Unit-4 Respiration, Catabolism & Biosynthesis of Nucleic acid

❖ Respiration

Respiration is the set of metabolic reactions and processes that take place in the cells of organisms to convert biochemical energy from nutrients into adenosine triphosphate (ATP), and then release waste products. The reactions involved in respiration are catabolic reactions, which break large molecules into smaller ones, releasing energy in the process, as weak so-called "high-energy" bonds are replaced by stronger bonds in the products. Respiration is one of the key ways a cell gains useful energy to fuel cellular activity. Cellular respiration is considered an exothermic redox reaction which releases heat. The overall reaction occurs in a series of biochemical steps, most of which are redox reactions themselves. Although technically, cellular respiration is a combustion reaction, it clearly does not resemble one when it occurs in a living cell due to slow release of energy from the series of reactions.

Nutrients that are commonly used by animal and plant cells in respiration include sugar, amino acids and fatty acids, and the most common oxidizing agent (electron acceptor) is molecular oxygen (O_2). The chemical energy stored in ATP (its third phosphate group is weakly bonded to the rest of the molecule and is cheaply broken allowing stronger bonds to form, thereby transferring energy for use by the cell) can then be used to drive processes requiring energy, including biosynthesis, locomotion or transportation of molecules across cell membranes.

➢ Aerobic respiration

Aerobic respiration requires oxygen (O_2) in order to generate ATP. Although carbohydrates, fats, and proteins are consumed as reactants, it is the preferred method of pyruvate breakdown in glycolysis and requires that pyruvate enter the mitochondria in order to be fully oxidized by the Krebs cycle. The products of this process are carbon dioxide and water, but the energy transferred is used to break strong bonds in ADP as the third phosphate group is added to form ATP (adenosine triphosphate), by substrate-level phosphorylation, NADH and $FADH_2$

Simplified reaction: $C_6H_{12}O_6$ (s) + 6 O_2 (g) → 6 CO_2 (g) + 6 H_2O (l) + heat

$\Delta G = -2880$ kJ per mol of $C_6H_{12}O_6$

The negative ΔG indicates that the reaction can occur spontaneously.

The potential of NADH and $FADH_2$ is converted to more ATP through an electron transport chain with oxygen as the "terminal electron acceptor". Most of the ATP produced by aerobic cellular respiration is made by oxidative phosphorylation. This works by the energy released in the consumption of pyruvate being used to create a chemiosmotic potential by pumping protons across a membrane. This potential is then used to drive ATP synthase and produce ATP from ADP and a phosphate group. Aerobic metabolism is up to 15 times more efficient than anaerobic metabolism.

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❖ Respiratory Electron Transport Chain (RETC)

An **electron transport chain** (ETC) is a series of compounds that transfer electrons from electron donors to electron acceptors via redox reactions, and couples this electron transfer with the transfer of protons (H^+ ions) across a membrane. This creates an electrochemical proton gradient that drives ATP synthesis, or the generation of chemical energy in the form of adenosine triphosphate (ATP). The final acceptor of electrons in the electron transport chain is molecular oxygen.

Electron transport chains are used for extracting energy via redox reactions from sunlight in photosynthesis or, such as in the case of the oxidation of sugars, cellular respiration. In eukaryotes, an important electron transport chain is found in the inner mitochondrial membrane where it serves as the site of oxidative phosphorylation through the use of ATP synthase. It is also found in the thylakoid membrane of the chloroplast in photosynthetic eukaryotes. In bacteria, the electron transport chain is located in their cell membrane.

In chloroplasts, light drives the conversion of water to oxygen and $NADP^+$ to NADPH with transfer of H^+ ions across chloroplast membranes. In mitochondria, it is the conversion of oxygen to water, NADH to NAD^+ and succinate to fumarate that are required to generate the proton gradient.

Electron transport chains are major sites of premature electron leakage to oxygen, generating superoxide and potentially resulting in increased oxidative stress.

- **Location**

Electron transport requires a membrane in order to work. In prokaryotic cells, those of bacteria and bacteria-like Archaeans, electron transport takes place in the plasma membrane, in folded areas called mesosomes.

In eukaryotic cells, the more evolutionarily advanced and complex cells of animals, plants and fungi, electron transport takes place in cellular organelles known as mitochondria, where these tiny eukaryotic power factories break down food to make ATP.

In addition to having mitochondria to turn food into ATP, plant cells also have organelles called chloroplasts with an internal thylakoid membrane, where electron transport chains use sunlight to make ATP.

- **Functions**

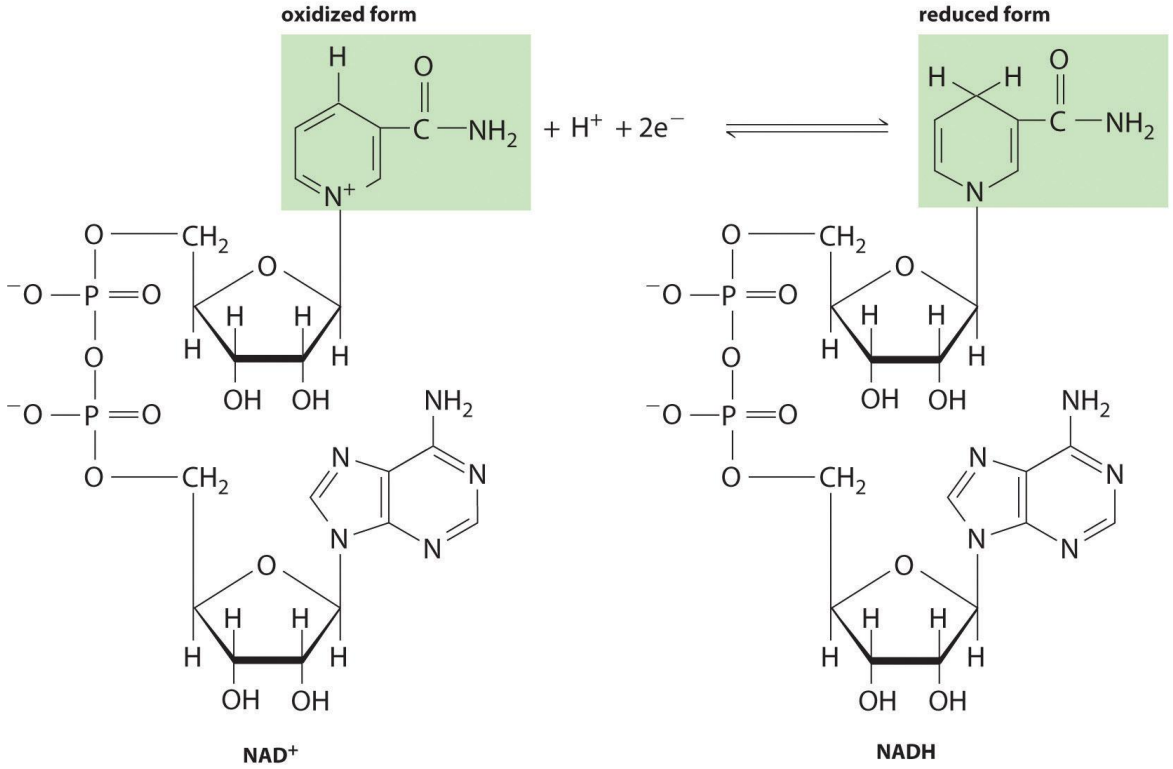
The function of the electron transport chain is to produce a proton electrochemical gradient across mitochondrial inner membrane as a result of the redox reactions. If protons flow back through the membrane, they enable mechanical work, such as rotating bacterial flagella. ATP synthase, an enzyme highly conserved among all domains of life, converts this mechanical work into chemical energy by producing ATP, which powers most cellular reactions.

- **Components of ETC**

The electron transport chain of the mitochondria is the means by which electrons are removed from the reduced carrier NADH and transferred to oxygen to yield H_2O.

1) NAD (Nicotinamide Adenine Dinucleotide)
2) FAD (Flavin Adenine Dinucleotide) / Flavoproteins
3) Coenzyme Q (CoQ) / Ubiquinone (UQ)
4) Cytochromes
5) Iron-Sulfur Proteins

1) NAD (Nicotinamide Adenine Dinucleotide)



NADH is generated in the matrix by the reactions of pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehyrogenase. The electron transport chain begins with reoxidizing NADH to form NAD+ and channeling the electrons into the formation of reduced coenzymes. Important to note that NADH transfers 2 electrons at a time in the form of a hydride.

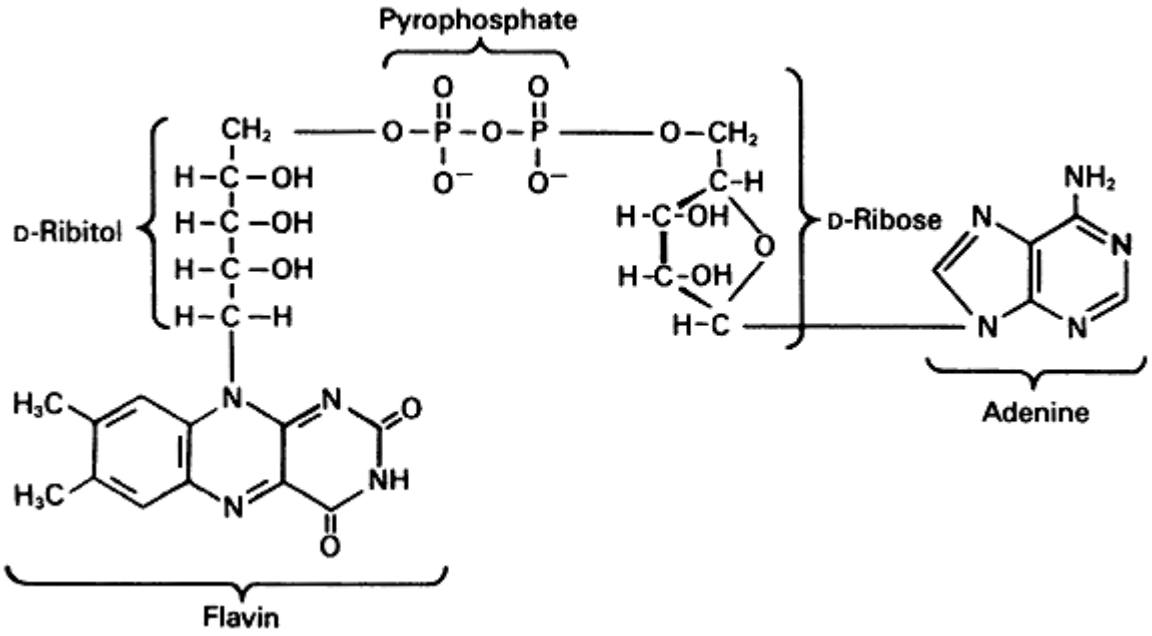
$$NAD^+ + 2e^- + H^+ \longrightarrow NADH$$

2) FAD (Flavin Adenine Dinucleotide) / Flavoproteins

Flavoproteins have either a FAD (flavin adenosine dinucleotide) or a FMN (flavin mononucleotide) prosthetic group. Flavoproteins can accept or donate electrons one at time or two at a time. Thus they are often intermediaries between two electron acceptors/donors and one electron acceptors/donors. For flavoproteins the typical standard reduction potentials are around 0 V.

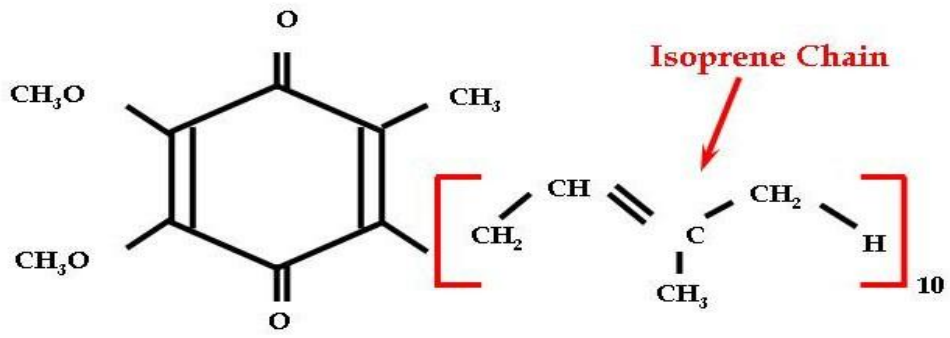
$FAD + 2e^- + 2H^+ \longrightarrow FADH_2$

$FMN + 2e^- + 2H^+ \longrightarrow FMNH_2$

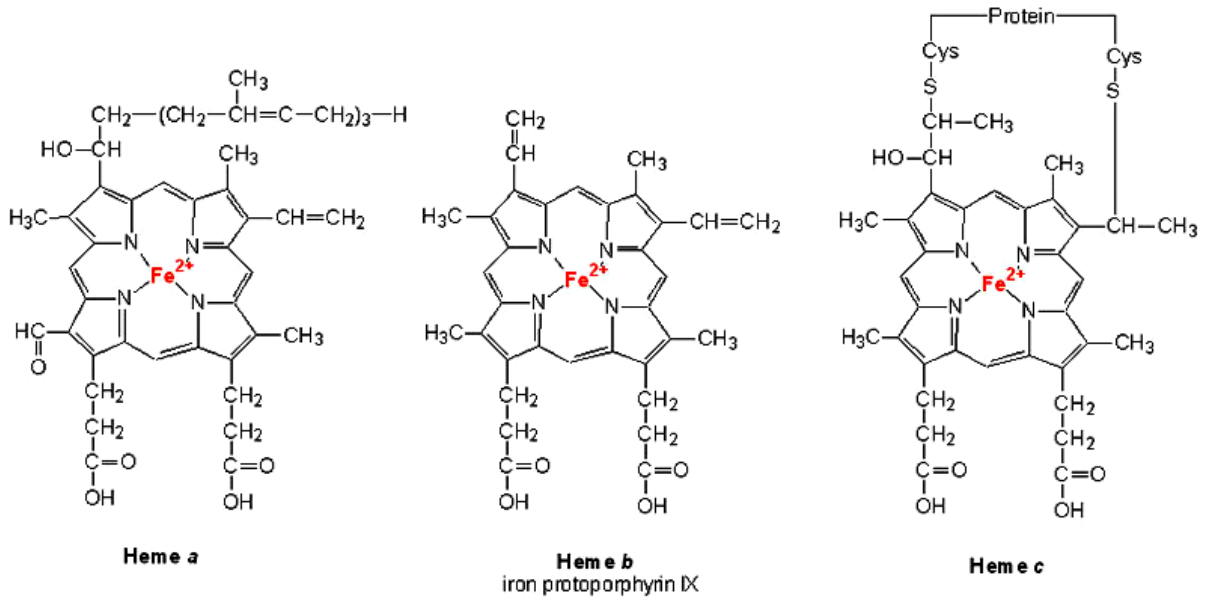


3) Coenzyme Q (CoQ) / Ubiquinone (UQ)

CoQ has ten repeating isoprene units which make it insoluble in water, but soluble in the hydrophobic lipid bylayer. Coenzyme Q is a versatile cofactor because it is a soluble electron carrier in the hydrophobic bilipid layer of the inner mitochondrial membrane. Like flavoproteins, CoQ can accept/donate electrons one at a time or two at a time.



4) Cytochromes



Cytochromes are proteins that contain heme prosthetic groups which function as one electron carriers. The heme iron is involved in one electron transfer s involving the Fe^{2+} and Fe^{3+} oxidation states.

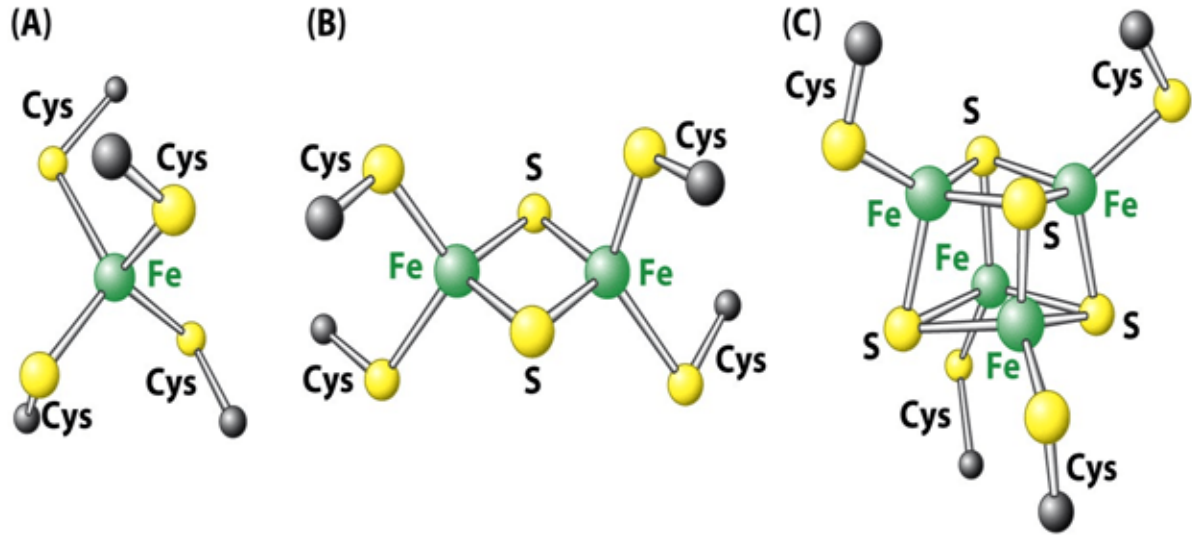
Cytochromes are named by their absorption spectra which depend on the porphyrin structure and environment. The example shown is the heme prosthetic group of cytochrome b. Cytochrome b contains the same iron porphyrin found in hemoglobin and myoglobin. Other cytochromes we will encounter in the electron transport complexes are cytochromes b, c, c_1, a and a_3.

5) Iron-Sulfur Proteins

In the electron transport chain we will encounter many iron-sulfur proteins which participate in one electron transfers involving the Fe^{2+} and Fe^{3+} oxidation states.

These are non-heme iron-sulfur proteins. The simplest iron-sulfur protein is FeS in which iron is tetrahedrally coordinated by four cysteines. The second form is Fe_2S_2 which contains two irons complexed to 2 cysteine residues and two inorganic sulfides. The third form is Fe_3S_4 which contains 3 iron atoms coordinated to three cysteine residues and 4 inorganic sulfides.

The last form is the most complicated Fe_4S_4which contains 4 iron atoms coordinated to 4 cysteine residues and 4 inorganic sulfides.



➢ **Electron transport chains in mitochondria**

Most eukaryotic cells have mitochondria, which produce ATP from products of the citric acid cycle, fatty acid oxidation, and amino acid oxidation. At the mitochondrial inner membrane, electrons from NADH and succinate pass through the electron transport chain to oxygen, which is reduced to water. The electron transport chain comprises an enzymatic series of electron donors and acceptors. Each electron donor passes electrons to a more electronegative acceptor, which in turn donates these electrons to another acceptor, a process that continues down the series until electrons are passed to oxygen, the most electronegative and terminal electron acceptor in the chain. Passage of electrons between donor and acceptor releases energy, which is used to generate a proton gradient across the mitochondrial membrane by actively "pumping" protons into the intermembrane space, producing a thermodynamic state that has the potential to do work. The entire process is called oxidative phosphorylation, since ADP is phosphorylated to ATP using the energy of hydrogen oxidation in many steps.

A small percentage of electrons do not complete the whole series and instead directly leak to oxygen, resulting in the formation of the free-radical superoxide, a highly reactive molecule that contributes to oxidative stress and has been implicated in a number of diseases and aging.

Mitochondrial redox carriers

Energy obtained through the transfer of electrons (blue arrows) down the ETC is used to pump protons (red arrows) from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient across the mitochondrial inner membrane (IMM) called $\Delta\Psi$. This electrochemical proton gradient allows ATP synthase (ATP-ase) to use the flow of H^+ through the enzyme back into the matrix to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. Complex I (NADH coenzyme Q reductase; labeled I) accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled Q), which also receives electrons from complex II (succinate dehydrogenase; labeled II). UQ passes electrons to complex III (cytochrome bc_1 complex; labeled III), which passes them to cytochrome *c* (cyt *c*). Cyt *c* passes electrons to Complex IV (cytochrome *c* oxidase; labeled IV), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex transmembrane structure that is embedded in the inner membrane. Three of them are proton pumps. The structures are electrically connected by lipid-soluble electron carriers and water-soluble electron carriers. The overall electron transport chain:

$$\mathbf{NADH+H^+} \rightarrow \textbf{\textit{Complex I}} \rightarrow \mathbf{Q} \rightarrow \textbf{\textit{Complex III}} \rightarrow \textbf{cytochrome } \textbf{\textit{c}} \rightarrow \textbf{\textit{Complex IV}} \rightarrow \mathbf{O_2}$$

$$\uparrow$$

$$\textbf{\textit{Complex II}}$$

$$\uparrow$$

$$\textbf{\textit{Succinate}}$$

Complex I

In *Complex I* (NADH:ubiquinone oxidoreductase, NADH-CoQ reductase, or NADH dehydrogenase), two electrons are removed from NADH and transferred to a lipid-soluble carrier, ubiquinone (Q). The reduced product, ubiquinol (QH_2), freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of superoxide.

The pathway of electrons is as follows:

NADH is oxidized to NAD^+, by reducing Flavin mononucleotide to $FMNH_2$ in one two-electron step. $FMNH_2$ is then oxidized in two one-electron steps, through a semiquinone intermediate. Each electron thus transfers from the $FMNH_2$ to an Fe-S cluster, from the Fe-S cluster to ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH_2. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space.

Complex II

In *Complex II* (succinate dehydrogenase or succinate-CoQ reductase) additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II consists of four protein subunits: SDHA, SDHB, SDHC, and SDHD. Other electron donors (e.g., fatty acids and glycerol 3-phosphate) also direct electrons into Q (via FAD). Complex 2 is a parallel electron transport pathway to complex 1, but unlike complex 1, no protons are transported to the intermembrane space in this pathway. Therefore, the pathway through complex 2 contributes less energy to the overall electron transport chain process.

Complex III

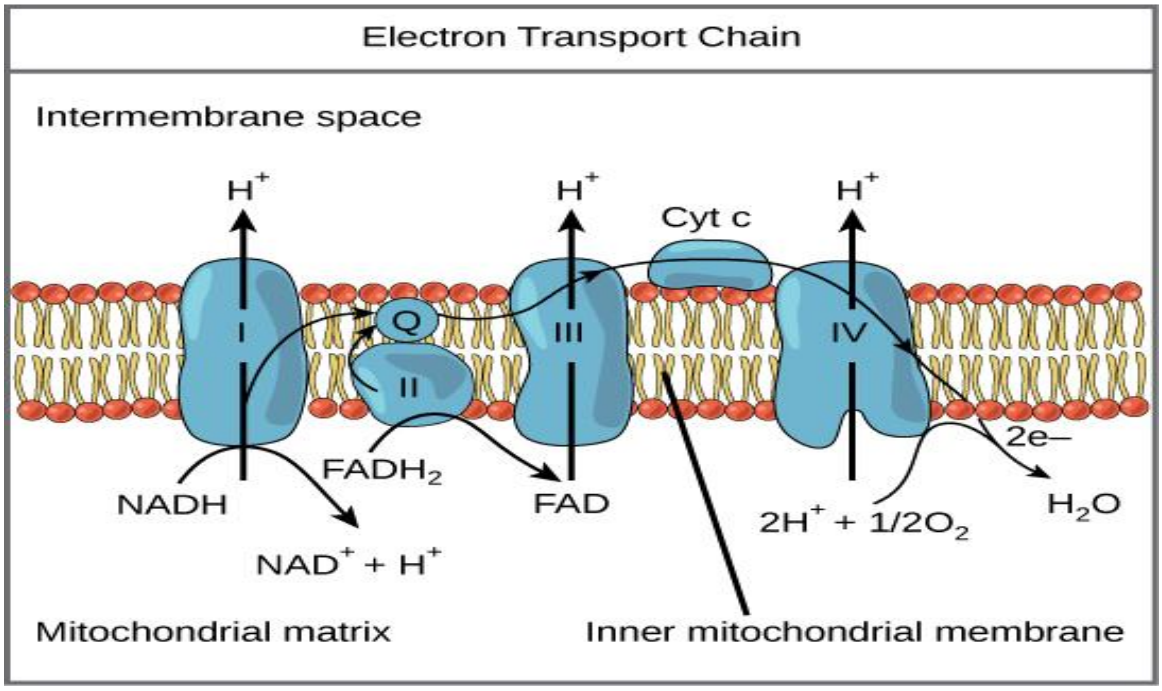
In *Complex III* (cytochrome bc_1 complex or $CoQH_2$-cytochrome *c* reductase), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH_2 at the Q_O site and sequentially transferred to two molecules of cytochrome *c*, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Q_i site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by one quinol (2H+2e-) oxidations at the Q_o site to form one quinol (2H+2e-) at the Q_i site. (in total four protons are translocated: two protons reduce quinone to quinol and two protons are released from two ubiquinol molecules).

$$QH2 + 2 \text{ cytochrome c (FeIII)} + 2\ H^+\text{in} \rightarrow Q + 2 \text{ cytochrome c (FeII)} + 4\ H^+\text{out}$$

When electron transfer is reduced (by a high membrane potential or respiratory inhibitors such as antimycin A), Complex III may leak electrons to molecular oxygen, resulting in superoxide formation.

Complex IV

In *Complex IV* (cytochrome *c* oxidase), sometimes called cytochrome A3, four electrons are removed from four molecules of cytochrome *c* and transferred to molecular oxygen (O_2), producing two molecules of water. At the same time, four protons are removed from the mitochondrial matrix (although only two are translocated across the membrane), contributing to the proton gradient. The activity of cytochrome c oxidase is inhibited by cyanide.



In the mitochondrial electron transport chain electrons move from an electron donor (NADH or QH_2) to a terminal electron acceptor (O_2) via a series of redox reactions. These reactions are coupled to the creation of a proton gradient across the mitochondrial inner membrane. There are three proton pumps: *I*, *III*, and *IV*. The resulting transmembrane proton gradient is used to make ATP via ATP synthase.

The reactions catalyzed by *Complex I* and *Complex III* work roughly at equilibrium. This means that these reactions are readily reversible, by increasing the concentration of the products relative to the concentration of the reactants (for example, by increasing the proton gradient). ATP synthase is also readily

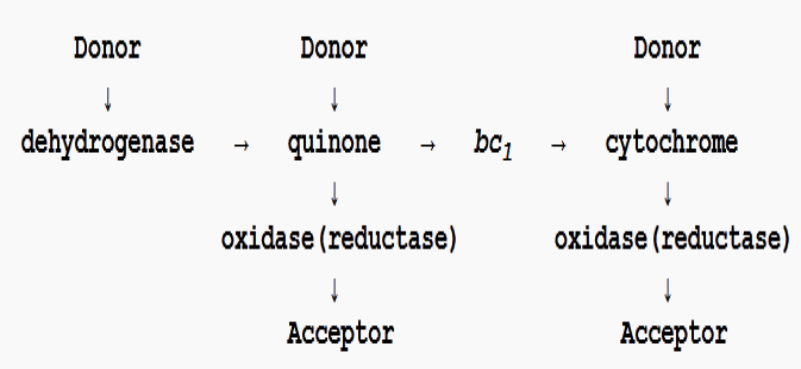
reversible. Thus ATP can be used to build a proton gradient, which in turn can be used to make NADH. This process of *reverse electron transport* is important in many prokaryotic electron transport chains.

- **Electron transport chains in bacteria**

In eukaryotes, NADH is the most important electron donor. The associated electron transport chain is

NADH → *Complex I* → Q → *Complex III* → cytochrome *c* → *Complex IV* → O_2 where *Complexes I, III* and *IV* are proton pumps, while Q and cytochrome *c* are mobile electron carriers. The electron acceptor is molecular oxygen.

In prokaryotes (bacteria and archaea) the situation is more complicated, because there are several different electron donors and several different electron acceptors. The generalized electron transport chain in bacteria is:



Note that electrons can enter the chain at three levels: at the level of a dehydrogenase, at the level of the quinone pool, or at the level of a mobile cytochrome electron carrier. These levels correspond to successively more positive redox potentials, or to successively decrease potential differences relative to the terminal electron acceptor. In other words, they correspond to successively smaller Gibbs free energy changes for the overall redox reaction

Donor → *Acceptor*.

Individual bacteria use multiple electron transport chains, often simultaneously. Bacteria can use a number of different electron donors, a number of different dehydrogenases, a number of different oxidases and reductases, and a number of different electron acceptors. For example, *E. coli* (when growing aerobically using glucose as an energy source) uses two different NADH dehydrogenases and two different quinol oxidases, for a total of four different electron transport chains operating simultaneously.

A common feature of all electron transport chains is the presence of a proton pump to create a transmembrane proton gradient. Bacterial electron transport chains may contain as many as three proton pumps, like mitochondria, or they may contain only one or two. They always contain at least one proton pump.

Electron donors

In the present day biosphere, the most common electron donors are organic molecules. Organisms that use organic molecules as an energy source are called *organotrophs*. Organotrophs (animals, fungi, protists) and *phototrophs* (plants and algae) constitute the vast majority of all familiar life forms.

Some prokaryotes can use inorganic matter as an energy source. Such organisms are called *lithotrophs* ("rock-eaters"). Inorganic electron donors include hydrogen, carbon monoxide, ammonia, nitrite, sulfur, sulfide, and ferrous iron. Lithotrophs have been found growing in rock formations thousands of meters below the surface of Earth. Because of their volume of distribution, lithotrophs may actually outnumber organotrophs and phototrophs in our biosphere.

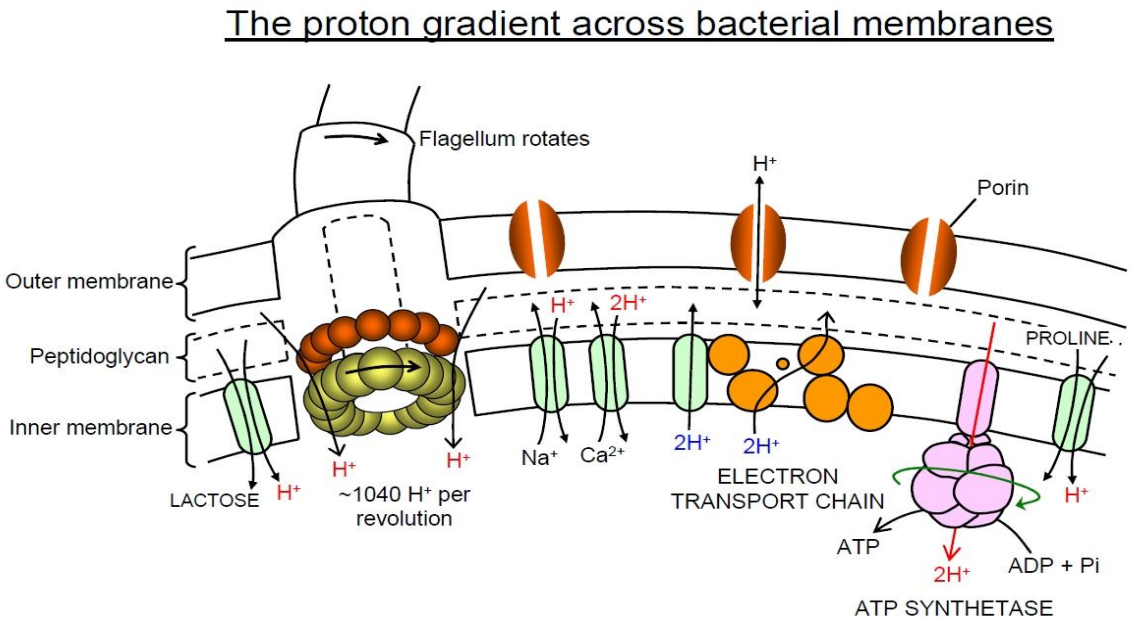
The use of inorganic electron donors as an energy source is of particular interest in the study of evolution. This type of metabolism must logically have preceded the use of organic molecules as an energy source.

Dehydrogenases

Bacteria can use a number of different electron donors. When organic matter is the energy source, the donor may be NADH or succinate, in which case electrons enter the electron transport chain via NADH dehydrogenase (similar to *Complex I* in mitochondria) or succinate dehydrogenase (similar to *Complex II*). Other dehydrogenases may be used to process different energy sources: formate dehydrogenase, lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, H_2 dehydrogenase (hydrogenase), etc. Some dehydrogenases are also proton pumps; others funnel electrons into the quinone pool. Most dehydrogenases show induced expression in the bacterial cell in response to metabolic needs triggered by the environment in which the cells grow.

Quinone carriers

Quinones are mobile, lipid-soluble carriers that shuttle electrons (and protons) between large, relatively immobile macromolecular complexes embedded in the membrane. Bacteria use *ubiquinone* (the same quinone that mitochondria use) and related quinones such as *menaquinone*. Another name for ubiquinone is *Coenzyme Q10.*



Protons are pumped out across the membrane by the electron transport system (ETS or electron transport chain, ETC) similar to the case in mitochondria (using NADH as energy source, derived from glycolysis and Kreb's cycle) create a proton gradient used in ion and metabolite transport as well as ATP synthesis and flagella rotation. (Based on Boyd 1988)

Proton pumps

A *proton pump* is any process that creates a proton gradient across a membrane. Protons can be physically moved across a membrane; this is seen in mitochondrial *Complexes I* and *IV*. The same effect can be produced by moving electrons in the opposite direction. The result is the disappearance of a proton from the cytoplasm and the appearance of a proton in the periplasm. Mitochondrial *Complex III* uses this second type of proton pump, which is mediated by a quinone (the Q cycle).

Some dehydrogenases are proton pumps; others are not. Most oxidases and reductases are proton pumps, but some are not. Cytochrome bc_1 is a proton pump found in many, but not all, bacteria (it is not found in *E. coli*). As the name implies, bacterial bc_1 is similar to mitochondrial bc_1 (*Complex III*).

Proton pumps are the heart of the electron transport process. They produce the transmembrane electrochemical gradient that enables ATP Synthase to synthesize ATP.

Cytochrome electron carriers

Cytochromes are pigments that contain iron. They are found in two very different environments.

Some cytochromes are water-soluble carriers that shuttle electrons to and from large, immobile macromolecular structures imbedded in the membrane. The mobile cytochrome electron carrier in mitochondria is cytochrome *c*. Bacteria use a number of different mobile cytochrome electron carriers.

Other cytochromes are found within macromolecules such as *Complex III* and *Complex IV*. They also function as electron carriers, but in a very different, intramolecular, solid-state environment.

Electrons may enter an electron transport chain at the level of a mobile cytochrome or quinone carrier. For example, electrons from inorganic electron donors (nitrite, ferrous iron, etc.) enter the electron transport chain at the cytochrome level. When electrons enter at a redox level greater than NADH, the electron transport chain must operate in reverse to produce this necessary, higher-energy molecule.

Terminal oxidases and reductases

When bacteria grow in aerobic environments, the terminal electron acceptor (O_2) is reduced to water by an enzyme called an *oxidase*. When bacteria grow in anaerobic environments, the terminal electron acceptor is reduced by an enzyme called a *reductase*.

In mitochondria the terminal membrane complex (*Complex IV*) is cytochrome oxidase. Aerobic bacteria use a number of different terminal oxidases. For example, *E. coli* does not have a cytochrome oxidase or a bc_1 complex. Under aerobic conditions, it uses two different terminal quinol oxidases (both proton pumps) to reduce oxygen to water.

Anaerobic bacteria, which do not use oxygen as a terminal electron acceptor, have terminal reductases individualized to their terminal acceptor. For example, *E. coli* can use fumarate reductase, nitrate reductase, nitrite reductase,

DMSO reductase, or trimethylamine-N-oxide reductase, depending on the availability of these acceptors in the environment.

Most terminal oxidases and reductases are *inducible*. They are synthesized by the organism as needed, in response to specific environmental conditions.

Electron acceptors

Just as there are a number of different electron donors (organic matter in organotrophs, inorganic matter in lithotrophs), there are a number of different electron acceptors, both organic and inorganic. If oxygen is available, it is invariably used as the terminal electron acceptor, because it generates the greatest Gibbs free energy change and produces the most energy.

In anaerobic environments, different electron acceptors are used, including nitrate, nitrite, ferric iron, sulfate, carbon dioxide, and small organic molecules such as fumarate.

Since electron transport chains are redox processes, they can be described as the sum of two redox pairs. For example, the mitochondrial electron transport chain can be described as the sum of the NAD^+/NADH redox pair and the O_2/H_2O redox pair. NADH is the electron donor and O_2 is the electron acceptor.

Not every donor-acceptor combination is thermodynamically possible. The redox potential of the acceptor must be more positive than the redox potential of the donor. Furthermore, actual environmental conditions may be far different from *standard* conditions (1 molar concentrations, 1 atm partial pressures, pH = 7), which apply to *standard* redox potentials. For example, hydrogen-evolving bacteria grow at an ambient partial pressure of hydrogen gas of 10^{-4} atm. The associated redox reaction, which is thermodynamically favorable in nature, is thermodynamic impossible under "standard" conditions.

Bacterial electron transport pathways are, in general, inducible. Depending on their environment, bacteria can synthesize different transmembrane complexes and produce different electron transport chains in their cell membranes. Bacteria select their electron transport chains from a DNA library containing multiple possible dehydrogenases, terminal oxidases and terminal reductases. The situation is often summarized by saying that electron transport chains in bacteria are *branched*, *modular*, and *inducible*.

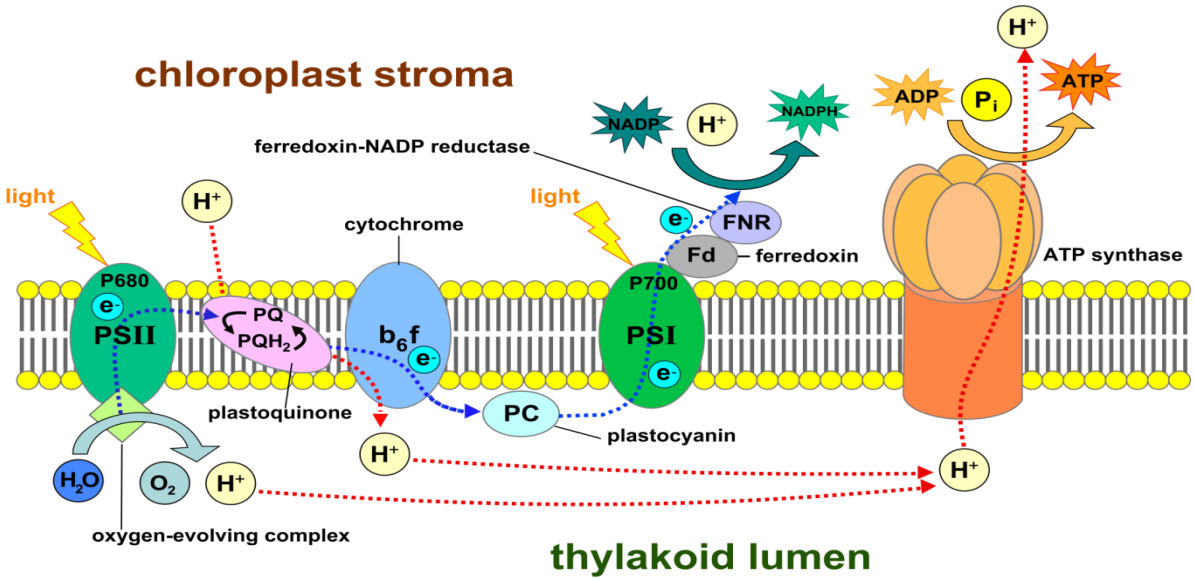
➢ Photosynthetic electron transport chains

In oxidative phosphorylation, electrons are transferred from a low-energy electron donor (e.g., NADH) to an acceptor (e.g., O_2) through an electron transport chain. In photophosphorylation, the energy of sunlight is used to *create* a high-energy electron donor and an electron acceptor. Electrons are then transferred from the donor to the acceptor through another electron transport chain.

Photosynthetic electron transport chains have many similarities to the oxidative chains discussed above. They use mobile, lipid-soluble carriers (quinones) and mobile, water-soluble carriers (cytochromes, etc.). They also contain a proton pump. It is remarkable that the proton pump in *all* photosynthetic chains resembles mitochondrial *Complex III*.

Photosynthetic electron transport chains are discussed in greater detail in the articles Photophosphorylation, Photosynthesis, Photosynthetic reaction center and Light-dependent reaction.

Electron transport chains are redox reactions that transfer electrons from an electron donor to an electron acceptor. The transfer of electrons is coupled to the translocation of protons across a membrane, producing a proton gradient. The proton gradient is used to produce useful work. About 30 work units are produced per electron transport.



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❖ Tricarboxylic acid (TCA) cycle / Kreb's cycle / Citric acid cycle

The **Citric acid cycle** – also known as the T**ricarboxylic acid** (**TCA**) **cycle** or the **Kreb's cycle** – is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetate derived from carbohydrates, fats and proteins into carbon dioxide and chemical energy in the form of adenosine triphosphate (ATP).

The cycle begins with the reaction between acetyl-CoA and the four-carbon oxaloacetate to form six-carbon citric acid. Through the next steps of the cycle, two of the six carbons of the citric acid leave as carbon dioxide to ultimately yield the four carbon product, oxaloacetate, which is used again in the first step of the next cycle. During the eight reactions that take place, for every molecule of acetyl-CoA the cycle produces three NADH and one flavin adenine dinucleotide ($FAD/FADH_2$), along with one molecule of ATP.

The name of this metabolic pathway is derived from citric acid (a type of tricarboxylic acid) that is consumed and then regenerated by this sequence of reactions to complete the cycle. In addition, the cycle consumes acetate (in the form of acetyl-CoA) and water, reduces NAD^+ to NADH, and produces carbon dioxide as a waste byproduct. The NADH generated by the TCA cycle is fed into the oxidative phosphorylation (electron transport) pathway. The net result of these two closely linked pathways is the oxidation of nutrients to produce usable chemical energy in the form of ATP.

In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. In prokaryotic cells, such as bacteria which lack mitochondria, the TCA reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the cell's surface (plasma membrane) rather than the inner membrane of the mitochondrion

Pyruvate dehydrogenase complex (PDC)

It is a complex of three enzymes that convert pyruvate into acetyl-CoA by a process called pyruvate decarboxylation. Acetyl-CoA may then be used in the citric acid cycle to carry out cellular respiration, and this complex links the glycolysis metabolic pathway to the citric acid cycle.

Enzyme	Abbrevation	Cofactor(s)
Pyruvate dehydrogenase	E1	TPP (Thiamine pyrophosphate)
Pihydrolipoyl transacetylase	E2	Lipoate Coenzyme A
Dihydrolipoyl dehydrogenase	E3	FAD NAD^+

- **Pyruvate dehydrogenase (E1)**

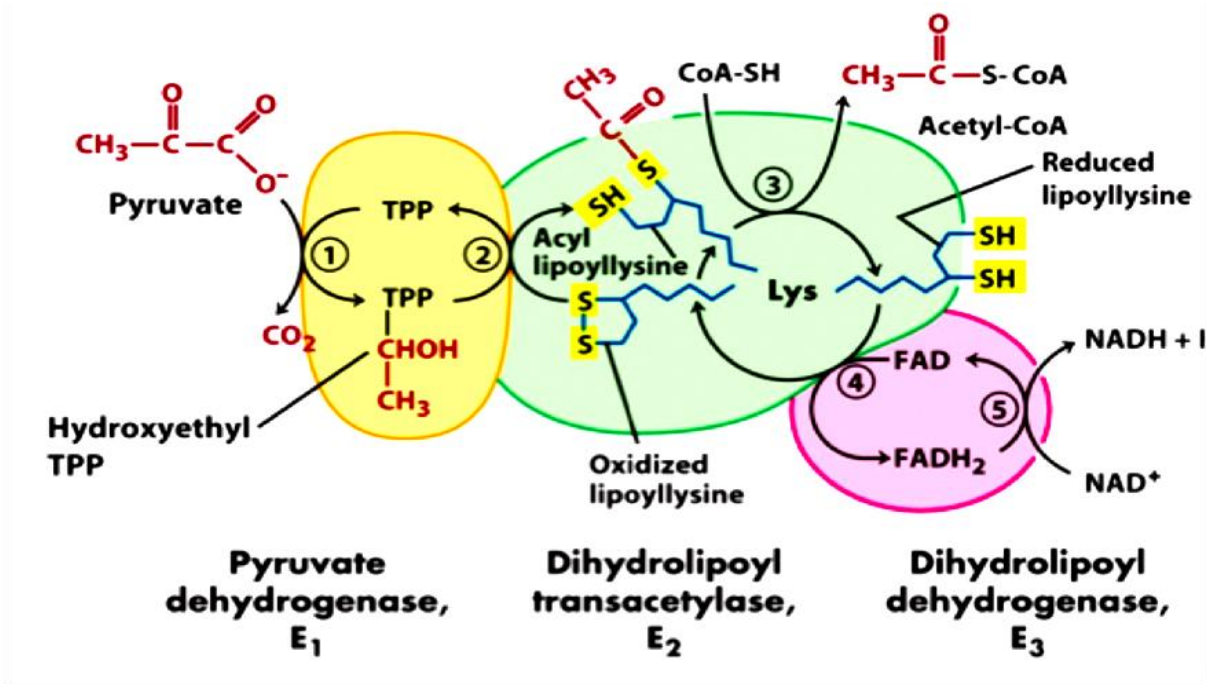
Initially, pyruvate and thiamine pyrophosphate (TPP or vitamin B_1) are bound by pyruvate dehydrogenase subunits. The thiazolium ring of TPP is in a zwitterionic form, and the anionic C2 carbon performs a nucleophilic attack on the C2 (ketone) carbonyl of pyruvate. The resulting hemithioacetal undergoes decarboxylation to produce an acyl anion equivalent. This anion attacks S1 of an oxidized lipoate species that is attached to a lysine residue. In a ring-opening S_N2-like mechanism, S2 is displaced as a sulfide or sulfhydryl moiety. Subsequent collapse of the tetrahedral hemithioacetal ejects thiazole, releasing the TPP cofactor and generating a thioacetate on S1 of lipoate. The E1-catalyzed process is the rate-limiting step of the whole pyruvate dehydrogenase complex.

- **Dihydrolipoyl transacetylase (E2)**

At this point, the lipoate-thioester functionality is translocated into the dihydrolipoyl transacetylase (E2) active site, where a transacylation reaction transfers the acetyl from the "swinging arm" of lipoyl to the thiol of coenzyme A. This produces acetyl-CoA, which is released from the enzyme complex and subsequently enters the citric acid cycle. E2 can also be known as lipoamide reductase-transacetylase.

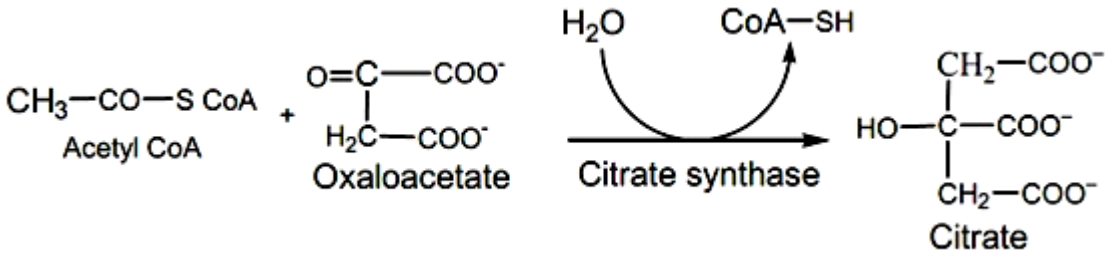
- **Dihydrolipoyl dehydrogenase (E3)**

The dihydrolipoate, still bound to a lysine residue of the complex, then migrates to the dihydrolipoyl dehydrogenase (E3) active site where it undergoes a flavin-mediated oxidation, identical in chemistry to disulfide isomerase. First, FAD oxidizes dihydrolipoate back to its lipoate resting state, producing $FADH_2$. Then, a NAD^+ cofactor oxidizes $FADH_2$ back to its FAD resting state, producing NADH.



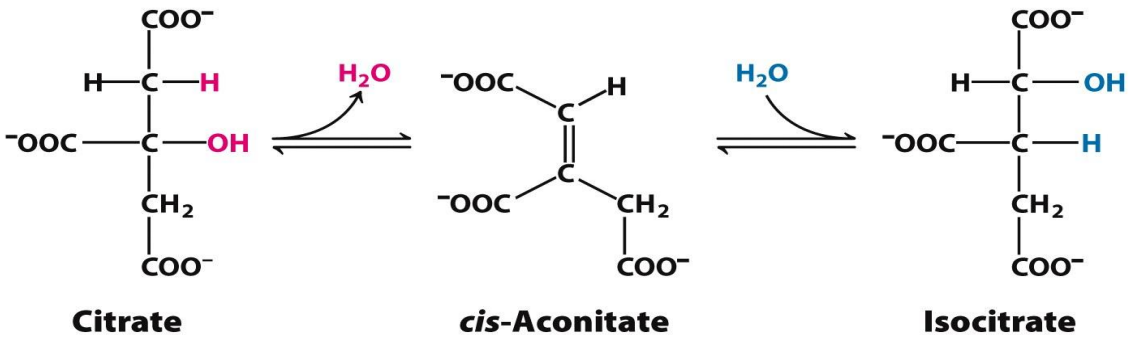
Reaction 1: Citrate Synthase

The first reaction of the citric acid cycle is catalyzed by the enzyme citrate synthase. In this step, oxaloacetate is joined with acetyl-CoA to form citric acid. Once the two molecules are joined, a water molecule attacks the acetyl leading to the release of coenzyme A from the complex.



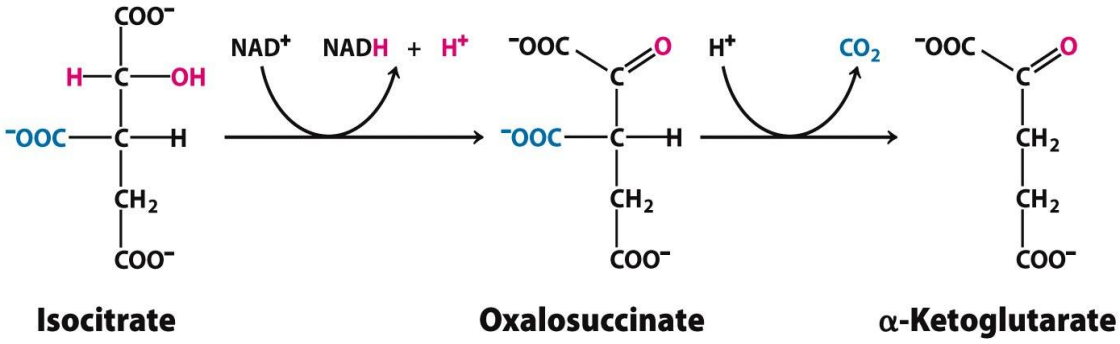
Reaction 2: Acontinase

The next reaction of the citric acid cycle is catalyzed by the enzyme aconitase. In this reaction, a water molecule is removed from the citric acid and then put back on in another location. The overall effect of this conversion is that the –OH group is moved from the 3' to the 4' position on the molecule. This transformation yields the molecule isocitrate.



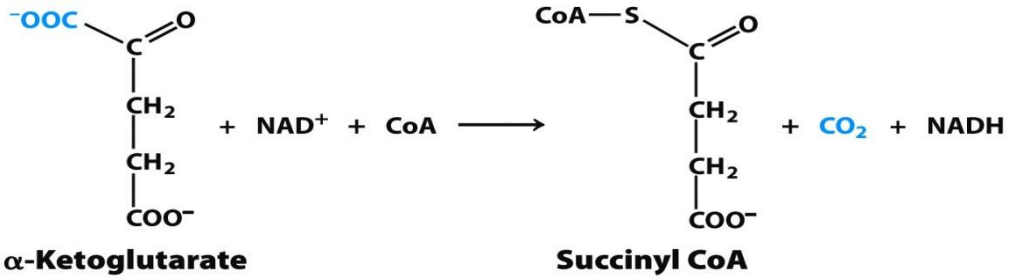
Reaction 3: Isocitrate Dehydrogenase

Two events occur in reaction 3 of the citric acid cycle. In the first reaction, we see our first generation of NADH from NAD. The enzyme isocitrate dehydrogenase catalyzes the oxidation of the –OH group at the 4' position of isocitrate to yield an intermediate which then has a carbon dioxide molecule removed from it to yield α-ketoglutarate.



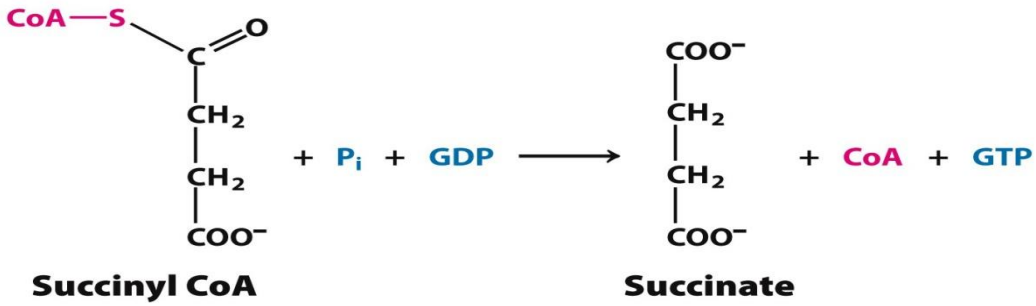
Reaction 4: α-ketoglutarate deydrogenase

In reaction 4 of the citric acid cycle, α-ketoglutarate loses a carbon dioxide molecule and coenzyme A is added in its place. The decarboxylation occurs with the help of NAD, which is converted to NADH. The enzyme that catalyzes this reaction is α-ketoglutarate dehydrogenase. The mechanism of this conversion is very similar to what occurs in the first few steps of pyruvate metabolism. The resulting molecule is called succinyl-CoA.



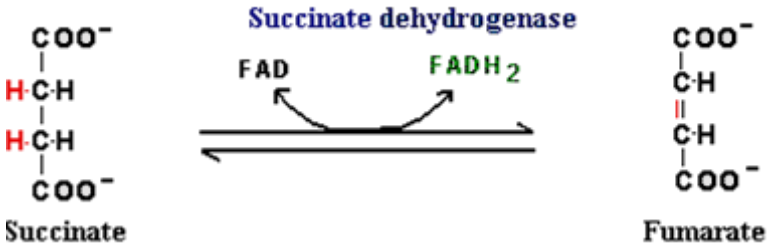
Reaction 5: Succinyl-CoA Synthetase

The enzyme succinyl-CoA synthetase catalyzes the fifth reaction of the citric acid cycle. In this step a molecule of guanosine triphosphate (GTP) is synthesized. GTP is a molecule that is very similar in its structure and energetic properties to ATP and can be used in cells in much the same way. GTP synthesis occurs with the addition of a free phosphate group to a GDP molecule (similar to ATP synthesis from ADP). In this reaction, a free phosphate group first attacks the succinyl-CoA molecule releasing the CoA. After the phosphate is attached to the molecule, it is transferred to the GDP to form GTP. The resulting product is the molecule succinate.



Reaction 6: Succinate Dehydrogenase

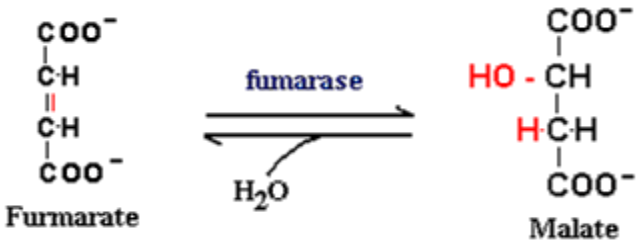
The enzyme succinate dehydrogenase catalyzes the removal of two hydrogens from succinate in the sixth reaction of the citric acid cycle. In the reaction, a molecule of FAD, a coenzyme similar to NAD, is reduced to FADH2 as it takes the hydrogens from succinate. The product of this reaction is fumarate.



FAD, like NAD, is the oxidized form while FADH2 is the reduced form. Although FAD and NAD perform the same oxidative and reductive roles in reactions, FAD and NAD work on different classes of molecules. FAD oxidizes carbon-carbon double and triple bonds while NAD oxidizes mostly carbon-oxygen bonds.

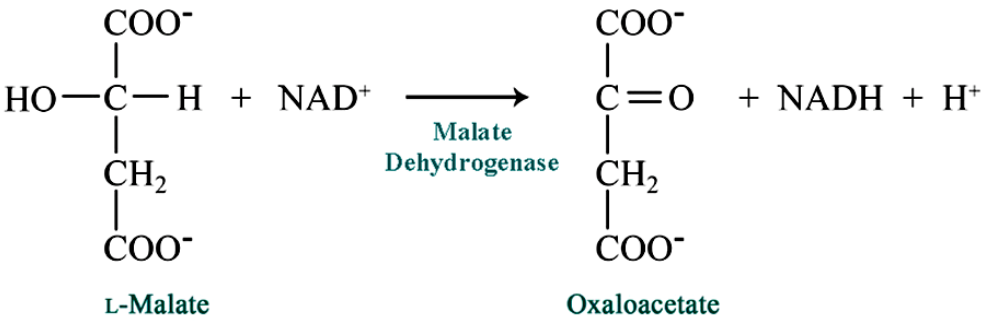
Reaction 7: Fumarase

In this reaction, the enzyme fumarase catalyzes the addition of a water molecule to the fumarate in the form of an –OH group to yield the molecule L-malate.



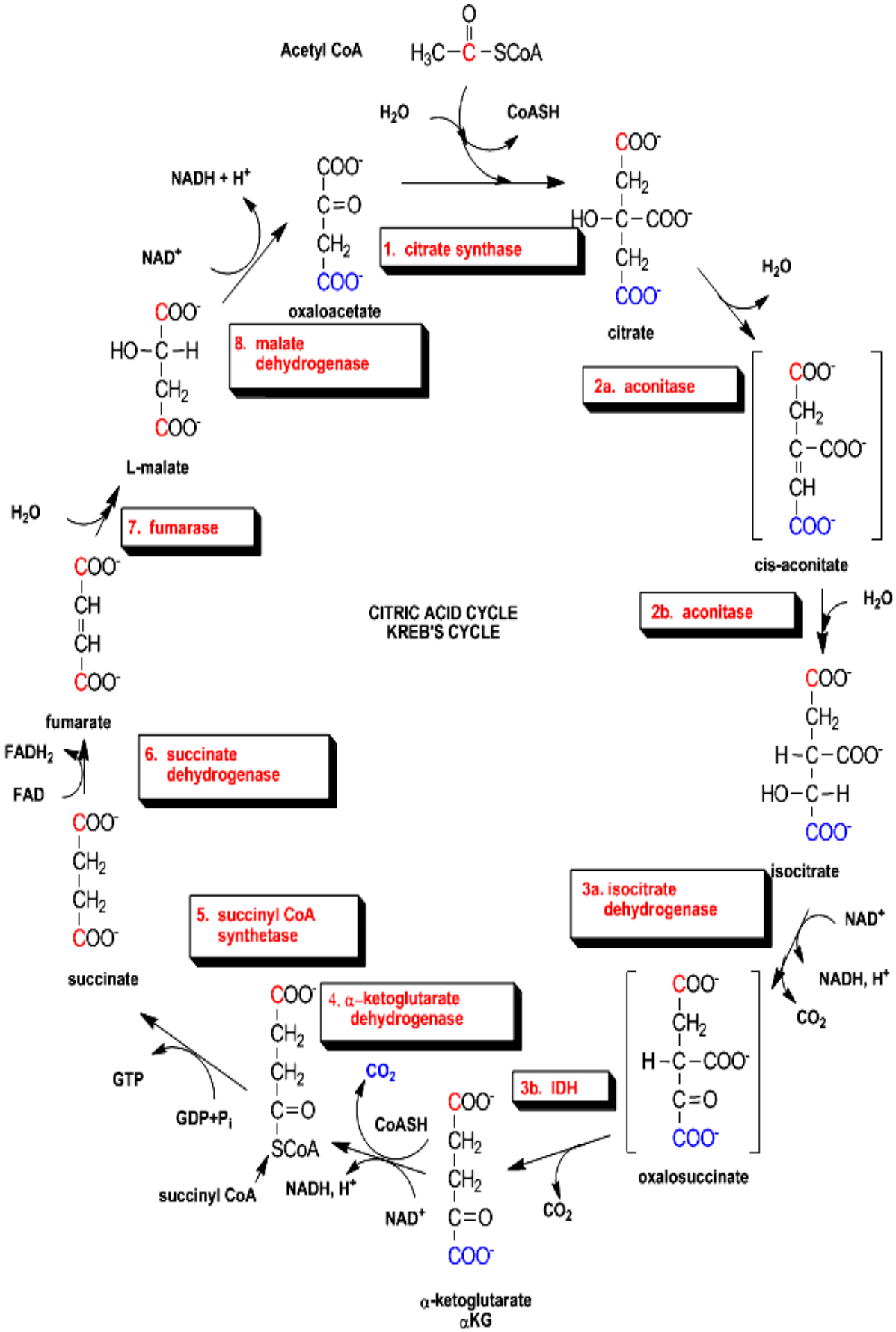
Reaction 8: Malate Dehydrogenase

In the final reaction of the citric acid cycle, oxaloacetate is regenerated by oxidizing L–malate with a molecule of NAD to produce NADH.



- The acetyl-CoA, has been oxidized to two molecules of carbon dioxide.
- Three molecules of NAD were reduced to NADH.
- One molecule of FAD was reduced to FADH2.
- One molecule of GTP (the equivalent of ATP) was produced.

NADH and FADH2 molecules act as electron carriers and are used to generate ATP in the next stage of glucose metabolism, oxidative phosphorylation.


Acetyl CoA
$H_3C-C(=O)-SCoA$
H_2O
CoASH
1. citrate synthase
citrate
H_2O
2a. aconitase
cis-aconitate
2b. aconitase
H_2O
isocitrate
3a. isocitrate dehydrogenase
NAD^+
$NADH, H^+$
CO_2
oxalosuccinate
3b. IDH
CO_2
NAD^+
$NADH, H^+$
CoASH
α-ketoglutarate
αKG
4. α-ketoglutarate dehydrogenase
succinyl CoA
GTP
$GDP+P_i$
succinate
5. succinyl CoA synthetase
$FADH_2$
FAD
fumarate
6. succinate dehydrogenase
H_2O
L-malate
7. fumarase
$NADH + H^+$
NAD^+
oxaloacetate
8. malate dehydrogenase
CITRIC ACID CYCLE
KREB'S CYCLE

- **Energetics of TCA cycle**

$$Acetyl\text{-}CoA + 2H_2O + 3NAD^+ + Pi + GDP + FAD \Rightarrow 2CO_2 + 3NADH + GTP + CoA\text{-}SH + FADH_2 + 2H^+$$

One turn of the citric acid cycle generates:

- One high-energy phosphate through substrate-level phosphorylation
- Three NADH
- One $FADH_2$

Catabolism of Glucose through Glycolysis and the Krebs Cycle

- Each molecule of Glucose produces two molecules of Pyruvate

$$Glucose + 2NAD^+ + 2ADP + 2Pi \Rightarrow 2Pyruvate + 2NADH + 2H^+ + 2H_2O + 2ATP$$

- Action of *Pyruvate Dehydrogenase* on Pyruvate:

$$Pyruvate + CoA\text{-}SH + NAD^+ \Rightarrow CO_2 + Acetyl\text{-}CoA + NADH$$

- The overall catabolism of Glucose to 2 Pyruvate molecules:

$$Glucose + 2NAD^+ + 2ADP + 2Pi \Rightarrow 2Pyruvate + 2NADH + 2H^+ + 2H_2O + 2ATP$$
$$2Pyruvate + 2CoA\text{-}SH + 2NAD^+ \Rightarrow 2CO_2 + 2Acetyl\text{-}CoA + 2NADH$$

$$Glucose + 4NAD^+ + 2ADP + 2CoA\text{-}SH + 2Pi \Rightarrow 2CO_2 + 2Acetyl\text{-}CoA + 4NADH + 2H^+ + 2H_2O + 2ATP$$

- The GTP formed in the animal *Succinyl-CoA Synthetase* reaction in the Krebs cycle is readily converted to ATP (by *Nucleoside Diphosphokinase*)

$$\cancel{2Acetyl\text{-}CoA} + 4H_2O + 6NAD^+ + 2Pi + 2ADP + 2FAD \Rightarrow 4CO_2 + 6NADH + 2ATP + \cancel{2CoA\text{-}SH} + 2FADH_2 + 4H^+$$
$$Glucose + 4NAD^+ + 2ADP + \cancel{2CoA\text{-}SH} + 2Pi \Rightarrow 2CO_2 + \cancel{2Acetyl\text{-}CoA} + 4NADH + 2H^+ + 2H_2O + 2ATP$$

$$Glucose + 10NAD^+ + 4ADP + 2H_2O + 4Pi + 2FAD \Rightarrow 6CO_2 + 10NADH + 4ATP + 2FADH_2 + 6H^+$$

Yield of ATP

At this point the yield of ATP is 4 moles per mole of Glucose as it passes through the Krebs cycle

- **Regulation of TCA cycle**

The regulation of the TCA cycle is largely determined by product inhibition and substrate availability. If the cycle were permitted to run unchecked, large amounts of metabolic energy could be wasted in overproduction of reduced coenzyme such as NADH and ATP. The major eventual substrate of the cycle is ADP which gets converted to ATP. A reduced amount of ADP causes accumulation of precursor NADH which in turn can inhibit a number of enzymes. NADH, a product of all dehydrogenases in the TCA cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits alpha-ketoglutarate dehydrogenase and citrate synthase. When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.[26]

Calcium is used as a regulator. Mitochondrial matrix calcium levels can reach the tens of micromolar levels during cellular activation.[27] It activates pyruvate dehydrogenase phosphatase which in turn activates the pyruvate dehydrogenase complex. Calcium also activates isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.[28] This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

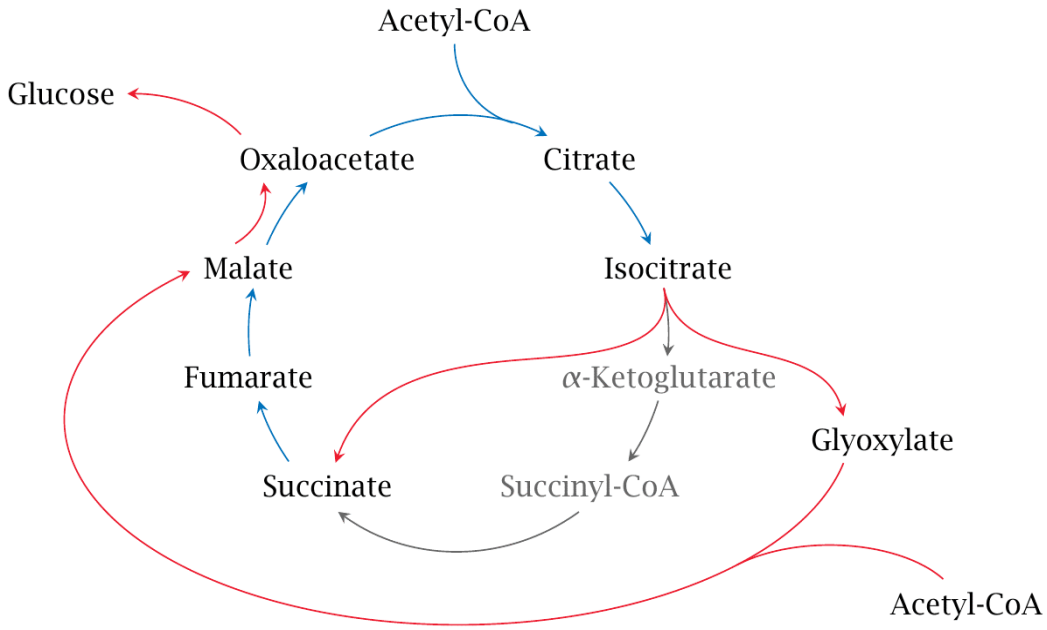
Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate,a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.

❖ Glyoxylate cycle

The glyoxylate cycle, a variation of the tricarboxylic acid cycle, is an anabolic pathway occurring in plants, bacteria, protists, and fungi. The glyoxylate cycle centers on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates.[1] In microorganisms, the glyoxylate cycle allows cells to utilize simple carbon compounds as a carbon source when complex sources such as glucose are not available.[2] The cycle is generally assumed to be absent in animals, with the exception of nematodes at the early stages of embryogenesis. In recent years, however, the detection of malate synthase (MS) and isocitrate lyase (ICL), key enzymes involved in the glyoxylate cycle, in some animal tissue has raised questions regarding the evolutionary relationship of enzymes in bacteria and animals.

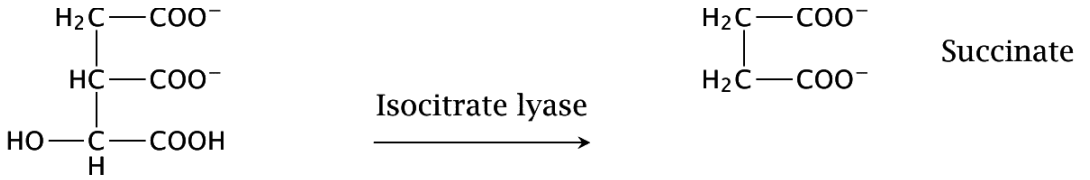
Carbon contained in fatty acids cannot be utilized efficiently for gluconeogenesis, since there is no efficient pathway to convert the acetyl-CoA that results from their breakdown into TCA cycle intermediates. Interestingly, however, plants *do* have a straightforward pathway to do just this, namely, the glyoxylate cycle, which is an ancillary road to the TCA cycle.

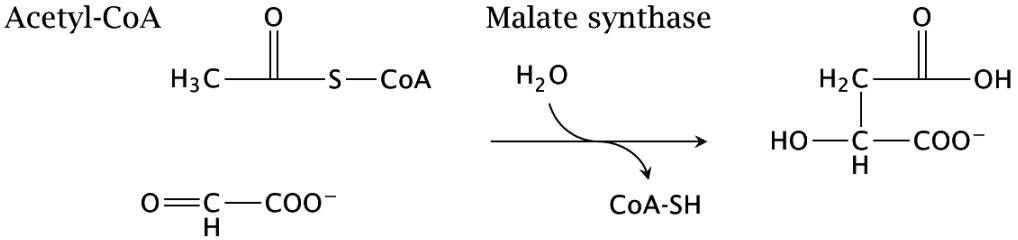
In the glyoxylate cycle, the two decarboxylation steps of the TCA cycle are skipped, and an entry point for a second molecule of acetyl-CoA is created. In this manner, plants are able to use two molecules of acetyl-CoA for the net synthesis of one C_4 TCA cycle intermediate.



The cycle involves two reactions, both of which are mechanistically similar to citrate synthase:

1. Isocitrate is split into succinate and glyoxylate by isocitrate lyase. Since the isocitrate dehydrogenase and the α-ketoglutarate dehydrogenase reactions are bypassed, the loss of two carbons as CO_2 is avoided; these carbons are retained in the form of glyoxylate.
2. Glyoxylate combines with the second acetyl-CoA molecule to form malate. This reaction is catalyzed by malate synthase, and like the citrate synthase reaction it is pushed forward by the concomitant hydrolysis of coenzyme A.





Many plant seeds are very rich in oil—that is, fat. The glyoxylate cycle enables plant seeds to store metabolic energy and carbon at high density as fat, and to use it for the synthesis of glucose and other carbohydrates during germination.

Similarities with TCA cycle

The glyoxylate cycle utilizes five of the eight enzymes associated with the tricarboxylic acid cycle: citrate synthase, aconitase, succinate dehydrogenase, fumarase, and malate dehydrogenase.

Difference with TCA cycle

The two cycles differ in that in the glyoxylate cycle, isocitrate is converted into glyoxylate and succinate by ICL instead of into α-ketoglutarate.

This bypasses the decarboxylation steps that take place in the TCA cycle, allowing simple carbon compounds to be used in the later synthesis of macromolecules, including glucose. Glyoxylate is subsequently combined with acetyl-CoA to produce malate, catalyzed by MS. Malate is also formed in parallel from succinate by the action of succinate dehydrogenase and fumarase.

Role of glyoxylate cycle

In gluconeogenesis

This pathway thus allows cells to obtain energy from fat. To utilize acetate from fat for biosynthesis of carbohydrates, the glyoxylate cycle, whose initial reactions are identical to the TCA cycle, is used.

Cell-wall containing organisms, such as plants, fungi, and bacteria, require very large amounts of carbohydrates during growth for the biosynthesis of complex structural polysaccharides, such as cellulose, glucans, and chitin. In these organisms, in the absence of available carbohydrates (for example, in certain microbial environments or during seed germination in plants), the glyoxylate cycle permits the synthesis of glucose from lipids via acetate generated in fatty acid β-oxidation.

The glyoxylate cycle bypasses the steps in the citric acid cycle where carbon is lost in the form of CO_2. Glyoxylate condenses with acetyl-CoA (a step catalyzed by malate synthase), yielding malate. Both malate and oxaloacetate can be converted into phosphoenolpyruvate, which is the product of phosphoenolpyruvate carboxykinase, the first enzyme in gluconeogenesis. The net result of the glyoxylate cycle is therefore the production of glucose from fatty acids. Succinate generated in the first step can enter into the citric acid cycle to eventually form oxaloacetate.

In plants

In plants the glyoxylate cycle occurs in special peroxisomes which are called glyoxysomes. This cycle allows seeds to use lipids as a source of energy to form the shoot during germination. This cycle allows plants to take in acetate both as a carbon source and as a source of energy.

In fungi

The glyoxylate cycle may serve an entirely different purpose in some species of pathogenic fungi. The levels of the main enzymes of the glyoxylate cycle, ICL and MS, are greatly increased upon contact with a human host.

In vertebrates

Vertebrates were once thought to be unable to perform this cycle because there was no evidence of its two key enzymes, isocitrate lyase and malate synthase. However, some research suggests that this pathway may exist in some, if not all, vertebrates.

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- **Anaplerotic reactions**

Anaplerotic reactions are chemical reactions that form intermediates of a metabolic pathway. Examples of such are found in the citric acid cycle (TCA cycle). In normal function of this cycle for respiration, concentrations of TCA intermediates remain constant; however, many biosynthetic reactions also use these molecules as a substrate. Anaplerosis is the act of replenishing TCA cycle intermediates that have been extracted for biosynthesis (in what are called **cataplerotic reactions**).

The TCA cycle is a hub of metabolism, with central importance in both energy production and biosynthesis. Therefore, it is crucial for the cell to regulate concentrations of TCA cycle metabolites in the mitochondria. Anaplerotic flux must balance cataplerotic flux in order to retain homeostasis of cellular metabolism.

Reactions of anaplerotic metabolism

There are 4 major reactions classed as anaplerotic, and it is estimated that the production of oxaloacetate from pyruvate has the most physiologic importance. The malate is created by PEP carboxylase and malate dehydrogenase in the cytosol. Malate, in the mitochondrial matrix, can be used to make pyruvate (catalyzed by malic enzyme) or oxaloacetic acid, both of which can enter the citric acid cycle.

From	To	Reaction	Notes
Pyruvate	oxaloacetate	pyruvate + HCO_3^- + ATP $\longrightarrow$ oxaloacetate + ADP + P_i + H_2O	This reaction is catalysed by pyruvate carboxylase, an enzyme activated by Acetyl-CoA, indicating a lack of oxaloacetate. It occurs in animal mitochondria. Most important anaplerotic reaction depending on severity, deficiency causes lactic acidosis, severe psychomotor deficiency or death in infancy Pyruvate can also be converted to L-malate, another intermediate, in a similar way.
Aspartate	oxaloacetate	-	This is a reversible reaction forming oxaloacetate from aspartate in a transamination reaction, via aspartate transaminase.
Glutamate	α-ketoglutarate	glutamate + NAD^+ + H_2O $\longrightarrow$ NH_4^+ + α-ketoglutarate + NADH + H^+.	This reaction is catalysed by glutamate-dehydrogenase.
β-Oxidation of fatty acids	succinyl-CoA	-	When odd-chain fatty acids are oxidized, one molecule of succinyl-CoA is formed per fatty acid. The final enzyme is methylmalonyl-CoA mutase. Triheptanoin (fat with three heptanoic (C7:0) fatty acids) may be used to treat pyruvate carboxylase deficiency
Adenylo-succinate	fumarate	Adenylo-succinate $\longrightarrow$AMP + fumarate	This reaction is catalysed by adenylosuccinate lyase and occurs in purine synthesis and purine nucleotide cycle. Defect of this enzyme causes psychomotor retardation.

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❖ Catabolism of saturated (16 C)and unsaturated (16C) fatty acids by β-oxidation

A **fatty acid** is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 12 to 28. Fatty acids are usually derived from triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. In particular, heart and skeletal muscle prefer fatty acids.

Length of free fatty acid chains

Fatty acid chains differ by length, often categorized as short to very long.

- Short-chain fatty acids are fatty acids with aliphatic tails of fewer than six carbons (e.g. butyric acid).
- Medium-chain fatty acids are fatty acids with aliphatic tails of 6–12 carbons, which can form medium-chain triglycerides.
- Long-chain fatty acids are fatty acids with aliphatic tails 13 to 21 carbons.
- Very long chain fatty acids are fatty acids with aliphatic tails longer than 22 carbons.

Types of fatty acids

Fatty acids that have carbon–carbon double bonds are known as unsaturated. Fatty acids without double bonds are known as saturated. They differ in length as well.

- **Unsaturated fatty acids**

Unsaturated fatty acids have one or more double bonds between carbon atoms. (Pairs of carbon atoms connected by double bonds can be saturated by adding hydrogen atoms to them, converting the double bonds to single bonds. Therefore, the double bonds are called unsaturated.)

The two carbon atoms in the chain that are bound next to either side of the double bond can occur in a *cis* or *trans* configuration.

A *cis* configuration means that the two hydrogen atoms adjacent to the double bond stick out on the same side of the chain. For example, oleic acid, with one double bond, has a "kink" in it, whereas linoleic acid, with two double bonds, has a more pronounced bend. Alpha-linolenic acid, with three double bonds, favors a hooked shape.

A *trans* configuration, by contrast, means that the adjacent two hydrogen atoms lie on *opposite* sides of the chain. As a result, they do not cause the chain to bend much, and their shape is similar to straight saturated fatty acids.

In most naturally occurring unsaturated fatty acids, each double bond has three *n* carbon atoms after it, for some n, and all are cis bonds. Most fatty acids in the *trans* configuration (trans fats) are not found in nature and are the result of human processing (e.g., hydrogenation).

The differences in geometry between the various types of unsaturated fatty acids, as well as between saturated and unsaturated fatty acids, play an important role in biological processes, and in the construction of biological structures (such as cell membranes).

Examples of Unsaturated Fatty Acids

Common name	**No. of C atoms**	**Chemical structure**
Myristoleic acid	14	$CH_3(CH_2)_3\mathbf{CH{=}CH}(CH_2)_7COOH$
Palmitoleic acid	16	$CH_3(CH_2)_5\mathbf{CH{=}CH}(CH_2)_7COOH$
Sapienic acid	16	$CH_3(CH_2)_8\mathbf{CH{=}CH}(CH_2)_4COOH$
Oleic acid	18	$CH_3(CH_2)_7\mathbf{CH{=}CH}(CH_2)_7COOH$
Linoleic acid	18	$CH_3(CH_2)_4\mathbf{CH{=}CH}CH_2\mathbf{CH{=}CH}(CH_2)_7COOH$

- **Saturated fatty acids**

Saturated fatty acids have no double bonds. Thus, saturated fatty acids are saturated with hydrogen (since double bonds reduce the number of hydrogens on each carbon). Because saturated fatty acids have only single bonds, each carbon atom within the chain has 2 hydrogen atoms (except for the omega carbon at the end that has 3 hydrogens).

Common name	No. of C atoms	Chemical structure
Caprylic acid	8	$CH_3(CH_2)_6COOH$
Capric acid	10	$CH_3(CH_2)_8COOH$
Lauric acid	12	$CH_3(CH_2)_{10}COOH$
Myristic acid	14	$CH_3(CH_2)_{12}COOH$
Palmitic acid	16	$CH_3(CH_2)_{14}COOH$

Essential fatty acids

Fatty acids that are required by the human body but cannot be made in sufficient quantity from other substrates, and therefore must be obtained from food, are called essential fatty acids. Two essential fatty acids are linoleic acid (LA) and α-linolenic acid (ALA). They are widely distributed in plant oils. The human body has a limited ability to convert ALA into the longer-chain *n*-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which can also be obtained from fish.

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❖ **β – Oxidation of saturated 16 Carbon fatty acid (Palmitic Acid)**

The oxidation of long-chain fatty acids to acetyl-CoA is a central energy-yielding pathway in animals, many protists and some bacteria. Complete combustion or oxidaton of a typical fatty acid, palmitic acid, yields 2,380 kcal per mole.

The process of beta oxidation is named after the carbon atom in the beta position of the fatty acyl-CoA which becomes the most oxidized during the cyclic redox reactions that remove C2 units in form of acetyl-CoA from the fatty acyl chain. The β carbon becomes the new carboxyl end of the shortened (n-2) fatty acyl-CoA.

The degradation of fatty acid proceeds step wise by series of reactions which remove 2 carbon atoms at a time from the carboxyl end of the carbon chain. This catabolic scheme or mechanism is known as Beta oxidation which was proposed by Knoop in 1905.

Lipid Metabolism is Summarized Below:

Lipids = Glycerol + Fatty Acid

↓↑ ↓↑

Glucose C2 unit ↔ Acetoacetate

↓↑

$CO_2 + H_2O$

The overall reaction is:

Fatty acid CoA + NAD^+ + FAD → Acetyl CoA + NADH + H^+ + $FADH_2$

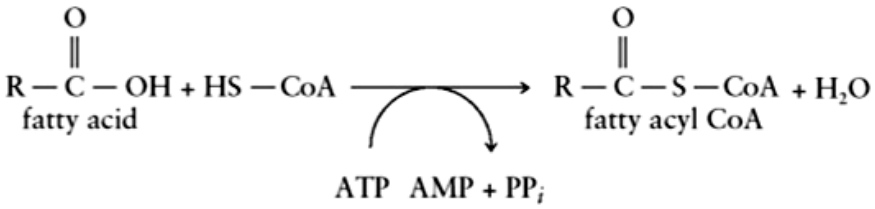
- **Steps in fatty acid oxidation**

A) Activation of fatty acids
B) Transport of fatty acids across mitochondrial membranes into mitochondrial matrix
C) β (Beta) oxidation of fatty acids.

A) Activation of fatty acids

Fatty Acids are activated to fatty acyl CoA by acyl CoA synthase (thiokinase) present in the cytoplasm, outer mitochondrial membrane or Endoplasmic Reticulum. By activation the relative stability of -C-C- bond in a Fatty Acid is overcome, which allows stepwise oxidation. There are different isoforms of Fatty acyl CoA synthase specific for different kind of Fatty Acids.

Fatty acid + ATP + CoA ⟶ Acyl-CoA + PPi + AMP



- FA + CoA + ATP ←→ fatty acyl-CoA + AMP + PPi

B) Transport of fatty acids across mitochondrial membranes into mitochondrial matrix

The activated long chain fatty acid is carried across the mitochondria membrane by an organic compound called carnitine.

C) β (Beta) oxidation of fatty acids.

Four enzyme-catalyzed reactions make up fatty acid oxidation.

i) **First step: Formation of unsaturated acyl-CoA.**

It produces a double bond between the C-2 and C-3 carbon atoms, yielding a trans-2-enoyl-CoA (the symbol 2 designates the position of the double bond) Note that the new double bond has the trans configuration, whereas the double bonds in naturally occurring unsaturated fatty acids are normally in the cis configuration. We consider the significance of this difference later. This first step is catalyzed by acyl-CoA dehydrogenase. This has flavo-proteins with FAD as a prosthetic group. The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the electron-transferring flavoprotein. The oxidation catalyzed by an acyl-CoA dehydrogenase is analogous to succinate dehydrogenation in the citric acid cycle; in both reactions the enzyme is bound to the inner membrane, a double bond is introduced into a carboxylic acid between the α and β carbons, FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O_2, with the concomitant synthesis of about 1.5 ATP molecules per electron pair.

ii) **Second step: Formation of β hydroxy Acyl CoA**

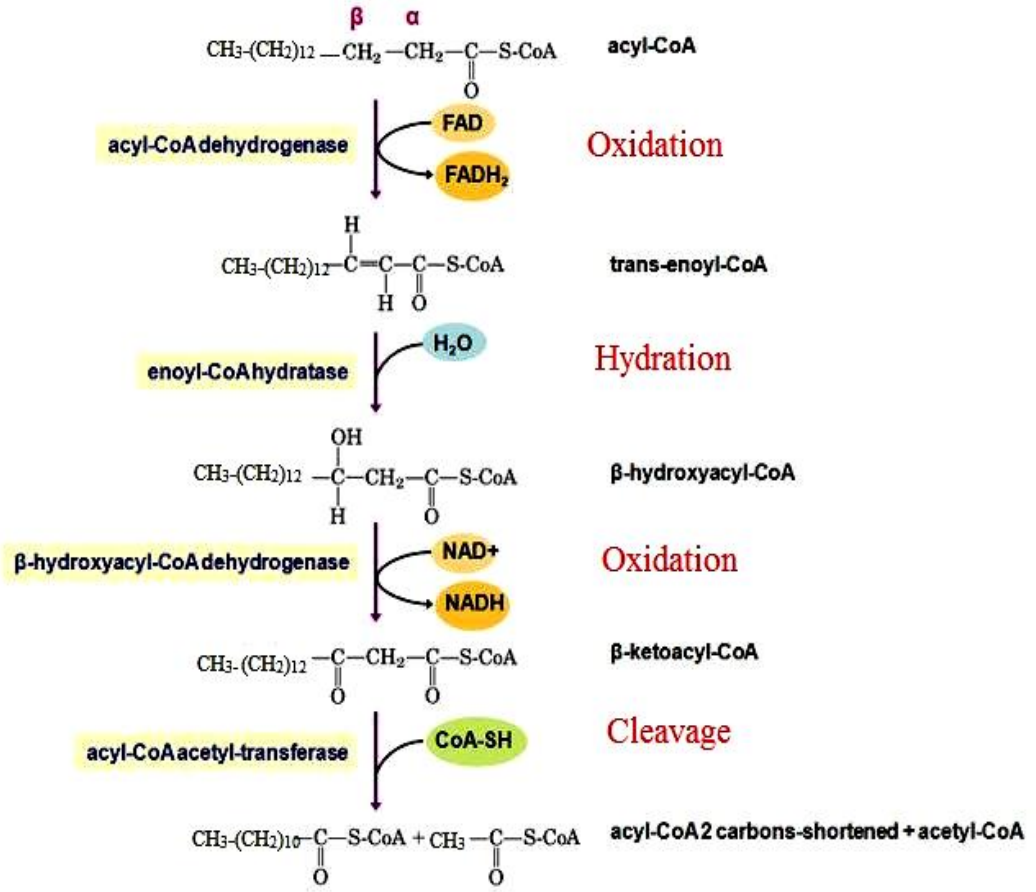
Water is added to the double bond of the trans-2-enoyl-CoA to form the L stereoisomer of -hydroxyacyl-CoA (3-hydroxyacyl-CoA).This reaction, catalyzed by enoyl-CoA hydratase, is formally analogous to the fumarase reaction in the citric acid cycle, in which H_2O adds across double bond.

iii) **Third step: Formation of β-keto acyl CoA**

L-hydroxyacyl-CoA is dehydrogenated to form -ketoacyl-CoA, by the action of hydroxyacyl-CoA dehydrogenase; NAD is the electron acceptor. This enzyme is absolutely specific for the L stereoisomer of hydroxyacyl-CoA. The NADH formed in the reaction donates its electrons to NADH dehydrogenase, an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to O_2. The reaction catalyzed by hydroxyacyl-CoA dehydrogenase is closely analogous to the malate dehydrogenase reaction of the citric acid cycle.

iv) **Fourth step**: **Thiolytlc cleavage of Acyl CoA**

This reaction is catalyzed by acyl-CoA acetyltransferase, more commonly called thiolase, which promotes reaction of ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms. This reaction is called thiolysis, by analogy with the process of hydrolysis, because the ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.



- **Energy from Oxidation of Fatty Acid:**

Ex. palmitic acid (16 carbon) yield 8 molecules of acetate and run through the Beta oxidation cycle 7 times. Oxidation of a carbon (one cycle) yield 5 ATP (7 x 5 = 35 ATP).
8 molecules of acetate where oxidized in TCA will produce (8 x 12 = 96).
Total ATP = 35 + 96 = 131.
Loss of 2 energy rich phosphate at activation step hence 131-2 = 129 ATP net gained.

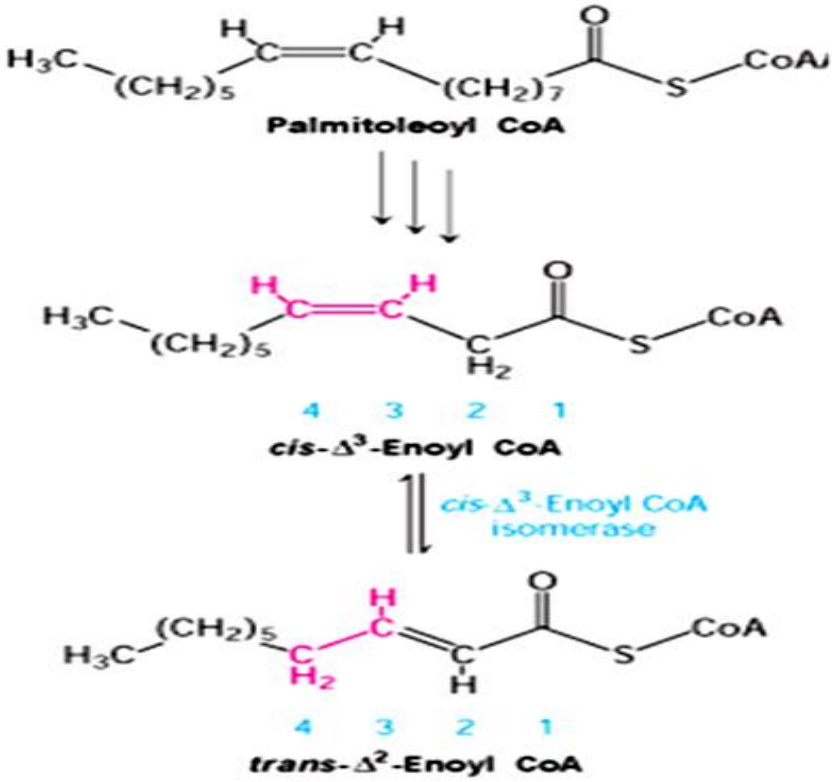
==========

❖ β – Oxidation of unsaturated 16 Carbon fatty acid (Palmitoleic Acid)

Unsaturated fatty acids are found in our high fat diets. The complete oxidation of unsaturated fatty acids presents some difficulties. Happily most of the reactions involved in the oxidation of these unsaturated fatty acids are the same as β-oxidation. In addition, we need two additional enzymes, an isomerase and a reductase.

Palmitoleic acid is a 16 carbon unsaturated fatty acid with a double bond between the C9 and the C10 carbons. This fatty acid is activated by acyl-CoA synthetase, transported across the mitochondrial inner membrane by carnitine and transferred back to CoA to form palmitoyl CoA. Palmitoyl CoA undergoes 3 cycles of β-oxidation to produce a cis-Δ3-enoyl CoA.

This cis-Δ3-enoyl CoA is not a substrate for acyl CoA dehydrogenase. The presence of the cis double bond between the C3 and C4 carbons prevents the formation of a double bond between the C2 and C3 carbons. Cis-Δ3-enoyl CoA isomerase converts this double bond into a trans-Δ2-double bond. This trans-Δ2-enoyl CoA is a substrate for acyl CoA dehydrogenase and proceeds through the β-oxidation pathway.



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❖ Degradation of proteins and amino acids: Proteolysis and Putrefaction

- **Proteolysis**

Proteolysis is the breakdown of proteins into smaller polypeptides or amino acids. Uncatalysed, the hydrolysis of peptide bonds is extremely slow, taking hundreds of years. Proteolysis is typically catalysed by cellular enzymes called proteases, but may also occur by intra-molecular digestion. Low pH or high temperatures can also cause proteolysis non-enzymatically.

Proteolysis in organisms serves many purposes; for example, digestive enzymes break down proteins in food to provide amino acids for the organism, while proteolytic processing of a polypeptide chain after its synthesis may be necessary for the production of an active protein. It is also important in the regulation of some physiological and cellular processes, as well as preventing the accumulation of unwanted or abnormal proteins in cells. Consequently, dis-regulation of proteolysis can cause diseases, and is used in some venoms to damage their prey.

Proteolysis is important as an analytical tool for studying proteins in the laboratory, as well as industrially, for example in food processing and stain removal.

Protein degradation may take place intracellularly or extracellularly. In digestion of food, digestive enzymes may be released into the environment for extracellular digestion whereby proteolytic cleavage breaks down proteins into smaller peptides and amino acids so that they may be absorbed and used by an organism.

Proteins in cells are also constantly being broken down into amino acids. This intracellular degradation of protein serves a number of functions: It removes damaged and abnormal protein and prevent their accumulation, and it also serves to regulate cellular processes by removing enzymes and regulatory proteins that are no longer needed. The amino acids may then be reused for protein synthesis.

- **Putrefaction**

It is one of seven stages in the decomposition of the body of a dead animal. It can be viewed, in broad terms, as the decomposition of proteins in a process that results in the eventual breakdown of cohesion between tissues and the

liquefaction of most organs. It is caused by bacterial or fungal decomposition of organic matter and results in production of noxious odours.
The bacterial digestion of the cell proteins weakens the tissues of the body. As the proteins are continuously broken down to smaller components, the bacteria excrete gases and organic compounds, such as the functional-group amines putrescine and cadaverine, which carry the noxious odor of rotten flesh.

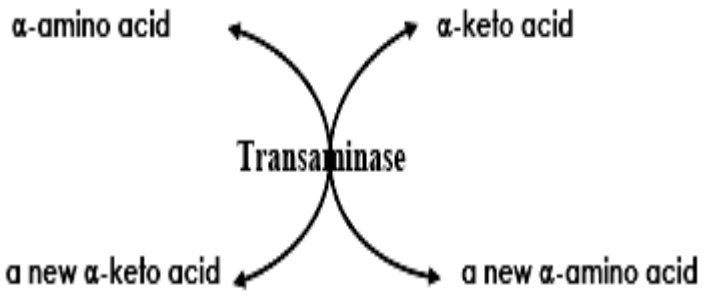
==========

Catabolic pathways of amino acids

1. Transamination
2. Deamination
3. Transamidination
4. Transamidation
5. Decarboxylation

1- Transamination

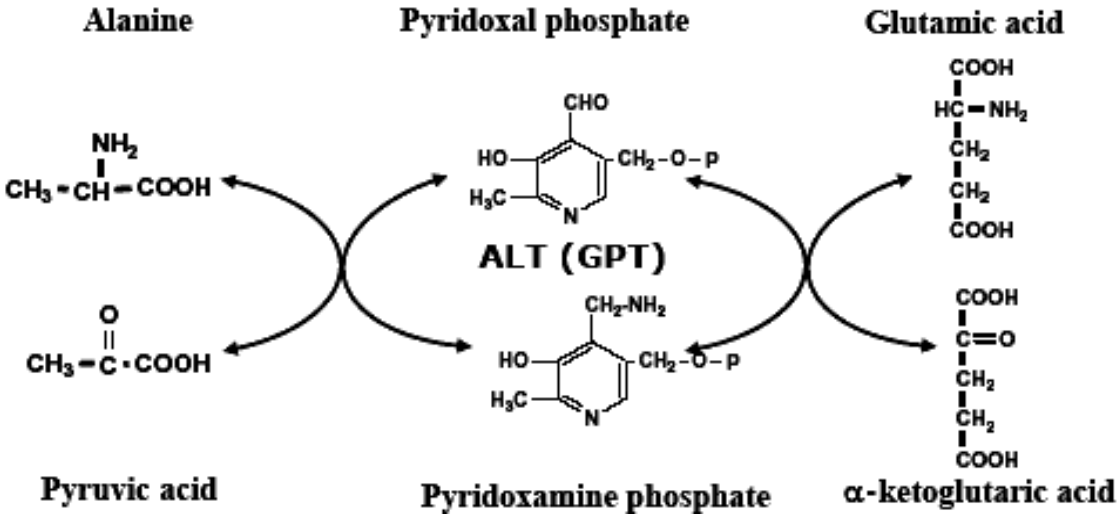
Transamination means transfer of amino group from α-amino acid to α-keto acid with formation of a new α-amino acid and a new α-keto acid. The liver is the main site for transamination. All amino acids can be transaminated except lysine, threonine, proline and hydroxy proline. All transamination reactions are reversible. It is catalyzed by aminotransferases (transaminases). It needs pyridoxal phosphate as a coenzyme.



Examples of transaminase

Alanine transaminase

- It is also called glutamic pyruvic transaminase (GPT).
- It catalyzes the transfer of amino group from glutamic acid to pyruvic acid to form alanine and α-ketoglutaric acid.
- It also catalyzes the reverse reaction.
- It needs pyridoxal phosphate as a coenzyme.
- It is present in the cytoplasm of liver cells.



2. Deamination

Deamination means the removal of amino group from α-amino acid in the form of ammonia with formation of α-keto acid. The liver and kidney are the main sites for deamination.

Deamination may be oxidative or non-oxidative

A. Oxidative deamination: It is catalyzed by one of the following enzymes:

1. L-amino acid oxidases
2. D-amino acid oxidases
3. Glutamate dehydrogenase

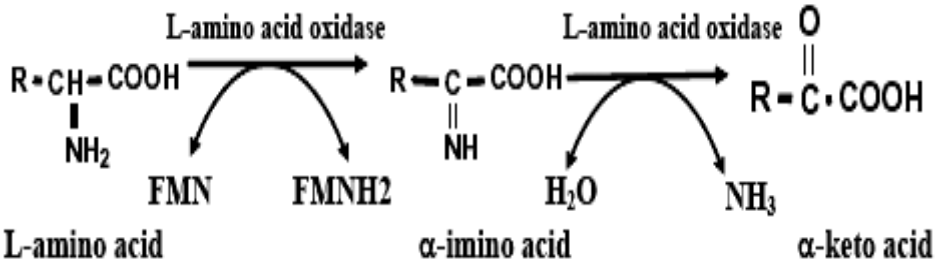
B. Non-oxidative deamination. It is catalyzed by one of the following enzymes:

1. Dehydratases
2. Desulfhydrases

A. Oxidative deamination

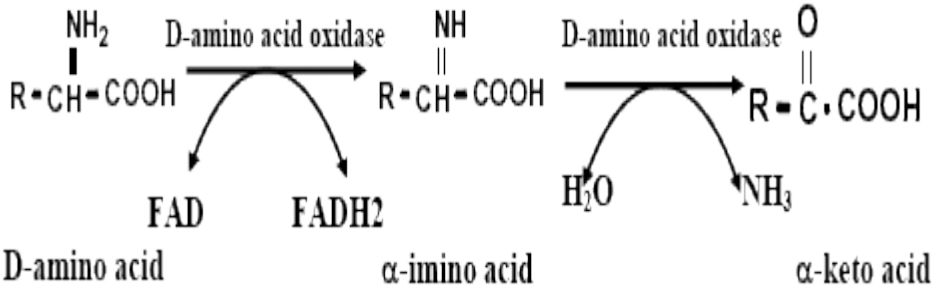
1. L amino acid oxidase

- This enzyme is present in the liver and kidney. Its activity is low.
- It is an aerobic dehydrogenase that needs FMN as a coenzyme.
- It deaminates most of the naturally occurring L-amino acids



2. D amino acid oxidase

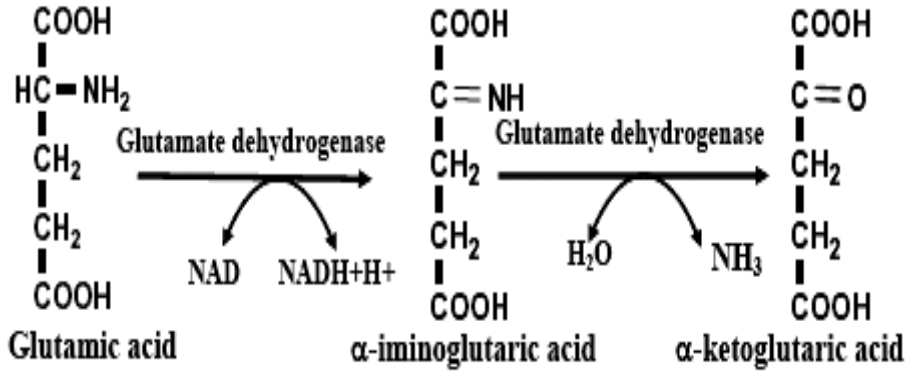
- D- amino acids are present in plants and bacterial cell wall.
- They are not used in protein biosynthesis in humans and animals.
- D-amino acids are deaminated by D-amino acid oxidase resulting in ammonia and α-keto acids.
- D-amino acid oxidase is present in the liver.
- It is an aerobic dehydrogenase.
- It needs FAD as a coenzyme.



3. Glutamate dehydrogenase

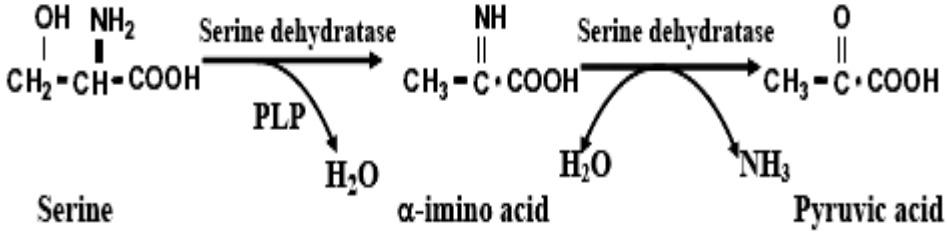
- This enzyme is present in most tissues
- It is present both in cytoplasm and mitochondria

- Its activity is high
- It is an anaerobic dehydrogenase
- It needs NAD or NADP as a coenzyme
- It deaminates glutamic acid resulting in α-ketoglutaric acid and ammonia

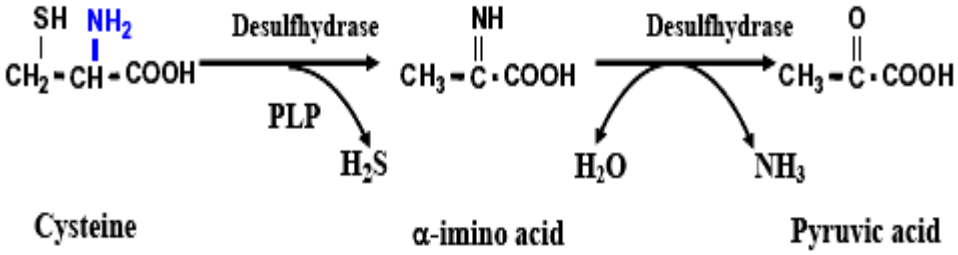


B. Non-oxidative deamination

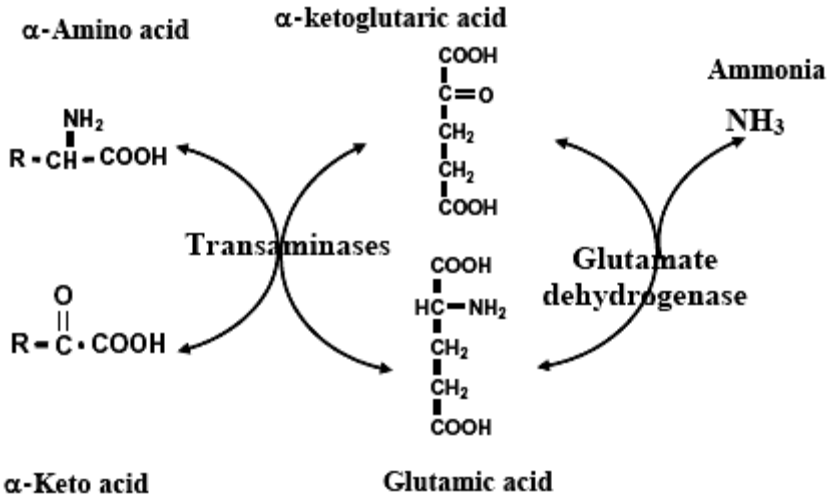
1. **Dehydratase**: This enzyme deaminates amino acids containing hydroxyl group e.g. serine, homoserine and threonine. It needs pyridoxal phosphate as coenzyme.



2. **Desulfhydrase**: This enzyme deaminates sulpher containing amino aids e.g. cysteine and cystine. It needs pyridoxal phosphate as a coenzyme.

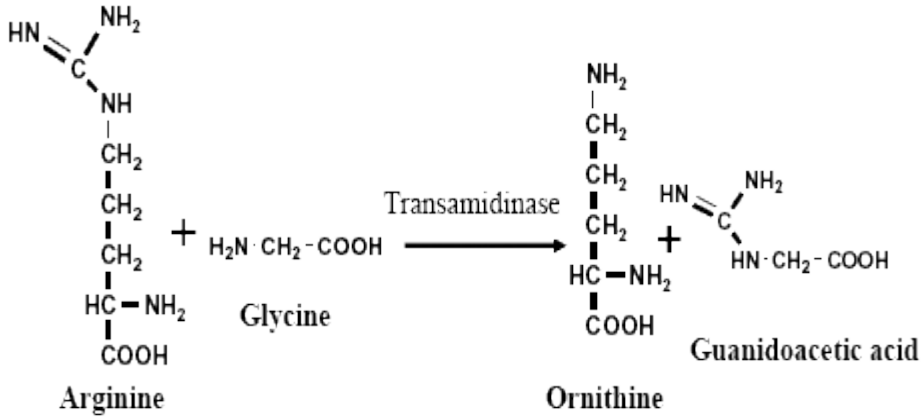


Most of the naturally occurring α-amino acids are catabolized by transamination with α-ketoglutaric acid followed by deamination of the produced glutamic acid, a condition called transdeamination.



3. Transamidination

Transamidination means the transfer of amidine group from a donor molecule to an acceptor molecule. It is catalyzed by transamidinase enzyme An example of transmidination reaction is the transfer of amidine group from arginine (donor) to glycine (acceptor) in creatine biosynthesis.



4-Transamidation

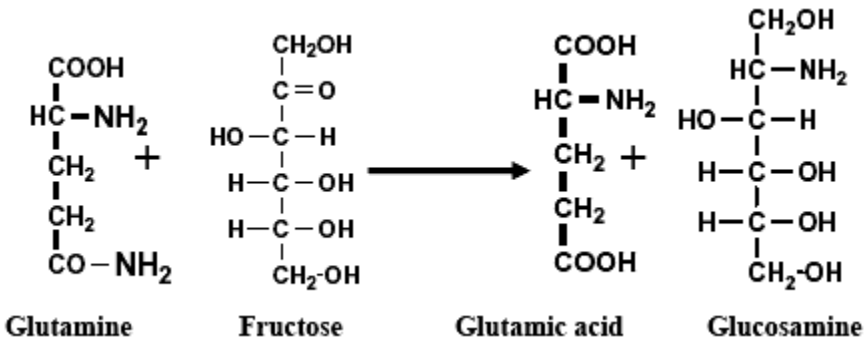
Transamidation means transfer of amide group nitrogen from a donor molecule to an acceptor molecule. It is catalyzed by transamidase enzyme

Examples of transmidation reaction include:

1. Transfer of amide nitrogen from glutamine (donor) to fructose (acceptor) to form glucosamine

2. Amide group nitrogen of glutamine is the source of N3 and N9 in purine bases

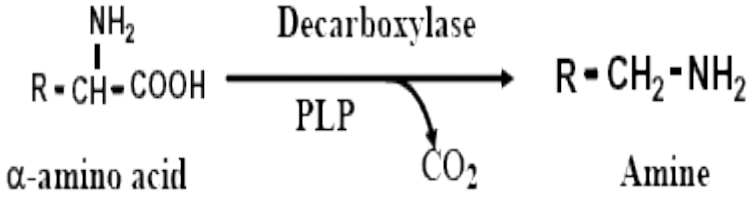
Glucosamine biosynthesis



5. Decarboxylation

Decarboxylation means removal of CO_2 from amino acid with formation of corresponding amines. It is catalyzed by decarboxylase enzyme. It needs pyridoxal phosphate as a coenzyme. Examples of decarboxylation reaction include:

1. Decarboxylation of histidine to form histamine
2. Decarboxylation of tyrosine to form tyramine



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❖ Nucleic acid catabolism

The breakdown of DNA and RNA is occurring continuously in the cell. Purine and pyrimidine nucleosides can either be degraded to waste products and excreted or can be salvaged as nucleotide components.

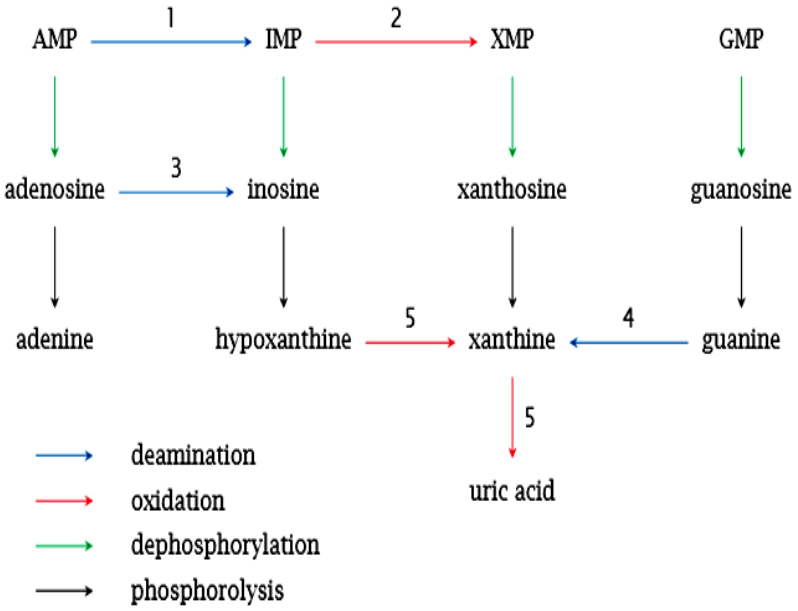
Purine catabolism

Purine degradation takes place mainly in the liver of humans and requires an assortment of enzymes to degrade purines to uric acid. First, the nucleotide will lose its phosphate through 5'-nucleotidase. The nucleoside, adenosine, is then deaminated and hydrolyzed to form hypoxanthine via adenosine deaminase and nucleosidase respectively. Hypoxanthine is then oxidized to form xanthine and then uric acid through the action of xanthine oxidase. The other purine nucleoside, guanosine, is cleaved to form guanine. Guanine is then deaminated via guanine deaminase to form xanthine which is then converted to uric acid. Oxygen is the final electron acceptor in the degradation of both purines. Uric acid is then excreted from the body in different forms depending on the animal.

Free purine and pyrimidine bases that are released into the cell are typically transported intercellularly across membranes and salvaged to create more nucleotides via nucleotide salvage.

For example, adenine + PRPP → MP + PPi. This reaction requires the enzyme adenine phosphoribosyltransferase. Free guanine is salvaged in the same way except it requires hypoxanthine-guanine phosphoribosyltransferase.

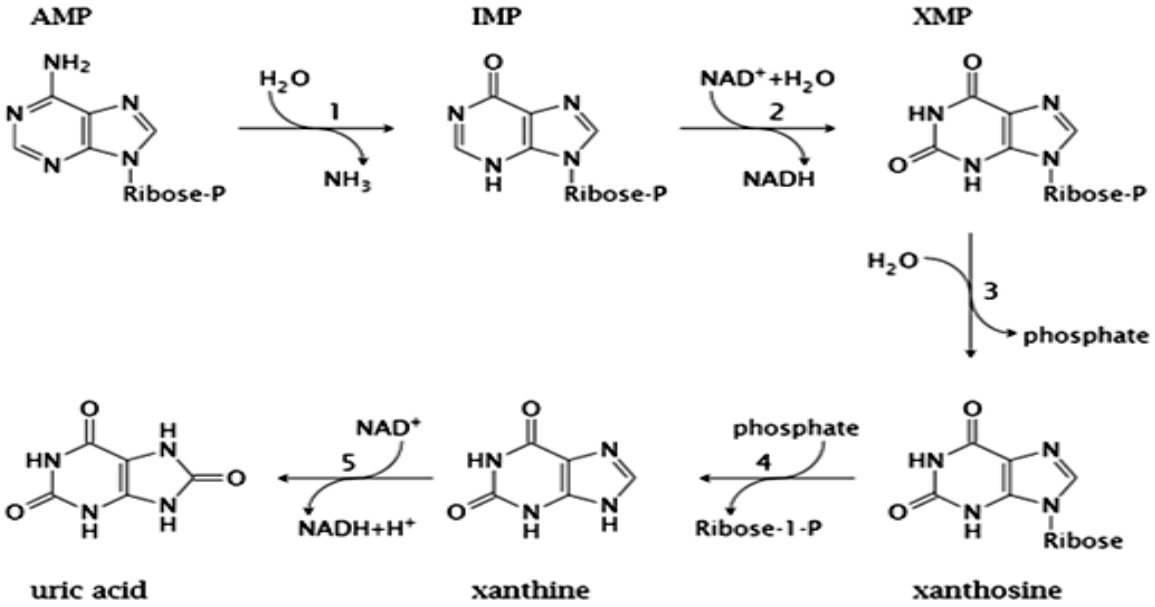
Defects in purine catabolism can result in a variety of diseases including gout, which stems from an accumulation of uric acid crystals in various joints, and adenosine deaminase deficiency, which causes immunodeficiency.



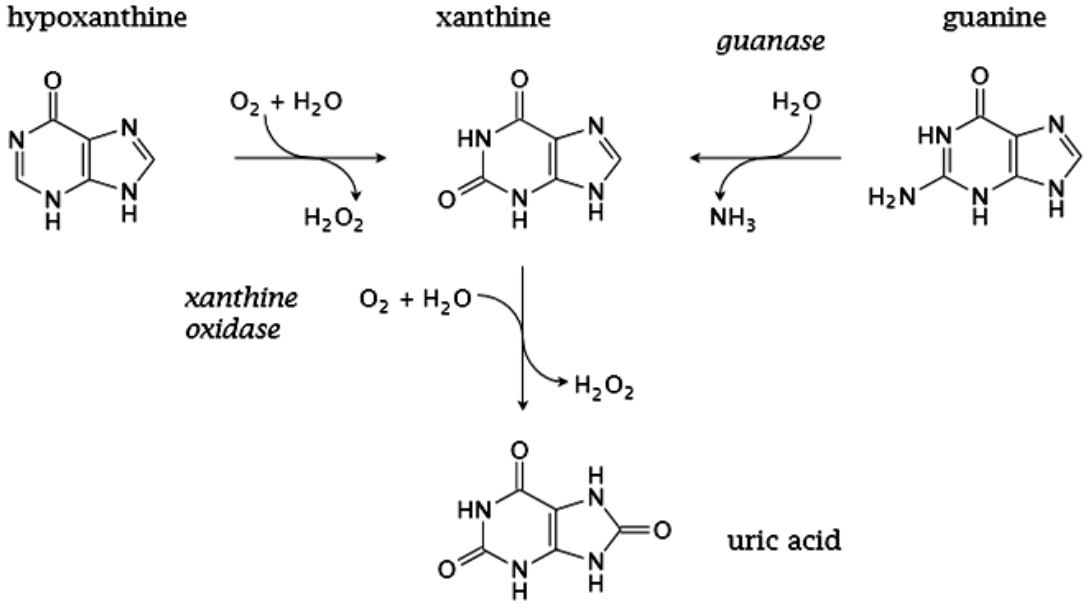
Notes: Enzymes: 1, AMP deaminase; 2, IMP dehydrogenase; 3, adenosine deaminase; 4, guanase; 5, xanthine dehydrogenase or oxidase.

Degradation of adenine

1) IMP dehydrogenase 2) 5'-nucleosidase 3) Purine nucleoside phosphorylase 4) Xanthine dehydrogenase/ oxidase



Degradation of Guanine



Pyrimidine catabolism

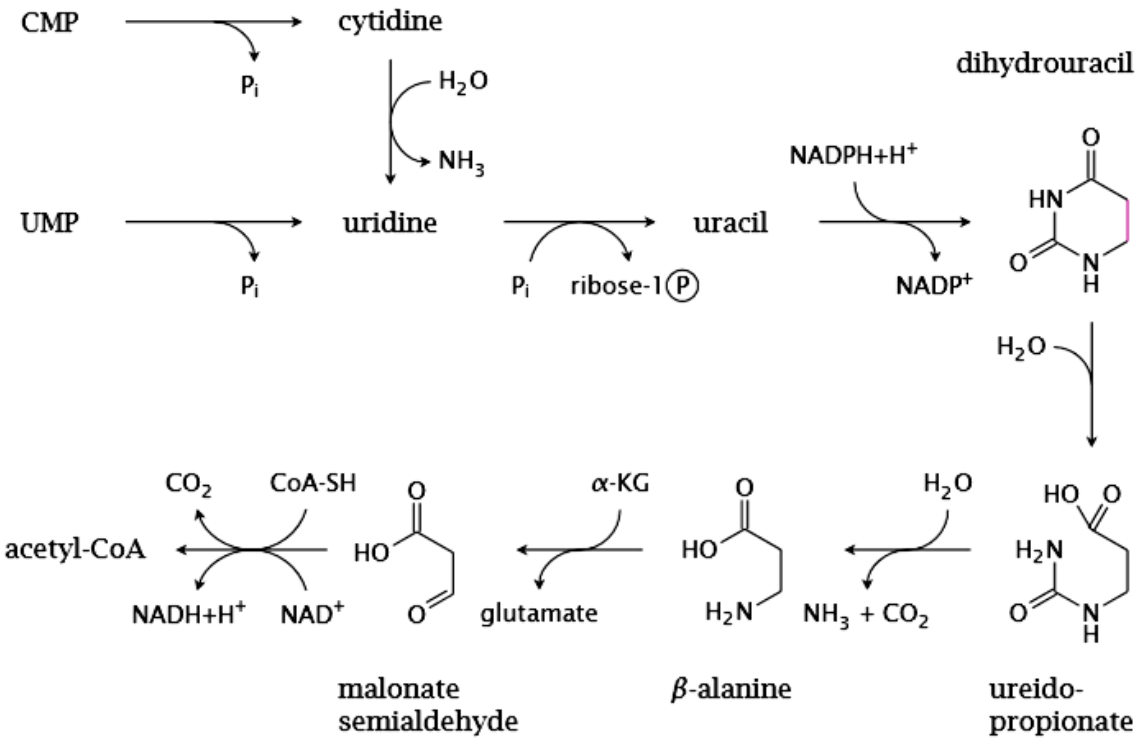
Cytosine and uracil are converted into beta-alanine and later to malonyl-CoA which is needed for fatty acid synthesis, among other things. Thymine, on the other hand, is converted into β-aminoisobutyric acid which is then used to form methylmalonyl-CoA. The leftover carbon skeletons such as acetyl-CoA and Succinyl-CoA can then by oxidized by the citric acid cycle. Pyrimidine

degradation ultimately ends in the formation of ammonium, water, and carbon dioxide. The ammonium can then enter the urea cycle which occurs in the cytosol and the mitochondria of cells.

Pyrimidine bases can also be salvaged. For example, the uracil base can be combined with ribose-1-phosphate to create uridine monophosphate or UMP. A similar reaction can also be done with thymine and deoxyribose-1-phosphate.

Deficiencies in enzymes involved in pyrimidine catabolism can lead to diseases such as Dihydropyrimidine dehydrogenase deficiency which has negative neurological effects.

Degradation of Pyrimidines



❖ Nucleotide Biosynthesis

I. Biosynthesis of purine nucleotides: -The precursors of the purine ring were first established in the laboratory of Buchanan by administering **labeled** isotopes of carbon and nitrogen compounds to pigeons. These studies revealed the following general picture of the origin of the purine nucleus. Carbon atoms 2 and 8 are derived from *format* or the 1-carbon unit arising from various compounds, e.g., *serine* and *glycine*. Carbon atom 6 is derived from CO**2**. Carbon atoms 4 and 5 come from *carboxyl* and *methylene carbons* of *glycine*, respectively. Nitrogen 7 is also derived from *glycine*. It is apparent that glycine is incorporated as a whole. Nitrogen atom 1 is derived from *amino nitrogen* of *aspartic acid* and nitrogens at position 3 and 9 come from the *amide nitrogen*

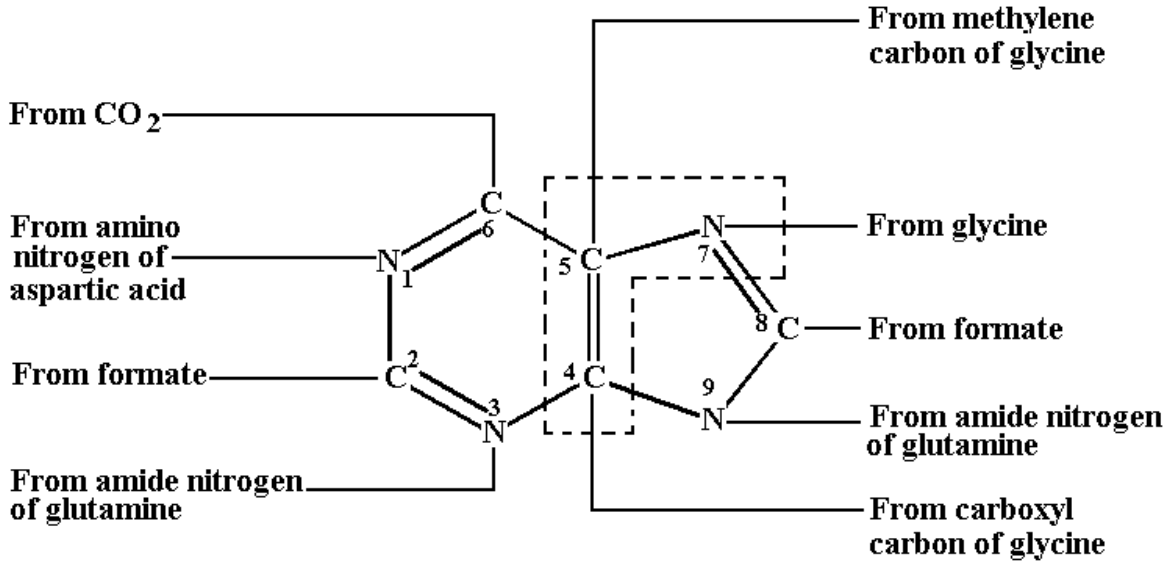


Fig. - Origin of carbon and nitrogen atoms of the purine ring from different precursors.

of *glutamine* (fig.).

- **De novo synthesis of inosinic acid.**

Inosinic acid is the first product formed with a complete purine ring structure in the biosynthesis of purine nucleotides. The general route for purine biosynthesis has been studied in many species and is essentially the same in all organisms. The detailed biosynthetic pathway, for which the enzymes have now been isolated and studied, is indicated in above Fig. The pathway consists of a series of successive reactions by which the purine ring system is formed on *carbon-1* of *ribose 5-phosphate*. This directly leads to the formation of

purine ribonucleotides. Free purines or nucleosides never appear as intermediates in this pathway.

(a) **5-Phosphoribosyl-1-pyrophosphate(PRPP):**

ATP-dependent pyrophosphorylation of ribose-5-phosphate gives *5-phosphoribosyl-1-pyrophosphate*. This compound is a key substance in the biosynthesis of both purine and pyrimidine nucleotides. The reaction is interesting in that it is catalysed by a *kinase that transfers pyrophosphate* rather than phosphate from ATP.

Ribose-5-phosphate+ATP $\xrightarrow{Mg^{2+}}$ **5 Phosphoribsyl-1-pyrophosphate + ATP.**

(b) **5-Phosphoribosyl-1-amine:**

The second step of this pathway involves the glutamine-dependent amination of PRPP, yielding a labile amino sugar, 5-*phosphoribosyl-1-amine* in presence of *glutamine phosphoribosyl pyrophosphate amido-transferase.* The reaction incorporates a nitrogen atom into position 9 of the purine ring. The amination step result in the inversion of the configuration at carbon 1 of ribose, since *purine ribosides* are of the β-configuration while *5-phosphoribiosyl-pyrophosphate has the* ∝-configuration. Hence, an N-glycosidic bond of proper stereochemistry is introduced early in the reaction sequence. This reaction is the "*committed step"* in purine biosynthesis, and is subject to feed back inhibition. The enzyme purified from *A. aerogenes* was inhibited by low concentrations of purine ribonucleotides.

PRPP+ Glutamine $\xrightarrow{Mg^{2+}}$ **5-Phosphoribosyl-1-amine + Glutamic acid +PPi**

(c) **Glycinamide ribonucleotide:**

The next reaction is catalysed by *phosphoribosyl Glycinamide synthetase.* This is an ATP-dependent reaction wherein the entire structure of glycine is conjugated through the formation of an amide linkage with 5-phosphoribosyl-1-amine.the enzyme isolated from A.*aerogenes* is not inhibited by purine ribonucleotides.

Phosphoribosyl amine + ATP+Glycine $\xrightarrow{Mg^{2+}}$ **Glycinamide ribonucleotide + ADP + Pi**

(d) N-Formylglycinamide ribonucleotide:

Glycinamide ribonucleotide is formulated to yield **α-N-formylglycinamide ribonucleotide**. This reaction is catalysed by *Glycinamide*

ribonucleotide transformylase, the enzyme transfers a formyl residue from N5, and N10-methenyltetrahydrofolate.

Glycinamide ribonucleotide + N^5, N^{10}-methenyltetrayrofolate+H_2O

$\longrightarrow$ ∝-N-formylglycinamide ribonucleotide+Tetrahydrofolate

(e) **∝-N-formylglycinamidine ribonucleotide:**

The next step involves transfer of the amide group from glutamine to ∝–*N-formylglycinamide ribonucleotide* to from ∝–*N-formylglyciamidine ribonucleotide.* This is the second glutamine-dependent amination, which inserts the nitrogen atom at position 3 of the purine ring.

∝-N-formylglycinamide ribonucleotide+Glutamine+ATP+H2O $\xrightarrow{Mg^{2+}}$

∝-N-formylglycinamidine ribonucleotide+Glutamic acid+ADP+Pi

(f) **5-Aminoimidazole ribonucleotide:**

∝-N-formylglycinamidine ribonucleotide has all the structural features of the imidazole ring of purines. This ring is closed by an ATP-dependent dehydration catalysed by the enzyme *5'-phosphoribosyl formylglycinamidine cycloligase* to yield *5-aminoimidazole ribonucleotide.*

∝-N-formylglycinamidine ribonucleotide+ATP $\xrightarrow{Mg^{2+}\ K^{+}}$

5 –Aminoimidazole ribonucleotide +ADP + Pi

(g) **5-Aminoimidazole-4-carboxylic acid ribonucleotide:**

A molecule of CO**2** is now incorporated through a reaction catalysed by *aminoimidazole ribonucleotide carboxylase* to yield *5-aminoimidazole-4-carboxylic acid ribonucleotide.*

5-Aminoimidazole ribonucleotide+CO_2 $\rightleftharpoons$

5-Aminoimidazole-4-carboxylic acid ribonucleotide.

(h) **5-Aminoimidazole-4-N- succinocarboxamide Ribonucleotide:**

The conversion of 5-aminoimidazole-4-carboxylic acid ribonucleotide to the corresponding amide occurs in a two-step process. First, an *amide linkage* between the substrate ad aspartic acid is introduced in an ATP-dependent

reaction catalysed by the enzyme *5'-phosphoribosyl-4-carboxy-5-aminoimidazole-L-aspartate ligase.* This produces the intermediate *5-aminoimidazole-4-N-succinocarboxamide ribonucleotide.*

5-Aminoimidazole-4-N-suceinocarboxamide ribonucleotide + Aspartic acid + ATP

$\xrightleftharpoons{Mg^{2+}}$ **5-Aminoimidazole-4-N- succinocarboxamide ribonucleotide + ADP + Pi**

(i) **5-Aminoimidazole-4-carboxamide ribonucleotide:**

5-Aminoimidazole-4-N-succinocarboxamide ribonucleotide subsequently undergoes an *elimination reaction* wherein the carbon skeleton of the asparatate molecule is eliminated as *fumarate*. This inserts the nitrogen atom at position 1 of the purine ring.

5-Aminoimidazole-4-N-succinocarboxamide ribonucleotide $\rightleftharpoons$

5-Aminoimidazole-4-carboxamide ribonucleotide + Fumarate.

(j) **5-Formamidoimidazole-4-carboxamide ribonucleotide:**

The last carbon atom required to yield all elements of the purine ring system is now introduced by the formylation of 5-aminoimidazole-4-carboxamide ribonucleotide. The reaction is catalysed by *transformylase* with N^{10}-formyltetrahdrofolate as formyl donor.

5-Aminoimidazole-4-carboxamide ribonucleotide + N^{10}-formyl tetrahydrofolic acid

$\xrightarrow{K+}$ **5- Formamidoimidazole- -4-carboxamideribonucleotide + tetrahydrofolic acid.**

(k) **Inosinic acid:**

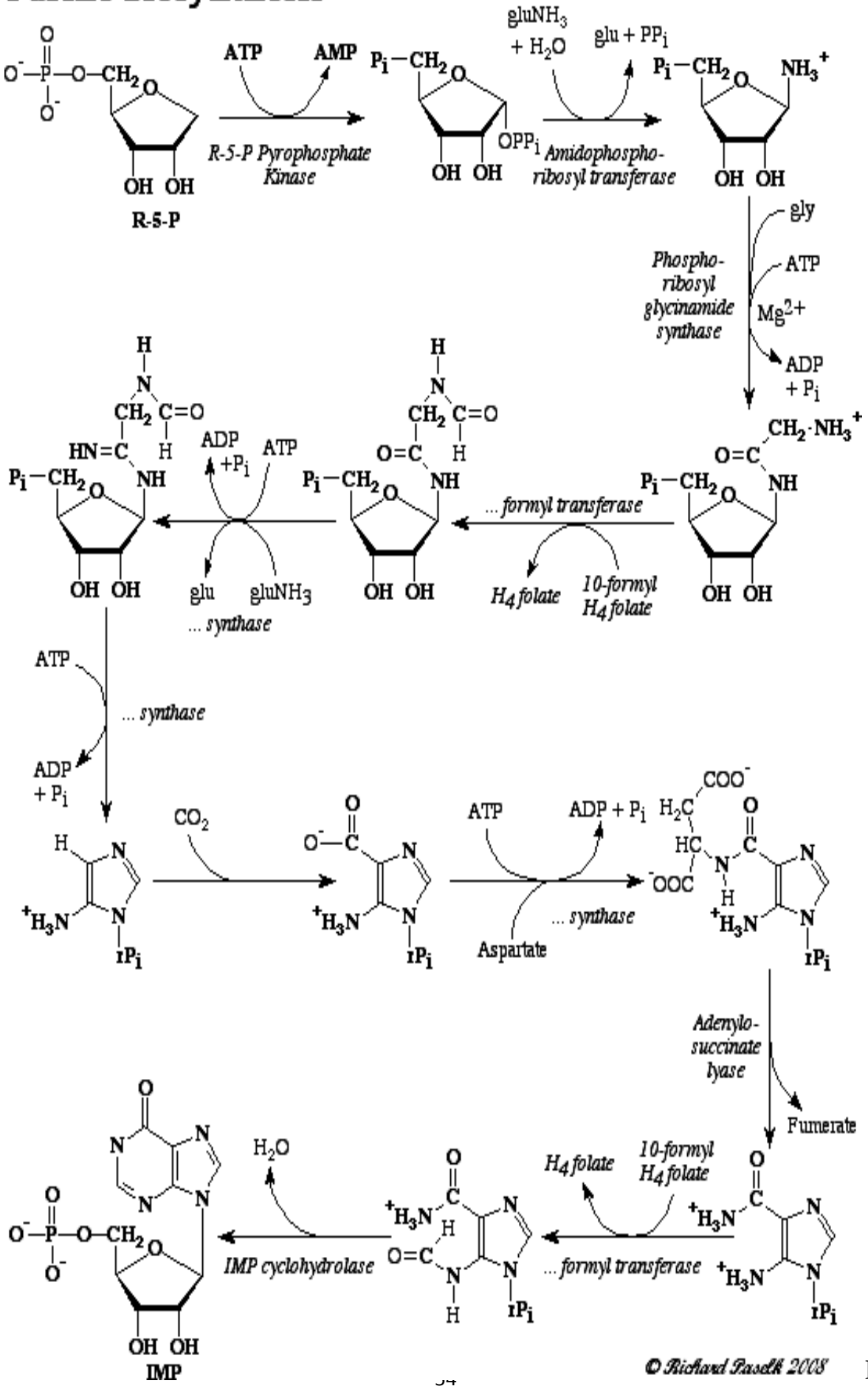
Closure of the ring with dehydration by the enzyme *inosinicase* yields inosinic acid (hypoxanthine ribonucleotide).

The synthesis of inosinic acid from elementary precursors can be regarded as the result of the sum of the following artificial, composite equations:

(a) $2Glutamine+2HCOO^{-}+HCO_3+Glycine + Aspartate +Ribose\ 5\text{-}phosphate$

$\longrightarrow 2Glutamate+Inosinicacid+Fumarate+7H_2O$

(b) $7ATP +7H_2O \longrightarrow 6ADP + 6Pi + AMP + PPi + 7H^{+}$


Purine Biosynthesis
R-5-P
ATP
AMP
R-5-P Pyrophosphate Kinase
gluNH3 + H2O
glu + PPi
Amidophospho-ribosyl transferase
gly
ATP
Mg2+
ADP + Pi
Phospho-ribosyl glycinamide synthase
... formyl transferase
H4folate
10-formyl H4folate
ADP +Pi
ATP
glu
gluNH3
... synthase
ATP
... synthase
ADP + Pi
CO2
ATP
ADP + Pi
... synthase
Aspartate
Adenylo-succinate lyase
Fumerate
H4folate
10-formyl H4folate
... formyl transferase
IMP cyclohydrolase
H2O
IMP
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B.

Conversion of inosinic acid to adenine and guanine ribonucleotides.

Formation of adenylic acid from inosinic acid proceeds via a two-step reaction. In the first step the formation of adenylosuccinate *(IMP: L-aspartate ligase)*, with participation of aspartic acid and GTP. In the second step adenylosuccinate is cleaved to yield adenylic acid and fumaric acid. This reaction is catalysed by adenylosuccinate (*adenylosuccinate AMP-lyase*). This is probably the same enzyme, which cleaves fumarate from 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide.

Conversion of inosinic acid to guanylic acid also proceeds in a two-step process. In the first step inosinic acid is oxidized to xanthylic acid by the enzyme *inosinic acid dehydrogenase*

(*IMP: NAD oxidoreductase*) with NAD as the electron acceptor. In the second step xanthylic acid is converted to guanylic aced with ATP and ammonia or glutamine. The reaction is catalysed by *guanylic acid synthetase* (xanthosine-5'-phosphate: ammonia ligase). The biochemical transformations converting inosinic acid to adenylic and guanylic acids are indicated in Fig.

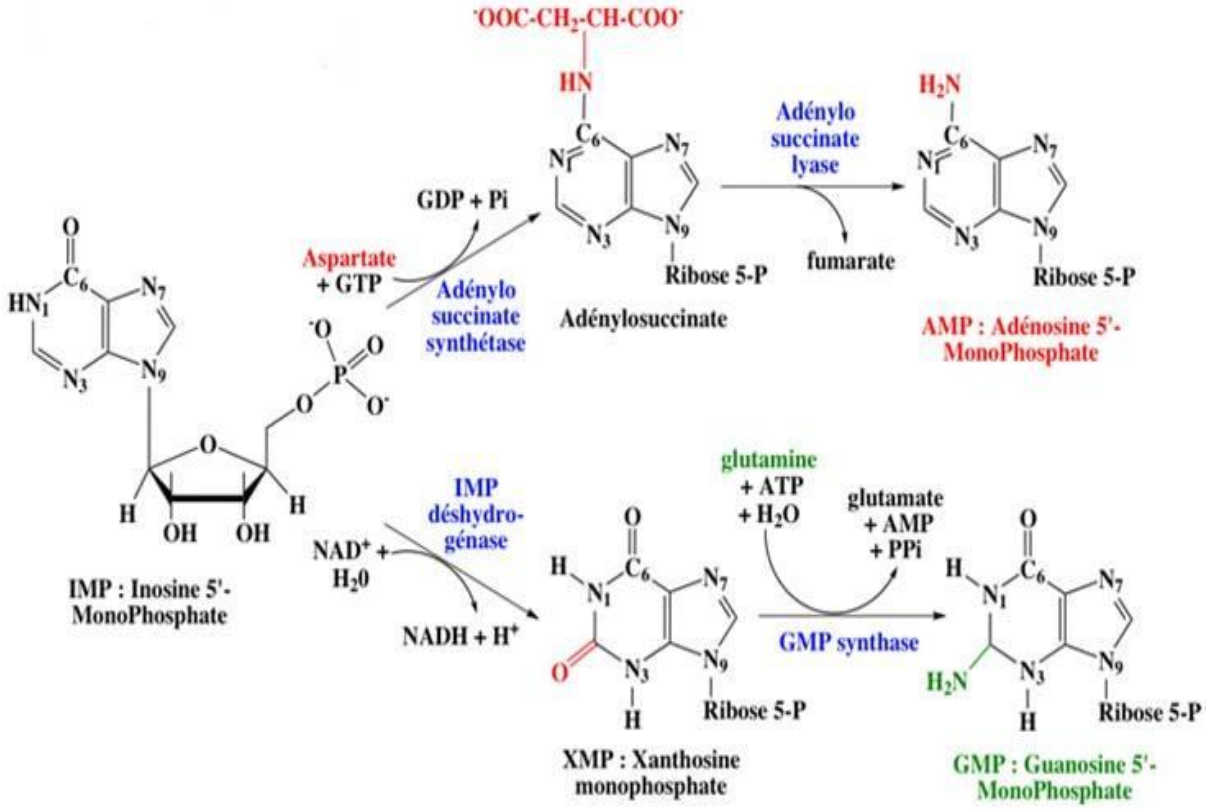


Fig. **Biosynthesis of adenylic and guanylic acids from inosinic acid.**

II. **BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES**

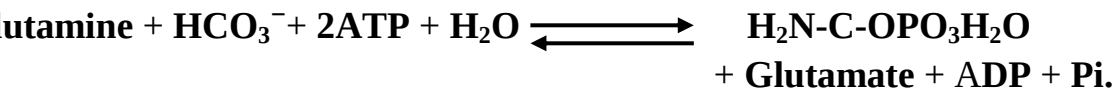
A major distinction between the metabolic routes leading to the synthesis of purine and pyrimidine nucleotides is the timing in the formation of the N-glycosidic bond. This bond is formed in the very early steps in purine synthesis, and the ring system is built upon this foundation. In contrast, the complete pyrimidine nucleus is first synthesized and then attached to ribose-5-phosphate. *Orotic acid,* which contains the pyrimidine nucleus, is the key intermediate introduced in the N-glycosidic linkage. The main enzymatic pathways leading to the formation of orotic acid and its conversion to uridylic acid have been elucidated.

A. **De novo synthesis of orotate.**

In the formation of orotic acid, ammonia is incorporated into the nitrogen atom at position 1 of orotate, carbon dioxide into position 2 and L-asparate into the remainder of the orotate molecule.

(a) Carbamoyl phosphate:

The initial step in pyrimidine biosynthesis is the formation of Carbamoyl phosphate. Enzymes utilizing glutamine as the amino group donor synthesize carbamoyl phosphate. *Carbamoyl phosphate synthetase* isolated from *E. coli* catalyses a reaction in which two molecules of ATP are consumed per molecule of Carbamoyl phosphate synthesized. The enzyme utilizes ammonia as well as glutamine as the amino group donor. However, the affinity for glutamine is much greater than for ammonia.



(b) L-4, 5-Dihydroortate:

N-Carbamoyl aspartate is converted to L-4, 5-dihydroorotic acid. This brings about ring closure catalysed by *dihydro-orotase.*

(c) Orotic acid:

L-4, 5-dihyroorotate is oxidised to orotic acid by the enzyme *dihydro-orotate dehydrogenase.* The enzyme is flavoproteins and transfers the electrons to NAD^+.

L-4, 5-Dihydroorostate +NAD^+ ⟷ Orotic acid + NADH + H^+

(d) Orotidine-5'-phosphate*:*

Orotic acid combines with a phosphoribosyl group from PRPP to form the first nucleotide orotidine-5'-phosphate. The reaction is catalysed by *orotate phosphoribosyl transferase.* The enzyme is specific for orotic and does not react with precursors of orotic acid or related pyrimidines.

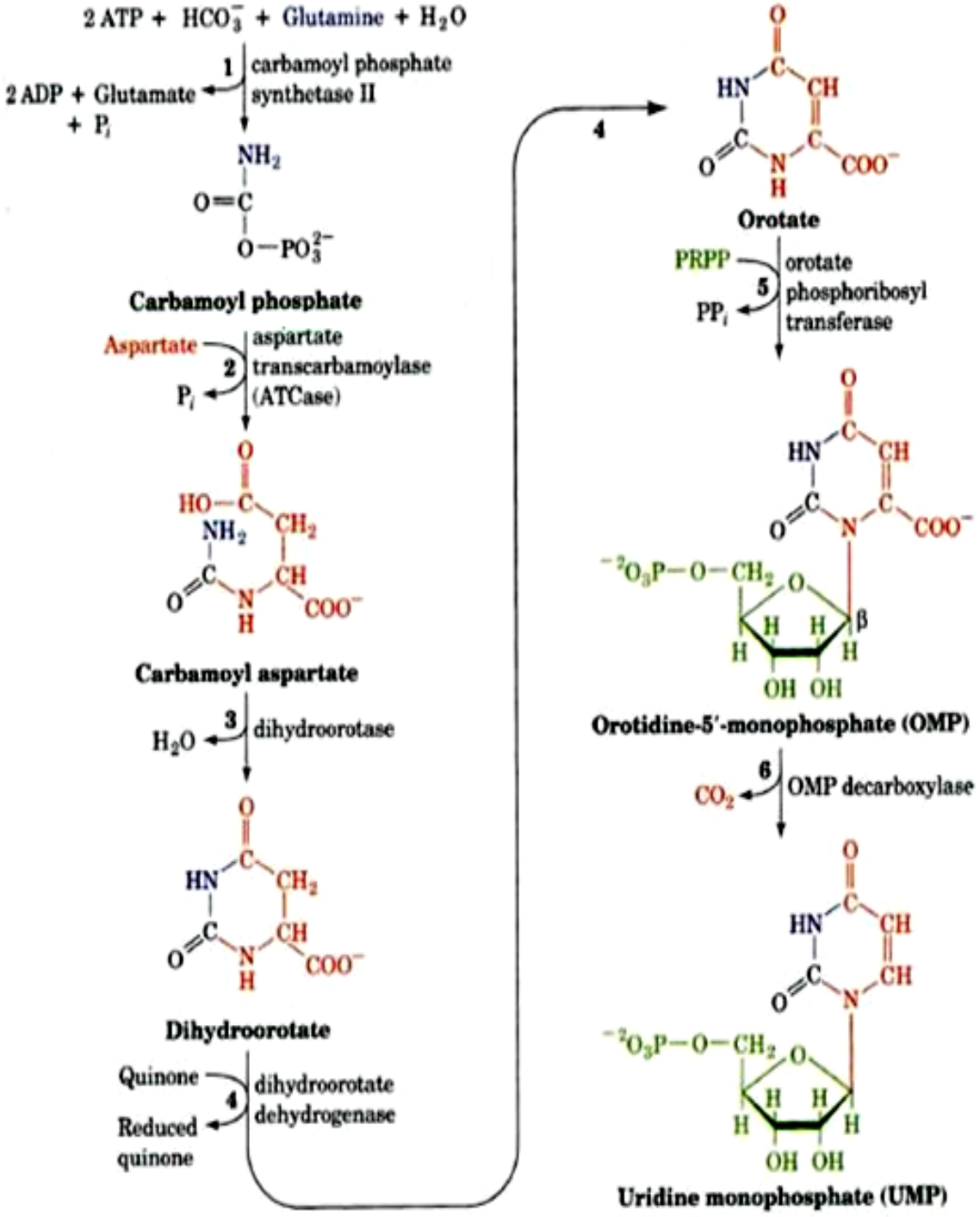
Orotic acid + PRPP $\xrightleftharpoons{Mg^{2+}}$ Orotidine-5'-phosphate + PPi.

(e) Uridylic acid:

Orotidine-5'-phosphate is decarboxylated to yield uridylic acid. This irreversible reaction is catalysed by *oretidine-5'-phosphate decarboxylase.*

Orotidine-5'-phosphate ⟶ Uridylic acid + CO_2

The metabolic pathway leading to the synthesis of uridylic acid is given in Fig.



III. Biosynthesis of deoxyribonucleotides

The purine and pyrimidine deoxyribonucleotides of DNA are derived by direct conversion from corresponding ribonucleotides. The formation of deoxyribonucleotides. Thus formation of deoxyribose from ribose occurs at the nucleotide level. Two distinct classes of *ribonucleotide reductases* have been

described. The enzyme isolated from E.coli, and the other by the enzyme represents one class from *L.leichmannii.* Both enzymes catalyse the substitution of the –OH group at position2' of ribose by a hydrogen, with retention of configuration at the carbon atom.

Reichard and his co-workers have investigated synthesis of deoxyribonucleotides in E. coli. The basic aspects of this system can be summarised as follows:

1. The substrates for reduction are *ribonucleoside diphosphates.*
2. The reduction is catalysed by *ribonucleoside reductase.*
3. The specific hydrogen donor for the reduction is *thioredoxin* in the sulphydryl form. Thioredoxin is a small protein (108 amino acids MW=1,200 in *E. coli),* containing an oxidation-reduction active disulphide with the sequence (-Cys-Gly-Pro-Cys). This sequence represents the active centre of thioredoxin isolated from all sources.
4. The oxidised form of thioredoxin is reduced in the cell by a specific FAD-protein, *thioredoxin reductase,* with NADPH as the hydrogen donor. *Thioredoxin reductase* from *E. coli* contains two moles of FAD, and has a molecular weight of 66,000, with the general structure $(FAD)_2$. Each polypeptide chain contains one oxidation-reduction active disulphide (-Cys-Ala-Thr-Cys). The reaction mechanism involves a stepwise reduction of FAD and oxidation-reduction disulphides. Since oxidation-reduction active disulphides also appear in *ribonucleotide reductase* the overall sequence of electron transfer from NADPH to ribonucleotides involves a shuttle of S_2 / $(SH)_2$ interchanges as described in Fig.
5. All four nucleoside diphosphates, ADP, GDP, CDP and UDP, are corresponding deoxyderivatives by the same *reductase system.*

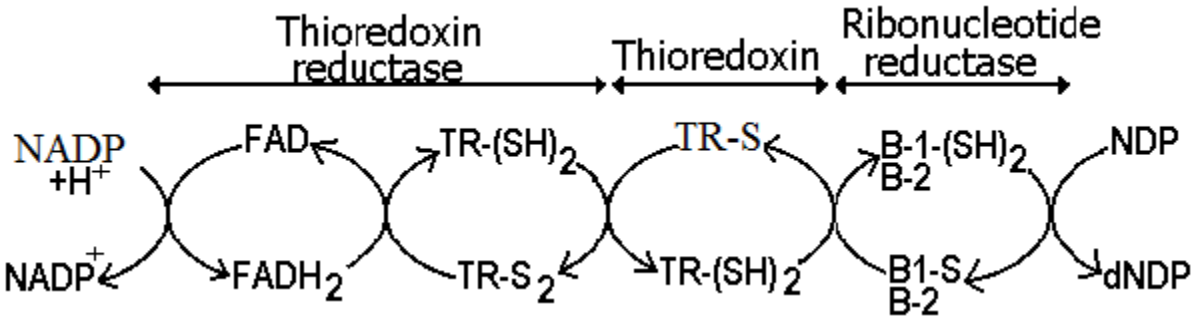


Fig. Involvement of oxidation-reduction active disulphides in ribonucleotide reduction in *E.coli.*