http://intranet.tdmu.edu.ua/data/kafedra/internal/chemistry/classes_stud/en/med/lik/ptn/2/02.%20Basic%20principles%20of%20metabolism%20catabolism,%20anabolism..htm

Differences between prokaryotic and eukaryotic cells

Feature	Prokyryotic cells	Eukaryotic cell
Organisms	Bacterie, cyanobacteria, unicellular	Protozoa, fungi, plants, animals, multicellular
Cell size (uM)	1 – 10 μm	10 – 20 μm
Separated nucleus	No	Yes
Subcellular organels	No	Yes
Character of Chromosomes	Circular DNA	Linear DNA
Ribosome size	70S	80S
Presence of cytoskeleton	No	Yes
Cell division	Lateral/binary fission	Mitosis
DNA	Free	Connected with protiens (histones)
Protein synthesis events	In cytoplasm	Cytoplasm, ER
Location of respiratory enzymes	In plasmatic membrane	In mitochondrial membrate (inner)

Assign the following processes to the corresponding cell compartments:
Transport of ions and small molecules, steroid synthesis, detoxification reactions, respiration, metabolism of glucose, protein biosynthesis, ATP synthesis, intracellular digestions, fatty acids oxidation, DNA synthesis, receptors for small molecules of hormones, modification and sorting of proteins, hydrogen peroxide degradation, protein degradation, RNA synthesis and RNA procesing

Cell membrane: Transport of ions and small molecules, receptors for small molecules of hormones
Cytoplasm: metabolism of glucose, protein biosynthesis, ATP synthesis,
Mitochondria: respiration, protein biosynthesis, ATP synthesis, fatty acids oxidation, DNA synthesis
Nucleus: DNA synthesis, RNA synthesis, RNA processiong, protein biosynthesis,
Rough ER: protein biosynthesis
Smooth ER: steroid synthesis, detoxification reactions,
Golgi apparatus: modification and sorting of proteins, glycosylation of proteins, export of proteins
Lysozome: intracellular digestions
Proteasome: protein degradation
Peroxisome: hydrogen peroxide degradation

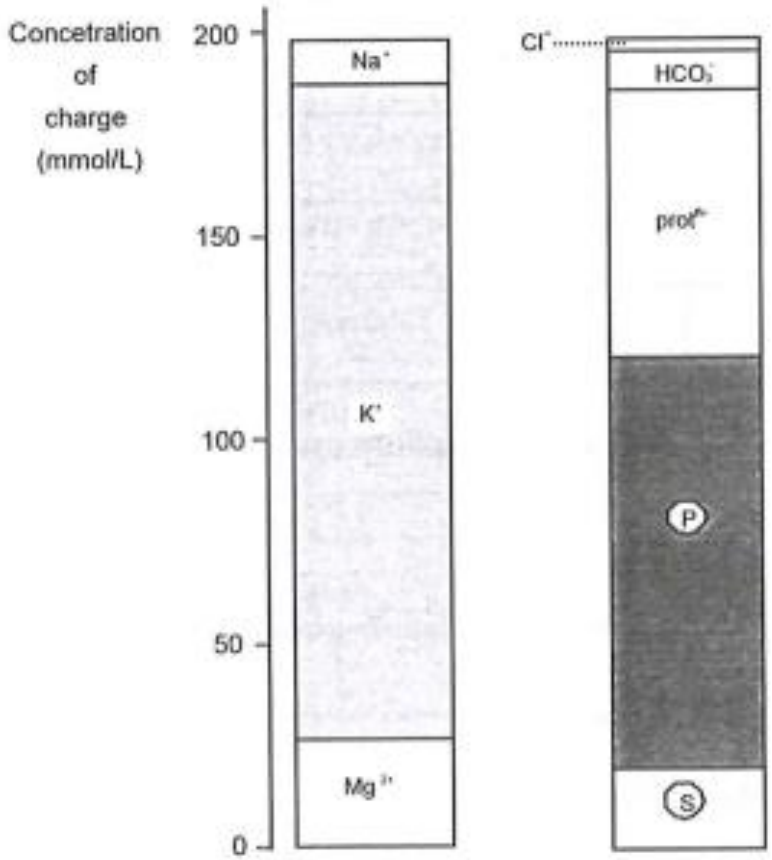
Cell membranes:

- plasmatic membrane

- The **cell membrane** (also known as the **plasma membrane** or **cytoplasmic membrane**) is a biological membrane that separates the interior of all cells from the outside environment.
- The cell membrane is selectively permeable to ions and organic molecules and controls the movement of substances in and out of cells.[3]
- The basic function of the cell membrane is to protect the cell from its surroundings. **It consists of the phospholipid bilayer with embedded proteins (receptors, ion chanells, transporters).**
- Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signalling and serve as the attachment surface for several extracellular structures, including the **cell wall**, **glycocalyx**, and intracellular cytoskeleton. Cell membranes can be artificially reassembled

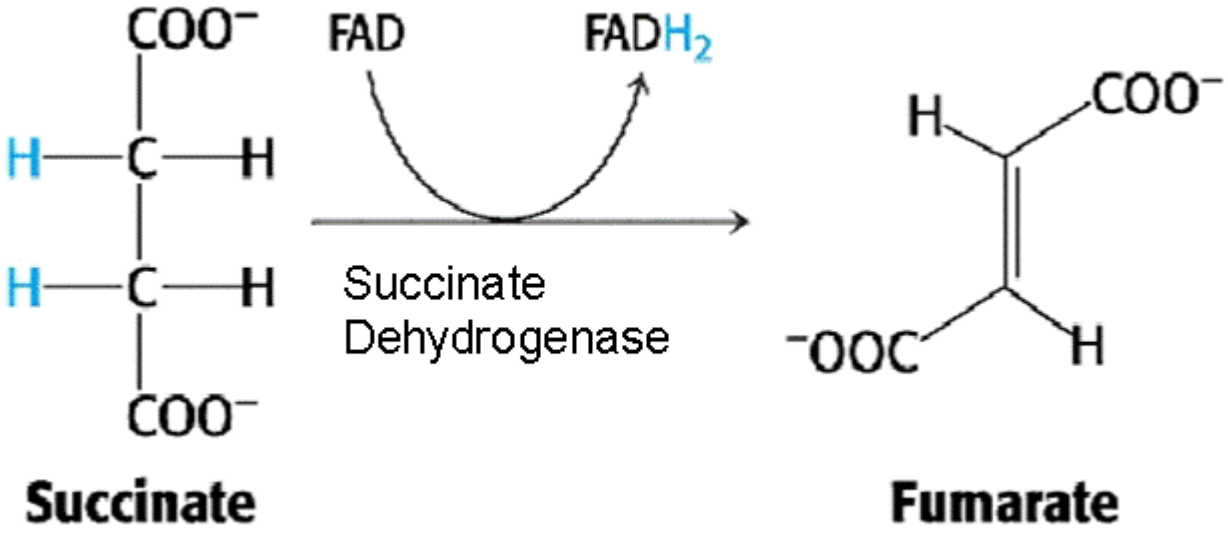
Cytoplasm

Main components of intracellular fluid (ICF)



- Cations
- Anions

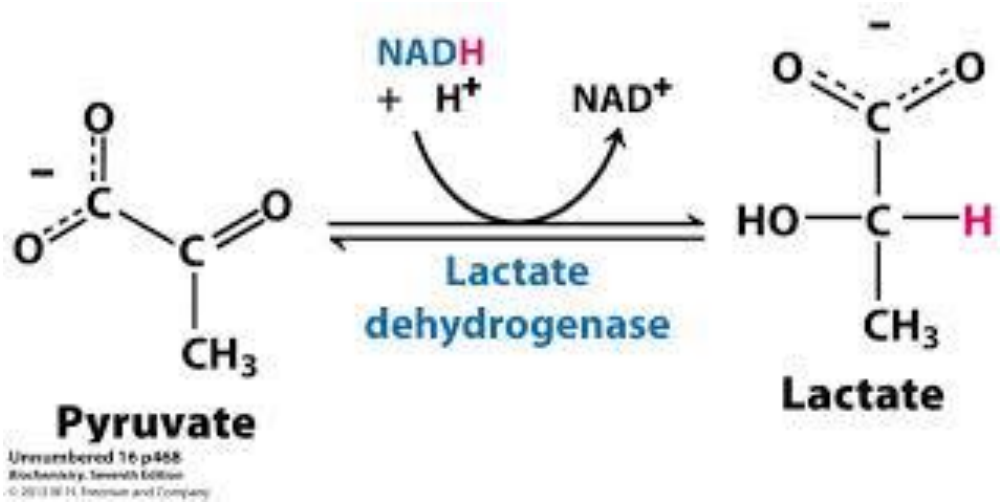
the equation of a reaction catalyzed by succinate dehydrogenase (structures, names, cofactors)



Succinate **Fumarate**

- The Succinate Dehydrogenase Complex of several polypeptides, an FAD prosthetic group and iron-sulfur clusters, **embedded in the inner mitochondrial membrane**. Electrons are transferred from succinate to FAD and then to ubiquinone (Q) in electron transport chain.

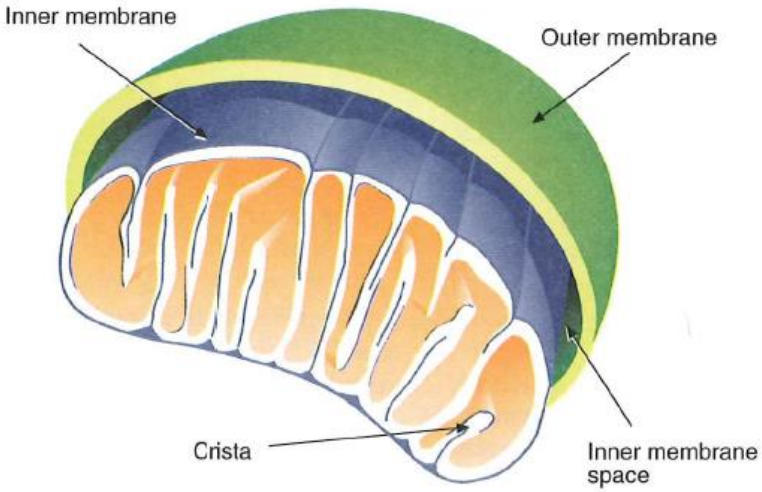
the equation of a reaction catalyzed by lactate dehydrogenase (structures, names, cofactors)



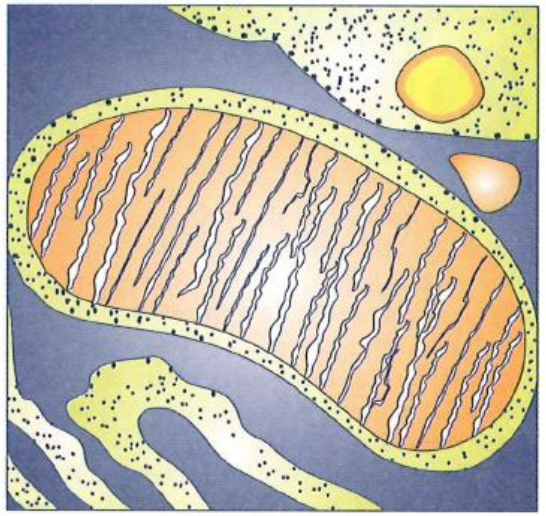
- LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another.
- Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. It converts pyruvate, the final product of glycolysis, to lactate when oxygen is absent or in short supply, and it performs the reverse reaction during the Cori cycle in the liver. At high concentrations of lactate, the enzyme exhibits feedback inhibition, and the rate of conversion of pyruvate to lactate is decreased. It also catalyzes the dehydrogenation of 2-Hydroxybutyrate, but it is a much poorer substrate than lactate.

Mitochondria

- Mitochondria are semi-autonomous organelles that contain their **own DNA, own proteosynthetic apparatus** and are wrapped in a **double membrane**.
- The outer membrane is relatively **high penetrability**, the inner membrane is almost impermeable and therefore contains a number of protein carriers, which allow transfer of the necessary substances.
- Besides the **transporters** are located **inner mitochondrial membrane** as well as **enzymes of the respiratory chain and ATP-synthase enzyme, which occurs aerobic ATP formation phosphorylation**.
- The material filling the content of mitochondria is called the **mitochondrial matrix**. It takes place in the series of important events, such as the **Krebs cycle, urea synthesis, heme synthesis, synthesis of ketones, β oxidation of fatty acids ...**

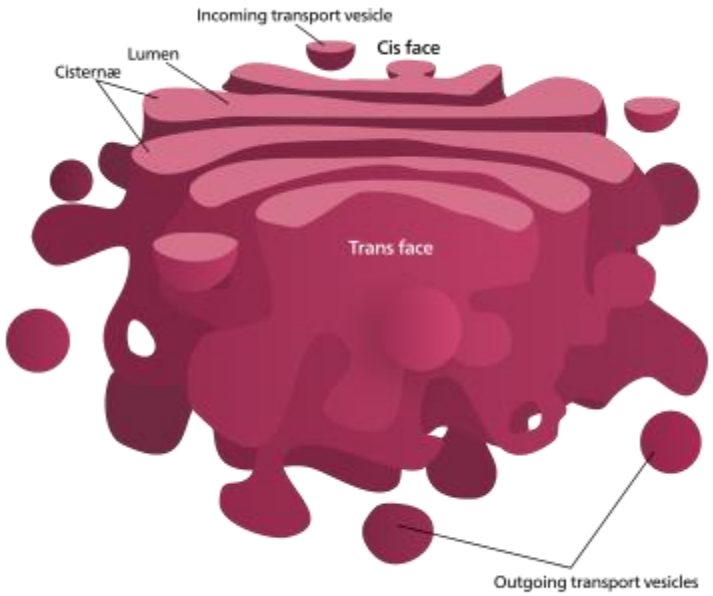


(a)


(b)

Golgi apparatus

- Golgi apparatus consisting of tanks and transport vesicles.
- This is a polarized organelle - we can distinguish the trans-side (which are received by agents - especially proteins - for editing and sorting) and cis-side on which these substances are also released.
- Part of the cellular endomembrane system, the Golgi apparatus packages proteins inside the cell before they are sent to their destination; it is particularly important in the processing of proteins for secretion.



It is of particular importance in processing proteins for secretion, containing a set of glycosylation enzymes that attach various sugar monomers to proteins as the proteins move through the apparatus.

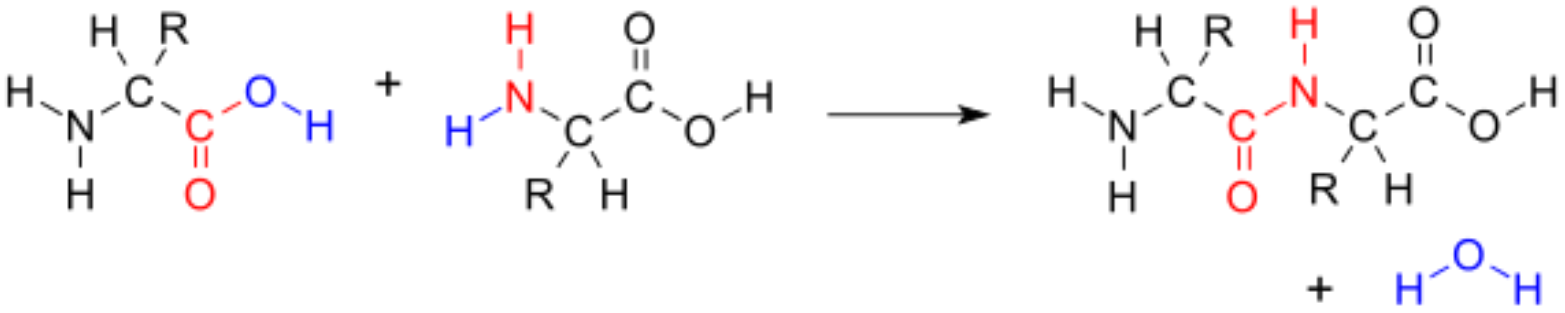
Lysosomes

- Lysosomes are organelles of the **cell digestion**.
- In its membrane comprises a **hydrogen pump**, which is involved in maintaining an **acidic pH** within them.
- Distinguish between primary and secondary lysosomes.
- Primary are those that have not participated in the digestion process, and do not contain remnants of organelles, proteins etc.., And their enzymes have not yet been used.
- Secondary lysosomes are those that are already involved in digestion. Most enzymes found in lysosomes, belongs to the group of **hydrolases**, and the cleavage of various bonds on different molecules.
- They are structurally and chemically spherical vesicles containing hydrolitic enzymes, which are capable of breaking down virtually all kinds of biomolecules, including proteins, nucleic acids, carbohydrates, lipids, and cellular debris. They are known to contain more than fifty different enzymes which are all active at an acidic environment of about pH 5.

The enzyme - type bonds

- α-glucosidase cleaves α-glycosidic linkage between the glucosamine
- β-galactosidase cleaves the β-glycosidic linkage between galactose
- Hyaluronidase cleaves a bond between molecules of hyaluronic acid
- arylsulphatase cleaves the bond sulfoesterovou
- Lysozyme cleaves the glycosidic bond
- cathepsin cleave a peptide bond (the protease)
- collagenase cleaves the triple helix collagen chain
- elastase cleaves a peptide bond (the protease)
- ribonuclease cleaves ribonucleotides diester linkage between
- lipase cleaves the ester bond between glycerol and fatty acids
- phosphatase cleaves the ester linkage (cleaves phosphate)
- ceramidasa cleave the ester bond between the ceramide, and fatty acid

Peptide bond



- When two amino acids form a *dipeptide* through a *peptide bond* it is called condensation. In condensation, two amino acids approach each other, with the acid moiety of one coming near the amino moiety of the other. One loses a hydrogen and oxygen from its carboxyl group (COOH) and the other loses a hydrogen from its amino group (NH_2). This reaction produces a molecule of water (H_2O) and two amino acids joined by a peptide bond (-CO-NH-). The two joined amino acids are called a dipeptide.
- The peptide bond is synthesized when the carboxyl group of one amino acid molecule reacts with the amino group of the other amino acid molecule, causing the release of a molecule of water (H_2O), hence the process is a dehydration synthesis reaction (also known as a condensation reaction).
- The formation of the peptide bond consumes energy, which, in living systems, is derived from ATP.[6] Polypeptides and proteins are chains of amino acids held together by peptide bonds. Living organisms employ enzymes to produce polypeptides, and ribosomes to produce proteins. Peptides are synthesized by specific enzymes. For example, the tripeptide glutathione is synthesized in two steps from free amino acids, by two enzymes: gamma-glutamylcysteine synthetase and glutathione synthetase.[7][8]

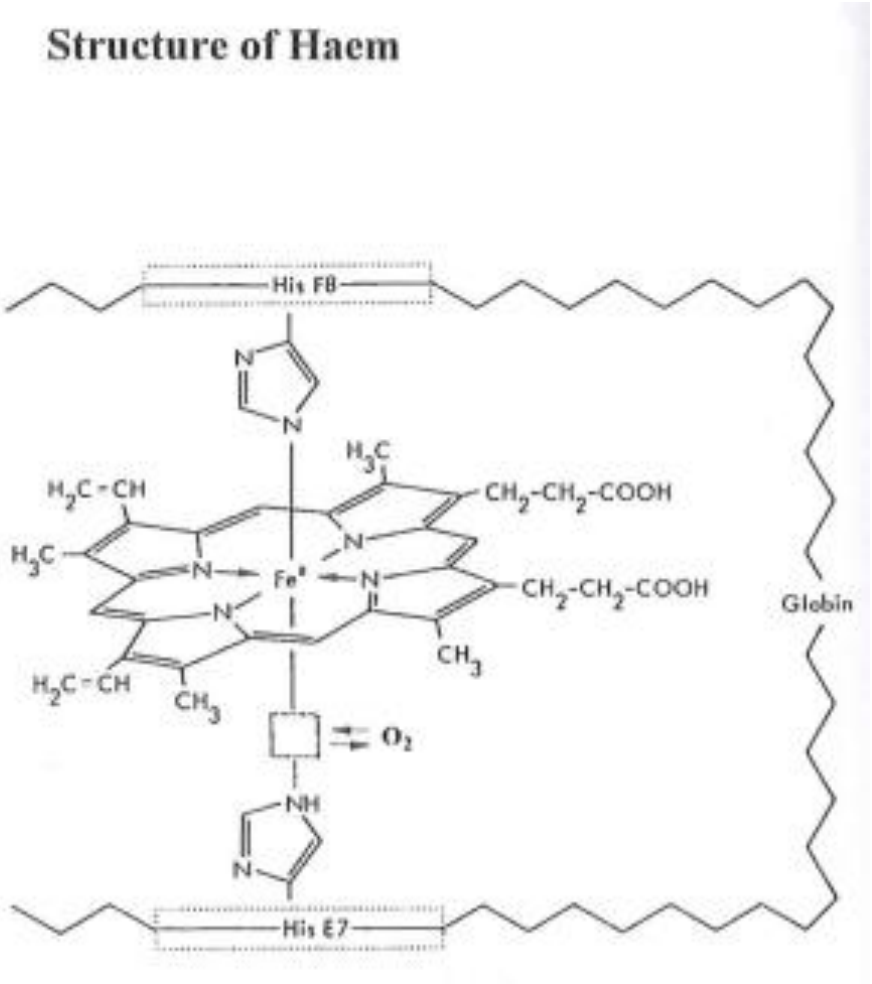
Non covalent interactions

- Coherence of cells and their interaction with each other between molecules (eg. interaction between molecules and receptor molecules and enzymes, etc.)
- And similar interactions are based on non covalent interactions The most important non covalent interactions are:
  - hydrogen bonds
  - electrostatic interactions
  - hydrophobic interactions

  Their main use in various situations described by the following table:

Structure / system	prevailing type of non-covalent interactions :
Proteins: Structure secondary	hydrogen bonding
Proteins: the tertiary structure	hydrophobic and electrostatic interactions
Proteins: Structure quaternary	electrostatic interactions
DNA	hydrogen bonds
Phospholipid bilayer	hydrophobic interactions
Binding of the enzyme-substrate	electrostatic interactions
Binding of antibody-antigen	electrostatic interactions

Characterize the structure of heam and its binding to the globin chain.

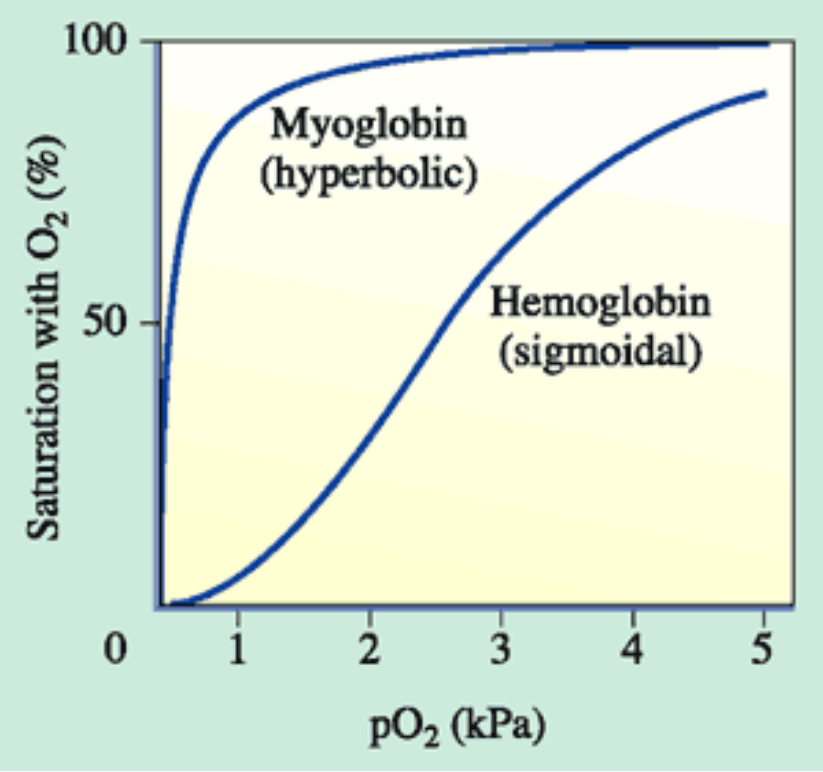


A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methine bridges) with the iron ion bound in the center.[36] The iron ion, which is the site of oxygen binding, coordinates with the four nitrogen atoms in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the N atoms of the imidazole ring of F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond,[37] completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds to Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

saturation curve of hemoglobin and myoglobin, mark areas corresponding to transport O_2 in lungs and mixed venous blood in graph.

The hemoglobin in the red blood cells and myoglobin inside tissues such as muscle cells have to act in tandem for effective oxygen transport. We can examine these coordinated activities using the oxygen binding curves of the two respiratory pigments. For hemoglobin the task is to become completely loaded with oxygen as the blood traverses through the capillaries of the lungs. It must then be able to efficiently off-load oxygen when the blood flows past the low oxygen environment of active tissues, such as heart muscles. The following graph illustrates the different oxygen affinities of myoglobin and hemoglobin at different concentrations of oxygen (given as partial pressure of O_2)

- Hemoglobin (tetramer, cooperation effect)
- Myoglobin (monomer)



Note how the hemoglobin dissociation curve is S-shaped, or sigmoidal, in character. This abrupt change in oxygen affinity over a small range of oxygen concentration is almost like a switch, allowing the hemoglobin to almost fully unload bound oxygen at the tissues where it is needed. We shall see later that a number of modifiers of hemoglobin function act by altering the nature of this binding curve, changing the affinity of the hemoglobin for oxygen. By contrast to hemoglobin, which is found in circulating red blood cells or erythrocytes, myoglobin is found intracellularly in body tissues. To perform its job, myoglobin (Mb) must effectively bind any oxygen released from hemoglobin (Hb). Myoglobin therefore needs to have a higher oxygen affinity than hemoglobin. The oxygen binding curves for myoglobin and hemoglobin are compared in the figure at left.

derivatives of hemoglobin

- hemoglobin (Hb),
- oxyhemoglobin (O2Hb),
- carboxyhemoglobin (COHb),
- methemoglobin (MetHb),
- and sulfhemoglobin (SHb)

Carbon monoxide poisoning (automobile exhaust, incomplete combustion, cigarette smoke) is caused by CO binding to hemoglobin (ferrohemoglobin, Fe^{2+}) forming **carbonylhemoglobin**, which cannot bind oxygen anymore, thus decreases oxygen supply to the tissues and generate tissue hypoxia.

Oxidizing agents oxidize Fe atom in hemoglobin from Fe^{2+} to Fe^{3+}, giving rise to **hemin** (methemoglobin, ferrihemoglobin) which, as a consequence, inhibits oxygen transport. Methemoglobinema arises from nitrite, chlorate, aniline, or nitro benzene poisoning, or from some drugs such as phenacetin and antipyrine.

Hemoglobin derivatives can be quickly identified according to their absorption spectra. **Oxygenated hemoglobin** (oxyhemoglobin, HbO2) has two characteristic maxima at λ=576 and 541nm. **Deoxygenated hemoglobin** (Hb) has only one characteristic maximum at λ=555nm. **Carbonyl hemoglobin** (HbCO) has two characteristic maxima at λ=572 and 539nm. **Methemoglobin** (MetHb) has only one characteristic maximum at λ=633nm. **Sulphurhemoglobin** (SHb) produced after hydrogen sulfid poisoning has only one characteristic maximum at λ=622nm. To distinguish between absorption spectra of HbO2 and HbCO, reduction reaction can be used. In comparison with HbCO, only HbO2 is reduced to Hb and so the absorption spectra differ.

Factors Affecting Rate of Enzyme Reactions

I. Substrate Concentration

The course of a mono-substrate enzyme reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

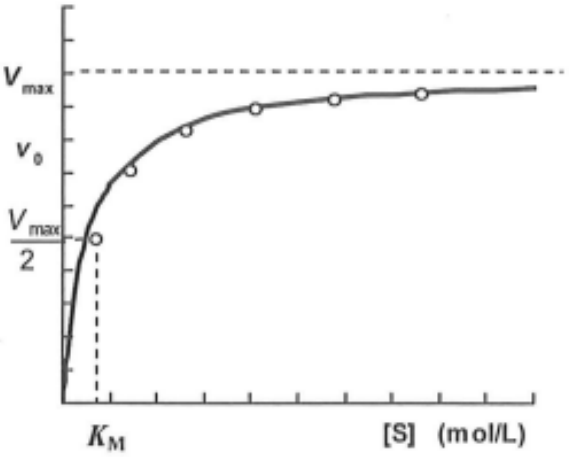
Saturation curve

Dependence of speed of reaction (velocity) on concetration of S (substrate)

Km

concetration of S (substrate) –1/2 of max. velocity

Michaelis Plot (Saturation Curve)



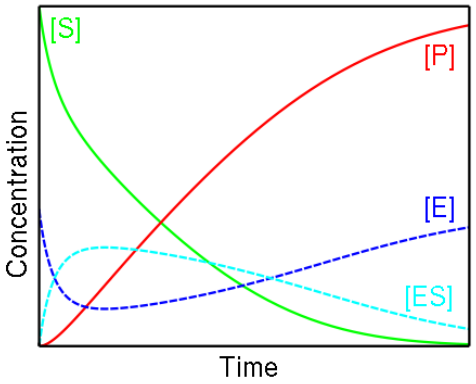
For steady state: **[ES]** = constant

Equation of Michaelis-Menten

$$v_0 = V_{max} \cdot \frac{[S]}{K_M + [S]}$$

[S] … initial concentration of substrate

V_{max} … maximal initial velocity

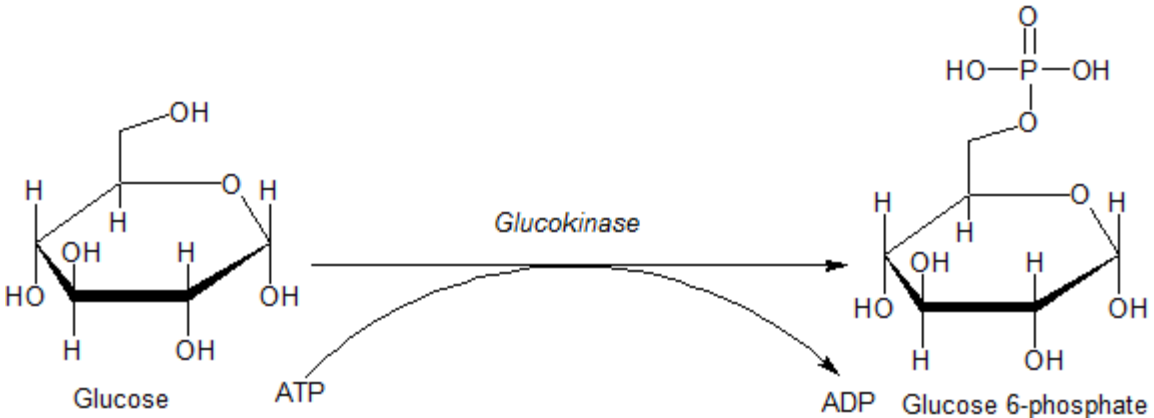


Kinetic curve

Dependence of concetration of S (substrate,) or P (product) on Time

Substrate specifity

- A **hexokinase** is an enzyme that phosphorylates hexoses (six-carbon sugars), forming hexose phosphate. In most organisms, glucose is the most important substrate of hexokinases, and glucose-6-phosphate is the most important product. Hexokinase can transfer an inorganic phosphate group from ATP to a substrate.
- Hexokinases should not be confused with glucokinase, which is a specific isoform of hexokinase. While other hexokinases are capable of phosphorylating several hexoses, glucokinase acts with a 50-fold lower substrate affinity and its only hexose substrate is glucose.
- Glucokinase (EC 2.7.1.2) is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate.
- Glucokinase (GK) is a hexokinase isozyme, related homologously to at least three other hexokinases.[1] All of the hexokinases can mediate phosphorylation of glucose to glucose-6-phosphate (G6P), which is the first step of both glycogen synthesis and glycolysis. However, glucokinase is coded by a separate gene and its distinctive kinetic properties allow it to serve a different set of functions. Glucokinase has a lower affinity for glucose than the other hexokinases do, and its activity is localized to a few cell types, leaving the other three hexokinases as more important preparers of glucose for glycolysis and glycogen synthesis for most tissues and organs. Because of this reduced affinity, the activity of glucokinase, under usual physiological conditions, varies substantially according to the concentration of glucose.[2]

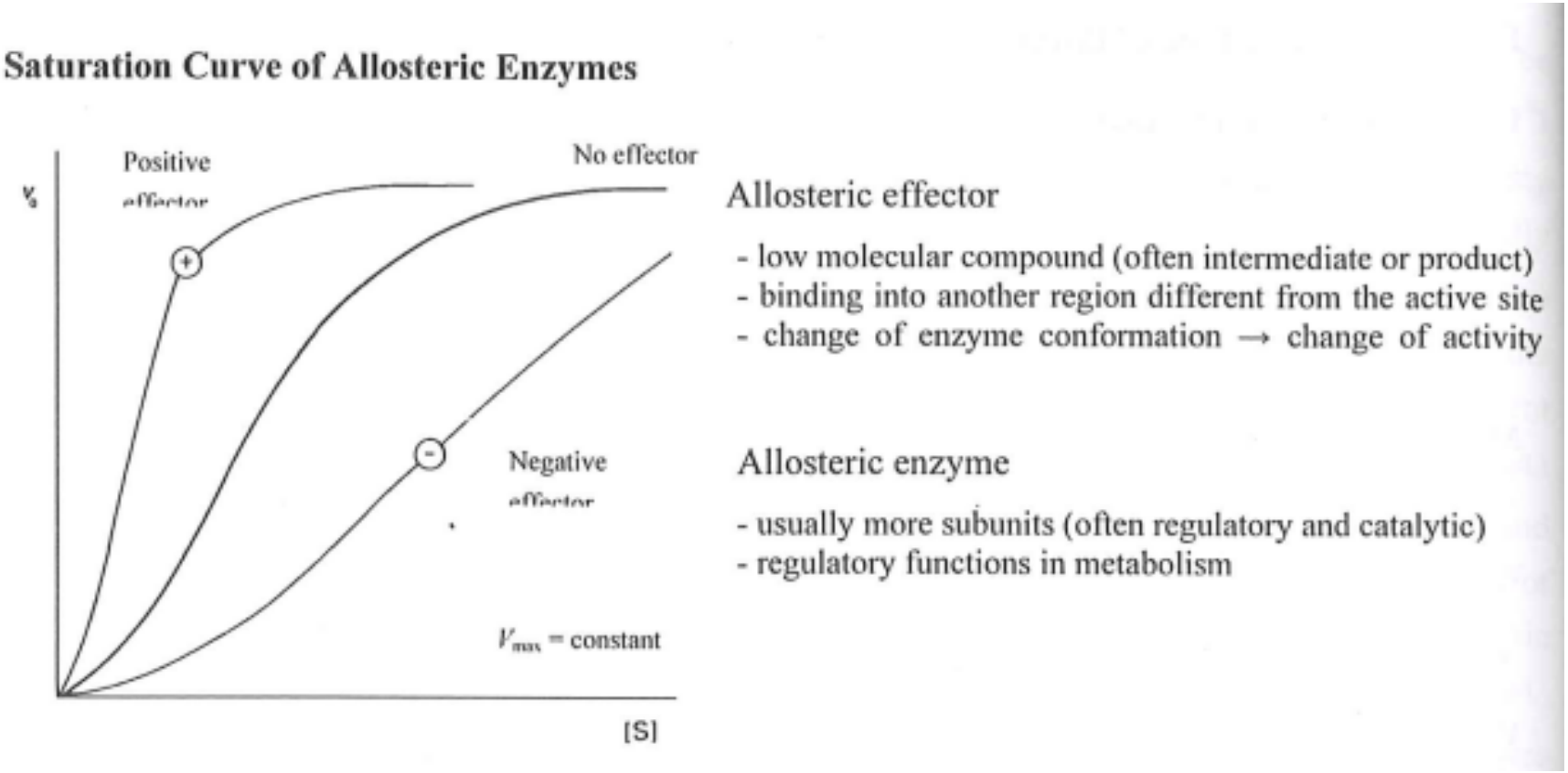


Allosteric enzymes

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector, which results in an apparent change in binding affinity at a different ligand binding site. This "action at a distance" through binding of one ligand affecting the binding of another at a distinctly different site, is the essence of the allosteric concept. **Allostery** plays a crucial role in many fundamental biological processes, including but not limited to cell signaling and the regulation of metabolism. Allosteric enzymes need not be oligomers as previously thought,[1] and in fact many systems have demonstrated allostery within single enzymes.[2] In biochemistry, allosteric regulation (or **allosteric control**) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site.

The site to which the effector binds is termed the *allosteric site*. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics. Effectors that enhance the protein's activity are referred to as *allosteric activators*, whereas those that decrease the protein's activity are called *allosteric inhibitors*.

Allosteric enzymes



- multiple subunits often regulatory and catalytic
- the enzyme binds effector structurally distinct from the substrate often product
- binds to the allosteric sites - other than the active site
- binding causes a conformational change in the enzyme activity …. change - allosteric activation or inhibition

Allosteric enzymes in methabolismus

- **citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase**
- **pyruvate dehydrogenase**
- **Phosphofructokinase (**PFK is an inducible, highly regulated, allosteric enzyme that is a key regulator of glycolysis.)

Enzyme Inhibitor Types

- Inhibitors of enzymes are generally molecules which resemble or mimic a particular enzymes substrate(s). Therefore, it is not surprising that many therapeutic drugs are some type of enzyme inhibitor. The modes and types of inhibitors have been classified by their kinetic activities and sites of actions.
- These include Reversible Competitive Inhibitors, Reversible Non-Competitive Inhibitors, and Irreversible Inhibitors

Reversible Competitive Inhibition

- Competitive inhibitors compete with the substrate for binding at the active site (as E + I). In the double reciprocal plot for a competitive inhibitor acting at the substrate site for the following reasons, notice with increasing concentration of inhibitor, the **V_{max} does not change; however, the K_m of the substrate is increased**. This also reflects the reversible nature of the inhibitor; there is always some concentration of substrate which can displace the inhibitor.

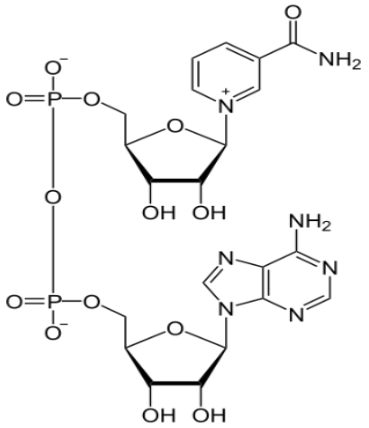
Reversible Non-Competitive Inhibition

- Non-competitive inhibitors combine with both the enzyme (E + I) and the enzyme-substrate (EI + S) complex. The inhibitor binds to a site other that the substrate site, and is thus independent of the presence or absence of substrate. This action results in a conformational change in the protein that affects a catalytic step and hence decreases or eliminates enzyme activity (formation of P). Notice in the reciprocal plot, a non-competitive inhibitor does not affect the binding of the substrate (K_m), but it **does result in a decrease in V_{max}**. This can be explained by the fact that since inhibitor bound to an enzyme inactivates it, the more EI formed will lower [ES] and thus lower the overall rate of the reaction V_{max}.

Irreversible Inhibitors

- Irreversible inhibitors generally result in the destruction or modification of an essential amino acid required for enzyme activity. Frequently, this is due to some type of covalent link between enzyme and inhibitor. These types of inhibitors range from fairly simple, broadly reacting chemical modifying reagents (like iodoacetamide that reacts with cysteines) to complex inhibitors that interact specifically and irreversibly with active site amino acids. (termed suicide inhibitors). These inhibitors are designed to mimic the natural substrate in recognition and binding to an enzyme active site. Upon binding and some catalytic modification, a highly reactive inhibitor product is formed that binds irreversibly and inactivates the enzyme. Use of suicide inhibitors have proven to be very clinically effective

Cofactor, describe function, vitamin form, type of reactions, where we can find it in cell

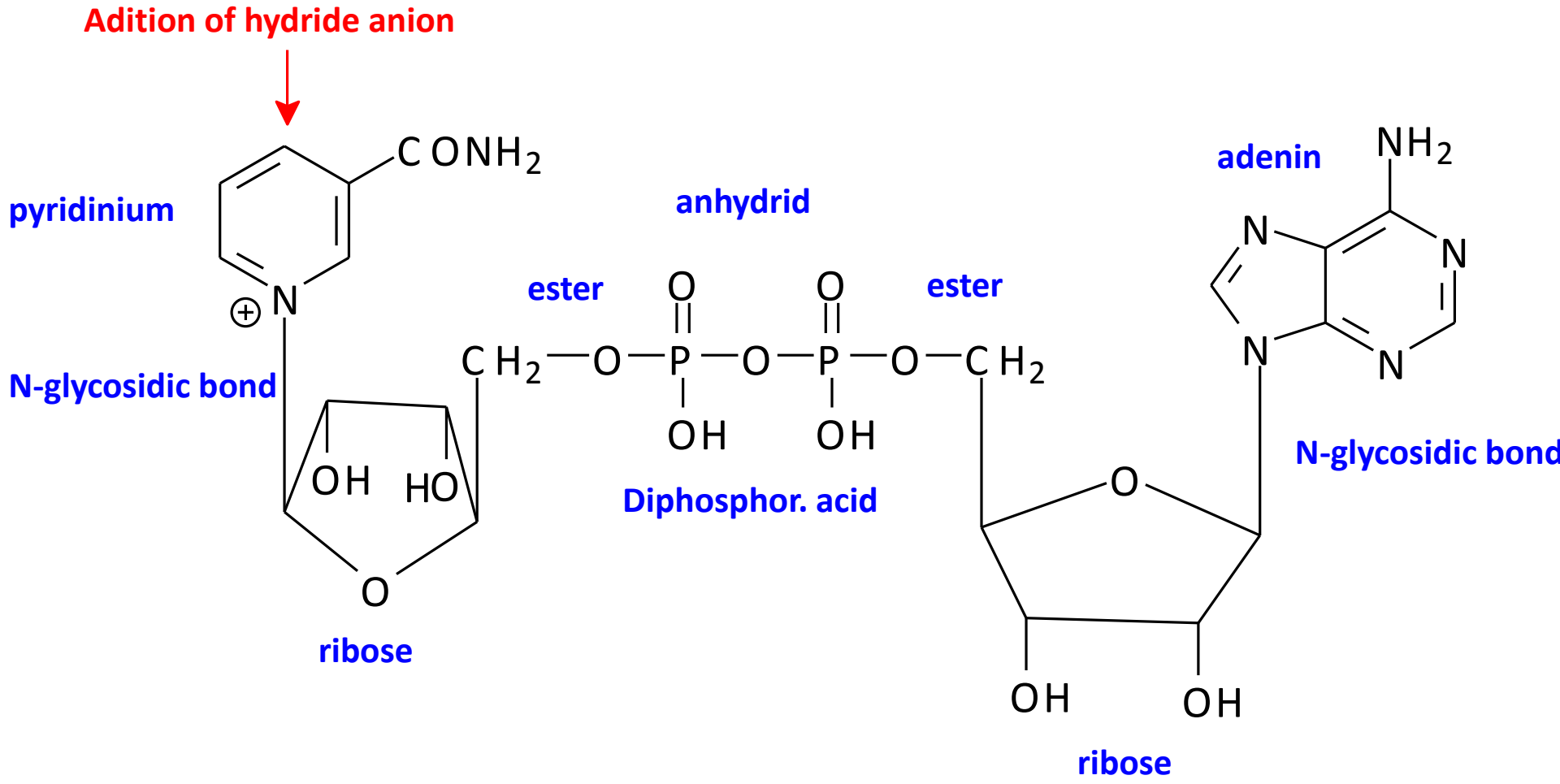


Nicotinamide adenine dinucleotide (**NAD**) is a cofactor found in all living cells. The compound is a dinucleotide, because it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide. Nicotinamide adenine dinucleotide exists in two forms, an oxidized and reduced form abbreviated as **NAD^+** and **NADH** respectively.

In organisms, NAD can be synthesized from simple building-blocks (*de novo*) from the amino acids tryptophan or aspartic acid. In an alternative fashion, more complex components of the coenzymes are taken up from food as the vitamin called niacin.

Nicotinamide adenine dinucleotide is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is, therefore, found in two forms in cells: NAD^+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced. This reaction forms NADH, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD. However, it is also used in other cellular processes, the most notable one being a substrate of enzymes that add or remove chemical groups from proteins, in posttranslational modifications.

Cofactors - NAD^+

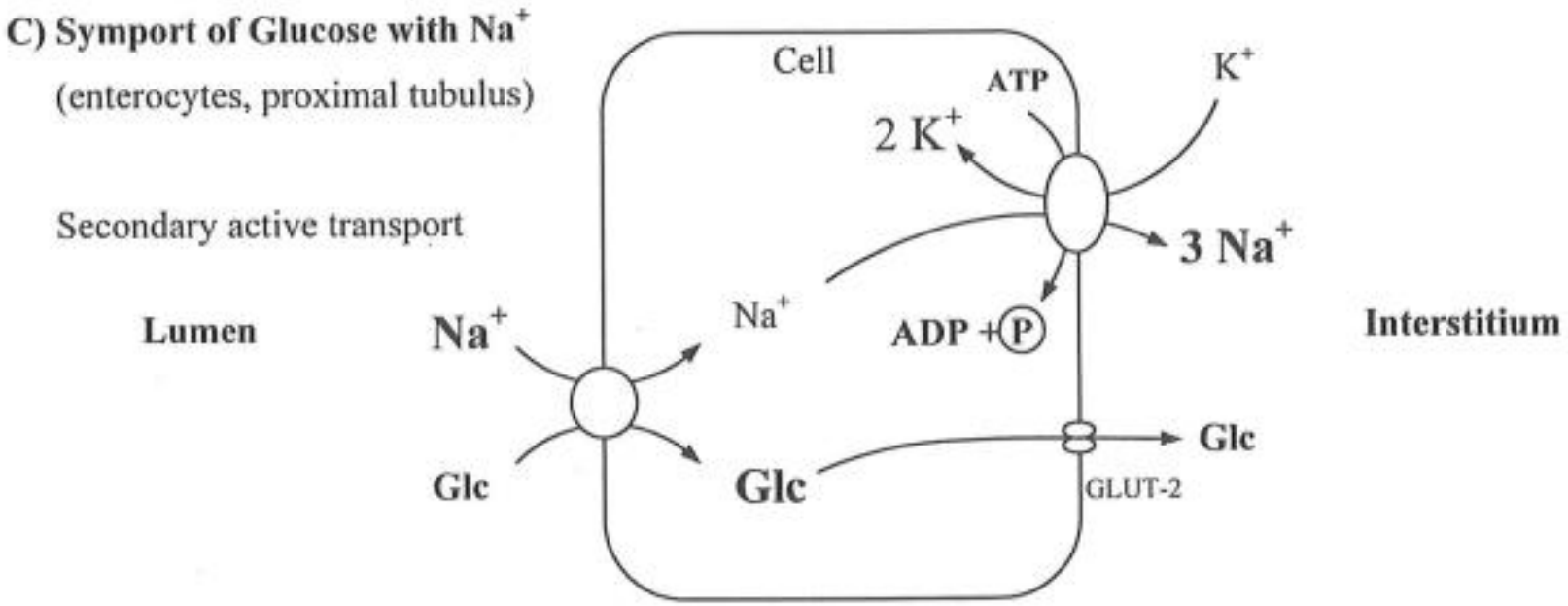


NOVÁK, Jan. Biochemie I.
Brno: Muni, 2009, Enzymy s. 8

chaperone

molecular chaperones are proteins that assist the covalent folding or unfolding and the assembly or disassembly of other macromolecular structures. Chaperones are present when the macromolecules perform their normal biological functions and have correctly completed the processes of folding and/or assembly. The chaperones are concerned primarily with protein folding. The first protein to be called a chaperone assists the assembly of nucleosomes from folded histones and DNA and such assembly chaperones, especially in the nucleus,[1][2] are concerned with the assembly of folded subunits into oligomeric structures.[3]

Mechanism of glucose transport into the cells - symport of glucose with Na+

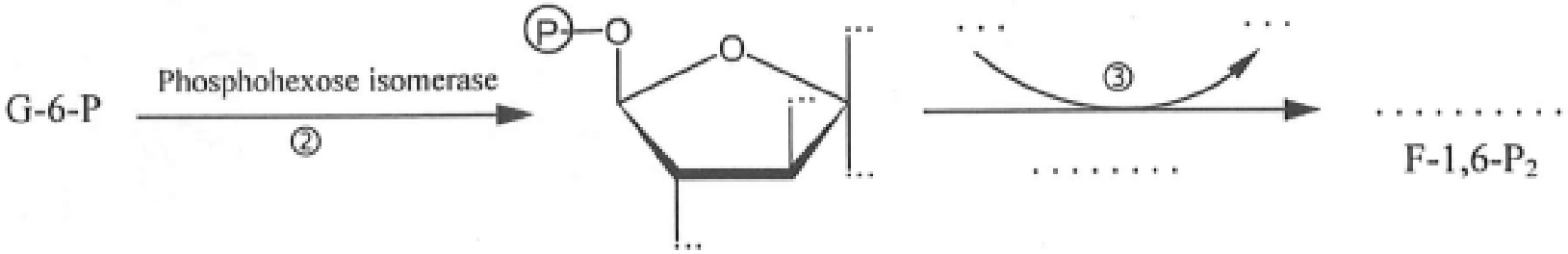


3. Describe various mechanisms of glucose transport into the cells using figures above.

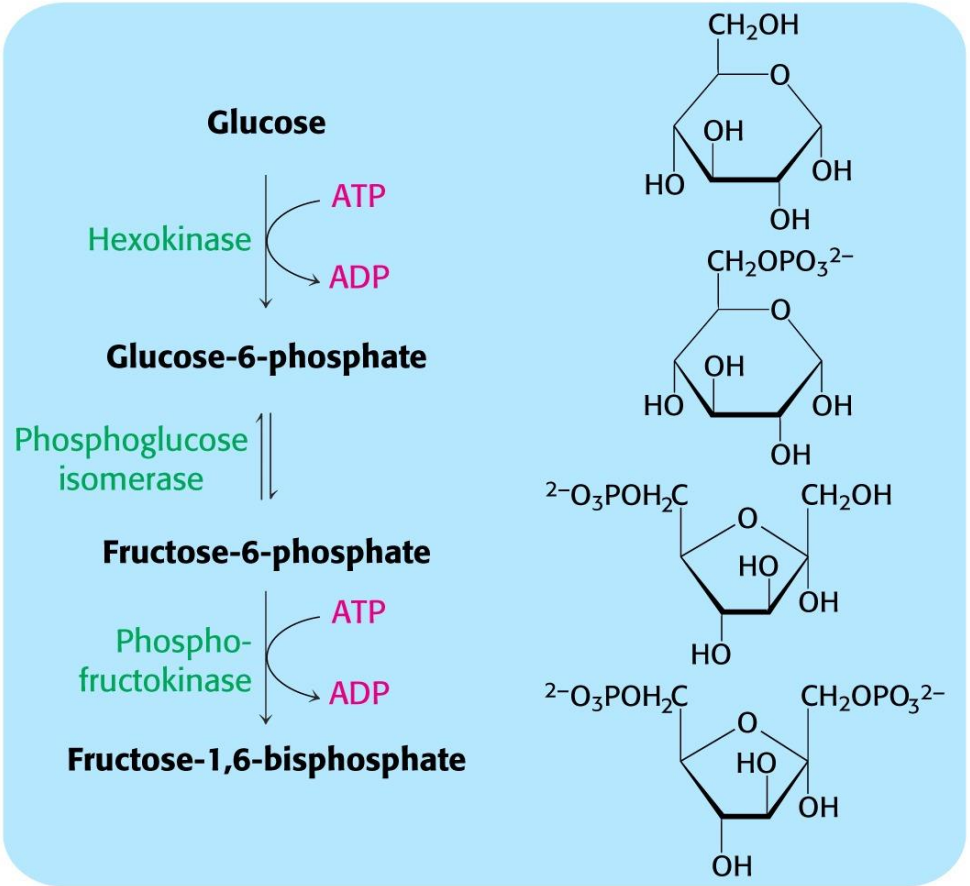
The secondary active transport of glucose in the kidney is Na^+ linked; therefore an Na^+ gradient must be established. This is achieved through the action of the Na^+/K^+ pump, the energy for which is provided through the hydrolysis of ATP. Three Na^+ ions are extruded from the cell in exchange for two K^+ ions entering through the intramembrane enzyme Na+/K+-ATPase; this leaves a relative deficiency of Na^+ in the intracellular compartment. Na^+ ions diffuse down their concentration gradient into the columnar epithelia, co-transporting glucose. Once inside the epithelial cells, glucose reenters the bloodstream through facilitated diffusion through GLUT2 transporters.

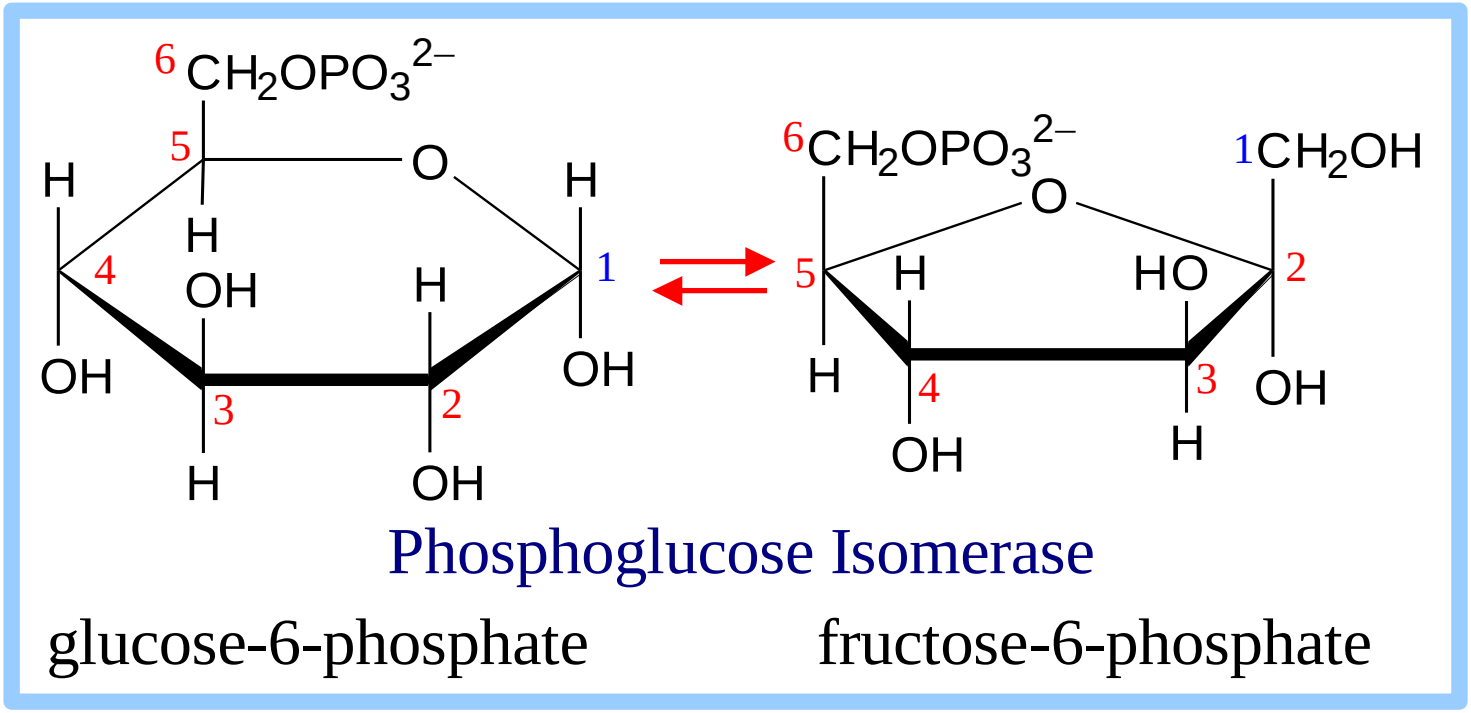
Glycolysis

7. Complete the following scheme:



Stage 1

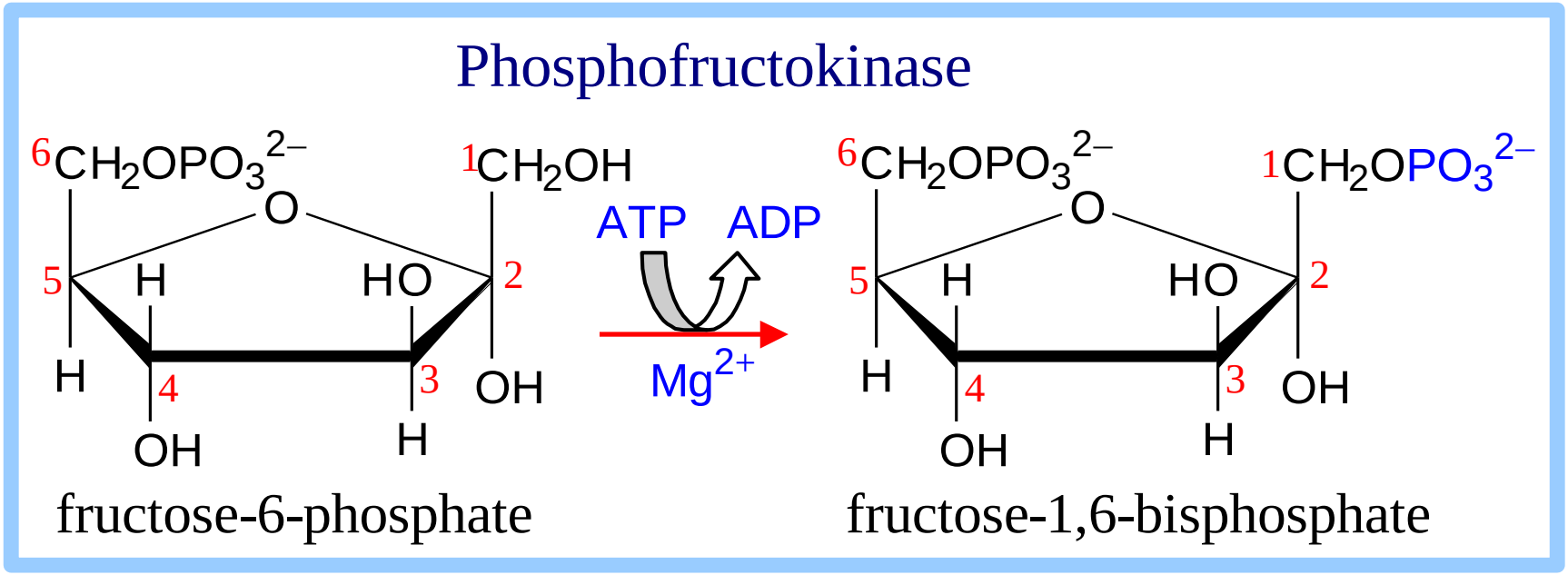




2. Phosphoglucose Isomerase catalyzes:

glucose-6-P (aldose) ←→ **fructose-6-P** (ketose)

The mechanism involves acid/base catalysis, with ring opening, isomerization via an **enediolate intermediate**, and then ring closure. A similar reaction catalyzed by Triosephosphate Isomerase will be presented in detail.



3. Phosphofructokinase catalyzes:

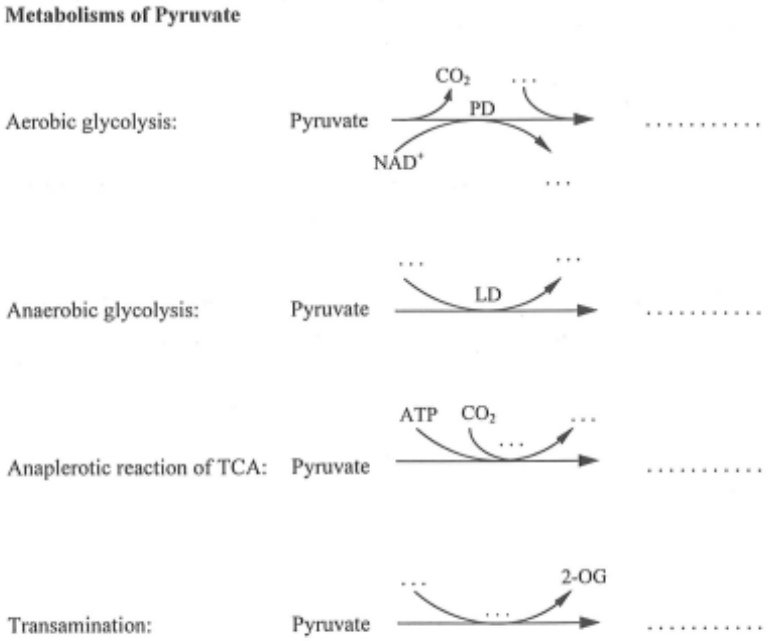
fructose-6-P + ATP → fructose-1,6-bisP + ADP

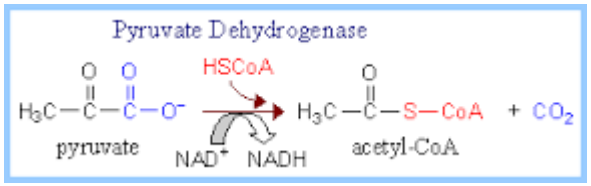
This highly **spontaneous** reaction has a mechanism similar to that of Hexokinase.

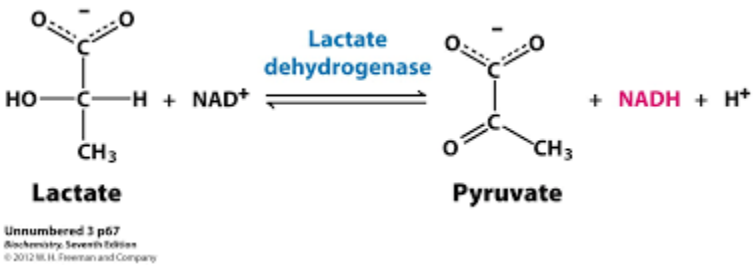
The Phosphofructokinase reaction is the **rate-limiting step** of Glycolysis.

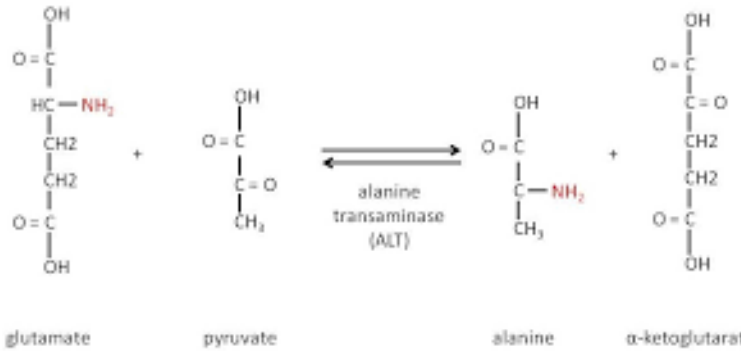
The enzyme is highly **regulated**, as will be discussed later.

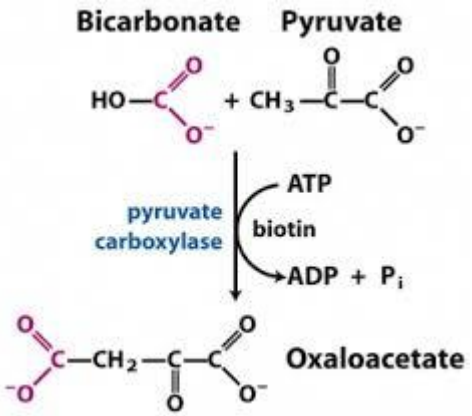
metabolism of pyruvate, name of process, structure and name of substrate and products, names of enzymes

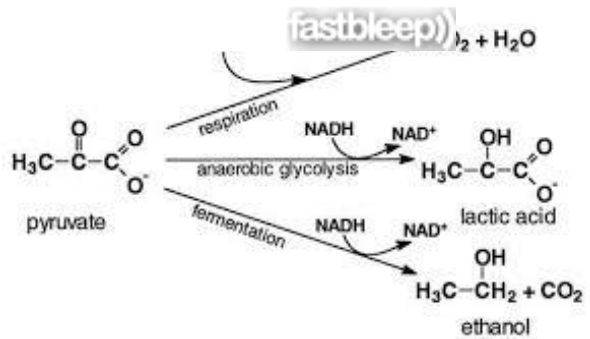












Total energy yield of aerobic glycolysis

Aerobic glycolysis till pyruvate:

Reaction	ATP yield
glucose $\rightarrow$2 pyruvate (substrate level phosporylation)	2
2 NADH $\rightarrow$ 2NAD$^+$	4-6*

Further conversions of pyruvate:

** Depending on shuttle type (see lecture Respiratory chain)*

Reakce	ATP yield
2 pyruvate $\rightarrow$ 2 acetylCoA + 2 NADH	6*
2 acetyl CoA $\rightarrow$ 2 CO_2 + 6 NADH + 2 $FADH_2$	2x 12
Total maximal energy yield	36-38 ATP

* (2x NADH to the resp. chain)

Energy yield of anaerobic glycolysis

Anaerobic glycolysis till pyruvate:

Reaction	ATP yield
glucose →2 x pyruvate (substrate-level phosphorylation)	2
2 NADH → 2NAD+	0

Formation and consumption of NADH at **anaerobic** glycolysis

Reaction	Yield/loss NADH
2 glyceraldehyde-3-P → 2 1,3-bisP-glycerát	+2
2 pyruvate → 2 lactate	-2
In sum	0

•The energy yield of anaerobic glycolysis is only 2 ATP from substrate level phosphorylation

- it is only small portion of the total energy conserved in molecule of glucose
- it has high significane at situations when
  - supply of oxygen is limited
  - tissue do not dispose of mitochondrias (ercs, leukocytes, ..)
  - it is necessary to spare lactate for gluconeogenesis

Gluconeogenesis (GNG)

is a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates. From breakdown of proteins, these substrates include glucogenic amino acids (although not ketogenic amino acids); from breakdown of lipids (such as triglycerides), they include glycerol (although not fatty acids); and from other steps in metabolism they include pyruvate and lactate.
Gluconeogenesis is one of several main mechanisms used by humans and many other animals to maintain blood glucose levels, avoiding low levels (hypoglycemia). Other means include the degradation of glycogen (glycogenolysis),[1] fatty acid breakdown.

gluconeogenesis takes place mainly in the liver and, to a lesser extent, in the cortex of the kidneys. In ruminants, this tends to be a continuous process.[3] In many other animals, the process occurs during periods of fasting, starvation, low-carbohydrate diets, or intense exercise. The process is highly endergonic until it is coupled to the hydrolysis of ATP or GTP, effectively making the process exergonic. For example, the pathway leading from pyruvate to glucose-6-phosphate requires 4 molecules of ATP and 2 molecules of GTP to proceed spontaneously. Gluconeogenesis is often associated with ketosis. Gluconeogenesis is also a target of therapy for type 2 diabetes, such as the antidiabetic drug, metformin, which inhibits glucose formation and stimulates glucose uptake by cells.[4] In ruminants, because metabolizable dietary carbohydrates tend to be metabolized by rumen organisms, gluconeogenesis occurs regardless of fasting, low-carbohydrate diets, exercise, etc.[5]

Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage

In humans, glycogen is made and stored primarily in the cells of the liver and the muscles hydrated with three or four parts of water.[3] Glycogen functions as the secondary long-term energy storage,[*citation needed*] with the primary energy stores being fats held in adipose tissue. Muscle glycogen is converted into glucose by muscle cells, and liver glycogen converts to glucose for use throughout the body including the central nervous system.

Glycogen synthesis is, unlike its breakdown, endergonic - it requires the input of energy. Energy for glycogen synthesis comes from uridine triphosphate (UTP), which reacts with glucose-1-phosphate, forming UDP-glucose, in a reaction catalysed by UTP—glucose-1-phosphate uridylyltransferase. Glycogen is synthesized from monomers of UDP-glucose initially by the protein glycogenin, which has two tyrosine anchors for the reducing end of glycogen, since glycogenin is a homodimer. After about eight glucose molecules have been added to a tyrosine residue, the enzyme glycogen synthase progressively lengthens the glycogen chain using UDP-glucose, adding α(1→4)-bonded glucose. The glycogen branching enzyme catalyzes the transfer of a terminal fragment of six or seven glucose residues from a nonreducing end to the C-6 hydroxyl group of a glucose residue deeper into the interior of the glycogen molecule. The branching enzyme can act upon only a branch having at least 11 residues, and the enzyme may transfer to the same glucose chain or adjacent glucose chains.

intracellular degradation of proteins

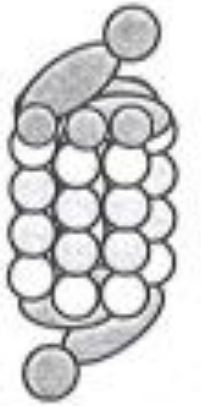
Intracellular Degradation of Proteins

a) Lysosomal Protein Turnover

- Proteins degraded: extracellular (accepted by endocytosis),
  membrane bonded,
  intracellular under the stress (autophagy)

b) Ubiquitin – Proteasome Pathway (cytoplasm, nucleus)

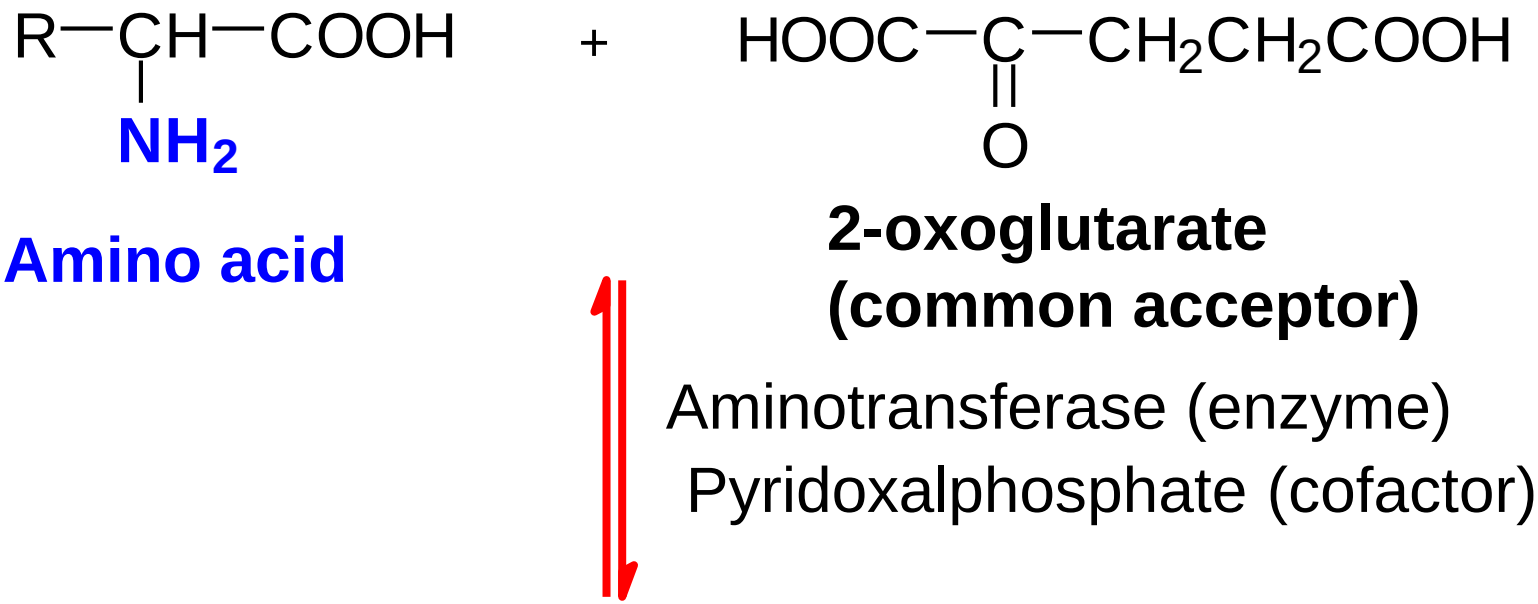
- Proteins degraded: damaged or misfolded intracellular proteins
  proteins coded by viruses and other intracellular parasites
  transcription factors
  cyclins and other regulation proteins
  proteins with the short half-life



Proteasome

- Important for cell processes (growth, differentiation, signal transduction, apoptosis).

General equation of transamination reaction

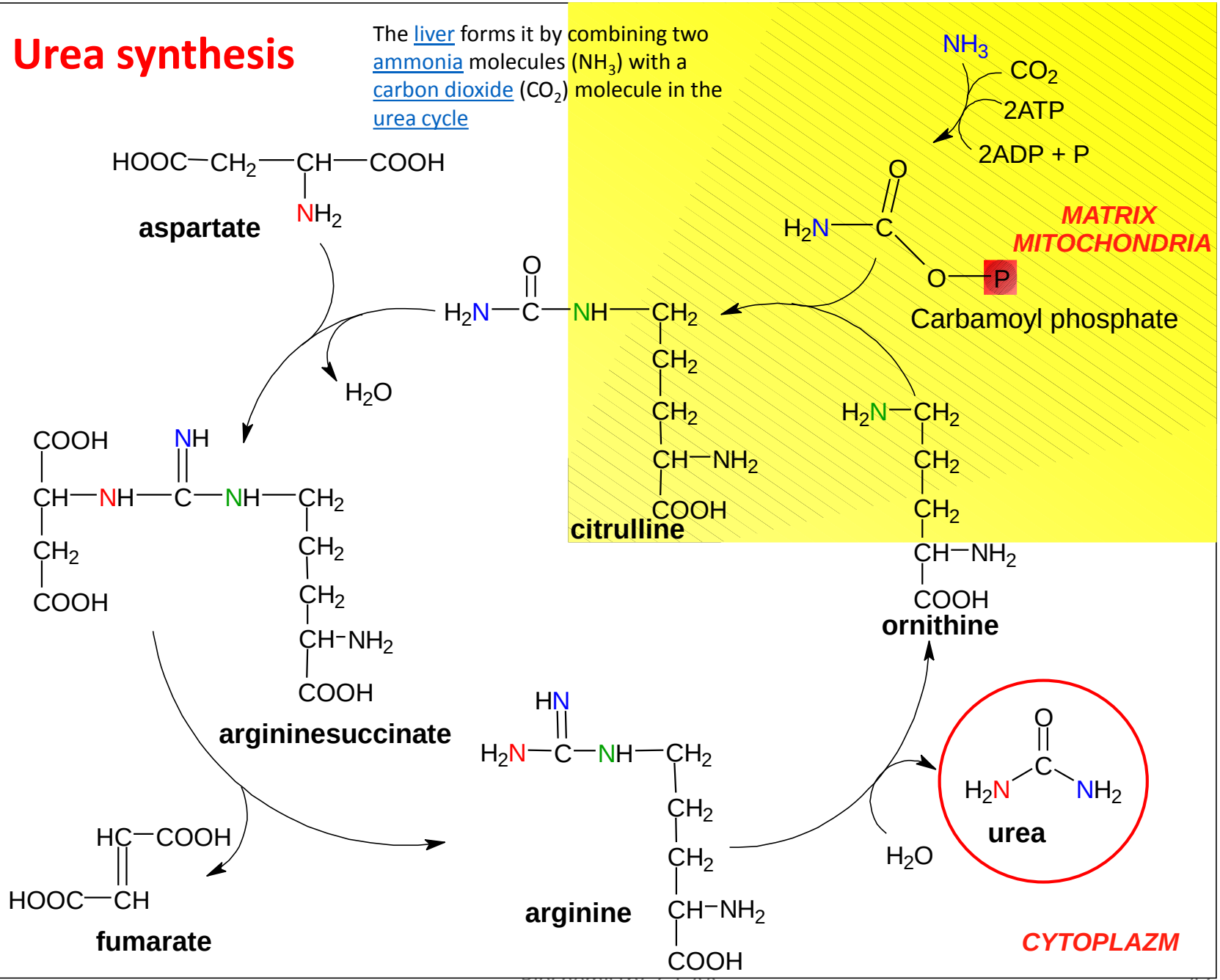


The transaminase enzymes

- are important in the production of various amino acids, and measuring the concentrations of various transaminases in the blood is important in the diagnosing and tracking many diseases. Transaminases require the coenzyme pyridoxal-phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid. Enzyme-bound pyridoxamine in turn reacts with pyruvate, oxaloacetate, or alpha-ketoglutarate, giving alanine, aspartic acid, or glutamic acid, respectively. Many transamination reactions occur in tissues, catalysed by transaminases specific for a particular amino/keto acid pair. The reactions are readily reversible, the direction being determined by which of the reactants are in excess. The specific enzymes are named from one of the reactant pairs, for example; the reaction between glutamic acid and pyruvic acid to make alpha ketoglutaric acid and alanine is called glutamic-pyruvic transaminase or GPT for short.
- Tissue transaminase activities can be investigated by incubating a homogenate with various amino/keto acid pairs. Transamination is demonstrated if the corresponding new amino acid and keto acid are formed, as revealed by paper chromatography. Reversibility is demonstrated by using the complementary keto/amino acid pair as starting reactants. After chromatogram has been taken out of the solvent the chromatogram is then treated with ninhydrin to locate the spots.
- Two important transaminase enzymes are AST (SGOT) and ALT (SGPT), the presence of elevated transaminases can be an indicator of liver damage. This discovery was made by Fernando De Ritis, Mario Coltorti and Giuseppe Giusti in 1955 at the University of Naples.[1][2][3]

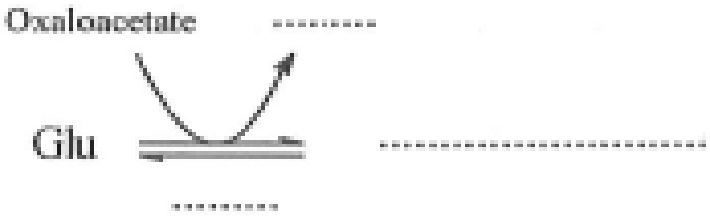
Urea synthesis

The liver forms it by combining two ammonia molecules (NH_3) with a carbon dioxide (CO_2) molecule in the urea cycle

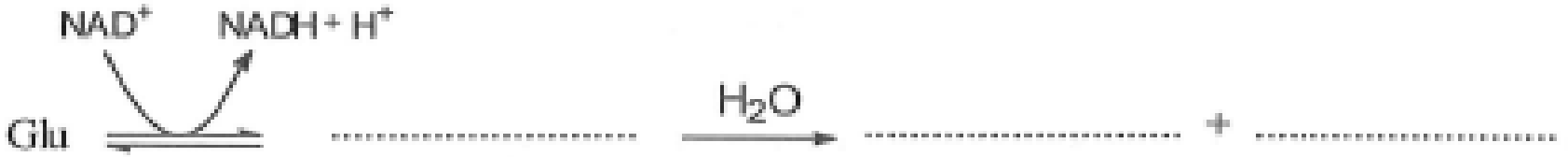


Glutamate

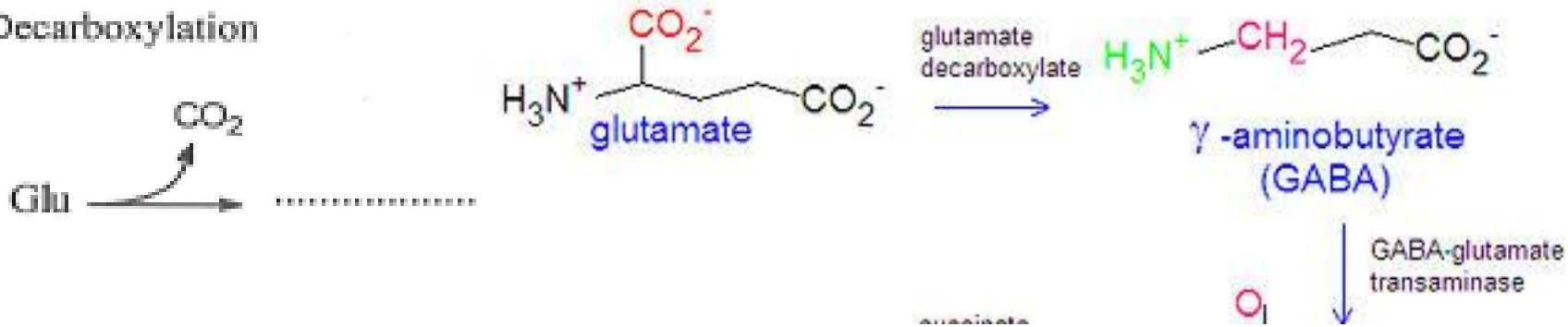
A. Transamination



B. Oxidative deamination



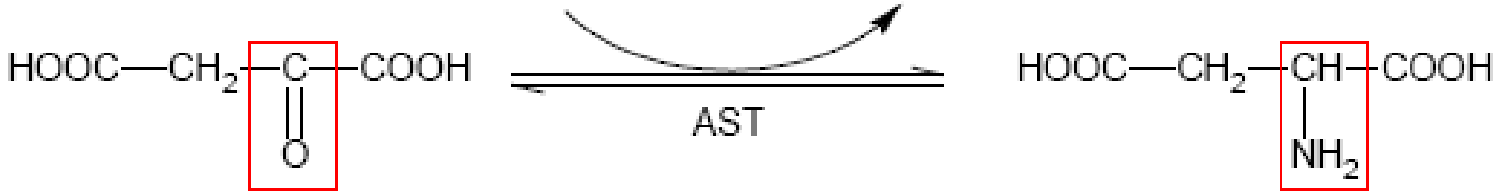
C. Decarboxylation



Regeneration of Aspartate

- PRODUCTION **transamination from oxalcetate**

(enzyme **AST – aspartateminotransferase**

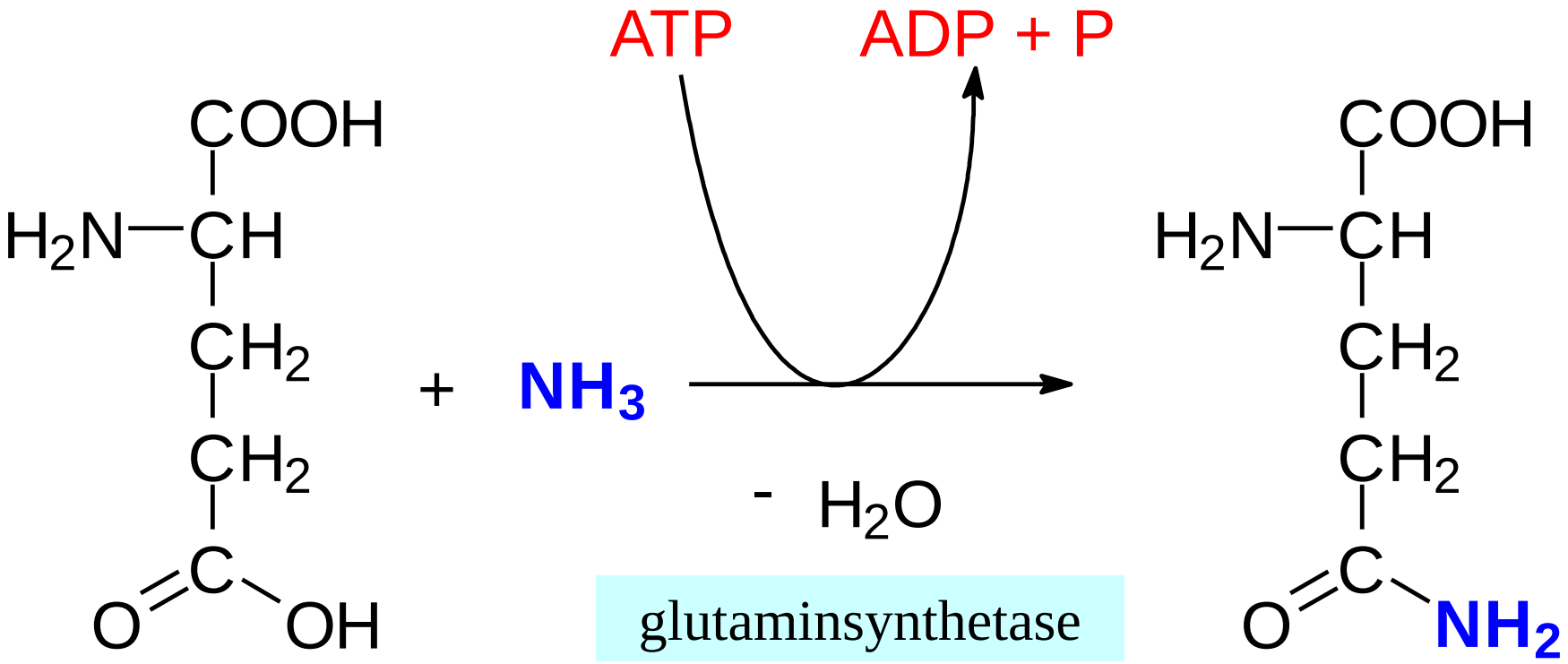


OA **Asp**

- OA- intermediate of CAC
- Substrate for TA
- Substrate for **2-oxoglutarate** (one reaction of CAC)

2. way detoxication NH_3

Synthesa glutamine



glutamate

glutamine

Deamination

e. g. oxidative deamination of Glu

Glu → α-ketoglutarate by **glutamate dehydrogenase**

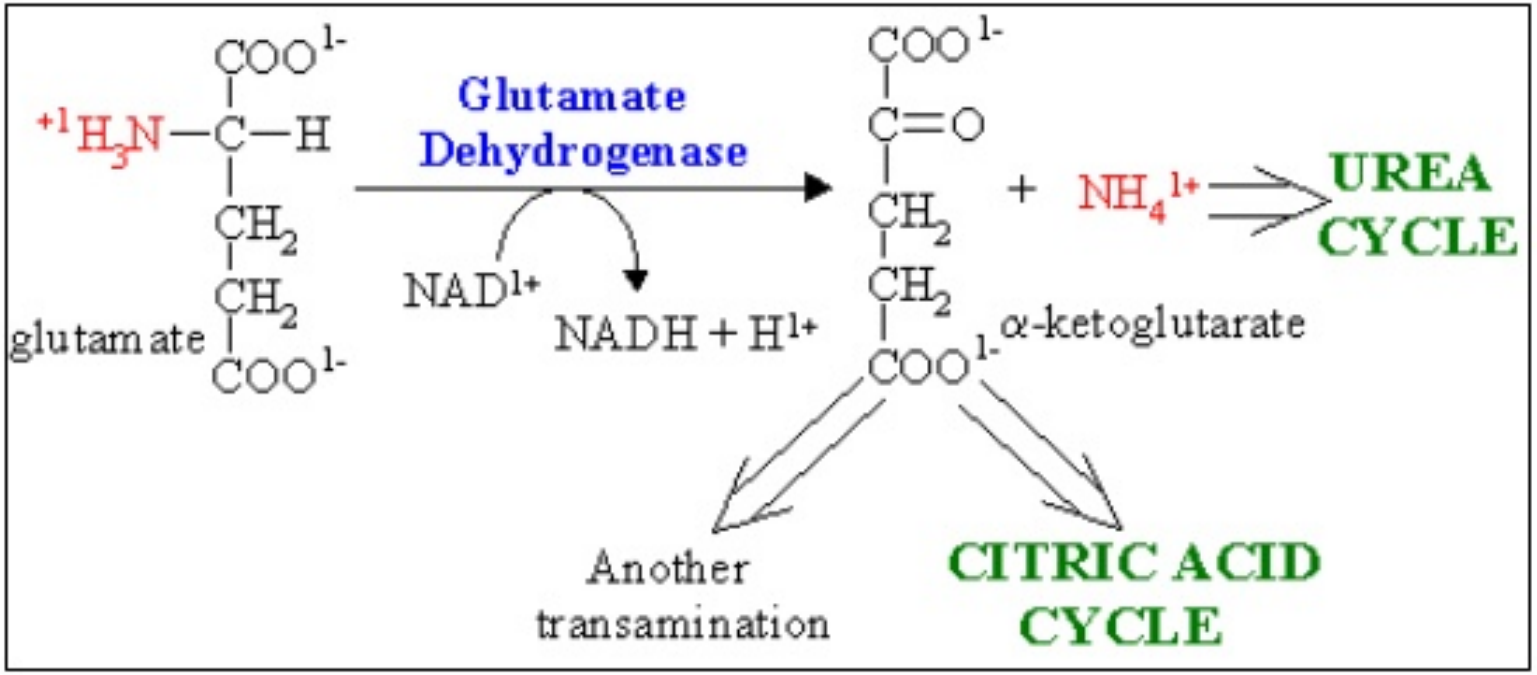
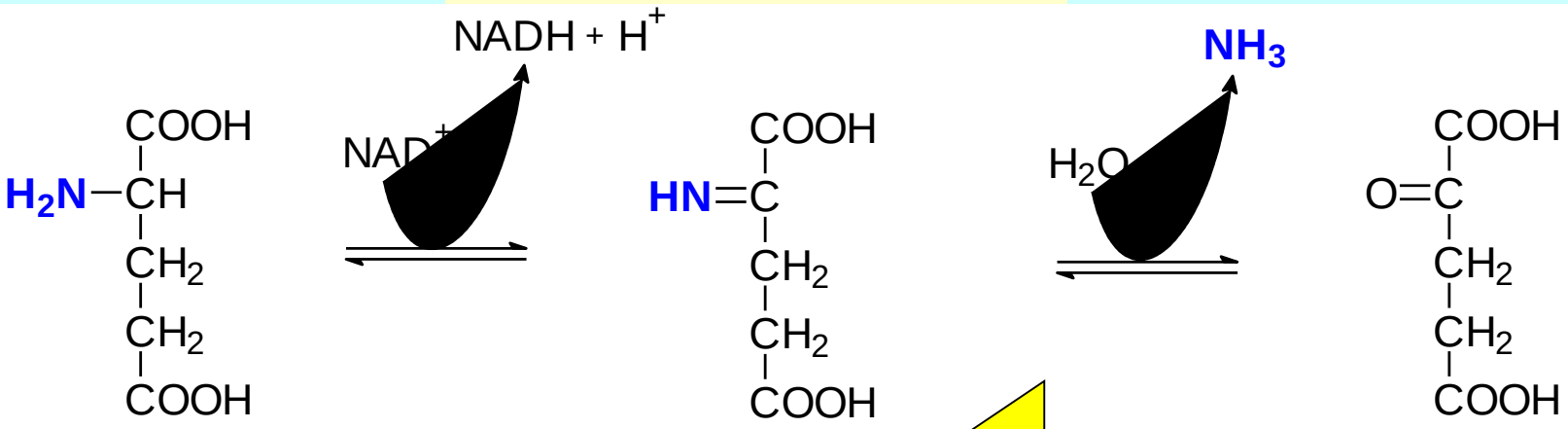


Figure is found at http://www.sbuniv.edu/~ggray/CHE3364/b1c25out.html

GMD r is reversibe reaction

Main NH3 formation in tissue

Dehydrogenation	deamination	glutamate
glutamate	2-iminoglutarate	



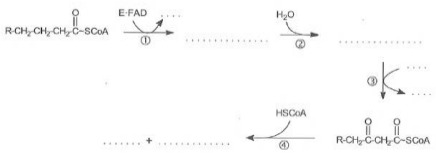
3. Way of detoxication of NH3

Hydrogenation	amination	2-oxoglutarate

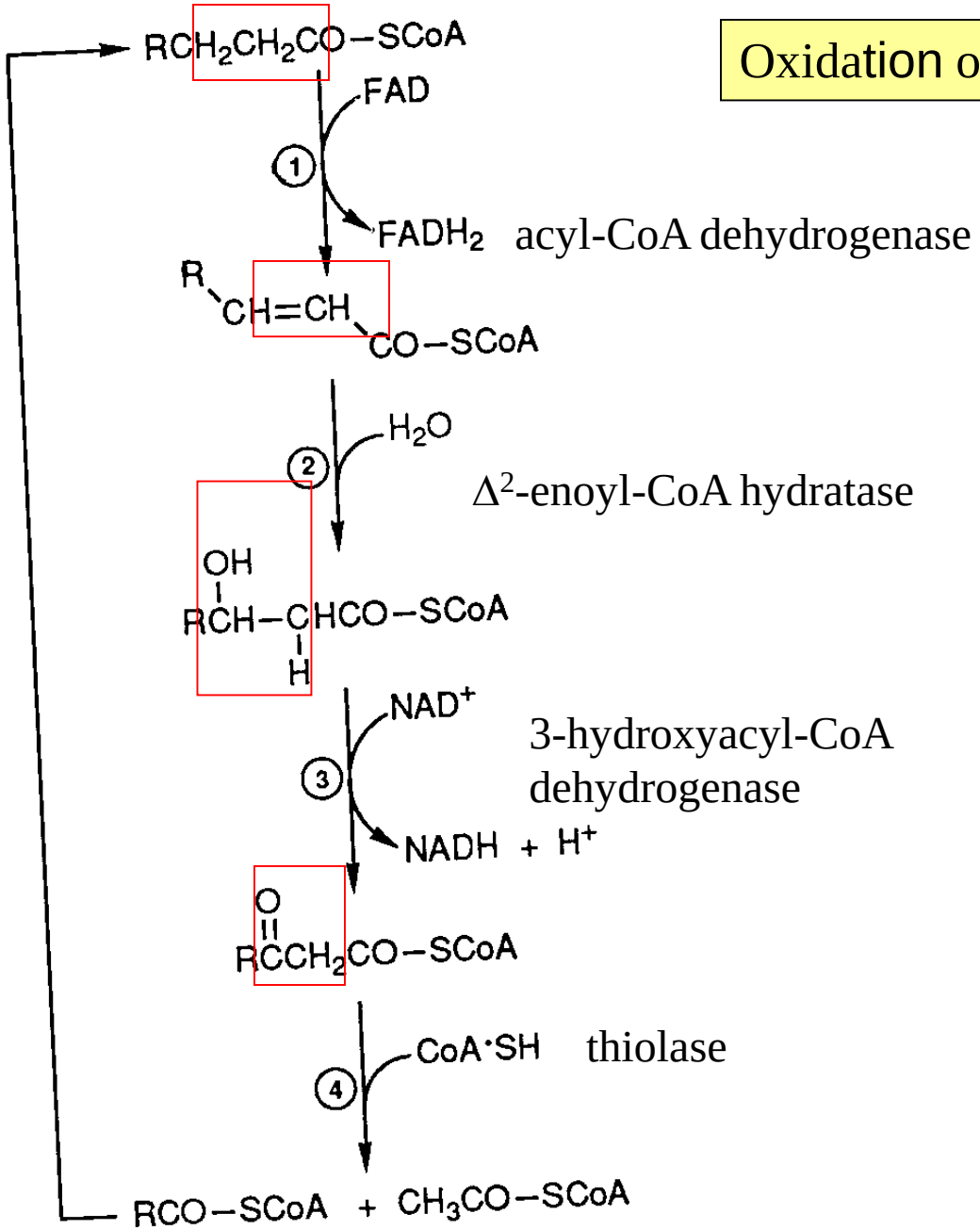
β-oxidation

Oxidation of FA

- 1.dehydrogenation (FAD)
- 2.hydratation



- 3 dehydrogenation (NAD^+)
- 4 transfer of acyl to CoASH



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Fatty Acid Synthesis

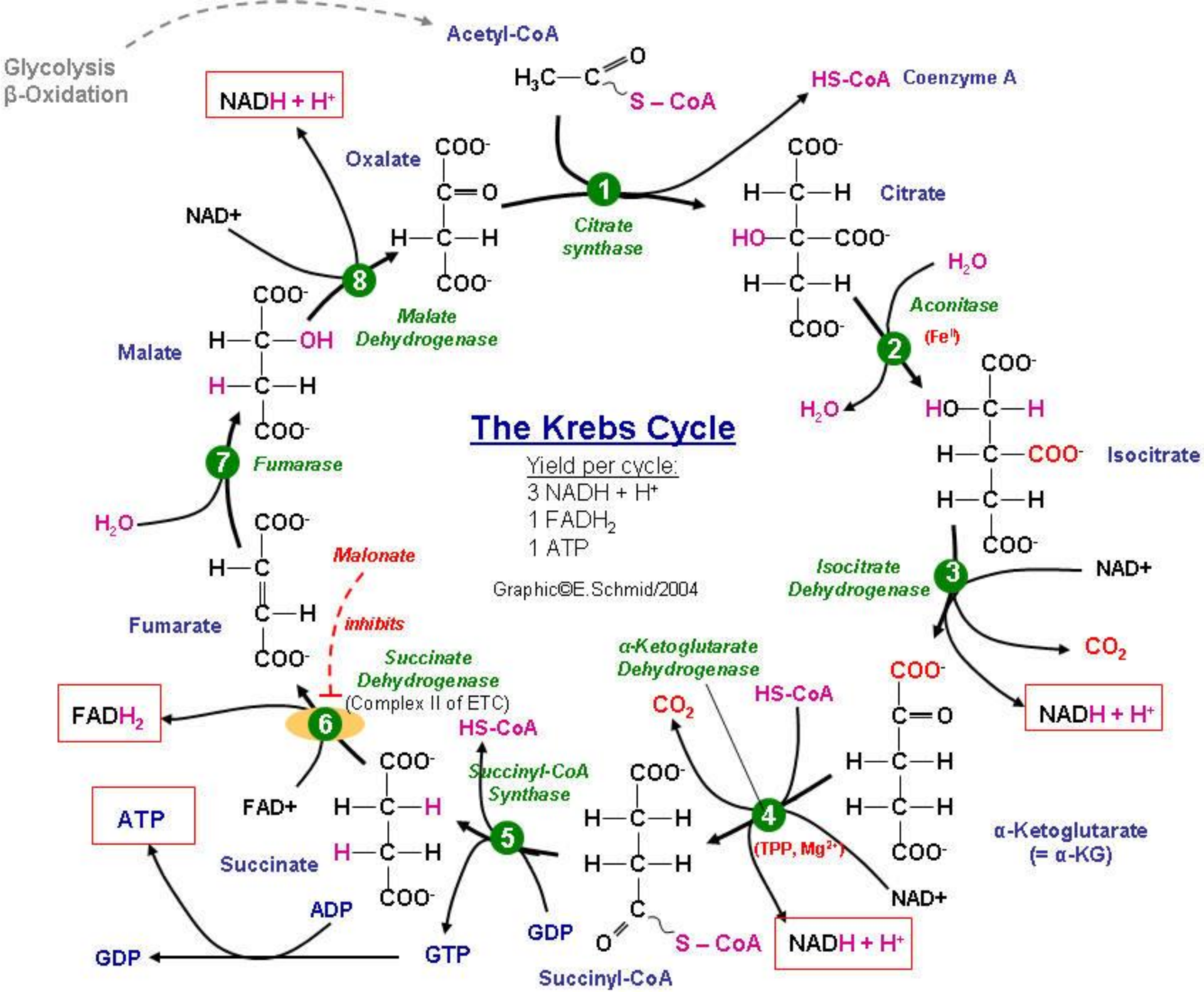
Fatty acids are synthesized by a cyclic pathway whose reactions appear very similar to a reversal of fatty acid oxidation reactions.

However, although the reactions appear to be similar, the pathways are very different, using different enzymes, different cofactors, and different regulatory controls.

Fatty acids are built from acetyl CoA molecules (two carbons at a time.) First, acetyl CoA is activated by adding CO_2 to form malonyl CoA:

Acetyl CoA → Malonyl CoA

+ ATP + HCO_3^- (& biotin cofactor)


Glycolysis
β-Oxidation
Acetyl-CoA
$H_3C-C(=O)-S-CoA$
HS-CoA Coenzyme A
NADH + H+
Oxalate
NAD+
Malate
Malate Dehydrogenase
Citrate
Citrate synthase
1
2
Aconitase
(Fe++)
H_2O
H_2O
Isocitrate
Isocitrate Dehydrogenase
3
NAD+
CO_2
NADH + H+
α-Ketoglutarate Dehydrogenase
CO_2
HS-CoA
4
(TPP, Mg^{2+})
NAD+
α-Ketoglutarate
(= α-KG)
NADH + H+
S – CoA
Succinyl-CoA
GDP
GTP
5
Succinyl-CoA Synthase
HS-CoA
ADP
ATP
GDP
FAD+
Succinate
6
Succinate Dehydrogenase
(Complex II of ETC)
inhibits
Malonate
$FADH_2$
Fumarase
7
8
Fumarate
H_2O
The Krebs Cycle
Yield per cycle:
3 NADH + H+
1 $FADH_2$
1 ATP
Graphic©E.Schmid/2004

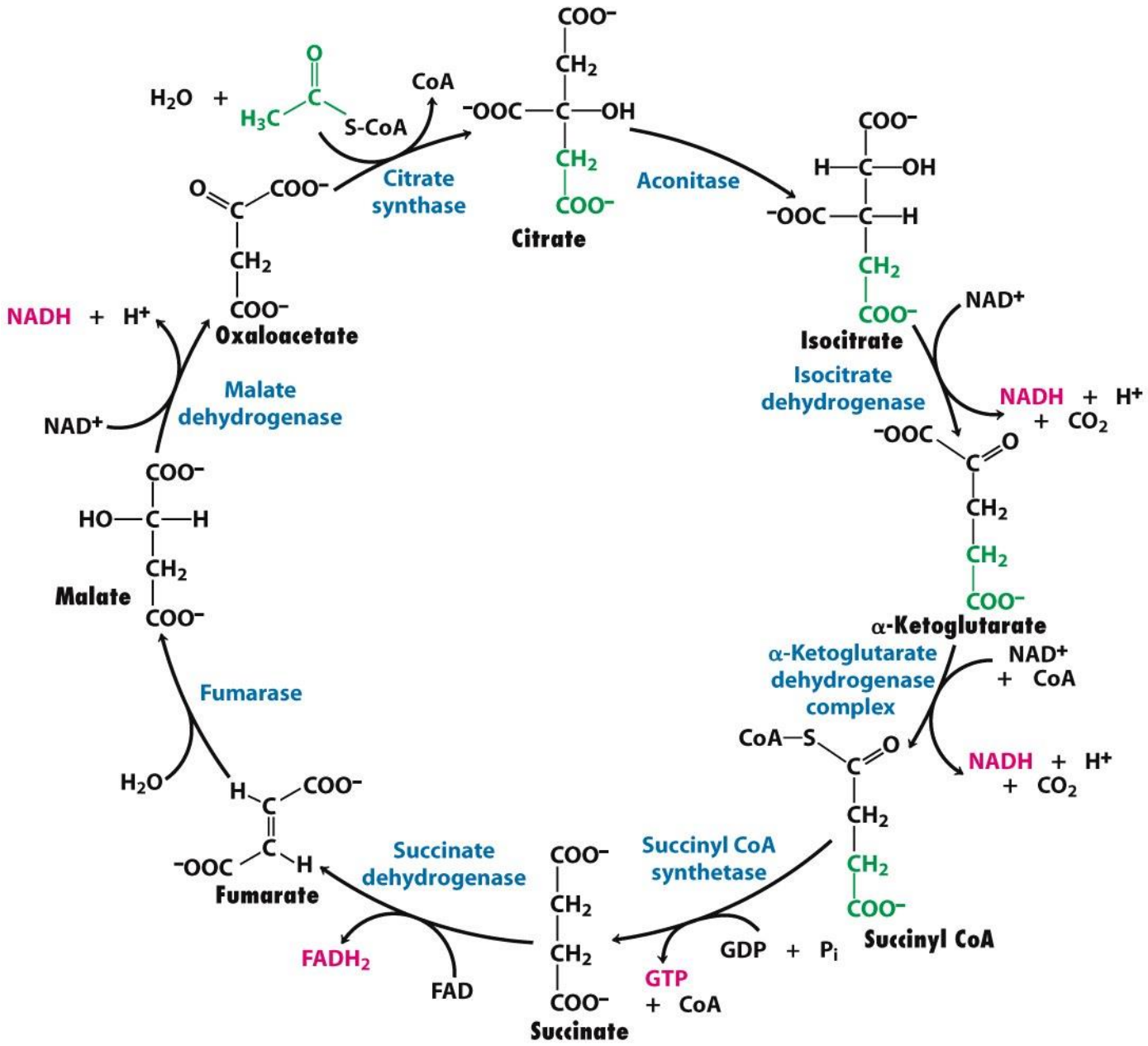
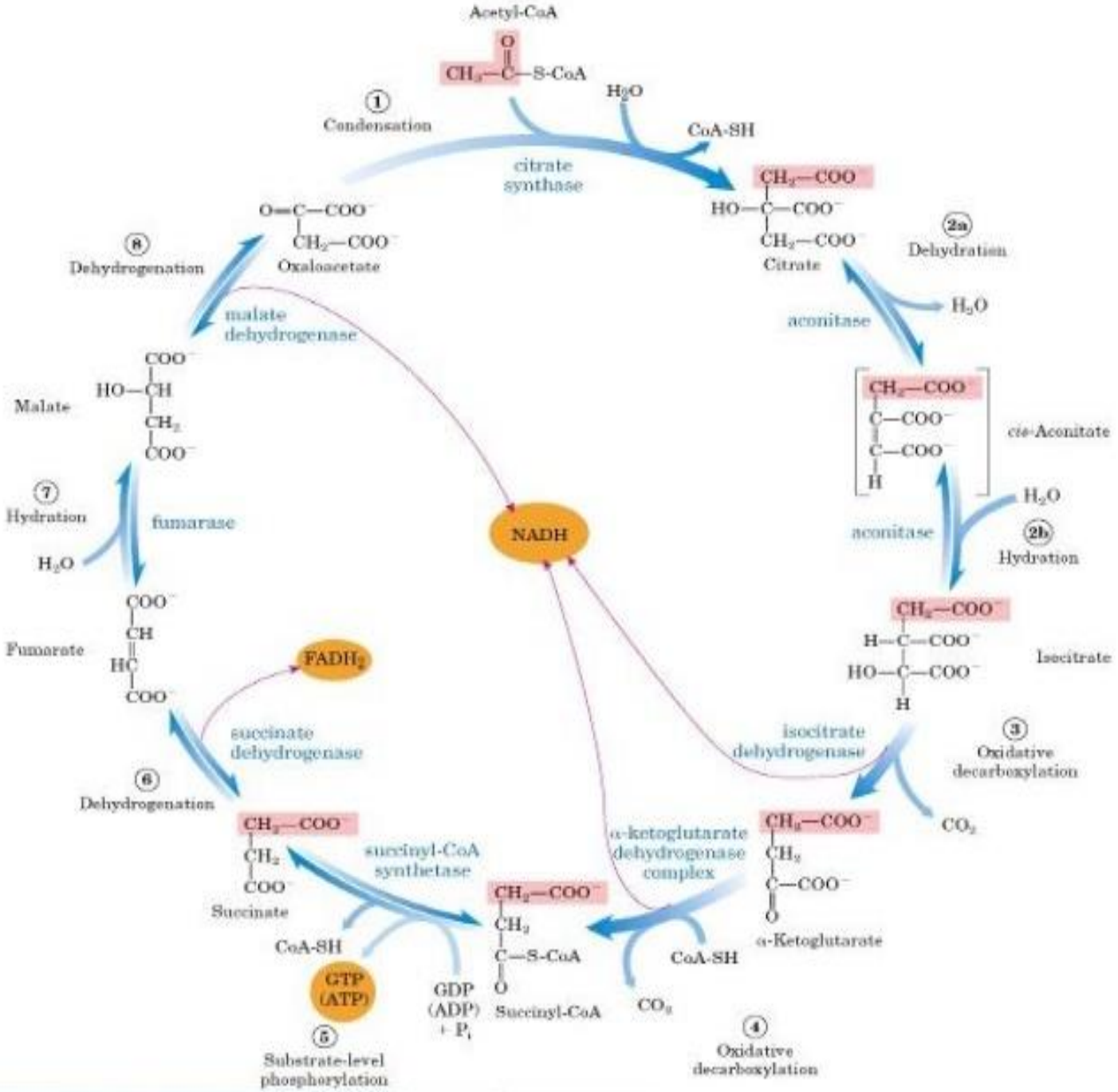
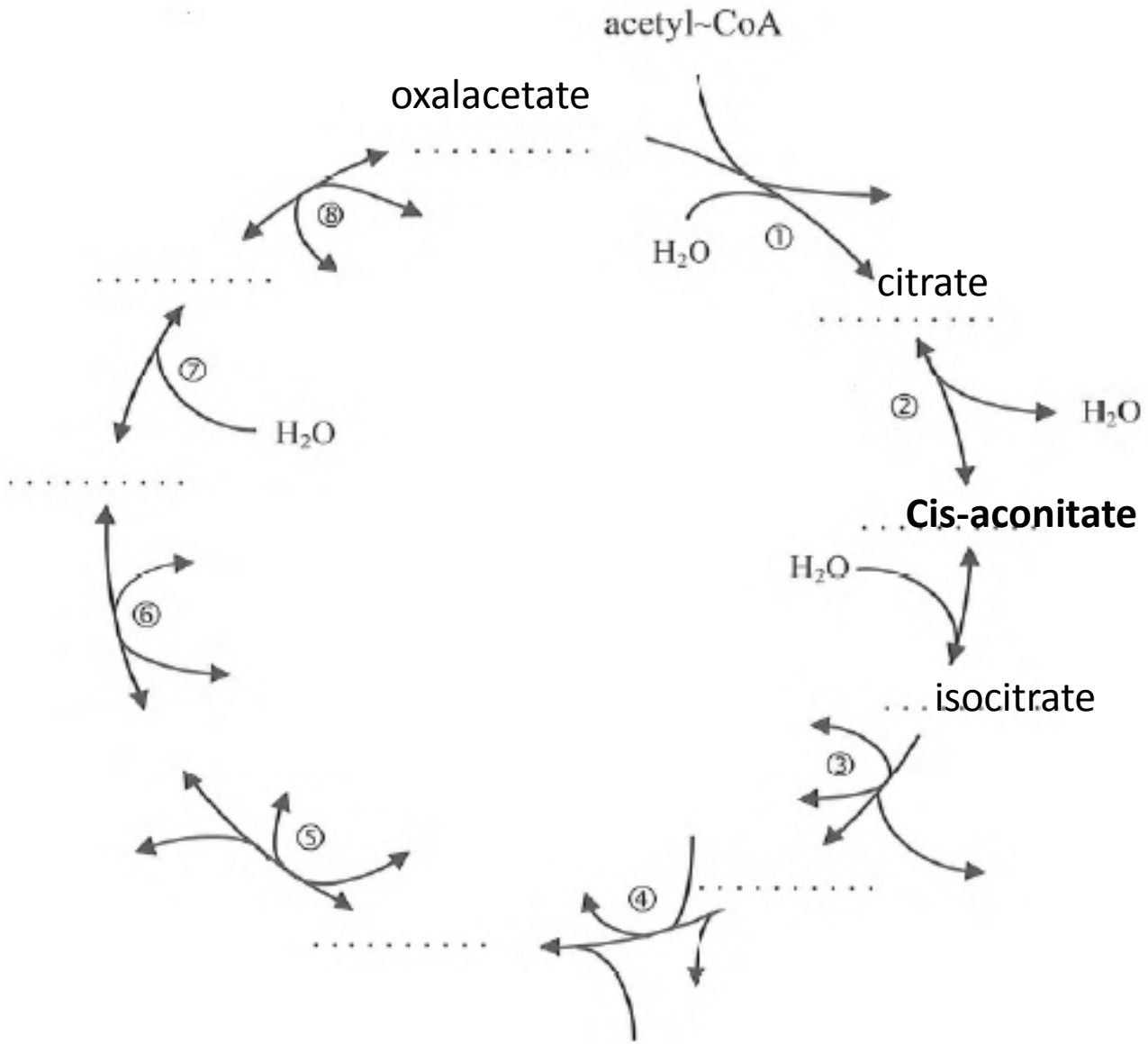
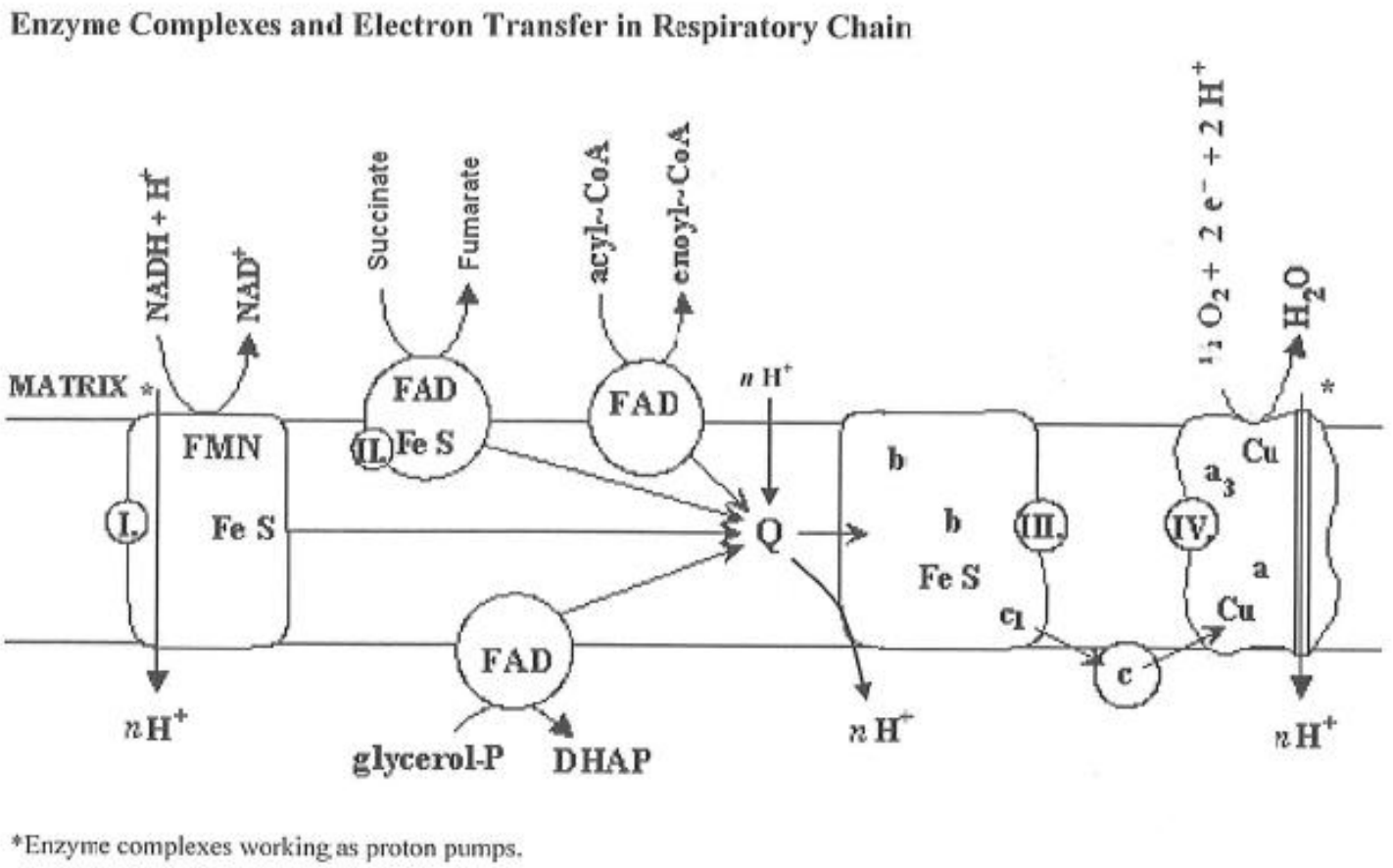


Figure 17.15
***Biochemistry,* Seventh Edition**

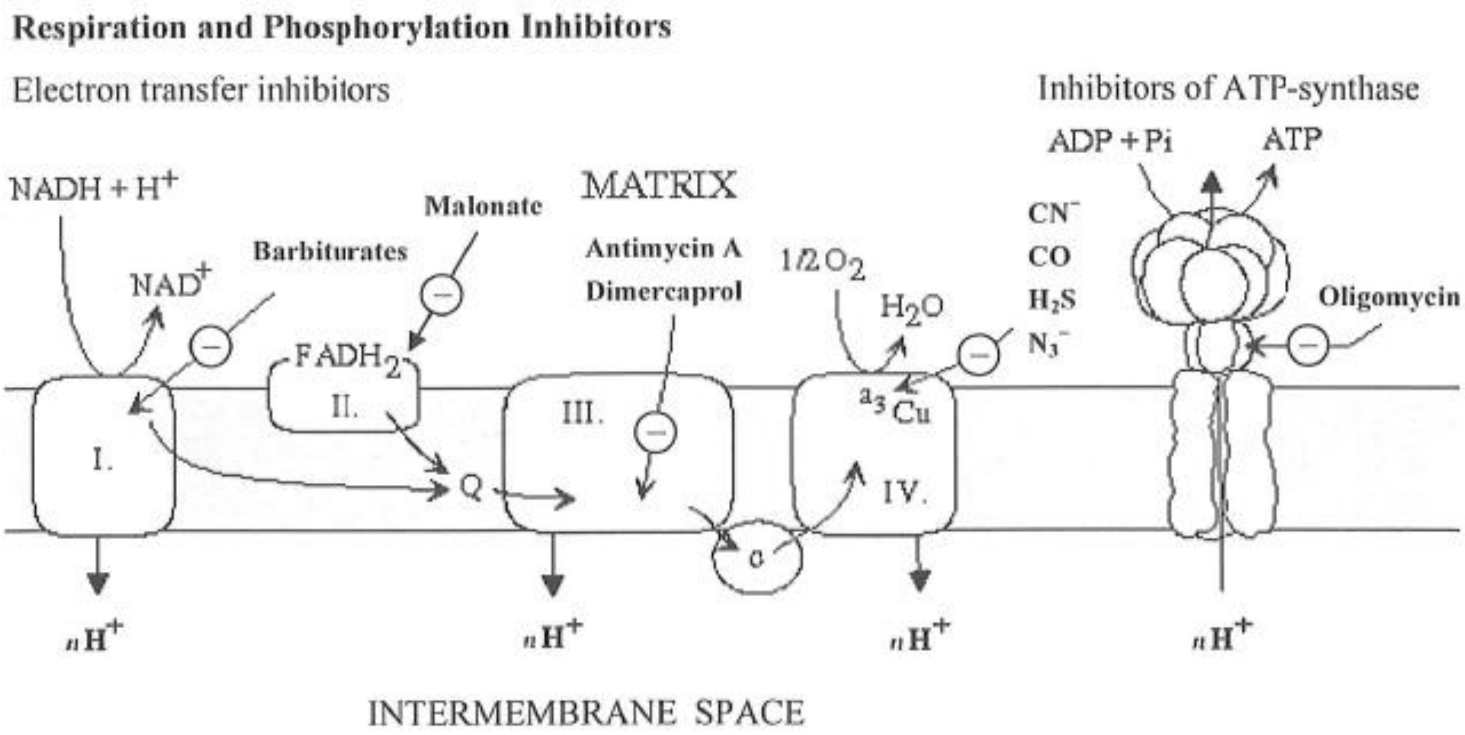

Acetyl-CoA
CH3—C—S-CoA
H2O
CoA-SH
1
Condensation
citrate synthase
CH2—COO
HO—C—COO
CH2—COO
Citrate
2a
Dehydration
H2O
aconitase
cis-Aconitate
H2O
2b
Hydration
aconitase
CH2—COO
H—C—COO
HO—C—COO
H
Isocitrate
isocitrate dehydrogenase
3
Oxidative decarboxylation
CO2
α-ketoglutarate dehydrogenase complex
CH2—COO
CH2
C—COO
O
α-Ketoglutarate
CoA-SH
CO2
4
Oxidative decarboxylation
succinyl-CoA synthetase
CH2—COO
CH2
C—S-CoA
O
Succinyl-CoA
CoA-SH
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
CH2—COO
CH2
COO
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
COO
CH
HC
COO
Fumarate
7
Hydration
H2O
fumarase
COO
HO—CH
CH2
COO
Malate
8
Dehydrogenation
malate dehydrogenase
O=C—COO
CH2—COO
Oxaloacetate
NADH


acetyl~CoA
oxalacetate
citrate
Cis-aconitate
isocitrate
H2O
H2O
H2O
H2O
①
②
③
④
⑤
⑥
⑦
⑧

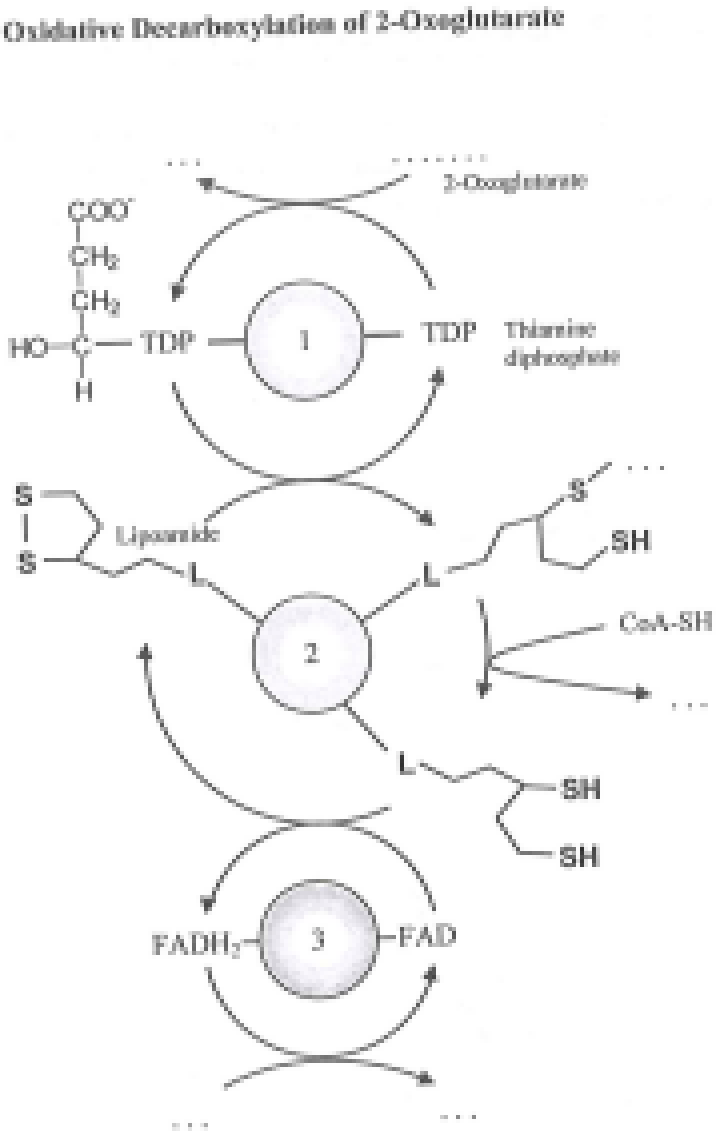


•**Complex I (NADH coenzyme Q reductase; labeled I)** accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled UQ), which also receives electrons from **complex II (succinate dehydrogenase; labeled II).** UQ passes electrons to **complex III (cytochrome bc1 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.

Uncouplers and Inhibitors



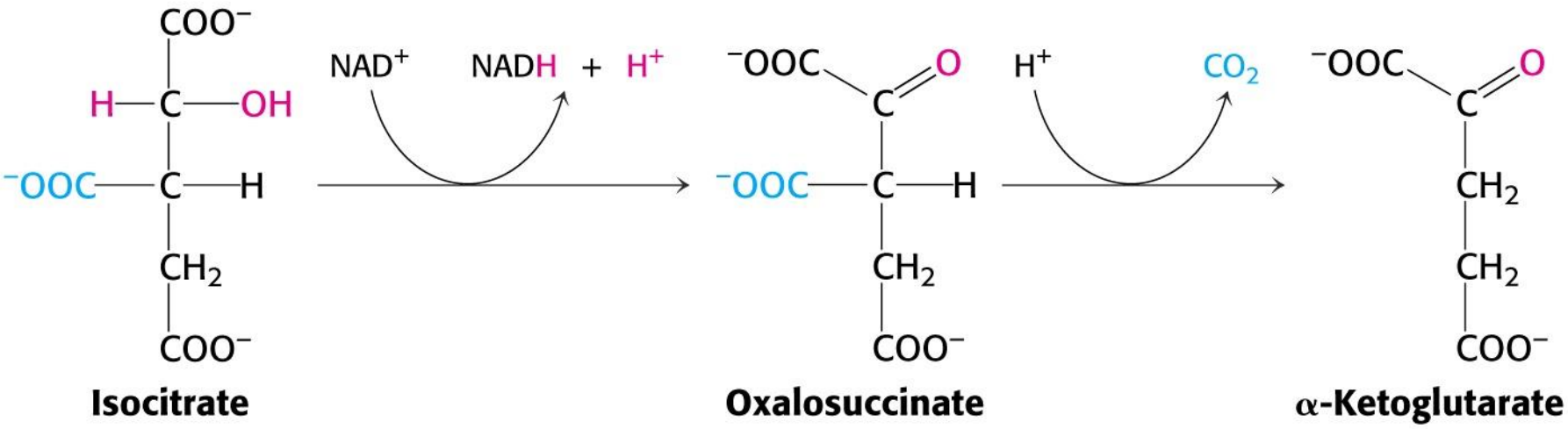
Specific inhibitors were used to distinguish the electron transport system from the phosphorylation system and helped to define the sequence of redox carriers along the respiratory chain. If the chain is blocked then all the intermediates on the substrate side of the block become more reduced, while all those on the oxygen side become more oxidized. It is easy to see what has happened because the oxidized and reduced carriers often differ in their spectral properties. If a variety of different inhibitors are available then many of the respiratory carriers can be placed in the correct order.



oxidative decarboxylation of 2-oxo glutharate (keto-glutharate), 5 cofactors

Isocitrate to α-ketoglutarate: step 3

Enzyme: isocitrate dehydrogenase



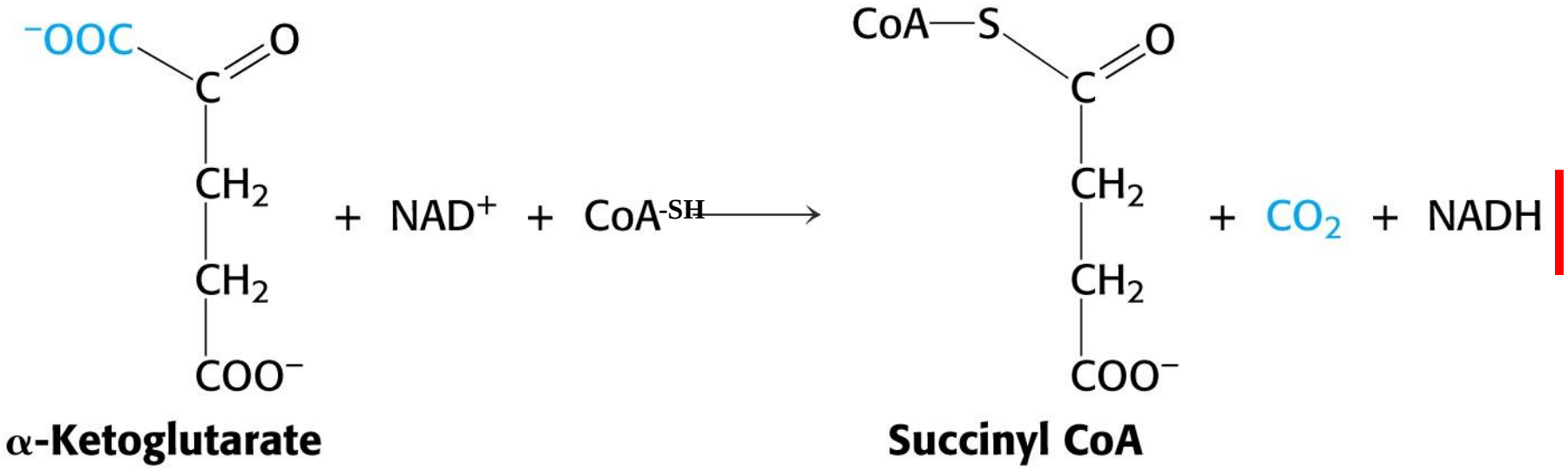
1st NADH produced

1st CO_2 removed

Oxidation of IC to aKG and CO2, Mn2+ in active site

Succinyl CoA formation: step4

Enzyme: α-ketoglutarate dehydrogenase



α-Ketoglutarate → **Succinyl CoA**

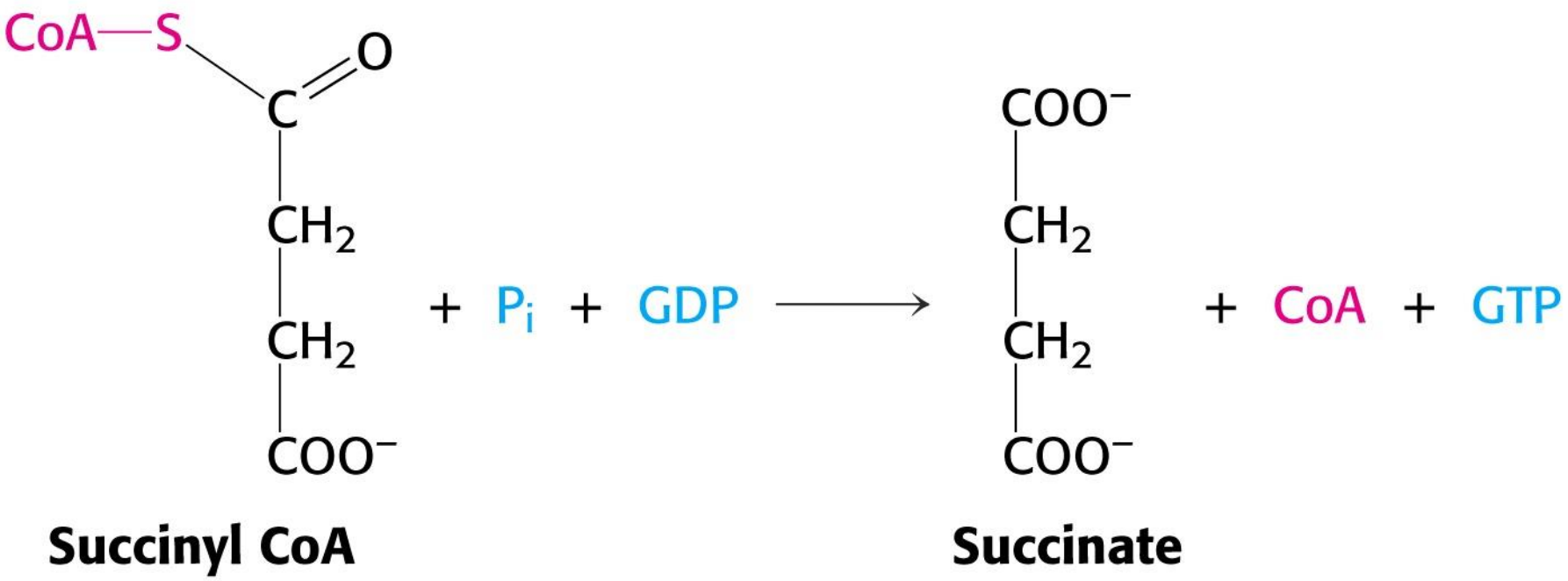
2nd NADH produced

2nd CO_2 removed

oxidation

Succinate formation: step5

Enzyme: succinyl CoA synthetase



GTP produced

GTP + ADP ⇒ GDP + ATP (NPTase)

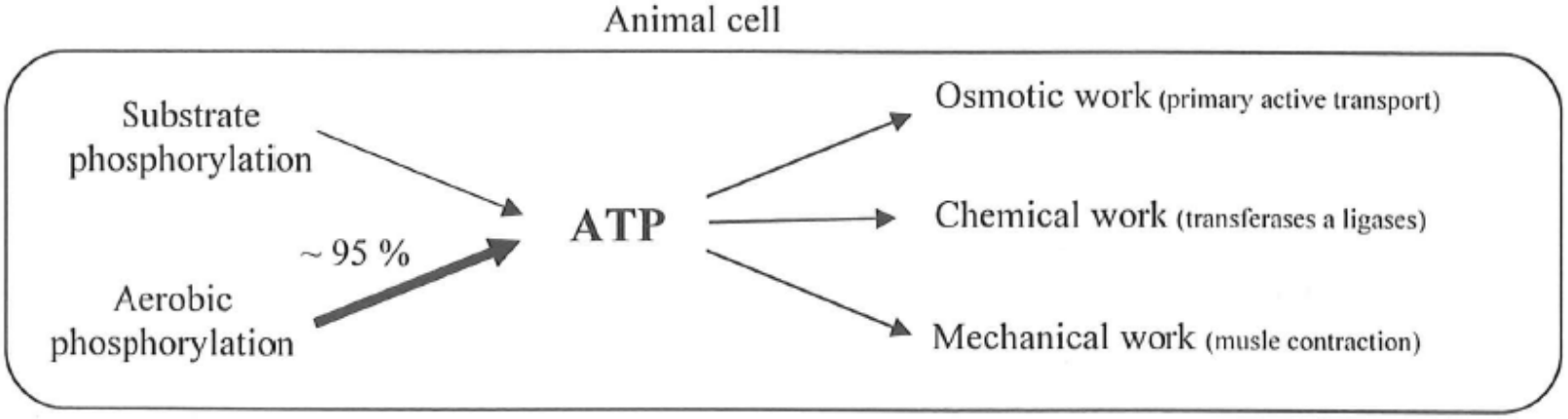
Metabolism of purines and pyrimidines

	purines	pyrimidines
PRPP	1st step	Last steps
product	IMP	UMP
localization	cytoplasm	cytoplasm + 1 enzyme in mitochondria
Degradation products	Uric acid, ammonia	CO_2, NH_4, β-Alanine, β-Aminoisobutyrate

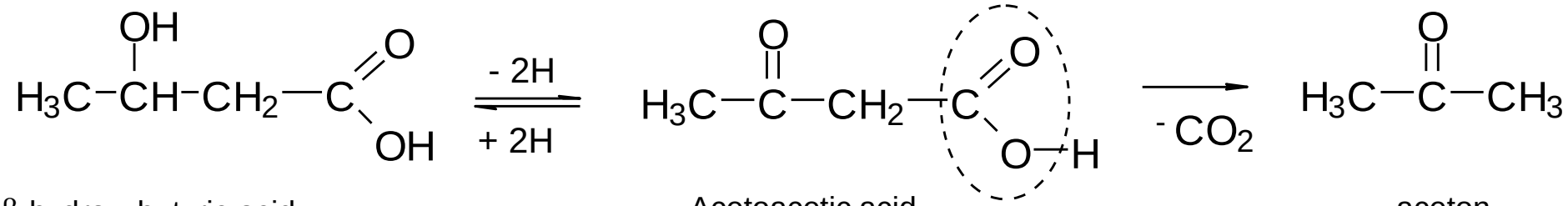
The covalent modification of proteins

- Many proteins are modified by the covalent linking of groups that can affect their function and/or localisation in the cell. Such **covalent modifications** occur after synthesis and folding of the polypeptide component. The main types of covalent modification and their functions are listed below.
- Methylation/acetylation of amino acids at the N-terminal tails of histone proteins in eukaryotes can affect the structure of chromatin and ultimately gene expression. Prokaryotes also use methylation as a means of directly regulating protein activities. For example, the methylation of specific proteins controlling flagellar movement is an important mechanism for the regulation of bacterial chemotaxis.
- Phosphorylation of proteins (catalysed by specific kinases) is a key regulatory mechanism in eukaryotic intracellular signalling and in metabolic pathways.
- Lipidation of proteins (i.e. addition of lipid tails) targets them to cell membranes (plasma membrane and cell organelles).
- Glycosylation is a feature of many extracellular proteins, whether secreted or on the cell surface, and may offer the protein some protection against proteases.
- These modifications are catalysed by specific enzymes and can be reversed, permitting regulation of the protein's function. This reversibility is particularly significant with respect to protein phosphorylation

Formation and Utilization of ATP



ketone bodies



β-hydroxybutyric acid

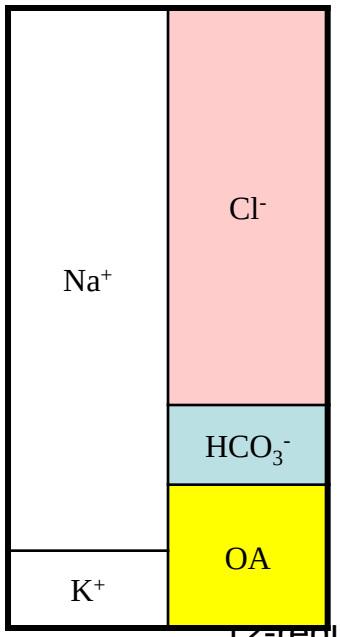
Acetoacetic acid

aceton

anion

anion

No electrolyte



Ketone bodies are three water-soluble molecules that are produced by the liver from fatty acids during periods of low food intake (fasting) or carbohydrate restriction for cells of the body to use as energy instead of glucose. Two of the three are used as a source of energy in the heart and brain while the third (acetone) is a degradation breakdown product of acetoacetic acid.

acetone, acetoacetic acid, and *beta*-hydroxybutyric acid

Ketone bodies as source of energy