heart of biochemistry, such as how energy flows in biological cells and how large molecules assemble into complex structures like the cell.

Bioenergetics, or biochemical thermodynamics, is the study of the energy changes accompanying biochemical reactions. Biologic systems are essentially **isothermic** and use chemical energy to power living processes. How an animal obtains suitable fuel from its food to provide this energy is basic to the understanding of normal nutrition and metabolism.

Gibbs free energy:

Gibbs change in free energy (ΔG) is that portion of the total energy change in a system that is available for doing work—that is, the useful energy, also known as the chemical potential. 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:

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

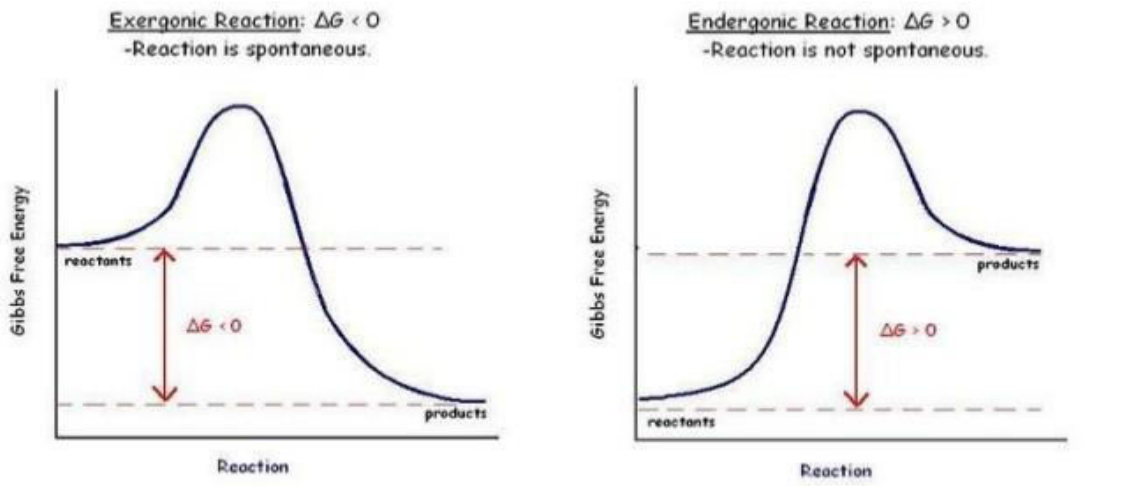
where ΔH is the change in **enthalpy** (heat) and T is the absolute temperature. In biochemical reactions, because ΔH is approximately equal to ΔE, the total change in internal energy of the reaction, the above relationship may be expressed in the following way:

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

GIBBS FREE ENERGY

A reaction will spontaneously occur if ΔG<0 (exergonic reaction)

A reaction will NOT spontaneously occur if ΔG>0 (endergonic reaction)



$\Delta_r H^\ominus$	$\Delta_r S^\ominus$	$\Delta_r G^\ominus$	**Description**
−	+	− (at all T)	Reaction spontaneous at all temperatures
−	−	− (at low T)	Reaction spontaneous at low temperature
−	−	+ (at high T)	Reaction non-spontaneous at high temperature
+	+	+ (at low T)	Reaction non-spontaneous at low temperature
+	+	− (at high T)	Reaction spontaneous at high temperature
+	−	+ (at all T)	Reaction non-spontaneous at all temperatures

Entropy and enthalpy:

The enthalpy of a reaction is a measure of how much heat is absorbed or given off when a chemical reaction takes place. It is represented by ΔH of a reaction and is found by subtracting the enthalpy of the reactants from the enthalpy of the products:

ΔHreaction = ΣΔH products - ΣΔH reactants

The Greek letter Σ, in mathematics, it is used to represent the phrase "to sum." Therefore, this equation is telling us to sum the enthalpy of the products and subtract the sum of the enthalpy of the reactants.

When the enthalpy of reaction is calculated, a negative value indicates the reaction is exothermic. A positive value indicates the reaction is endothermic.

ENTROPY

The entropy change for a reaction, or $S_{reaction}$, is a measure of the dispersal of energy and matter that takes place during a reaction. As far as identifying an increase in dispersal of matter, there are two things that indicate an increase in entropy:

- Have more total moles of products than total moles of reactants.
- Have products that are in states of matter that exhibit high amounts of freedom for their particles, namely gases and aqueous compounds.

The entropy of a reaction can be calculated using a formula similar to the enthalpy of reaction:

ΔSreaction = ΣΔSproducts - ΔΣSreactants

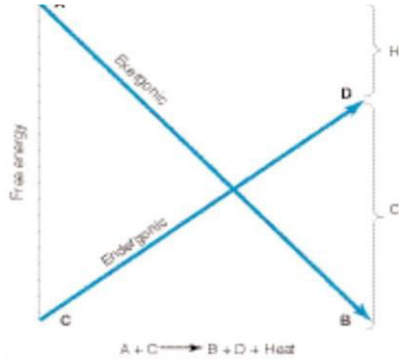
Endergonic and exergonic processes:

If ΔG is negative, the reaction proceeds spontaneously with loss of free energy; that is it is **exergonic.** If, in addition, ΔG is of great magnitude, the reaction goes virtually to completion and is essentially irreversible.

On the other hand, if ΔG is positive, the reaction proceeds only if free energy can be gained; that is, it is **endergonic.** If, in addition, the magnitude of ΔG is great, the system is stable, with little or no tendency for a reaction to occur.

If ΔG is zero, the system is at equilibrium and no net change takes place.

The vital processes—eg, synthetic reactions, muscular contraction, nerve impulse conduction, and active transport—obtain energy by chemical linkage, or **coupling,** to oxidative reactions. In its simplest form, this type of coupling can be represented as follows;



The conversion of metabolite A to metabolite B occurs with release of free energy. It is coupled to another reaction, in which free energy is required to convert metabolite C to metabolite D. The terms **exergonic** and **endergonic** rather than the normal chemical terms "exothermic" and "endothermic" are used to indicate that a process is accompanied by loss or gain, respectively, of free energy in any form, not necessarily as heat.

In practice, an endergonic process cannot exist independently but must be a component of a coupled exergonic-endergonic system where the overall net change is exergonic. The exergonic reactions are termed **catabolism** (generally, the breakdown or oxidation of fuel molecules), whereas the synthetic reactions that build up substances are termed **anabolism.** The combined catabolic and anabolic processes constitute **metabolism.**

If the reaction shown in figure is to go from left to right, then the overall process must be accompanied by loss of free energy as heat. One possible mechanism of coupling could be envisaged if a common obligatory intermediate (I) took part in both reactions, that is,

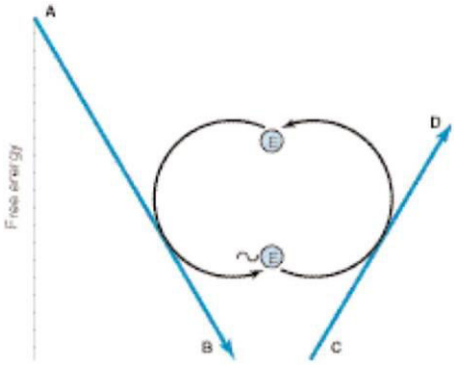
$$A + C \rightarrow I \rightarrow B + D$$

Some exergonic and endergonic reactions in biologic systems are coupled in this way. This type of system has a built-in mechanism for biologic control of the rate of oxidative processes

since the common obligatory intermediate allows the rate of utilization of the product of the synthetic path (D) to determine by mass action the rate at which A is oxidized.

Indeed, these relationships supply a basis for the concept of **respiratory control,** the process that prevents an organism from burning out of control. An extension of the coupling concept is provided by dehydrogenation reactions, which are coupled to hydrogenations by an intermediate carrier.

An alternative method of coupling an exergonic to an endergonic process is to synthesize a compound of high-energy potential in the exergonic reaction and to incorporate this new compound into the endergonic reaction, thus effecting a transference of free energy from the exergonic to the endergonic pathway.



The biological advantage of this mechanism is that the compound of high potential energy, ΔE, unlike in the previous system, need not be structurally related to A, B, C, or D, allowing ~E to serve as a transducer of energy from a wide range of exergonic reactions to an equally wide range of endergonic reactions or processes, such as biosyntheses, muscular contraction, nervous excitation, and active transport. In the living cell, the principal high-energy intermediate or carrier compound (designated ~E) is **adenosine triphosphate (ATP).**

Reversible irreversible reactions:

It was believed that all chemical reactions were irreversible until 1803, when French chemist Claude Louis Berthollet introduced the concept of reversible reactions. Initially he observed that sodium carbonate and calcium chloride react to yield calcium carbonate and sodium

chloride; however, after observing sodium carbonate formation around the edges of salt lakes, he realized that large amount of salts in the evaporating water reacted with calcium carbonate to form sodium carbonate, indicating that the reverse reaction was occurring.

Chemical reactions are represented by chemical equations. These equations typically have a unidirectional arrow (right arrow) to represent irreversible reactions. Other chemical equations may have a bidirectional harpoons (right left harpoons) that represent reversible reactions (not to be confused with the double arrows (left right arrow) used to indicate resonance structures).

Irreversible Reactions

A fundamental concept of chemistry is that chemical reactions occurred when reactants reacted with each other to form products. These unidirectional reactions are known as irreversible reactions, reactions in which the reactants convert to products and where the products cannot convert back to the reactants. These reactions are essentially like baking. The ingredients, acting as the reactants, are mixed and baked together to form a cake, which acts as the product. This cake cannot be converted back to the reactants (the eggs, flour, etc.), just as the products in an irreversible reaction cannot convert back into the reactants.

An example of an irreversible reaction is combustion. Combustion involves burning an organic compound—such as a hydrocarbon—and oxygen to produce carbon dioxide and water. Because water and carbon dioxide are stable, they do not react with each other to form the reactants. Combustion reactions take the following form:

$$[\ C_xH_y + O_2 \rightarrow CO_2 + H_2O\ ]$$

Reversible Reactions

In reversible reactions, the reactants and products are never fully consumed; they are each constantly reacting and being produced. A reversible reaction can take the following summarized form:

$$A + B \rightleftharpoons C + D$$

This reversible reaction can be broken into two reactions.

Reaction 1:

$A + B \rightarrow C+D$

Reaction 2:

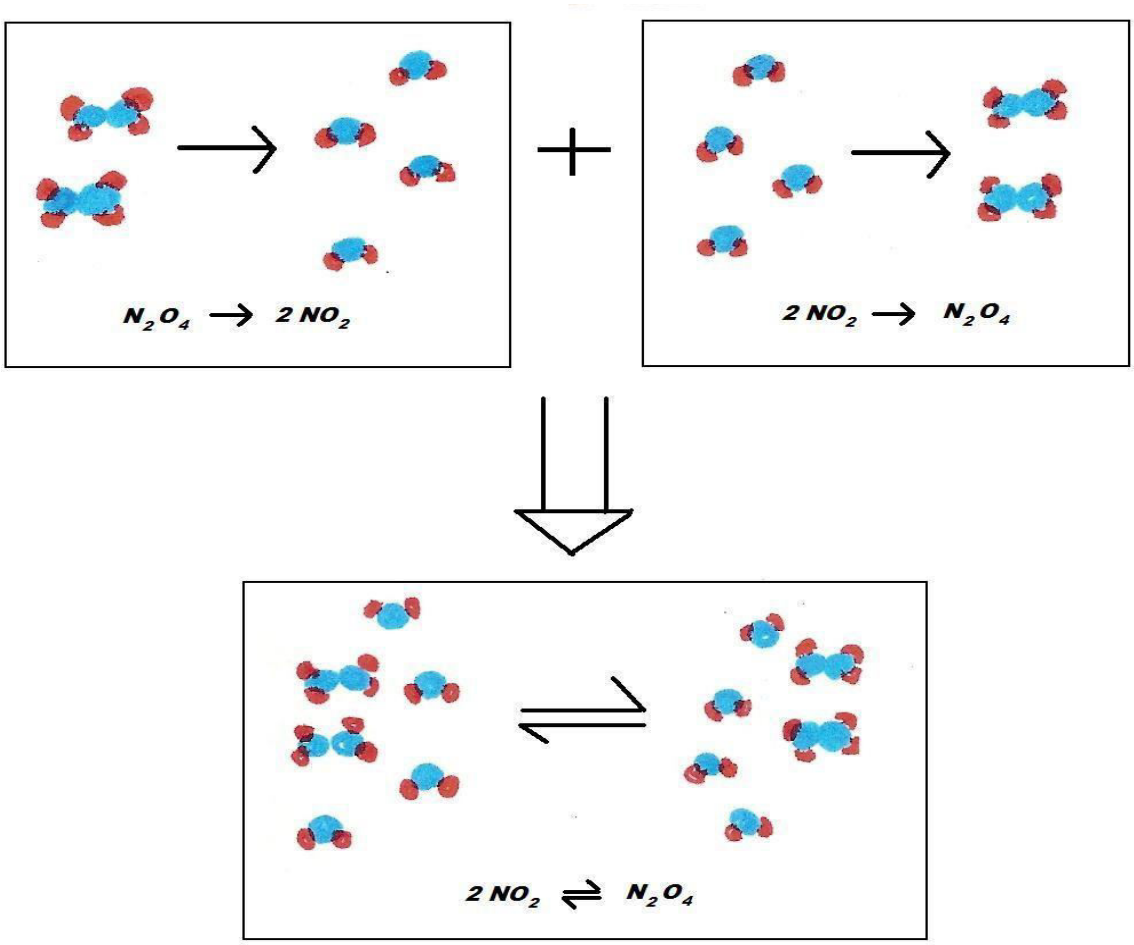
$C + D \rightarrow A+B$

These two reactions are occurring simultaneously, which means that the reactants are reacting to yield the products, as the products are reacting to produce the reactants. Collisions of the reacting molecules cause chemical reactions in a closed system. After products are formed, the bonds between these products are broken when the molecules collide with each other, producing sufficient energy needed to break the bonds of the product and reactant molecules.

Below is an example of the summarized form of a reversible reaction and a breakdown of the reversible reaction

$N_2O_4 \rightleftharpoons 2NO_2$

Reaction 1 and Reaction 2 happen at the same time because they are in a closed system. (Blue: Nitrogen Red: Oxygen)



Unlike irreversible reactions, reversible reactions lead to equilibrium: in reversible reactions, the reaction proceeds in both directions whereas in irreversible reactions the reaction proceeds in only one direction.

If the reactants are formed at the same rate as the products, a dynamic equilibrium exists. For example, if a water tank is being filled with water at the same rate as water is leaving the tank, the amount of water remaining in the tank remains constant.

Connection to Biology

There are four binding sites on a hemoglobin protein. Hemoglobin molecules can either bind to carbon dioxide or oxygen. As blood travels through the alveoli of the lungs, hemoglobin

molecules pick up oxygen-rich molecules and bind to the oxygen. As the hemoglobin travels through the rest of the body, it drops off oxygen at the capillaries for the organ system to use oxygen.

After expelling the oxygen, it picks up carbon dioxide. Because this process is constantly carried out through the body, there are always hemoglobin molecules picking or expelling oxygen and other hemoglobin molecules that are picking up or expelling carbon dioxide. Therefore, the hemoglobin molecules, oxygen, and carbon dioxide are reactants while the hemoglobin molecules with oxygen or carbon dioxide bound to them are the products. In this closed system, some reactants convert into products as some products are changing into reactants, making it similar to a reversible reaction.

Reversible and irreversible reactions

It is a common observation that most of the reactions when carried out in closed vessels do not go to completion, under a given set of conditions of temperature and pressure. In fact in all such cases, in the initial state, only the reactants are present but as the reaction proceeds, the concentration of reactants decreases and that of products increases. Finally a stage is reached when no further change in concentration of the reactants and products is observed.

This state at which the concentration of reactants and products do not change with time is called a state of chemical equilibrium. The amount of reactants unused depend on the experimental conditions such as concentration, temperature, pressure and the nature of the reaction.

If a mixture of gaseous hydrogen and iodine vapours is made to react at 717K in a closed vessel for about 2 - 3 hours, gaseous hydrogen iodide is produced according to the following equation:

$$H_{2(g)} + I_{2(g)} \longrightarrow 2HI_{(g)}$$

But along with gaseous hydrogen iodide, there will be some amount of unreacted gaseous hydrogen and gaseous iodine left.

On the other hand if gaseous hydrogen iodide is kept at 717K in a closed vessel for about 2 - 3 hours it decomposes to give gaseous hydrogen and gaseous iodine.

$$2HI_{(g)} \longrightarrow H_{2(g)} + I_{2(g)}$$

In this case also some amount of gaseous hydrogen iodide will be left unreacted.

This means that the products of certain reactions can be converted back to the reactants. These types of reactions are called reversible reactions. Thus, in reversible reactions the products can react with one another under suitable conditions to give back the reactants.

In other words, in reversible reactions the reaction takes place in both the forward and backward directions. The reversible reaction may be expressed as:

$$H_{2(g)} + I_{2(g)} \Leftrightarrow 2HI_{(g)}$$

These reversible reactions never go to completion if performed in a closed container. For a reversible chemical reaction, an equilibrium state is attained when the rate at which a chemical reaction is proceeding in forward direction equals the rate at which the reverse reaction is proceeding.

At equilibrium,

Rate of forward reaction = Rate of reverse reaction

Consider the reversible reaction

$$N_2(g) + 3H_2(g) \Leftrightarrow 2NH_3(g)$$

When this reaction is performed at high pressure and temperature in a close container, at equilibrium,

Rate of formation of ammonia = Rate of decomposition of ammonia

Now, the question arises whether all the ammonia molecules are remaining intact and not decomposing? Are all the molecules of nitrogen and hydrogen becoming inactive and not combining? If this is the case, we would say a static equilibrium is attained.

To understand the concept of static equilibrium, let us consider two children sitting on a see-saw. At balance point (i.e., the equilibrium position) no movement of children on the see-saw occurs.

Static Equilibrium

In the case of reversible reaction, however a static equilibrium is not being established.

In the case of ammonia, using deuterium, D (an isotope of hydrogen) it has been proved that even at equilibrium, decomposition of ammonia into hydrogen and nitrogen and combination of hydrogen and nitrogen into ammonia continues. This equilibrium is dynamic in nature and is therefore called dynamic equilibrium.

A dynamic steady state can be compared with the equilibrium of water in a reservoir, which is being simultaneously filled and discharged. If the rate of water flowing in is equal to the rate of water flowing out, the quantity of water in the reservoir will remain unchanged like the quantities of substances in a state of chemical equilibrium.

Dynamic Equilibrium

Rate of water entering = Rate of water leaving

Hence the level of water is constant.

Similarly, some other reversible reactions are:

$$N_{2(g)} + O_{2(g)} \Leftrightarrow 2NO_{(g)}$$

$$2SO_{2(g)} + O_{2(g)} \Leftrightarrow 2SO_{3(g)}$$

On the other hand, the chemical reaction in which the products formed do not combine to give the reactants are known as irreversible reactions.

For e.g., potassium chlorate decomposes on heating to form potassium chloride and oxygen.

$$2KClO_{3(s)} \xrightarrow{\Delta} 2KCl_{(s)} + 3O_{2(g)}$$

However the products cannot combine to form potassium chlorate. In case of irreversible physical and chemical processes, the change occurs only in one direction and the processes go to completion. However, the reversible processes do not go to completion and appear to stop (attain state of chemical equilibrium) even though some starting materials are remaining.

Some examples of irreversible reactions are:

$$AgNO_{3(aq)} + NaCl_{(aq)} \longrightarrow AgCl_{(s)} + NaNO_{3(aq)}$$

$$2Mg_{(s)} + O_{2(g)} \longrightarrow 2MgO_{(s)}$$

It may be noted that for reversible reactions the symbol  is used between the reactants and products. For the irreversible reactions, single headed arrow $\longrightarrow$ is used.

Chemical equilibrium:

Principles of Chemical Equilibrium

Chemical equilibrium is a state in which the rate of the forward reaction equals the rate of the backward reaction. In other words, there is no net change in concentrations of reactants and products. This kind of equilibrium is also called dynamic equilibrium.

Introduction

Given the following elementary reaction:

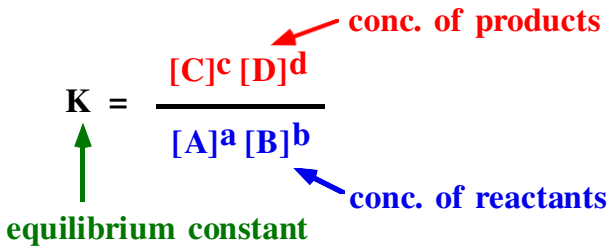
$$aA + bB \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} cC + dD$$

where k_1 and k_{-1} are the reaction constants for the forward and reverse reactions, respectively. Note: the constant k can vary among different reactions at different temperatures.

When the rate is zero, the net concentrations of A, B, C, and D are in equilibrium with each other. Thus, if there is a change in the system due to changes in concentration, temperature, or pressure, the equilibrium will shift to offset the change and re-establish equilibrium according to Le Chatelier's principle.

Le Chatelier's principle indicates whether more reactants or more products will be made. Note that the presence of a catalyst does not shift the equilibrium; it only causes the reaction to reach equilibrium faster. This is due to the fact that catalysts only lower activation energies.

Dynamic equilibrium is useful in predicting whether the forward or reverse reaction is spontaneous or nonspontaneous. To explain how this works, three quantities must be introduced: the equilibrium constant, K; the reaction quotient, Q; and the Gibbs Free Energy for a certain reaction, dG_r. The equations for K and Q are given below:



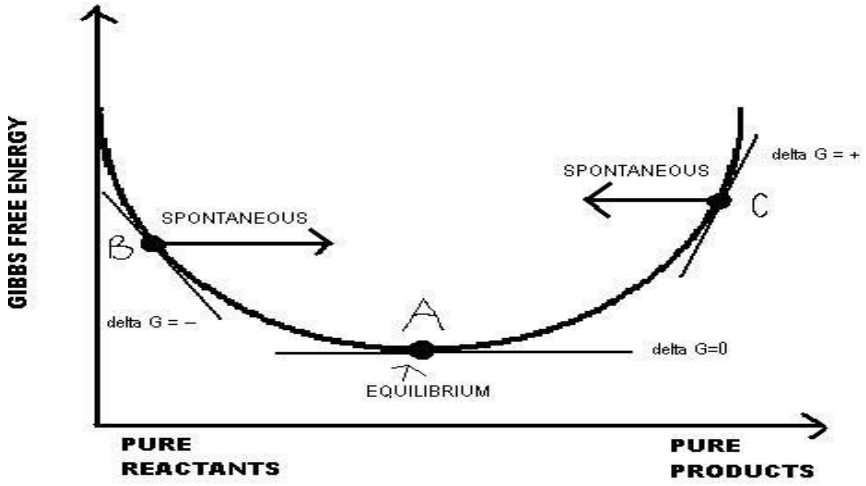
The Gibbs free energy of reaction, ΔG_r, relates to Q and T (temperature) in the following equation:

$$\Delta G = \Delta G^{\circ} + RT \ln Q$$

where ΔG_r^{o} is the Gibbs free energy for the reaction at standard conditions.

When ΔG_r is negative, the forward reaction is spontaneous and the reverse reaction nonspontaneous. When ΔG_r is positive, the forward reactions is nonspontaneous and the reverse reaction spontaneous. This is due to Q's deviation from K and Le Chatelier's principle

at standard temperature. When Q is larger than K, more products are present initially than at equilibrium, according to Le Chatelier's principle, the equilibrium shifts toward the reactants. In other words, the reverse reaction is spontaneous, which corresponds to a positive ΔG_r value. When Q is smaller than K, more reactants are present initially than at equilibrium, the reaction shifts towards the products, thus the forward reaction is spontaneous. This corresponds to a negative ΔG_r value. The relationship between Q and ΔG_r can be represented by the following graph:



At point A, the system is in equilibrium; therefore, Q=K and $\Delta G_r=0$. At point B, Q<K; the forward reaction is therefore spontaneous in order to reach equilibrium, and $\Delta G_r<0$. At point C, Q>K, and thus the reverse reaction is spontaneous in order to reach equilibrium; $\Delta G_r>0$.

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEEING

DEPARTMENT OF BIOTECHNOLOGY

UNIT II –INTERMEDIARY METABOLISM - SBB 1202

In cells, chemical reactions rarely occur in isolation, but rather are organized into multistep sequences called pathways, such as that of glycolysis. In a pathway, the product of one reaction serves as the substrate of the subsequent reaction. Different pathways can also intersect, forming an integrated and purposeful network of chemical reactions. These are collectively called metabolism, which is the sum of all the chemical changes occurring in a cell, a tissue, or the body.

Most pathways can be classified as either catabolic (degradative) or anabolic (synthetic). Catabolic reactions break down complex molecules, such as proteins, polysaccharides, and lipids, to a few simple molecules, for example, CO2, NH3 (ammonia), and water. Anabolic pathways form complex end products from simple precursors, for example, the synthesis of the polysaccharide, glycogen, from glucose. Pathways that regenerate a component are called cycles.

Glycolysis

The conversion of glucose to pyruvate occurs in two stages. The first five reactions of glycolysis correspond to an energy investment phase in which the phosphorylated forms of intermediates are synthesized at the expense of ATP. The subsequent reactions of glycolysis constitute an energy generation phase in which a net of two molecules of ATP are formed by substrate-level phosphorylation per glucose molecule metabolized.

Glycolysis involves ten individual steps, including three isomerizations and four phosphate transfers. The only redox reaction takes place in step [6].

[1] Glucose, which is taken up by animal cells from the blood and other sources, is first phosphorylated to glucose 6-phosphate, with ATP being consumed. The glucose 6-phosphate is not capable of leaving the cell.

[2] In the next step, glucose 6-phosphate is isomerized into fructose 6-phosphate.

[3] Using ATP again, another phosphorylation takes place, giving rise to fructose 1,6- bisphosphate. Phosphofructokinase is the most important key enzyme in glycolysis.

[4] Fructose 1,6-bisphosphate is broken down by aldolase into the C3 compounds glyceraldehydes 3-phosphate (also known as glyceral 3-phosphate) and glycerone 3- phosphate (dihydroxyacetone 3-phosphate).

[5] The latter two products are placed in fast equilibrium by triosephosphate isomerase.

[6] Glyceraldehyde 3-phosphate is now oxidized by glyceraldehyde-3-phosphate dehydrogenase, with NADH + H+ being formed.

In this reaction, inorganic phosphate is taken up into the molecule (substrate-level phosphorylation and 1,3-bisphosphoglycerate is produced. This intermediate contains a mixed acid–anhydride bond, the phosphate part of which is at a high chemical potential.

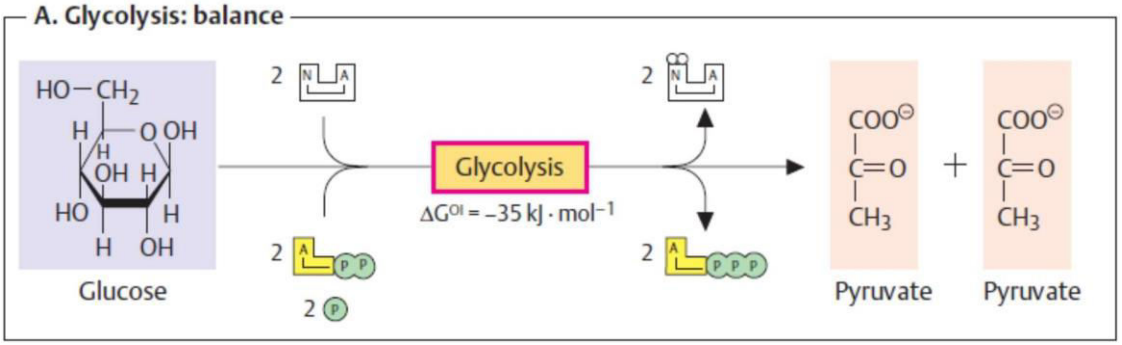
[7] Catalyzed by phosphoglycerate kinase, this phosphate residue is transferred to ADP, producing 3-phosphoglycerate and ATP. The ATP balance is thus once again in equilibrium.

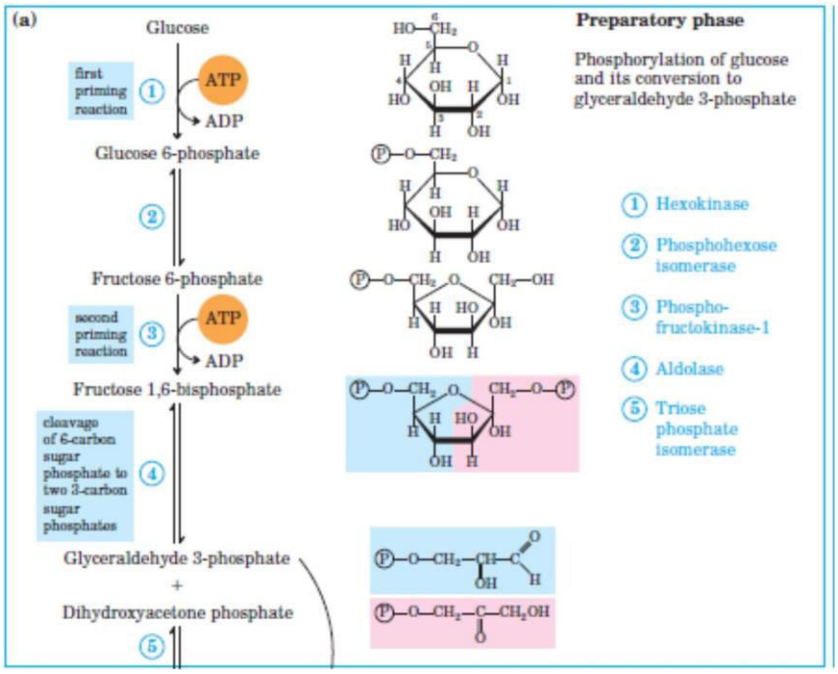
[8] As a result of shifting of the remaining phosphate residue within the molecule, the isomer 2-phosphoglycerate is formed.

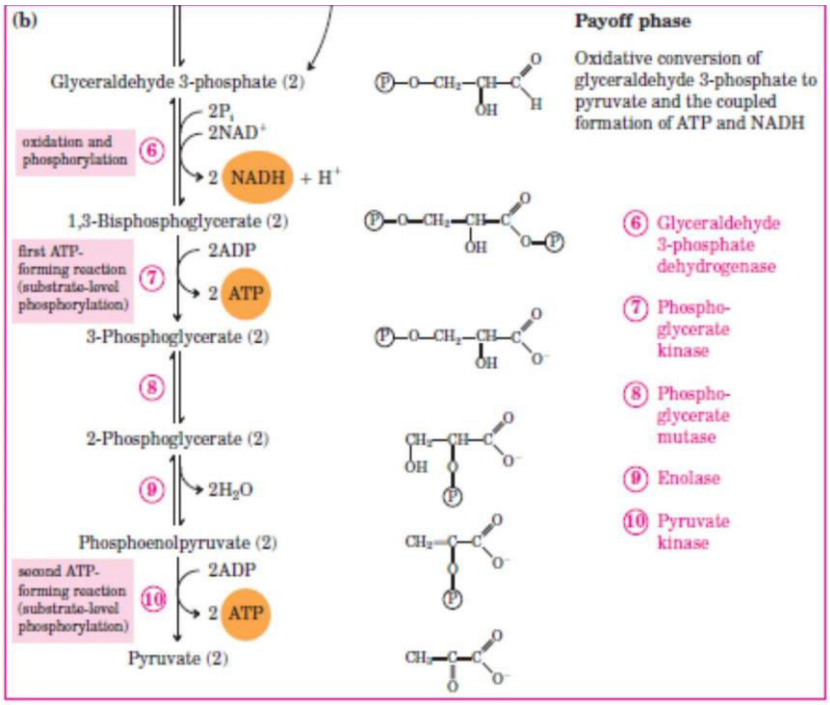
[9] Elimination of water from 2-phosphoglycerate produces the phosphate ester of the enol form of pyruvate—phosphoenolpyruvate (PEP). This reaction also raises the second phosphate residue to a high potential.

[10] In the last step, pyruvate kinase transfers this residue to ADP. The remaining enol pyruvate is immediately rearranged into pyruvate, which is much more stable. Along with step [7] and the thiokinase reaction in the tricarboxylic acid cycle, the pyruvate kinase reaction is one of the three reactions in animal metabolism that are able to produce ATP independently of the respiratory chain.

In glycolysis, two molecules of ATP are initially used for activation ([1], [3]). Later, two ATPs are formed per C3 fragment. Overall, therefore, there is a small net gain of 2 mol ATP per mol of glucose.





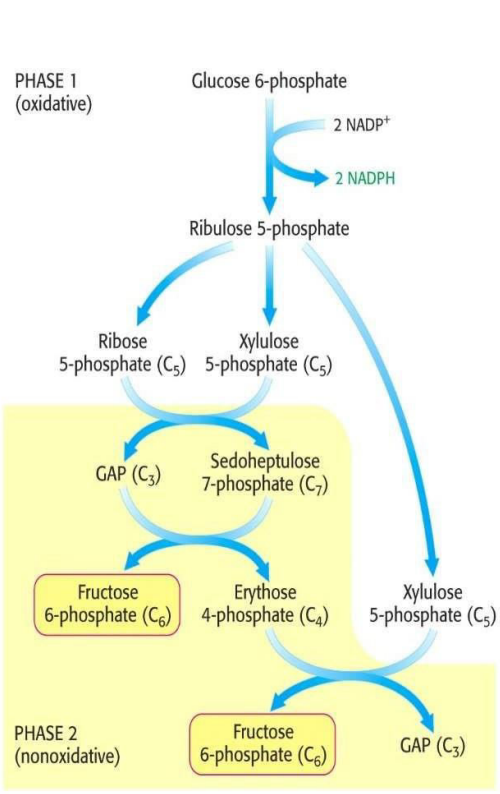


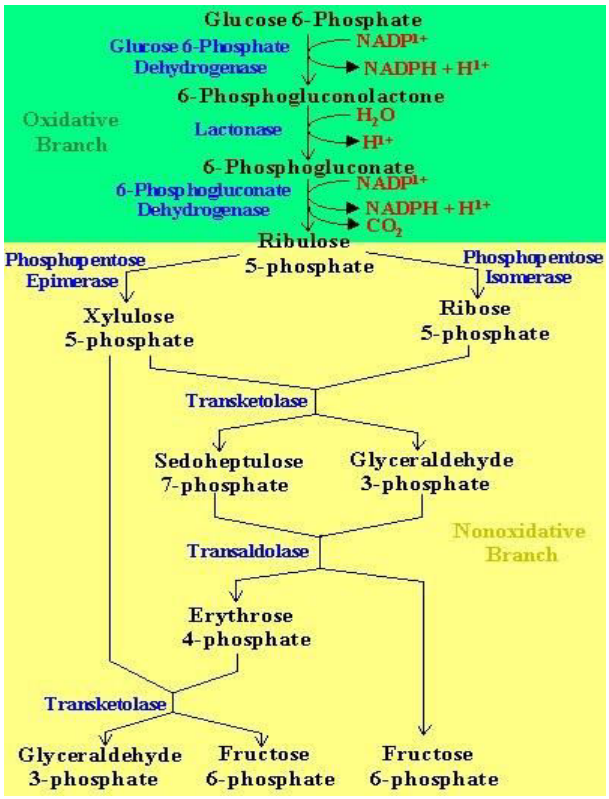
Pentose phosphate pathway

The pentose phosphate pathway (also called the hexose monophosphate shunt, or 6- phosphogluconate pathway) occurs in the cytosol of the cell. It includes two, irreversible oxidative reactions, followed by a

series of reversible sugar–phosphate interconversions. No ATP is directly consumed or produced in the cycle.

Carbon one of glucose 6-phosphate is released as CO2, and two NADPH are produced for each glucose 6-phosphate molecule entering the oxidative part of the pathway. The rate and direction of the reversible reactions of the pentose phosphate pathway are determined by the supply of and demand for intermediates of the cycle. The pathway provides a major portion of the body's NADPH, which functions as a biochemical reductant. It also produces ribose 5-phosphate, required for the biosynthesis of nucleotides, and provides a mechanism for the metabolic use of five-carbon sugars obtained from the diet or the degradation of structural carbohydrates in the body.





Reactions

[1] The oxidative part starts with the oxidation of glucose 6-phosphate by glucose-6- phosphate dehydrogenase. This forms NADPH + H+ for the first time. The second product, 6-phosphogluconolactone, is an intramolecular ester (lactone) of 6-phosphogluconate.

[2] A specific hydrolase then cleaves the lactone, exposing the carboxyl group of 6-phosphogluconate.

[3] The last enzyme in the oxidative part is phosphogluconate dehydrogenase, which

releases the carboxylate group of 6-phosphogluconate as CO_2 and at the same time oxidizes the hydroxyl group at C3 to an oxo group. In addition to a second NADPH + H+, this also produces the ketopentose ribulose 5-phosphate. This is converted by an isomerase to ribose 5-phosphate, the initial compound for nucleotide synthesis.

The function of the regenerative branch is to adjust the net production of NADPH + H+ and pentose phosphates to the cell's current requirements. Normally, the demand for NADPH + H+ is much higher than that for pentose phosphates. In these conditions, the reaction steps shown first convert six ribulose 5-phosphates to five molecules of fructose 6-phosphate and then, by isomerization, regenerate five glucose 6-phosphates. These can once again supply NADPH + H+ to the oxidative part of the PPP. Repeating these reactions finally results in the oxidation of one glucose 6-phosphate into six CO_2.

Twelve NADPH+H+ arise in the same process. In sum, no pentose phosphates are produced via this pathway. In the recombination of sugar phosphates in the regenerative part of the PPP, there are two enzymes that are particularly important:

[5] Transaldolase transfers C3 units from sedoheptulose 7-phosphate, a ketose with seven C atoms, to the aldehyde group of glyceraldehyde 3-phosphate.

[4] Transketolase, which contains thiamine diphosphate, transfers C2 fragments from one sugar phosphate to another.

The reactions in the regenerative segment of the PPP are freely reversible. It is therefore easily possible to use the regenerative part of the pathway to convert hexose phosphates into pentose phosphates. This can occur when there is a high demand for pentose phosphates—e. g., during DNA replication in the S phase of the cell cycle.

Gluconeogenesis

Some tissues, such as the brain, red blood cells, kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for only ten to eighteen hours in the absence of dietary intake of carbohydrate.

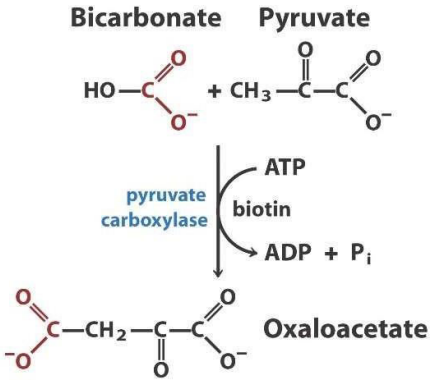
During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols), and α-ketoacids (derived from the catabolism of glucogenic amino acids). The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation.

Instead, glucose is synthesized by a special pathway, gluconeogenesis, that requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately ninety percent of gluconeogenesis occurs in the liver, with the kidneys providing ten percent of the newly synthesized glucose molecules. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated forty percent of the total glucose production.

Many of the reaction steps involved in gluconeogenesis are catalyzed by the same enzymes that are used in glycolysis. Other enzymes are specific to gluconeogenesis and are only synthesized, under the influence of cortisol and glucagon when needed. Glycolysis takes place exclusively when needed in the cytoplasm, but gluconeogenesis also involves the mitochondria and the endoplasmic reticulum.

Gluconeogenesis consumes 4 ATP (3 ATP + 1 GTP) per glucose—i.e., twice as many as glycolysis produces.

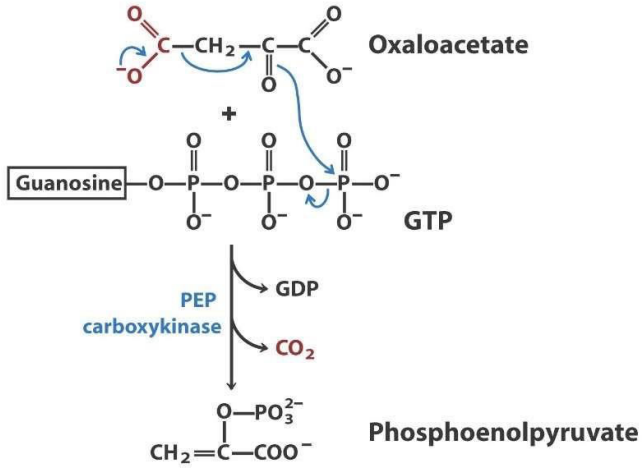
[1] Lactate as a precursor for gluconeogenesis is mainly derived from muscle (Cori cycle) and erythrocytes. LDH oxidizes lactate to pyruvate, with NADH + H+ formation.



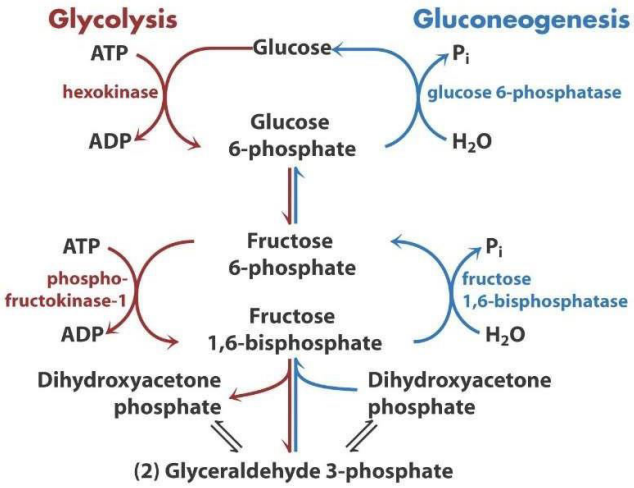
[2] The first steps of actual gluconeogenesis take place in the mitochondria. The reason for this "detour" is the equilibrium state of the pyruvate kinase reaction. Even coupling to ATP hydrolysis would not be sufficient to convert pyruvate directly into phosphoenol pyruvate (PEP). Pyruvate derived from lactate or amino acids is therefore initially transported into the mitochondrial matrix, and—in a biotin-dependent reaction catalyzed by pyruvate carboxylase—is carboxylated there to oxaloacetate. Oxaloacetate is also an intermediate in the tricarboxylic acid cycle. Amino acids with breakdown products that enter the cycle or supply pyruvate can therefore be converted into glucose.

[3] The oxaloacetate formed in the mitochondrial matrix is initially reduced to malate, which can leave the mitochondria via inner membrane transport systems.

[4] In the cytoplasm, oxaloacetate is reformed and then converted into phosphoenol pyruvate by a GTP-dependent PEP carboxykinase.



The subsequent steps up to fructose 1,6-bisphosphate represent the reverse of the corresponding reactions involved in glycolysis. One additional ATP per C3 fragment is used for the synthesis of 1,3-bisphosphoglycerate. Two gluconeogenesis-specific phosphatases then successively cleave off the phosphate residues from fructose 1,6- bisphosphate.



In between these reactions lies the isomerization of fructose 6-phosphate to glucose 6-phosphate—another glycolytic reaction.

[5] The reaction catalyzed by fructose 1,6-bisphosphatase is an important regulation point in gluconeogenesis.

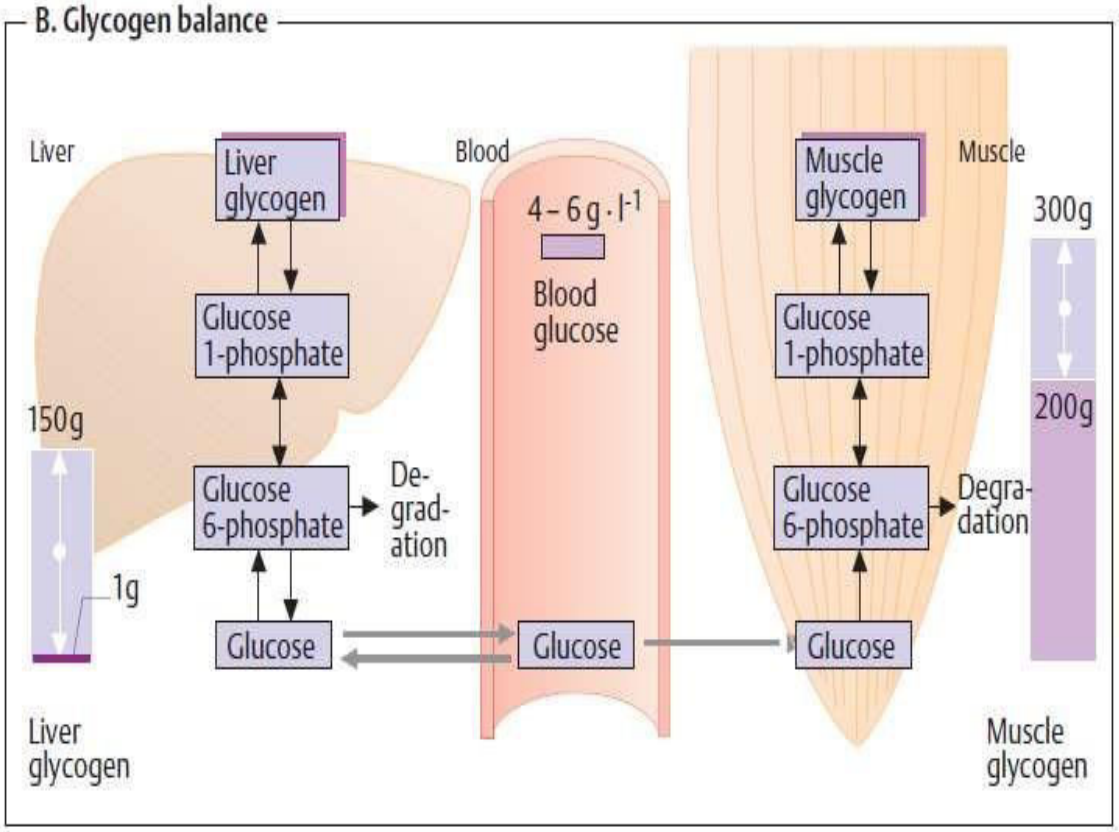
[6] The last enzyme in the pathway, glucose 6-phosphatase, occurs in the liver, but not in muscle. It is located in the interior of the smooth endoplasmic reticulum. Specific transporters allow glucose 6-phosphate to enter the ER and allow the glucose formed there to return to the cytoplasm. From there, it is ultimately released into the blood.

Glycogen metabolism

A constant source of blood glucose is an absolute requirement for human life. Glucose is the greatly preferred energy source for the brain, and the required energy source for cells with few or no mitochondria, such as mature erythrocytes. Glucose is also essential as an energy source for exercising muscle, where it is the substrate for anaerobic glycolysis. Blood glucose can be obtained from three primary sources: the diet, degradation of glycogen, and gluconeogenesis.

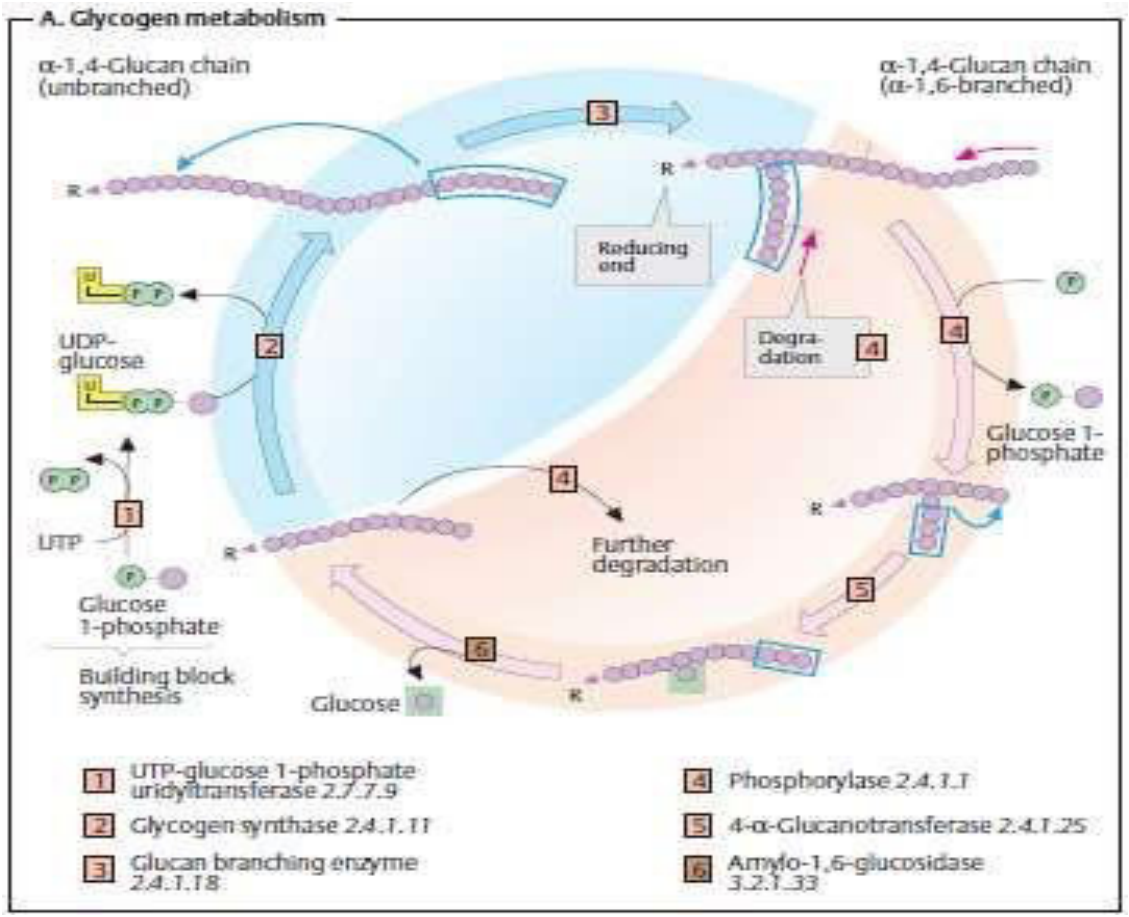
Dietary intake of glucose and glucose precursors, such as starch, monosaccharides, and disaccharides, is sporadic and, depending on the diet, is not always a reliable source of blood glucose. In contrast, gluconeogenesis can provide sustained synthesis of glucose, but it is somewhat slow in responding to a falling blood glucose level.

Therefore, the body has developed mechanisms for storing a supply of glucose in a rapidly mobilizable form, namely, glycogen. In the absence of a dietary source of glucose, this compound is rapidly released from liver and kidney glycogen. Similarly, muscle glycogen is extensively degraded in exercising muscle to provide that tissue with an important energy source. When glycogen stores are depleted, specific tissues synthesize glucose de novo, using amino acids from the body's proteins as a primary source of carbons for the gluconeogenic pathway.



The main stores of glycogen in the body are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen

is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast.



Animal glycogen, like amylopectin in plants, is a branched homopolymer of glucose. The glucose esidues are linked by an α 1_4-glycosidic bond. Every tenth or so glucose residue has an additional α 1_6 bond to another glucose. These branches are extended by additional α 1_4- linked glucose residues.

This structure produces tree-shaped molecules consisting of up to 50000 residues (M > 1 107 Da). Hepatic glycogen is never completely degraded. In general, only the nonreducing ends of the “tree” are shortened, or when glucose is abundant elongated. The reducing end of the tree is linked to a special protein, glycogenin.

Glycogenin carries out autocatalytic covalent bonding of the first glucose at one of its tyrosine residues and elongation of this by up to seven additional glucose residues. It is only at this point that glycogen synthase becomes active to supply further elongation.

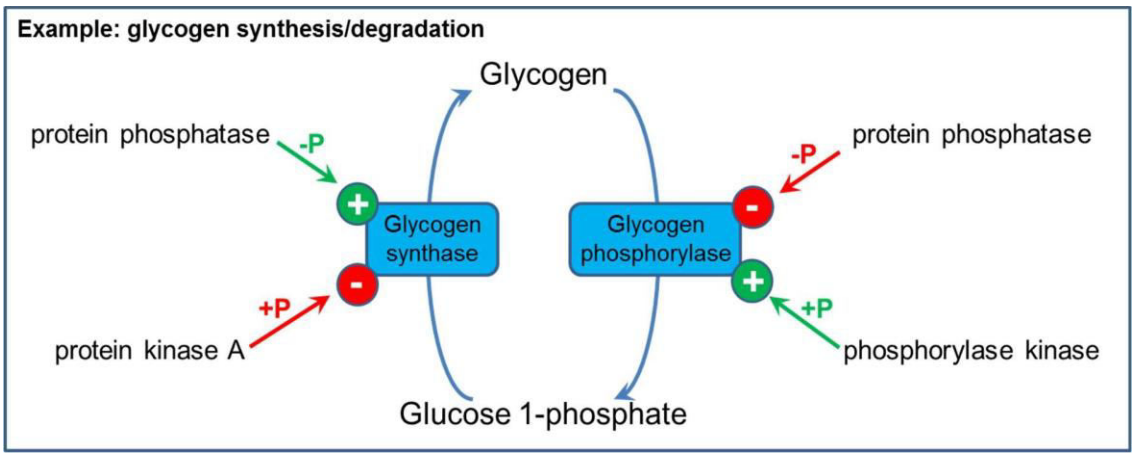
[1] The formation of glycosidic bonds between sugars is endergonic. Initially, therefore, the activated form UDP-glucose is synthesized by reaction of glucose 1-phosphate with UTP.

[2] Glycogen synthase now transfers glucose residues one by one from UDP-glucose to the non-reducing ends of the available “branches.”

[3] Once the growing chain has reached a specific length (> 11 residues), the branching enzyme cleaves an oligosaccharide consisting of 6–7 residues from the end of it, and adds this into the interior of the same chain or a neighbouring one with α 1_6 linkage. These branches are then further extended by glycogen synthase.

[4] The branched structure of glycogen allows rapid release of sugar residues. The most important degradative enzyme, glycogen phosphorylase, cleaves residues from a non-reducing end one after another as glucose 1-phosphate. The larger the number of these ends, the more phosphorylase molecules can attack simultaneously. The formation of glucose 1-phosphate instead of glucose has the advantage that no ATP is needed to channel the released residues into glycolysis or the PPP.

Due to the structure of glycogen phosphorylase, degradation comes to a halt four residues away from each branching point. Two more enzymes overcome this blockage. First, a glucanotransferase moves a trisaccharide fromthe side chain to the end of the main chain [5]. A 1,6-glucosidase [6] then cleaves the single remaining residue as a free glucose and leaves behind an unbranched chain that is once again accessible to phosphorylase.

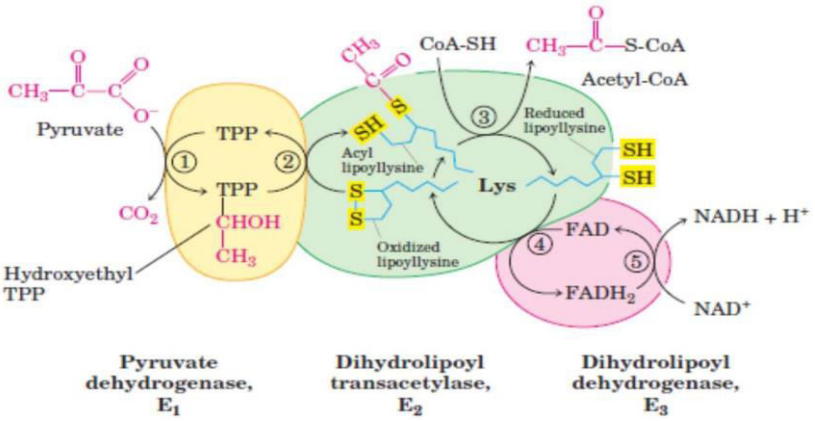


Citric acid cycle

The tricarboxylic acid cycle (TCA cycle, also called the Krebs cycle or the citric acid cycle) plays several roles in metabolism. It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO2. This oxidation provides energy for the production of the majority of ATP in most animals, including humans.

The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes produced by the cycle. The TCA cycle is an aerobic pathway, because O2 is required as the final electron acceptor. Most of the body's catabolic pathways converge on the TCA cycle. Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic reactions.

The citric acid cycle also participates in a number of important synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of some amino acids, and it



provides building blocks for the synthesis of some amino acids and heme. Therefore, this cycle should not be viewed as a closed circle, but instead as a traffic circle with compounds entering and leaving as required.

Oxidative decarboxylation of pyruvate

Pyruvate, the end product of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle. This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane. Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex. Strictly speaking, the pyruvate dehydrogenase complex is not part of the TCA cycle proper, but is a major source of acetyl CoA the two-carbon substrate for the cycle.

The pyruvate dehydrogenase complex is a multimolecular aggregate of three enzymes, pyruvate dehydrogenase (E1, also called a decarboxylase), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Each is present in multiple copies, and each catalyzes a part of the overall reaction. Their physical association links the reactions in proper sequence without the release of intermediates. In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the complex also

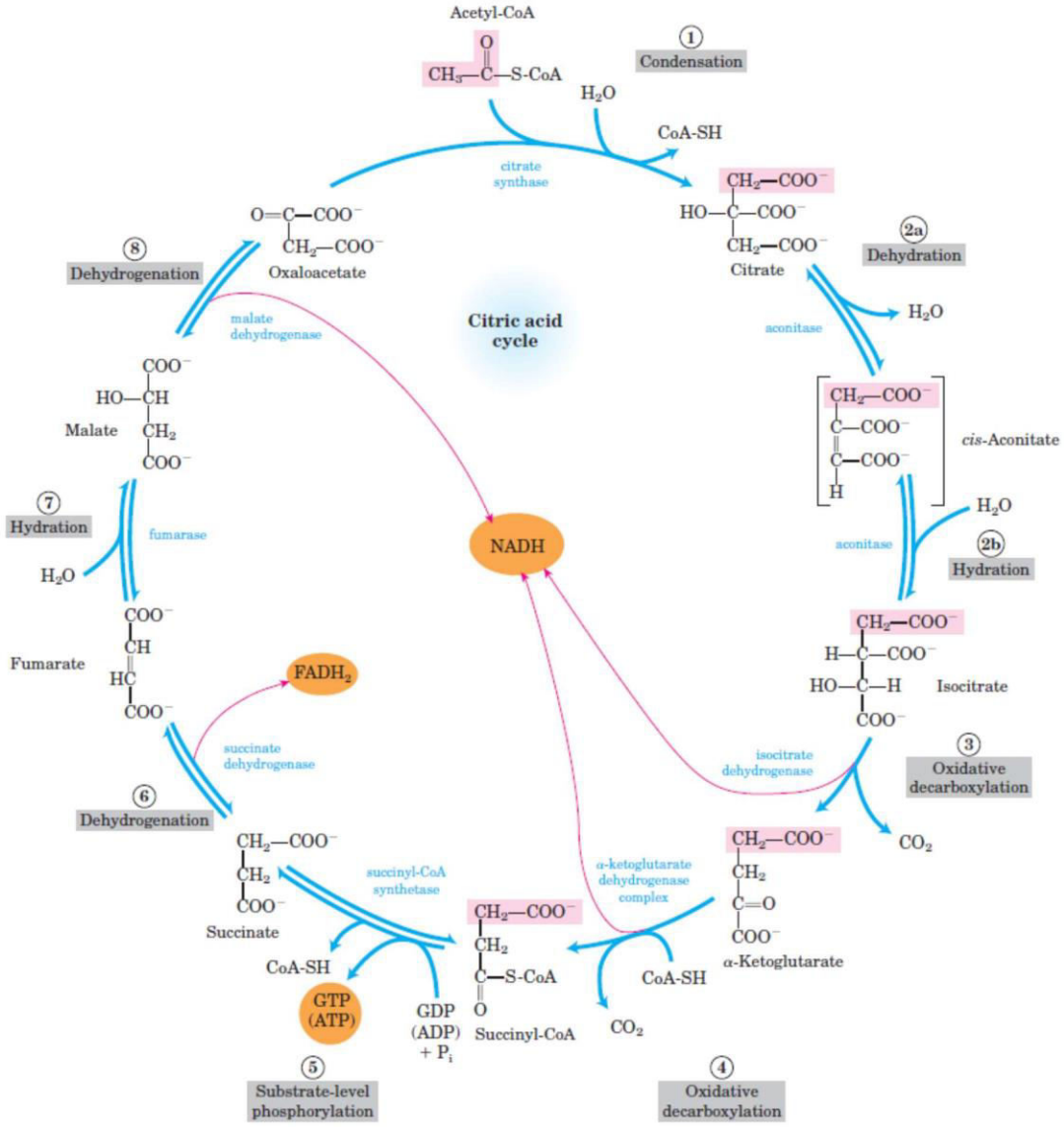
contains two tightly bound regulatory enzymes, pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase.

The acetyl-CoA that supplies the cycle with acetyl residues is mainly derived from β- oxidation of fatty acids and from the pyruvate dehydrogenase reaction. Both of these processes take place in the mitochondrial matrix.

[1] In the first step of the cycle, citrate synthase catalyzes the transfer of an acetyl residue from acetyl CoA to a carrier molecule, oxaloacetic acid. The product of this reaction, tricarboxylic acid, gives the cycle its name.

[2] In the next step, tricarboxylic acid undergoes isomerization to yield isocitrate. In the process, only the hydroxyl group is shifted within the molecule. The corresponding enzyme is called aconitate hydratase ("aconitase"), because unsaturated aconitate arises as an enzyme- bound intermediate during the reaction.

Due to the properties of aconitase, the isomerization is absolutely stereospecific. Although citrate is not chiral, isocitrate has two chiral centers, so that it could potentially appear in four isomeric forms. However, in the tricarboxylic acid cycle, only one of these stereoisomers, (2R,3S)-isocitrate, is produced.



[3] The first oxidative step now follows. Isocitrate dehydrogenase oxidizes the hydroxyl group of isocitrate into an oxo group. At the same time, a carboxyl group is released as CO2, and 2- oxoglutarate (also known as α-ketoglutarate) and NADH + H+ are formed.

[4] The next step, the formation of succinyl CoA, also involves one oxidation and one decarboxylation. It is catalyzed by 2-oxoglutarate dehydrogenase, a multienzyme complex closely resembling the PDH complex. NADH + H+ is once again formed in this reaction.

[5] The subsequent cleavage of the thioester succinyl CoA into succinate and coenzyme A by

succinic acid-CoA ligase (succinyl CoA synthetase, succinic thiokinase) is strongly exergonic and is used to synthesize a phosphoric acid anhydride bond ("substrate level phosphorylation ").

However, it is not ATP that is produced here as is otherwise usually the case, but instead guanosine triphosphate (GTP). However, GTP can be converted into ATP by a nucleoside diphosphate kinase.

[6] Via the reactions described so far, the acetyl residue has been completely oxidized to CO2. At the same time, however, the carrier molecule oxaloacetate has been reduced to succinate.

Three further reactions in the cycle now regenerate oxaloacetate from succinate. Initially, succinate dehydrogenase oxidizes succinate to fumarate. In contrast to the other enzymes in the cycle, succinate dehydrogenase is an integral protein of the inner mitochondrial membrane. It is therefore also assigned to the respiratory chain as complex II. Although succinate dehydrogenase contains FAD as a prosthetic group, ubiquinone is the real electron acceptor of the reaction.

[7] Water is now added to the double bond of fumarate by fumarate hydratase ("fumarase"), and chiral (2S)-malate is produced.

[8] In the last step of the cycle, malate is again oxidized by malate dehydrogenase into

oxaloacetate, with NADH + H+ again being produced.

With this reaction, the cycle is complete and can start again from the beginning. As the equilibrium of the reaction lies well on the side of malate, the formation of oxaloacetic acid by reaction [8] depends on the strongly exergonic reaction [1], which immediately removes it from the equilibrium.

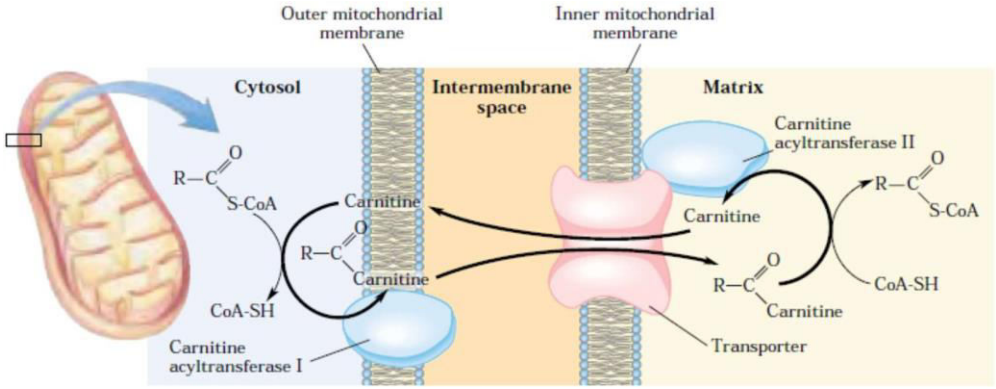
The net outcome is that each rotation of the tricarboxylic acid cycle converts one acetyl residue and two molecules of H2O into two molecules of CO2. At the same time, one GTP, three NADH

+ H+ and one reduced ubiquinone (QH2) are produced. By oxidative phosphorylation, the cell obtains around nine molecules of ATP from these reduced coenzymes. Together with the directly formed GTP, this yields a total of 10 ATP per acetyl group.

Fatty acid oxidation and biosynthesis

Fatty acid degradation: β-oxidation

After uptake by the cell, fatty acids are activated by conversion into their CoA derivatives **acyl CoA** is formed. This uses up two energy-rich anhydride bonds of ATP per fatty acid. For channeling into the mitochondria, the acyl residues are first transferred to *carnitine* and then transported across the inner membrane as **acyl carnitine**.



The degradation of the fatty acids occurs in the mitochondrial matrix through an oxidative cycle in which C2 units are successively cleaved off as **acetyl CoA** (*activated acetic acid*). Before the release of the acetyl groups, each CH_2 group at C-3 of the acyl residue (the β-C atom) is oxidized to the keto group hence the term **β-oxidation** for this metabolic pathway. Both spatially and functionally, it is closely linked to the tricarboxylic acid cycle and to the respiratory chain.

[1] The first step is dehydrogenation of **acyl CoA** at C-2 and C-3. This yields an unsaturated β2-enoyl-CoA derivative with a *trans*-configured double bond. The two hydrogen atoms are initially transferred from FAD-containing *acyl CoA dehydrogenase* to the **electron-transferring flavoprotein (ETF)**. *ETF dehydrogenase* [5] passes them on from ETF to ubiquinone (coenzyme Q), a component of the *respiratory chain*.

Other FAD-containing mitochondrial dehydrogenases are also able to supply the respiratory chain with electrons in this fashion.

There are three *isoenzymes* of *acyl CoA dehydrogenase* that are specialized for long-chain fatty acids (12–18 C atoms), medium-chain fatty acids (4–14), and short chain fatty acids (4–8).

[2] The next step in fatty acid degradation is the addition of a water molecule to the double bond of the enoyl CoA (*hydration*), with formation of **β-hydroxyacyl CoA**.

[3] In the next reaction, the OH group at C-3 is oxidized to a carbonyl group (*dehydrogenation*). This gives rise to **β-ketoacyl CoA**, and the reduction equivalents are transferred to NAD+, which also passes them on to the *respiratory chain*.

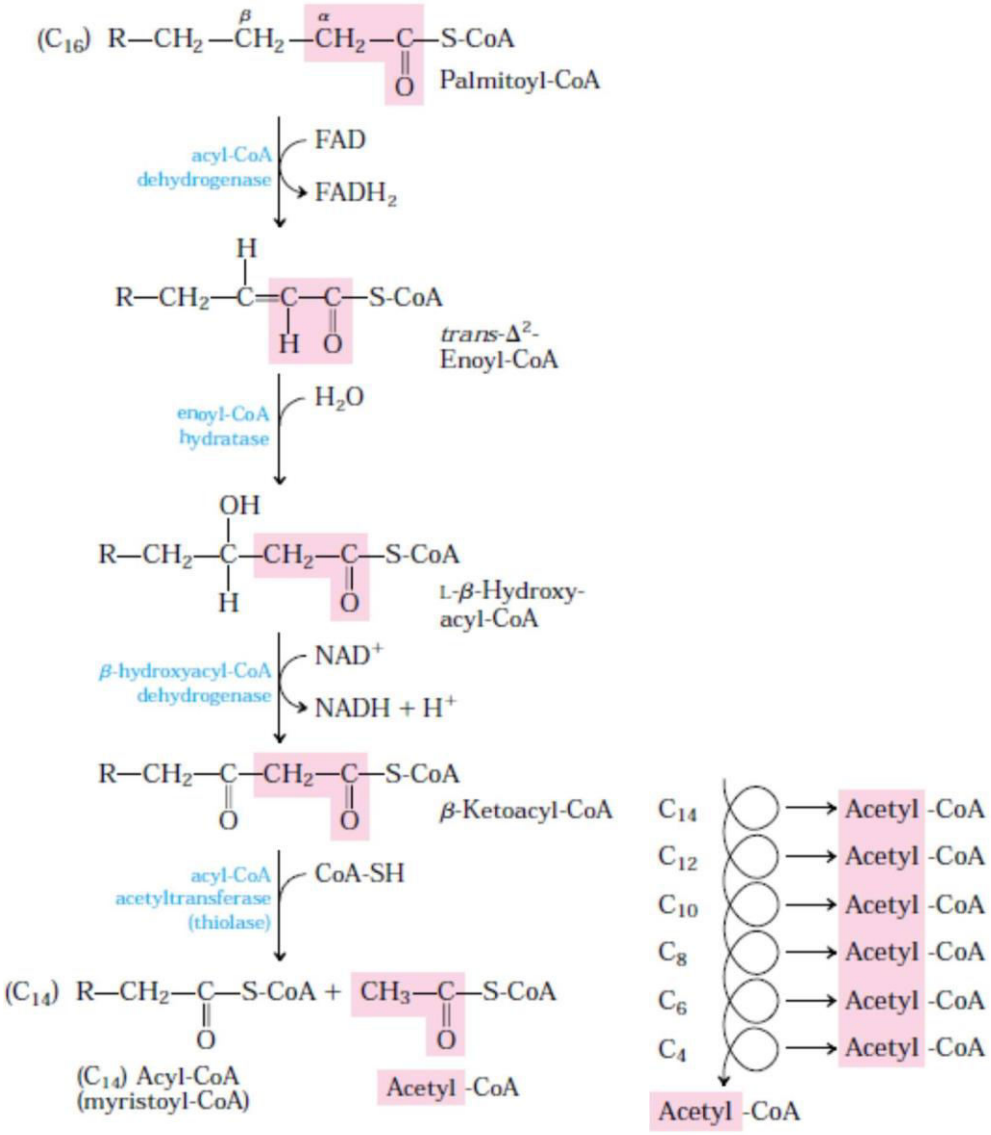
[4] β-Ketoacyl-CoA is now broken down by an *acyl transferase* into **acetyl CoA** and an **acyl CoA shortened by 2 C atoms** (*"thioclastic cleavage"*).

Several cycles are required for complete degradation of long-chain fatty acids—eight cycles in the case of stearyl-CoA (C18:0), for example. The acetyl CoA formed can then undergo further metabolism in the *tricarboxylic acid cycle*, or can be used for biosynthesis. When there is an excess of acetyl CoA, the liver can also form ketone bodies.

When oxidative degradation is complete, one molecule of palmitic acid supplies around 106 molecules of ATP, corresponding to an energy of 3300 kJ mol–1. This high energy yield makes fats an ideal form of storage for metabolic energy. Hibernating animals such as polar bears can meet their own energy requirements for up to 6 months solely by fat degradation, while at the same time producing the vital water they need via the respiratory chain ("respiratory water").

The overall equation for the oxidation of palmitoyl-CoA to eight molecules of acetyl-CoA, including the electron transfers and oxidative phosphorylations, is

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



Fatty acid synthesis

In the vertebrates, biosynthesis of fatty acids is catalyzed by *fatty acid synthase,* a multifunctional enzyme. Located in the cytoplasm, the enzyme requires acetyl CoA as a starter molecule. In a cyclic reaction, the acetyl residue is elongated by one C2 unit at a time for seven cycles. NADPH + H^+ is used as a reducing agent in the process. The end product of the reaction is the saturated C16 acid, *palmitic acid.*

A. Fatty acid synthase

Fatty acid synthase in vertebrates consists of two identical peptide chains i. e., it is a homodimer. Each of the two peptide chains, which are shown here as hemispheres, catalyzes all seven of the partial reactions required to synthesize palmitate. The spatial compression of several successive reactions into a single multifunctional enzyme has advantages in comparison with separate enzymes.

Competing reactions are prevented, the individual reactions proceed in a coordinated way as if on a production line, and due to low diffusion losses they are particularly efficient. Each subunit of the enzyme binds acetyl residues as thioesters at two different SH groups: at one peripheral *cysteine residue* (CysSH) and one central *4_-phosphopantetheine group* (Pan-SH). Pan-SH, which is very similar to coenzyme A, is covalently bound to a protein segment of the synthase known as the *acyl-carrier protein* (ACP).

This part functions like a long arm that passes the substrate from one reaction center to the next. The two subunits of fatty acid synthase cooperate in this process; the enzyme is therefore only capable of functioning as a dimer. Spatially, the enzyme activities are arranged into three different domains.

Domain 1 catalyzes the entry of the substrates acetyl CoA and malonyl CoA by *[ACP]-S acetyltransferase* [1] and *[ACP]-S malonyl transferase* [2] and subsequent condensation of the two partners by *3-oxoacyl-[ACP]-synthase* [3].

Domain 2 catalyzes the conversion of the 3-oxo group to a CH2 group by *3-oxoacyl-[ACP]-reductase* [4], *3-hydroxyacyl-[ACP]-dehydratase* [5], and *enoyl-[ACP]-reductase* [6].

Finally, **domain 3** serves to release the finished product by *acyl-[ACP]-hydrolase* [7] after seven steps of chain elongation.

B. Reactions of fatty acid synthase

The key enzyme in fatty acid synthesis is **acetyl CoA carboxylase**, which precedes

the synthase and supplies the malonyl-CoA required for elongation. Like all carboxylases, the enzyme contains covalently bound *biotin* as a prosthetic group and is hormone dependently *inactivated* by phosphorylation or *activated* by dephosphorylation. The precursor *citrate* is an allosteric activator, while *palmitoyl-CoA* inhibits the end product of the synthesis pathway.

[1] The first cycle (n = 1) starts with the transfer of an acetyl residue from acetyl CoA to the peripheral cysteine residue (Cys-SH). At the same time,
[2] a malonyl residue is transferred from malonyl CoA to 4-phosphopantetheine (Pan-SH).
[3] By condensation of the acetyl residue or (in later cycles) the acyl residue—with the malonyl group, with simultaneous decarboxylation, the chain is elongated.

[4]–[6] The following three reactions (reduction of the 3-oxo group, dehydrogenation of the 3-hydroxyl derivative, and renewed reduction of it) correspond in principle to a reversal of β-oxidation, but they are catalyzed by other enzymes and use NADPH + H^+ instead of NADH + H^+ for reduction.

They lead to an acyl residue bound at Pan-SH with 2n + 2 C atoms (n = the number of the cycle). Finally, depending on the length of the product, [1] The acyl residue is transferred back to the peripheral cysteine, so that the next cycle can begin again with renewed loading of the ACP with a malonyl residue, or:

[7] After seven cycles, the completed **palmitic acid** is hydrolytically released.

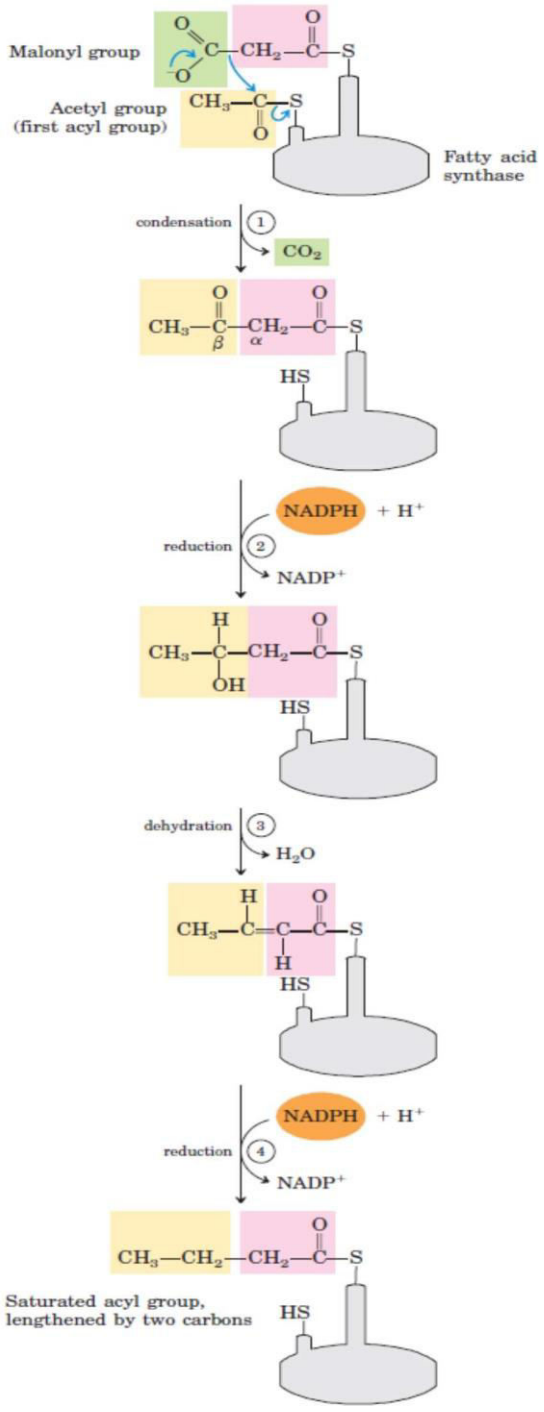
In all, one acetyl-CoA and seven malonyl-CoA are converted with the help of 14 NADPH + H^+ into one palmitic acid, 7 CO_2, 6 H_2O, 8 CoA and 14 NADP+. Acetyl CoA carboxylase also uses up seven ATP.

Addition of two carbons to a growing fatty acyl chain: a four-step sequence. Each malonyl group and acetyl (or longer acyl) group is activated by a thioester that links it to fatty acid synthase, a multienzyme complex.

1 Condensation of an activated acyl group (an acetyl group from acetyl-CoA is the first acyl group) and two carbons derived from malonyl-CoA, with elimination of CO_2 from the malonyl group, extends the acyl chain by two carbons. The mechanism of the first step of this reaction is given to illustrate the role of decarboxylation in facilitating condensation. The β-keto product of this condensation is then reduced in three more steps nearly identical to the reactions of β oxidation, but in the reverse sequence.

2 the β-keto group is reduced to an alcohol,

3 elimination of H_2O creates a double bond, and 4 the double bond is reduced to form the corresponding saturated fatty acyl group.


Malonyl group
Acetyl group
(first acyl group)
Fatty acid
synthase
condensation
1
CO2
HS
NADPH
+ H+
reduction
2
NADP+
dehydration
3
H2O
reduction
4
NADP+
Saturated acyl group,
lengthened by two carbons

SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited "A" Grade by NAAC | 12B Status by UGC | Approved by AICTE
www.sathyabama.ac.in

SCHOOL OF BIO AND CHEMICAL ENGINEEING
DEPARTMENT OF BIOTECHNOLOGY

UNIT IV –INTERMEDIARY METABOLISM - SBB 1202

Outline of amino acid biosynthesis

Amino acid biosynthesis: Overview

The proteinogenic amino acids can be divided into **five families** in relation to their biosynthesis. The members of each family are derived from common precursors, which are all produced in the tricarboxylic acid cycle or in catabolic carbohydrate metabolism.

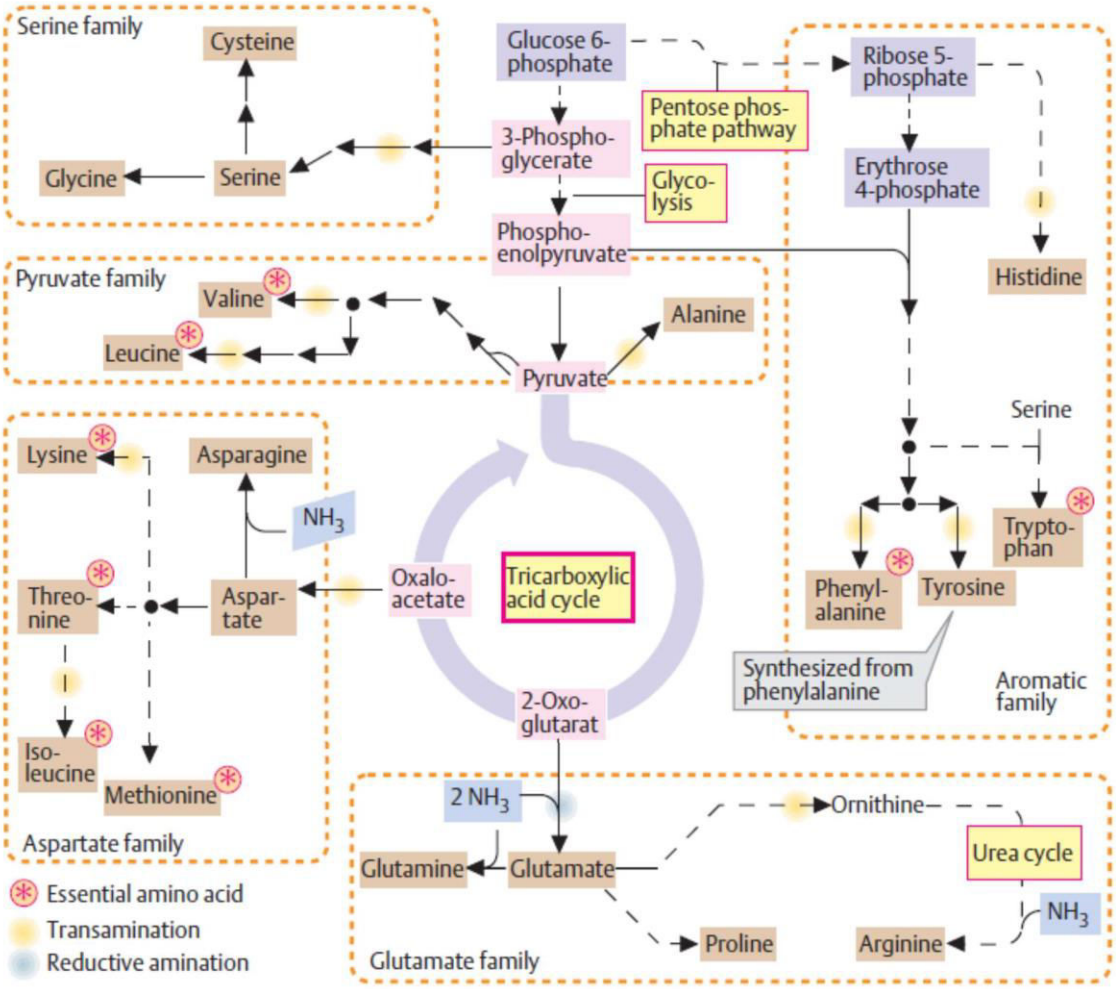
Plants and microorganisms are able to synthesize all of the amino acids from scratch, but during the course of evolution, mammals have lost the ability to synthesize approximately half of the 20 proteinogenic amino acids. These **essential amino acids** therefore have to be supplied in food. For example, animal metabolism is no longer capable of carrying out de-novo synthesis of the **aromatic amino acids** (tyrosine is only non-essential because it can be formed from phenylalanine when there is an adequate supply available).

The **branched-chain amino acids** (valine, leucine, isoleucine, and threonine) as well as **methionine** and **lysine**, also belong to the essential amino acids. Histidine and arginine are essential in rats; whether the same applies in humans is still amatter of debate. A supply of these amino acids in food appears to be essential at least during growth.

The nutritional value of proteins is decisively dependent on their essential amino acid content. Vegetable proteins—e. g., those from cereals—are low in lysine and methionine, while animal proteins contain all the amino acids in balanced proportions. As mentioned earlier, however, there are also plants that provide high-value protein. These include the soy bean, one of the plants that is supplied with NH_3 by symbiotic N_2 fixers.

Non-essential amino acids are those that arise by transamination from 2-oxoacids in the intermediary metabolism. These belong to the **glutamate family** (Glu, Gln, Pro, Arg, derived from 2-oxoglutarate), the **aspartate family** (only Asp and Asn in this group, derived from oxaloacetate), and **alanine**, which can be formed by transamination from pyruvate.

The amino acids in the **serine family** (Ser, Gly, Cys) and **histidine**, which arise from intermediates of glycolysis, can also be synthesized by the human body.



Amino acid degradation

A large number of metabolic pathways are available for amino acid degradation.

Amino acid degradation : overview

During the degradation of most amino acids, the α-amino group is initially removed by **transamination** or **deamination**. Various mechanisms are available for this. The carbon skeletons that are left over after deamination undergo further degradation in various ways. During degradation, the 20 proteinogenic amino acids produce only seven different **degradation products** (highlighted in pink and violet).

Five of these metabolites (2-oxoglutarate, succinyl CoA, fumarate, oxaloacetate, and pyruvate) are precursors for gluconeogenesis and can therefore be converted into glucose by the liver and kidneys. Amino acids whose degradation supplies one of these five metabolites are therefore referred to as **glucogenic amino acids**.

The first four degradation products listed are already intermediates in the tricarboxylic acid cycle, while pyruvate can be converted into oxaloacetate by *pyruvate carboxylase* and thus made available for gluconeogenesis. With two exceptions (lysine and leucine), all of the proteinogenic amino acids are also glucogenic. Quantitatively, they represent themost important precursors for gluconeogenesis. At the same time, they also have an **anaplerotic** effect—i. e., they replenish the tricarboxylic acid cycle in order to feed the anabolic reactions that originate in it.

Two additional degradation products (acetoacetate and acetyl CoA) cannot be channeled into gluconeogenesis in animal metabolism, as there is no means of converting them into precursors of gluconeogenesis. However, they can be used to synthesize ketone bodies, fatty acids, and isoprenoids. Amino acids that supply acetyl CoA or acetoacetate are therefore known as **ketogenic amino acids.** Only leucine and lysine are *purely* ketogenic. Several amino acids yield degradation products that are both *glucogenic and ketogenic*. This group includes phenylalanine,
tyrosine, tryptophan, and isoleucine.

Degradation of acetoacetate to acetyl CoA takes place in two steps. First, acetoacetate and succinyl CoA are converted into acetoacetyl CoA and succinate (enzyme: *3-oxoacid-CoA transferase 2.8.3.5*). Acetoacetyl CoA is then broken down by β-oxidation into two molecules of acetyl CoA, while succinate can be further metabolized via the tricarboxylic acid cycle.

Deamination

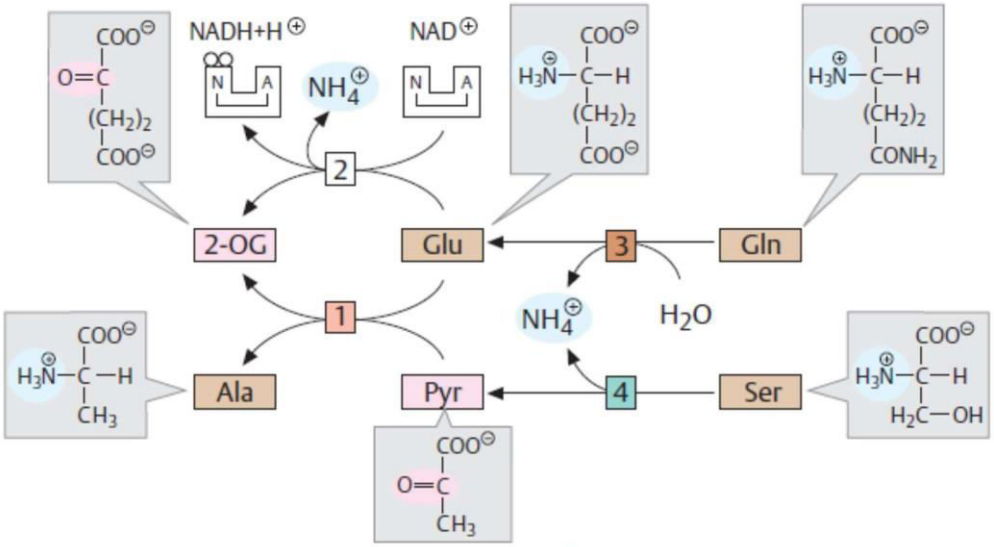
There are various ways of releasing ammonia (NH3) from amino acids, and these are illustrated here using the example of the amino acids glutamine, glutamate, alanine, and serine.

[1] In the branched-chain amino acids (Val, Leu, Ile) and also tyrosine and ornithine, degradation starts with a **transamination**. For alanine and aspartate, this is actually the only degradation step

[2] **Oxidative deamination**, with the formation of NADH + H^+, only applies to glutamate in animal metabolism. The reaction mainly takes place in the liver and releases NH_3 for urea formation.

[3] Two amino acids—asparagine and glutamine—contain acid–amide groups in the side chains, from which NH_3 can be released by hydrolysis (**hydrolytic deamination**). In the blood, glutamine is the most important transport molecule for amino nitrogen. Hydrolytic deamination of glutamine in the liver also supplies the urea cycle with NH_3.

[4] **Eliminating deamination** takes place in the degradation of histidine and serine. H_2O is first eliminated here, yielding an unsaturated intermediate. In the case of serine, this intermediate is first rearranged into an imine, which is hydrolyzed in the second step into NH_3 and pyruvate, with H_2O being taken up. H_2O does not therefore appear in the reaction equation.

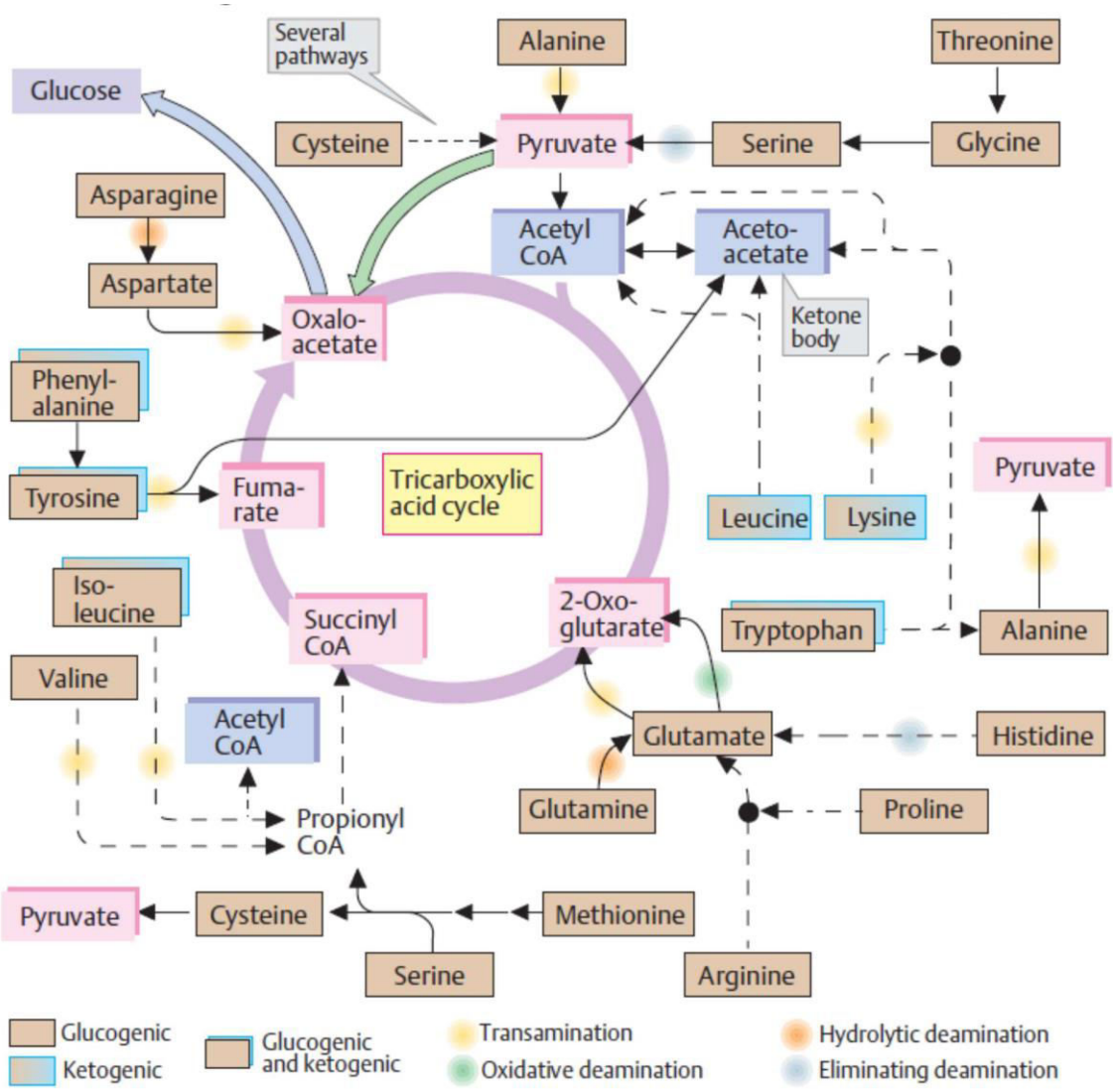


1 Alanine transaminase [PLP] *2.6.1.2*

2 Glutamate dehydrogenase *1.4.1.2*

3 Glutaminase *3.5.1.2*

4 Serine dehydratase [PLP] *4.2.1.13*



Urea Cycle

Urea is the major disposal form of amino groups derived from amino acids, and accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free NH_3, and the other nitrogen by aspartate. [Note: Glutamate is the immediate precursor of both ammonia (through oxidative deamination by glutamate dehydrogenase) and aspartate nitrogen (through transamination of oxaloacetate by AST).] The carbon and oxygen of urea are derived from CO_2. Urea is produced by the liver, and then is transported in the blood to the kidneys for excretion in the urine.

Amino acids are mainly broken down in the liver. Ammonia is released either directly or indirectly in the process. The degradation of nucleobases also provides significant amounts of ammonia.

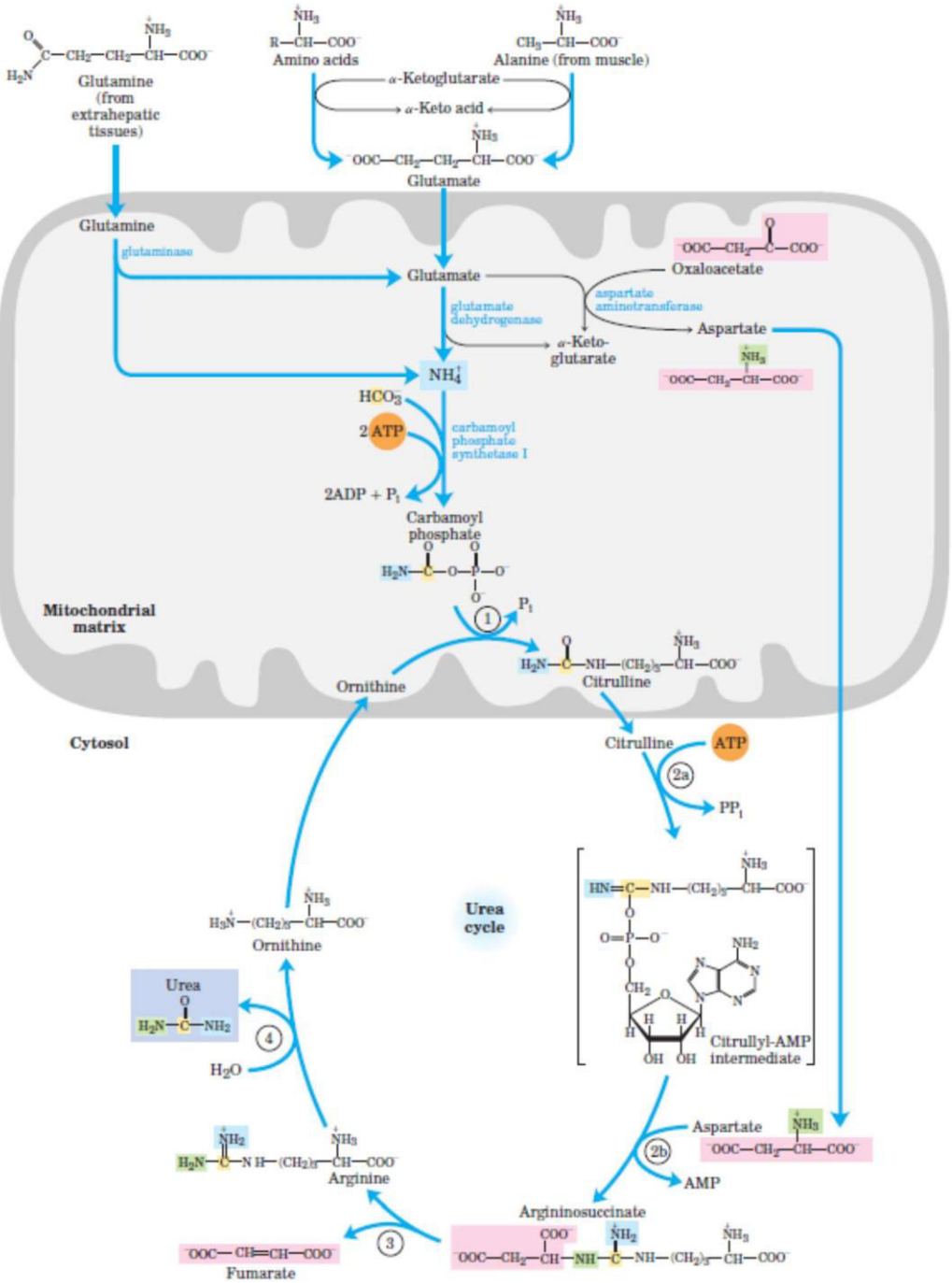
Ammonia (NH_3) is a relatively strong **base**, and at physiological pH values it is mainly present in the form of the **ammonium ion NH_4^+**. NH_3 and NH_4^+ are toxic, and at higher concentrations cause brain damage in particular. Ammonia therefore has to be effectively inactivated and excreted. This can be carried out in various ways.

Aquatic animals can excrete NH_4^+ directly. For example, fish excrete NH_4^+ via the gills (*ammonotelic animals*). Terrestrial vertebrates, including humans, hardly excrete any NH_3, and instead, most ammonia is converted into urea before excretion (*ureotelic animals*). Birds and reptiles, by contrast, form *uric acid,* which is mainly excreted as a solid in order to save water (*uricotelic animals*).

Urea ($H_2N–CO–NH_2$) is the diamide of carbonic acid. In contrast to ammonia, it is **neutral** and therefore relatively **non-toxic**. The reason for the lack of basicity is the molecule's mesomeric characteristics. The free electron pairs of the two nitrogen atoms are *delocalized* over the whole structure, and are therefore no longer able to bind protons. As a small, uncharged molecule, urea is able to cross biological membranes easily. In addition, it is easily transported in the blood and excreted in the urine.

Urea is produced **only in the liver**, in a cyclic sequence of reactions (the **urea cycle**) that starts in the mitochondria and continues in the cytoplasm. The two nitrogen atoms are derived from **NH_4^+** (the second has previously been incorporated into aspartate).

The keto group comes from **hydrogen carbonate** (HCO_3^-), or CO_2 that is in equilibrium with HCO_3^- .


Glutamine (from extrahepatic tissues)
Amino acids
Alanine (from muscle)
α-Ketoglutarate
α-Keto acid
Glutamate
Glutamine
glutaminase
Glutamate
Oxaloacetate
glutamate dehydrogenase
aspartate aminotransferase
Aspartate
α-Keto-glutarate
NH_4^+
HCO_3^-
2 ATP
carbamoyl phosphate synthetase I
2ADP + P_i
Carbamoyl phosphate
Mitochondrial matrix
P_i
1
Ornithine
Citrulline
Cytosol
Citrulline
ATP
2a
PP_i
Urea cycle
Ornithine
Urea
4
H_2O
Citrullyl-AMP intermediate
Aspartate
2b
AMP
Argininosuccinate
Arginine
3
Fumarate

[1] In the first step, **carbamoyl phosphate** is formed in the mitochondria from hydrogen carbonate (HCO_3^-) and NH_4^+, with two ATP molecules being consumed. In this compound, the carbamoyl residue (–O–CO–NH2) is at a high chemical potential.

[2] In the next step, the carbamoyl residue is transferred to the non-proteinogenic amino acid **ornithine**, converting it into **citrulline**, which is also non-proteinogenic. This is passed into the cytoplasm via a transporter.

[3] The second NH2 group of the later urea molecule is provided by **aspartate**, which condenses with citrulline into **argininosuccinate**. ATP is cleaved into AMP and diphosphate (PPi) for this endergonic reaction. To shift the equilibrium of the reaction to the side of the product, diphosphate is removed from the equilibrium by hydrolysis.

[4] Cleavage of fumarate from argininosuccinate leads to the proteinogenic amino acid **arginine**, which is synthesized in this way in animal metabolism.

[5] In the final step, isourea is released from the guanidinium group of the arginine by hydrolysis, and is immediately rearranged into **urea**. In addition, ornithine is regenerated and returns via the ornithine transporter into the mitochondria, where it becomes available for the cycle once again.

The **fumarate** produced is converted via malate to oxaloacetate, from which **aspartate** is formed again by transamination. The glutamate required is derived from the glutamate dehydrogenase reaction, which fixes the second NH_4^+ in an organic bond.

However, in urea formation they take place in the cytoplasm, where the appropriate isoenzymes are available. The rate of urea formation is mainly controlled by reaction [1]. *N*-**acetyl glutamate**, as an allosteric effector, activates *carbamoyl phosphate synthase*. In turn, the concentration of acetyl glutamate depends on arginine and ATP levels, as well as other factors.

Nucleotide metabolism

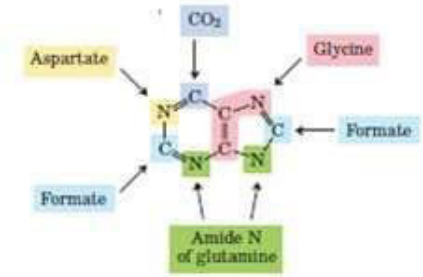
Ribonucleoside and deoxyribonucleoside phosphates (nucleotides) are essential for all cells. Without them, neither DNA nor RNA can be produced and, therefore, proteins cannot be synthesized or cells proliferate. Nucleotides also serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and proteins, and are structural components of several essential coenzymes, for example, coenzyme A, FAD, NAD^+, and $NADP^+$.

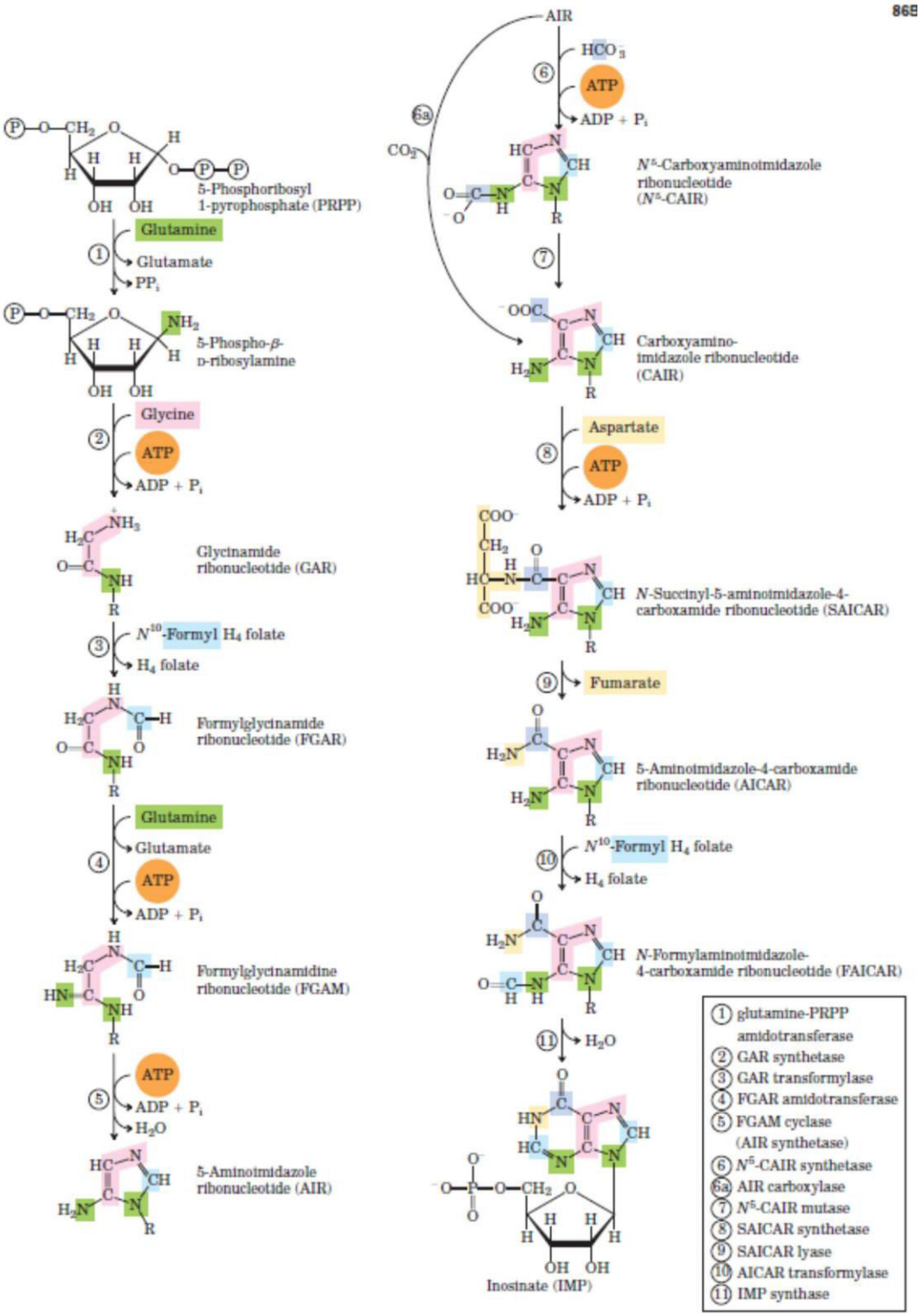
Nucleotides, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), serve as second messengers in signal transduction pathways. In addition, nucleotides play an important role as "energy currency" in the cell. Finally, nucleotides are important regulatory compounds for many of the pathways of intermediary metabolism, inhibiting or activating key enzymes. The purine and pyrimidine bases found in nucleotides can be synthesized de novo, or can be obtained through salvage pathways that allow the reuse of the preformed bases resulting from normal cell turnover or from the diet.

Biosynthesis of nucleotides

Synthesis of Purine Nucleotides

The atoms of the purine ring are contributed by a number of compounds, including amino acids (aspartic acid, glycine, and glutamine), CO_2, and N^{10}–formyl tetrahydrofolate. The purine ring is constructed by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate.




865
5-Phosphoribosyl 1-pyrophosphate (PRPP)
Glutamine
Glutamate
PPi
5-Phospho-β-D-ribosylamine
Glycine
ATP
ADP + Pi
Glycinamide ribonucleotide (GAR)
N10-Formyl H4 folate
H4 folate
Formylglycinamide ribonucleotide (FGAR)
Glutamine
Glutamate
ATP
ADP + Pi
Formylglycinamidine ribonucleotide (FGAM)
ATP
ADP + Pi
H2O
5-Aminoimidazole ribonucleotide (AIR)
AIR
HCO3
6
ATP
ADP + Pi
6a
CO2
N5-Carboxyaminoimidazole ribonucleotide (N5-CAIR)
7
Carboxyamino-imidazole ribonucleotide (CAIR)
Aspartate
8
ATP
ADP + Pi
N-Succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)
9
Fumarate
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)
N10-Formyl H4 folate
10
H4 folate
N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)
11
H2O
Inosinate (IMP)
1 glutamine-PRPP amidotransferase
2 GAR synthetase
3 GAR transformylase
4 FGAR amidotransferase
5 FGAM cyclase (AIR synthetase)
6 N5-CAIR synthetase
6a AIR carboxylase
7 N5-CAIR mutase
8 SAICAR synthetase
9 SAICAR lyase
10 AICAR transformylase
11 IMP synthase

A. Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

PRPP is an "activated pentose" that participates in the synthesis and salvage of purines and pyrimidines. Synthesis of PRPP from ATP and ribose 5-phosphate is catalyzed by PRPP synthetase (ribose phosphate pyrophosphokinase). This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotides (end-product inhibition). [Note: The sugar moiety of PRPP is ribose, and therefore ribonucleotides are the end products of de novo purine synthesis. When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced]

B. Synthesis of 5′-phosphoribosylamine

Synthesis of 5′-phosphoribosylamine from PRPP and glutamine is shown in diagram. The amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP. The enzyme, glutamine:phosphoribosyl pyrophosphate amidotransferase, is inhibited by the purine 5′-nucleotides AMP, GMP, and inosine monophosphate (IMP)—the end products of the pathway. This is the committed step in purine nucleotide biosynthesis. The rate of the reaction is also controlled by the intracellular concentration of PRPP. [Note: The intracellular concentration of PRPP is normally far below the K_m for the amidotransferase. Therefore, any small change in the PRPP concentration causes a proportional change in the rate of the reaction]

C. Synthesis of inosine monophosphate, the "parent" purine nucleotide

The next nine steps in purine nucleotide biosynthesis leads to the synthesis of IMP (whose base is hypoxanthine). This pathway requires four ATP molecules as an energy source. Two steps in the pathway require N^{10}-formyltetrahydrofolate.

D. Synthetic inhibitors of purine synthesis

Some synthetic inhibitors of purine synthesis (for example, the sulfonamides), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions. Other purine synthesis inhibitors, such as structural analogs of folic acid (for example, methotrexate), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA. Trimethoprim,

another folate analog, has potent antibacterial activity because of its selective inhibition of bacterial dihydrofolate reductase.

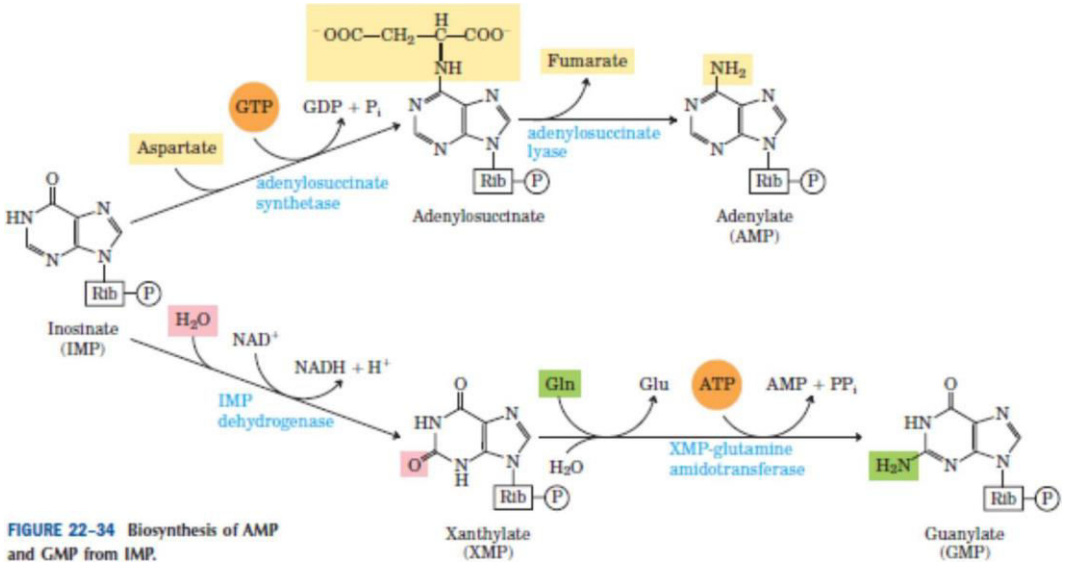


FIGURE 22–34 Biosynthesis of AMP and GMP from IMP.

E. Conversion of IMP to AMP and GMP

The conversion of IMP to either AMP or GMP uses a two-step, energy-requiring pathway. Note that the synthesis of AMP requires guanosine triphosphate (GTP) as an energy source, whereas the synthesis of GMP requires ATP. Also, the first reaction in each pathway is inhibited by the end product of that pathway. This provides a mechanism for diverting IMP to the synthesis of the species of purine present in lesser amounts. If both AMP and GMP are present in adequate amounts, the de novo pathway of purine synthesis is turned off at the amidotransferase step.

Nucleoside diphosphates (NDP) are synthesized from the corresponding nucleoside monophosphates (NMP) by base-specific nucleoside monophosphate kinases. [Note: These kinases do not discriminate between ribose or deoxyribose in the substrate.] ATP is generally the source of the transferred phosphate, because it is present in higher concentrations than the other nucleoside triphosphates. Adenylate kinase is particularly active in liver and muscle, where the turnover of energy from ATP is high. Its function is to maintain an equilibrium

among AMP, ADP, and ATP. Nucleoside diphosphates and triphosphates are interconverted by nucleoside diphosphate kinase—an enzyme that, unlike the monophosphate kinases, has broad specificity.

IV. Synthesis of Deoxyribonucleotides

The nucleotides described thus far all contain ribose (ribonucleotides). The nucleotides required for DNA synthesis, however, are 2′-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle.

A. Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is composed of two nonidentical dimeric subunits, R1 and R2, and is specific for the reduction of nucleoside diphosphates (ADP, guanidine diphosphate (GDP), cytosine diphosphate (CDP), and uridine triphosphate (UDP) to their deoxy-forms (dADP, dGDP, dCDP, and dUDP). The immediate donors of the hydrogen atoms needed for the reduction of the 2′-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond.

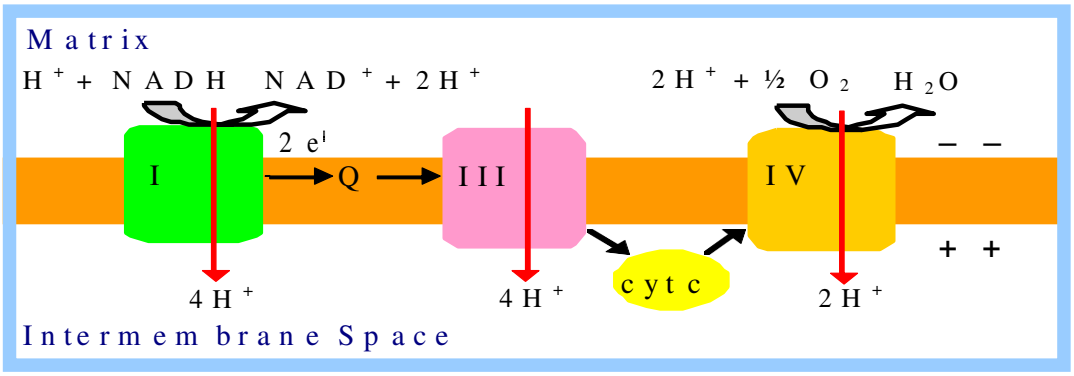
- Regeneration of reduced enzyme: In order for ribonucleotide reductase to continue to produce deoxyribonucleotides, the disulfide bond created during the production of the 2′-deoxy carbon must be reduced. The source of the reducing equivalents for this purpose is thioredoxin—a peptide coenzyme of ribonucleotide reductase. Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, in the process forming a disulfide bond.

- Regeneration of reduced thioredoxin: Thioredoxin must be converted back to its reduced form in order to continue to perform its function. The necessary reducing

equivalents are provided by NADPH + H^+, and the reaction is catalyzed by thioredoxin reductase.

Oxidative phosphorylation, Electron transfer reactions in mitochondria:

The **respiratory chain** is one of the pathways involved in *oxidative phosphorylation*. It catalyzes the steps by which electrons are transported from NADH + H^+ or reduced ubiquinone (QH_2) to molecular oxygen. Due to the wide difference between the redox potentials of the donor (NADH + H^+ or QH_2) and the acceptor (O_2), this reaction is strongly exergonic. Most of the energy released is used to establish a proton gradient across the inner mitochondrial membrane, which is then ultimately used to synthesize ATP with the help of *ATP synthase.*



A. Components of the respiratory chain

The **electron transport chain** consists of three protein complexes (**complexes I, III, and IV**), which are integrated into the inner mitochondrial membrane, and two mobile carrier molecules—**ubiquinone** (coenzyme Q) and **cytochrome *c***. *Succinate dehydrogenase,* which actually belongs to the tricarboxylic acid cycle, is also assigned to the respiratory chain as **complex II**. *ATP synthase* is sometimes referred to as **complex V**, although it is not involved in electron transport. With the

exception of complex I, detailed structural information is now available for every complex of the respiratory chain.

All of the complexes in the respiratory chain are made up of numerous polypeptides and contain a series of different protein bound **redox coenzymes**. These include *flavins* (FMN or FAD in complexes I and II), *iron–sulfur clusters* (in I, II, and III), and *heme groups* (in II, III, and IV). Of the more than 80 polypeptides in the respiratory chain, only 13 are coded by the mitochondrial genome. The remainder are encoded by nuclear genes, and have to be imported into the mitochondria after being synthesized in the cytoplasm.

Electrons enter the respiratory chain in various different ways. In the oxidation of NADH + H^+ by *complex I,* electrons pass via FMN and Fe/S clusters to ubiquinone (Q). Electrons arising during the oxidation of succinate, acyl CoA, and other substrates are passed to ubiquinone by *succinate dehydrogenase* or other *mitochondrial dehydrogenases* via enzyme-bound $FADH_2$ and the electron-transporting flavoprotein.

Ubiquinol passes electrons on to *complex III,* which transfers them via two b-type heme groups, one Fe/S cluster, and heme c1 to the small heme protein *cytochrome c*. Cytochrome *c* then transports the electrons to complex IV—*cytochrome c oxidase.* Cytochrome *c* oxidase contains redox-active components in the form of two copper centers (CuA and CuB) and hemes a and a3, through which the electrons finally reach *oxygen*.

As the result of the two-electron reduction of O_2, the strongly basic O_2^- anion is produced (at least formally), and this is converted into water by binding of two protons. The electron transfer is coupled to the **formation of a proton gradient** by complexes I, III, and IV.

Organization

Proton transport via complexes I, III, and IV takes place *vectorially* from the matrix into the intermembrane space. When electrons are being transported through the respiratory chain, the H+ concentration in this space increases—i. e., the pH value there is reduced by about one pH unit. For each H_2O molecule formed, around 10 H^+ ions are pumped into the intermembrane space. If the inner membrane is intact, then generally only *ATP synthase* can allow protons to

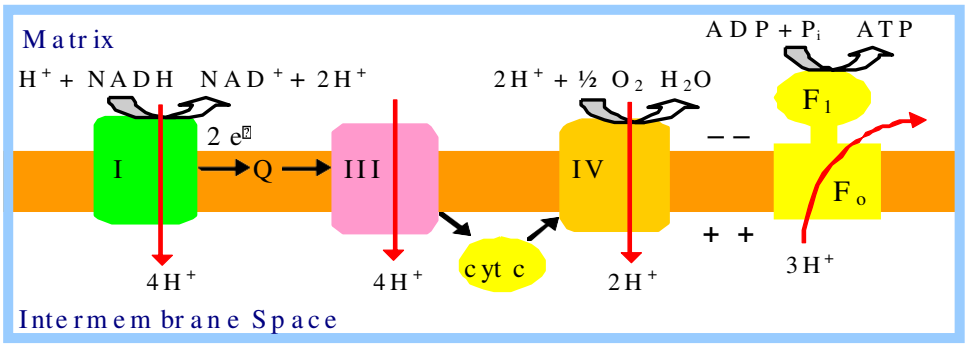
flow back into the matrix. This is the basis for the coupling of electron transport to ATP synthesis, which is important for regulation purposes.

As mentioned, although complexes I through V are all integrated into the inner membrane of themitochondrion, they are not usually in contact with one another, since the electrons are transferred by ubiquinone and cytochrome *c*. With its long apolar side chain, ubiquinone is freely mobile within the membrane.

Cytochrome *c* is water-soluble and is located on the *outside* of the inner membrane. NADH oxidation via complex I takes place on the *inside* of the membrane—i. e., in the matrix space, where the tricarboxylic acid cycle and β-oxidation (the most important sources of NADH) are also located. O_2 reduction and ATP formation also take place in the matrix.

ATP synthesis

In the **respiratory chain**, electrons are transferred from NADH or ubiquinol (QH_2) to O_2. The energy obtained in this process is used to establish a proton gradient across the inner mitochondrial membrane. ATP synthesis is ultimately coupled to the return of protons from the intermembrane space into the matrix.



A. Redox systems of the respiratory chain

The electrons provided by NADH do not reach oxygen directly, but instead are transferred to it in various steps. They pass through at least 10 intermediate redox systems,most of which are bound as **prosthetic groups** in complexes I, III, and IV. The large number of coenzymes

involved in electron transport may initially appear surprising. However, in redox reactions, the *change in free enthalpy* ΔG—i. e., the chemical work that is done—depends only on the difference in redox potentials ΔE between the donor and the acceptor.

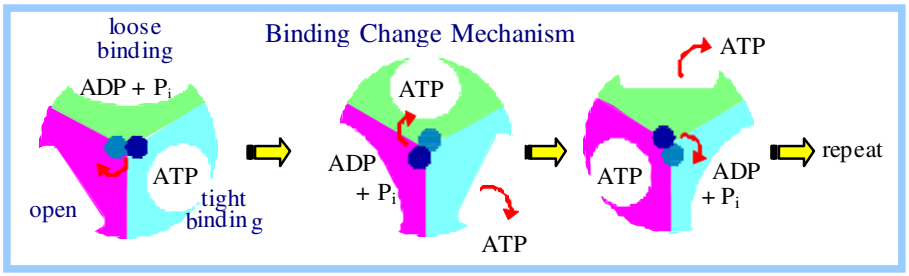
Introducing additional redox systems does not alter the reaction's overall energy yield. In the case of the respiratory chain, the difference between the normal potential of the donor ($NAD^+/NADH + H^+$, $E_0^- = -0.32$ V) and that of the acceptor (O_2/H_2O, $E_0^- = +0.82$ V) corresponds to an energy difference ΔG^0 of more than 200 kJ mol^{-1}. This large amount is divided into smaller, more manageable "packages," the size of which is determined by the difference in redox potentials between the respective *intermediates*. It is assumed that this division is responsible for the astonishingly high energy yield (about 60%) achieved by the respiratory chain. The illustration shows the important redox systems involved in mitochondrial electron transport and their approximate redox potentials.

These potentials determine the path followed by the electrons, as the members of a **redox series** have to be arranged in order of increasing redox potential if transport is to occur spontaneously. In complex 1, the electrons are passed from *NADH + H^+* first to *FMN* and then on to several *iron–sulfur (Fe/S) clusters.* These redox systems are only stable in the interior of proteins. Depending on the type, Fe/S clusters may contain two to six iron ions, which form complexes with inorganic sulfide and the SH groups of cysteine residues. *Ubiquinone* (coenzyme Q) is a mobile carrier that takes up electrons from complexes I and II and from reduced ETF and passes them on to complex III.

Heme groups are also involved in electron transport in a variety of ways. Type b hemes correspond to that found in hemoglobin. Heme c in cytochrome *c* is covalently bound to the protein, while the tetrapyrrole ring of heme a is isoprenylated and carries a formyl group. In complex IV, a *copper ion* (CuB) and heme a3 react directly with oxygen.

B. ATP synthase

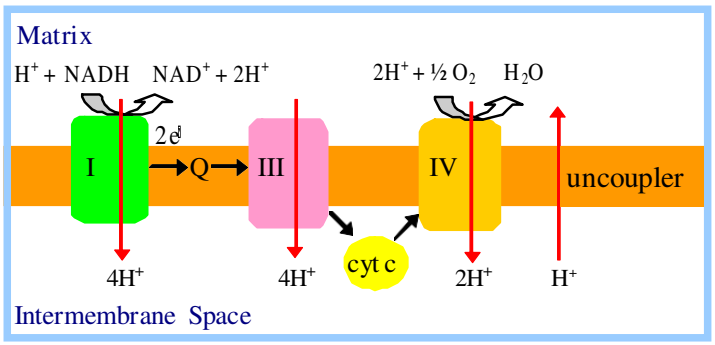
The ATP synthase (*EC 3.6.1.34,* complex V) that transports H^+ is a complex molecular machine. The enzyme consists of two parts—a *proton channel* (F_o, for "oligomycin-sensitive") that is integrated into the membrane; and a *catalytic unit* (F_1) that protrudes into the matrix. The F_o part consists of 12 membrane spanning c-peptides and one a-subunit. The "head" of the F1 part is composed of three α and three β subunits, between which there are three active centers. The "stem" between F_o and F_1 consists of one γ and one ε subunit. Two more polypeptides, b and δ, form a kind of "stator," fixing the α and β subunits relative to the F_o part.



The catalytic cycle can be divided into three phases, through each of which the three active sites pass in sequence. First, ADP and Pi are bound (**1**), then the anhydride bond forms (**2**), and finally the product is released (**3**). Each time protons pass through the F_o channel protein into the matrix, all three active sites change from their current state to the next. It has been shown that the energy for proton transport is initially converted into a rotation of the γ subunit, which in turn cyclically alters the conformation of the α and β subunits, which are stationary relative to the F_o part, and thereby drives ATP synthesis.

Uncouplers

Substances that functionally separate oxidation and phosphorylation from one another are referred to as uncouplers. They break down the proton gradient by allowing H^+ ions to pass from the intermembrane space back into the mitochondrial matrix without the involvement of ATP synthase.



Uncoupling effects are produced by **mechanical damage** to the inner membrane or by lipid-soluble substances that can transport protons through the membrane, such as **2,4-dinitrophenol** (DNP, **2**). **Thermogenin** (uncoupling protein-1, UCP-1, **3**)—an ion channel in mitochondria of *brown fat* tissue—is a naturally occurring uncoupler.

Brown fat is found, for example, in newborns and in hibernating animals, and serves exclusively to generate heat. In cold periods, norepinephrine activates the *hormone-sensitive lipase*. Increased lipolysis leads to the production of large quantities of free fatty acids. Like DNP, these bind H^+ ions in the intermembrane space, pass the UCP in this form, and then release the protons in the matrix again. This makes fatty acid degradation independent of ADP availability—i. e., it takes place at maximum velocity and only produces heat. It is becoming increasingly clear that there are also UCPs in other cells, which are controlled by hormones such as thyroxine. This regulates the ATP yield and what is known as the basal metabolic rate.