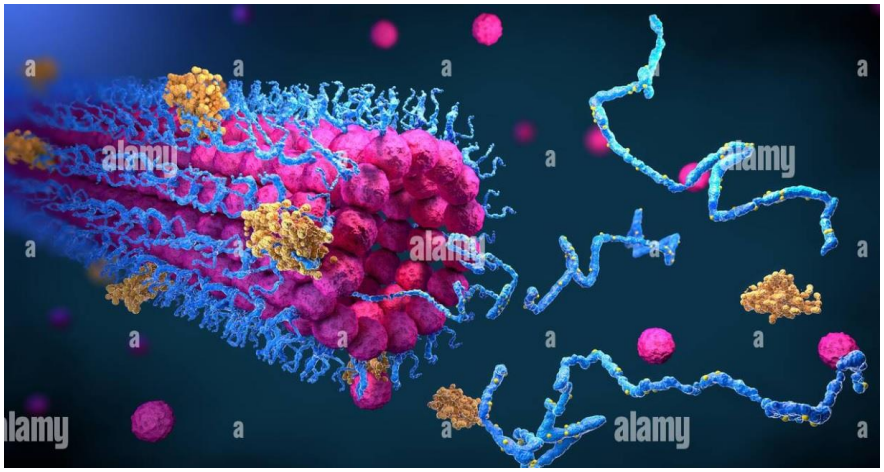
Enzyme

Enzyme, a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.

Enzymes do two important things: ***They recognize very specific substrates***, and ***They perform specific chemical reactions on them at fantastic speeds***. The way they accomplish all this can be described by a number of different models, each one of which accounts for some of the behavior that enzymes exhibit. Most enzymes make use of all these different mechanisms of specificity and/or catalysis. In the real world, some or all of these factors go into making a given enzyme work with exquisite specificity and blinding speed.



Enzymes help speed up chemical reactions in the human body. They bind to molecules and alter them in specific ways. They are essential for respiration, digesting food, muscle and nerve function, among thousands of other roles. Enzymes are built of proteins folded into complicated shapes; they are present throughout the body.

The chemical reactions that keep us alive - our metabolism - rely on the work that enzymes carry out. Enzymes speed up (catalyze) chemical reactions; in some cases, enzymes can make a chemical reaction millions of times faster than it would have been without it. A substrate binds to the active site of an enzyme and is converted into products. Once the products leave the active site, the enzyme is ready to attach to a new substrate and repeat the process. Many inherited diseases and conditions of humans result from a deficiency of one enzyme. Albinism, for example, results from an inherited lack of ability to synthesize the enzyme tyrosinase, which catalyzes one step in the pathway by which the pigment for hair and eye colour is formed.

Factors affecting catalytic activity of enzymes

1. Enzyme Concentration

The transient bonds between enzymes and their substrates catalyze the reactions by **decreasing the activation energy and stabilizing the transition state**. Given the exceeding amount of substrates and the necessary cofactors, enzymatic reactions possessing higher enzyme concentrations will reach equilibrium before those with the same enzyme but at lower concentrations.

Simply put, higher enzyme concentration indicates that more enzyme molecules are available to process the substrate. The high levels of enzyme-substrate complex result in a higher initial catalytic rate, which gives the reaction a headstart in the shift toward reactant-product equilibrium.

2. Substrate Concentration

The enzyme catalytic activity occurs when a geometrically and electronically complementary substrate can access the enzyme's *catalytic* or *active site*. There, the active residues transiently bond with the substrate, catalyzing the transformation of the substrate into a product. Thus, the more substrate-occupied active sites, the higher the catalytic activity and the faster the shift toward enzyme-product equilibrium.

Most enzymes follow the Michaelis-Menten kinetics, which describes the relationship between enzyme activity and substrate concentration in two stages. At the initial stage, the relationship between the two is a linear association and plateaus when the number of unbound active sites decreases.

Another group of enzymes, allosteric enzymes, display a sigmoidal kinetic. Initially, the relationship between the rate of an allosteric enzyme-catalyzed reaction is exponential. However, this becomes linear as the catalysis progresses and finally plateaus when the number of substrate-bound enzymes becomes saturated.

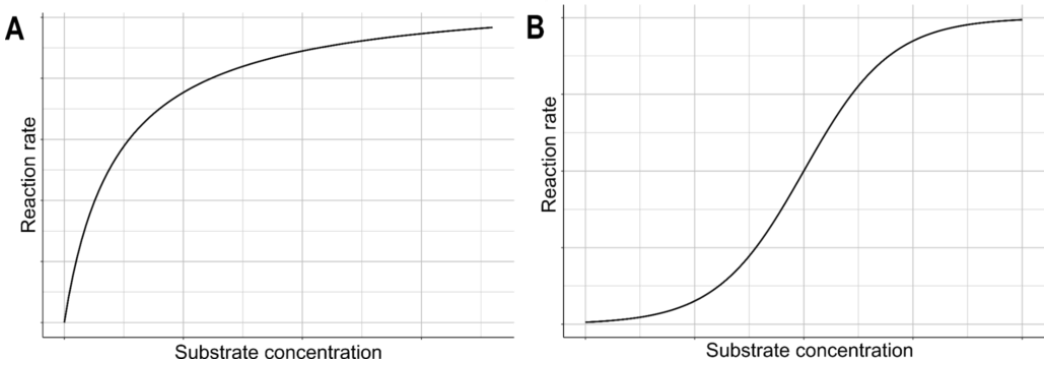
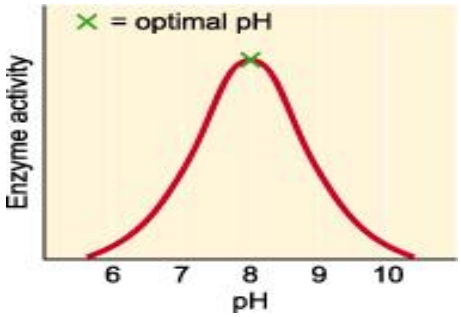


Figure 1: The relationship between substrate concentration and the rate of enzyme-catalyzed reaction follows the Michaelis-Menten kinetic in most enzymes (A) but a sigmoid curve in allosteric enzymes (B).

3. pH Value

As a chain of amino acids, proteins such as enzymes contain electrical charges from the sequence of their amino acid residues. Most amino acids in the chain are the basis for the intramolecular interactions that give the enzyme its three-dimensional structure. Few others act as functional residues at the enzyme's active site.



Altogether, the amino acids determine the substrate specificity and restrict the enzyme activity only to a narrow range of pH. Most enzymes function optimally in slightly acidic or basic pH. However, a few enzymes are native to extreme acidic or basic environments; hence, most active in these pH ranges.

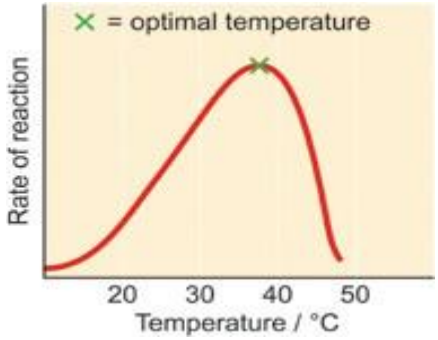
For this reason, a change in the pH value, either acidic or basic, affects the ionization of amino acid residues, leading to changes in the three-dimensional structure of the enzyme. The alteration in the enzyme conformation affects its interaction with its substrate, thus reducing its activity.

Another effect of pH change is in the enzyme's catalytic capability. In acid-base and covalent catalysis mechanisms, pH change can hinder or suppress catalytic activity. In extreme cases, it can denature the enzyme, destroy its three-dimensional structure, and render it permanently non-functional.

4. Temperature

In the same way that pH affects enzymes, temperature also influences the stability of their intramolecular bonds. For this reason, enzyme activity is generally more active at their optimal temperature.

Nonetheless, a few degree shifts from the optimal temperature only cause a minor decrease in the enzyme activity.



5. Effector or Inhibitor

Many enzymes require non-substrate and non-enzyme molecules to regulate or initiate their catalytic function. For example, certain enzymes rely on metal ions or ***cofactors*** to establish their catalytic activity. Many rely on ***effectors*** to activate their catalytic activities, promote or inhibit their successive binding to the substrates, as seen in allosteric enzymes.

Along the same line, ***inhibitors*** may bind to the enzyme or its substrate to inhibit the ongoing enzymatic activity and prevent successive catalytic events. The effect on enzyme activity is ***irreversible*** when the inhibitors form strong bonds to the enzyme's functional group, leaving the enzyme permanently inactive.

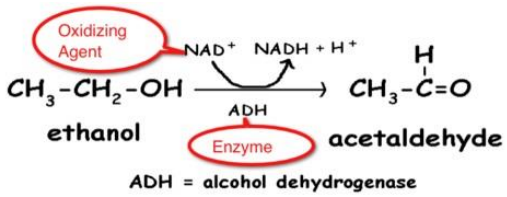
In contrast to ***irreversible inhibitors, reversible inhibitors*** only render the enzymes inactive when bound to the enzyme. ***Competitive*** inhibitors compete with the substrates for binding to the residues of the enzyme functional group at the catalytic sites. Other types of inhibitors do not bind to the catalytic site, but they bind to the non-substrate binding ***allosteric site***.

If an inhibitor binds to the enzyme concurrently with the enzyme-substrate binding, it is ***non-competitive***. If an inhibitor binds only to a substrate-occupied enzyme, it is ***uncompetitive***.

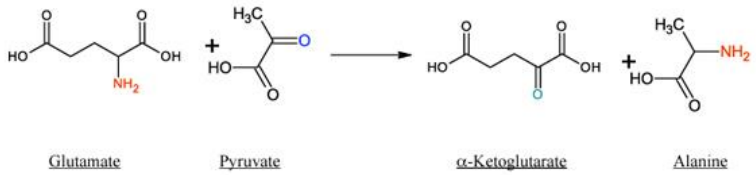
Classification of Enzymes:

The biochemical reactions occurring in the body are basically of 6 types and the enzymes that bring about these reactions are named accordingly:

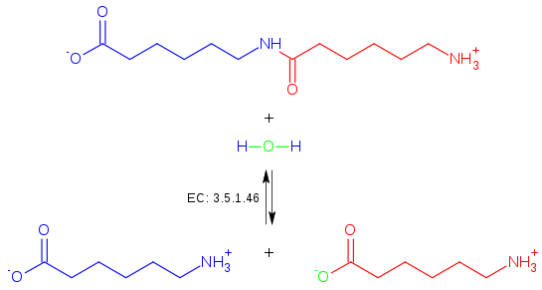
- **Oxidoreductases:** These enzymes bring about oxidation and reduction reactions and hence are called oxidoreductases. In these reactions, electrons in the form of hydride ions or hydrogen atoms are transferred. When a substrate is being oxidized, these enzymes act as the hydrogen donor. These enzymes are called dehydrogenases or reductases. When the oxygen atom is the acceptor, these enzymes are called oxidases.



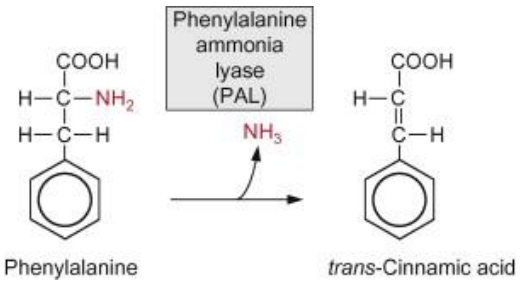
- **Transferases:** These enzymes are responsible for transferring functional groups from one molecule to another. Example: alanine aminotransferase which shuffles the alpha- amino group between alanine and aspartate etc. Some transferases also transfer phosphate groups between ATP and other compounds, sugar residues to form disaccharides such as hexokinase in glycolysis.



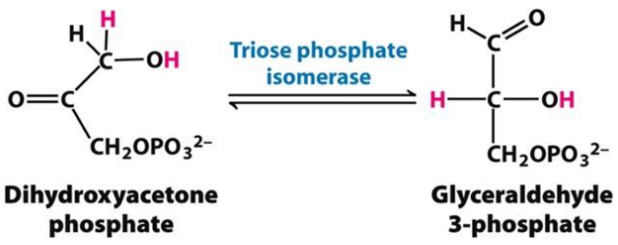
- **Hydrolases:** These enzymes catalyze reactions that involve the process of hydrolysis. They break single bonds by adding water. Some hydrolases function as digestive enzymes because they break the peptide bonds in proteins. Hydrolases can also be a type of transferases as they transfer the water molecule from one compound to another. Example: Glucose-6-phosphatase that removes the phosphate group from glucose-6-phosphate, leaving glucose and H_3PO_4.



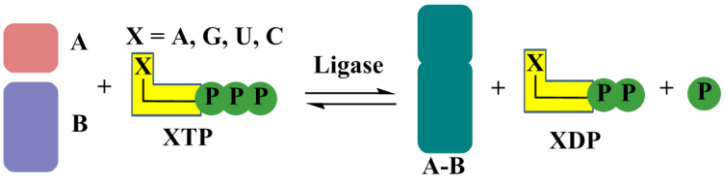
- **Lyases:** These enzymes catalyze reactions where functional groups are added to break double bonds in molecules or where double bonds are formed by the removal of functional groups. Example: Pyruvate decarboxylase is a lyase that removes CO_2 from pyruvate. Other examples include deaminases and dehydratases.



- **Isomerases:** These enzymes catalyze the reactions where a functional group is moved to another position within the same molecule such that the resulting molecule is actually an isomer of the earlier molecule. Example: triosephosphate isomerase and phosphoglucose isomerase for converting glucose 6-phosphate to fructose 6-phosphate.



- **Ligases:** These enzymes perform a function that is opposite to that of the hydrolases. Where hydrolases break bonds by adding water, ligases form bonds by removal of the water component. There are different subclasses of ligases which involve the synthesis of ATP.

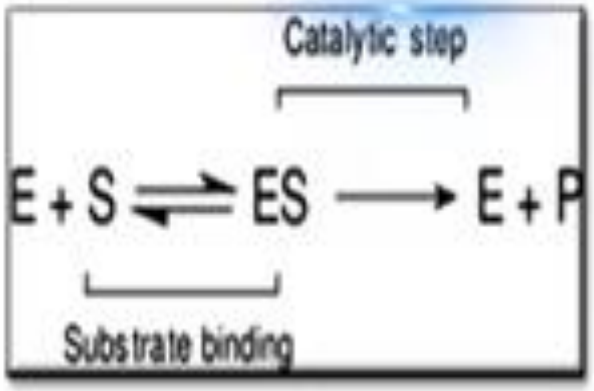


Introduction to Enzymes

An enzyme is a **biocatalyst**, which enhances the rate of thermodynamically favourable biological reactions to several thousand to million folds.

Enzymes are highly specialized catalysts with extraordinary catalytic power and also with remarkable specificity, catalysing almost all cellular reactions. Therefore they are known as the basis of life.

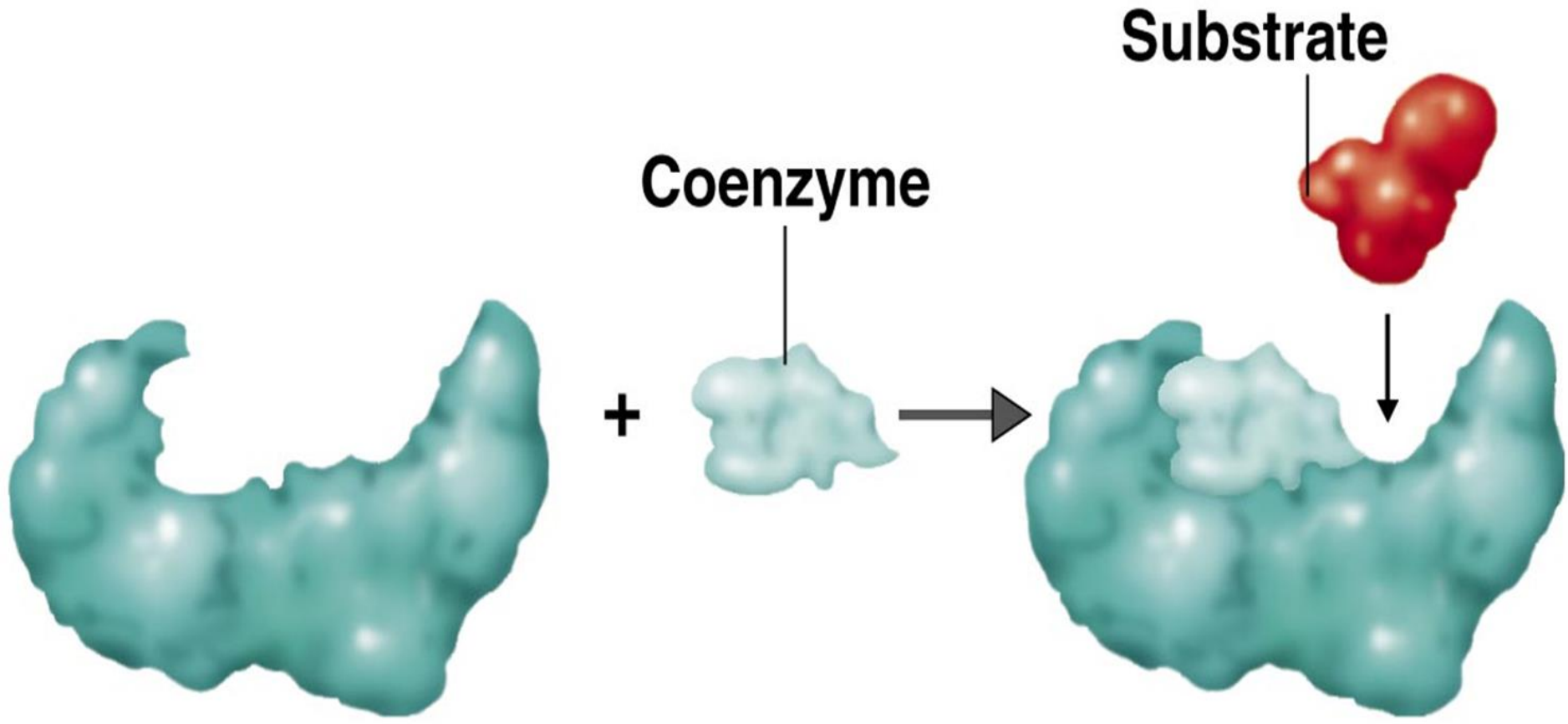
- All enzymes are proteins which act as biological catalysts.
- They catalyse the rate of biochemical reactions occurring in various vital life processes.
- The enzymes, during biocatalysis, themselves do not undergo any chemical change but are regenerated at the end of the reaction.
- The substances on which the enzymes act to yield products are called as "Substrates".



- Enzymes that are synthesised within the cell are intracellular/endoenzymes. Extracellular/exo enzymes are those secreted from the cells into the environment

They are divided into two general categories: **Simple enzymes**, which consists entirely of amino acids and **Conjugated enzymes**, contains a non-protein group called a **cofactor**, which is required for biological activity.

Removal of the cofactor from a conjugated enzyme produces a simple enzyme, called an **apoenzyme**, which generally is biologically inactive. The complete, biologically active conjugated enzyme (simple enzyme plus cofactor) is called **holoenzyme**.



Apoenzyme (protein portion), inactive

Cofactor (nonprotein portion), activator

Holoenzyme (whole enzyme), active

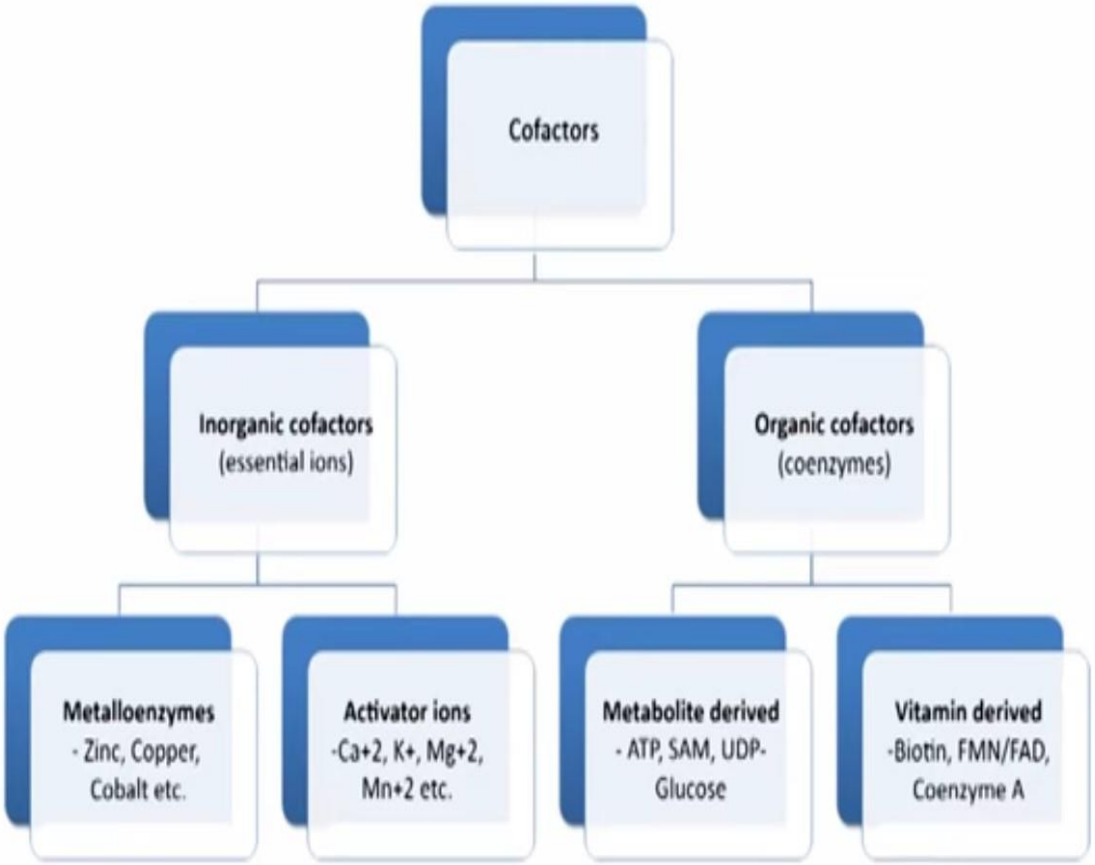
A **cofactor** can be linked to the protein portion of the enzyme either covalently or non-covalently.

Some cofactors are simple metal ions and other cofactors are complex organic groups, which are also called **coenzymes**. Cofactors which are tightly associated with the protein covalently or non-covalently are called **prosthetic group**.

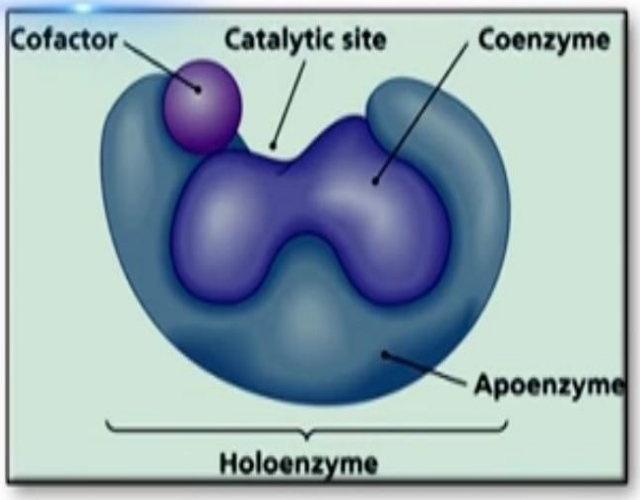
What are cofactors?

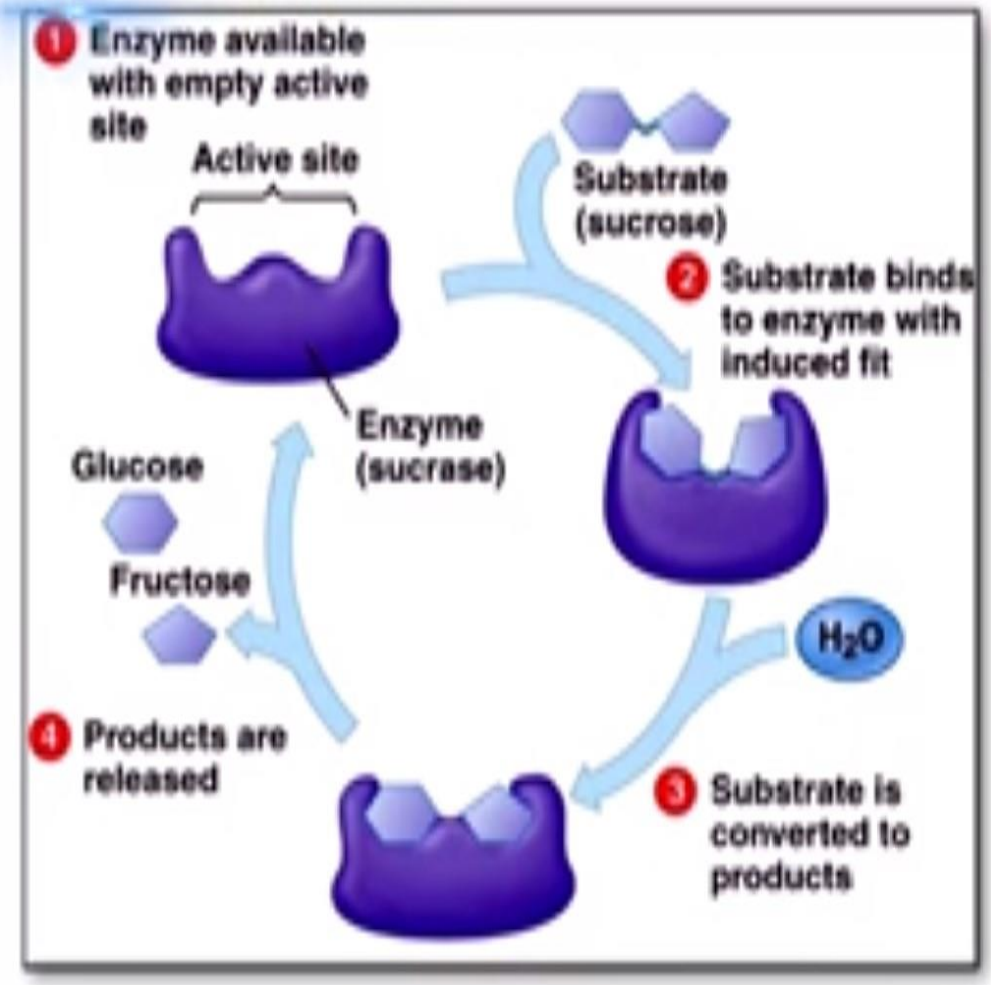
- Cofactors are the non-protein components of an enzyme.
- A cofactor may be an organic or inorganic molecule.
- An inorganic cofactor is called an essential ion, whereas, an organic cofactor is called a coenzyme.
- A cofactor required in fixed quantity for reaction to occur is called a co-substrate.

Types of cofactors



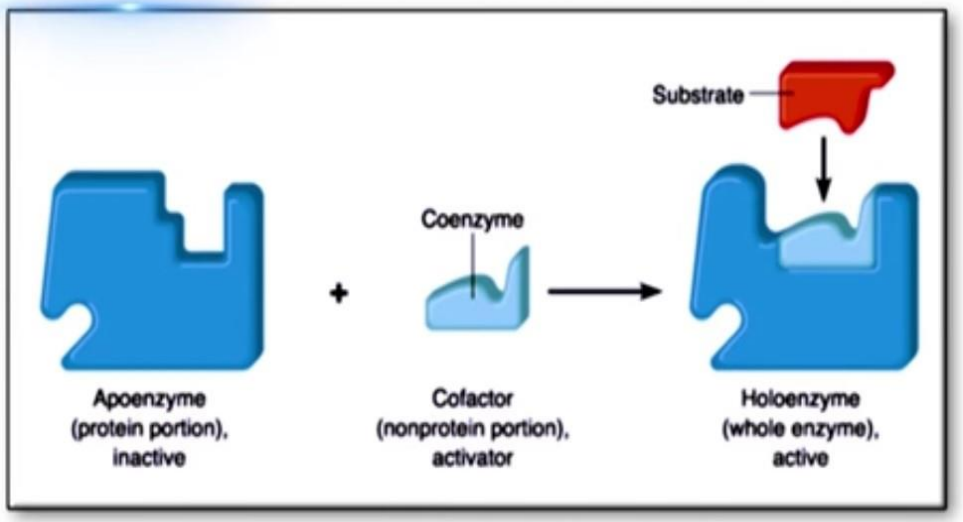
- Apoenzyme = Enzyme – Cofactor
- Holoenzyme = Enzyme + Cofactor
- A reaction requires many components in correct amount and arrangement. These components include - the enzyme, its substrate and co-substrate, the enzyme cofactor.




1 Enzyme available with empty active site
Active site
Enzyme (sucrase)
Substrate (sucrose)
2 Substrate binds to enzyme with induced fit
H_2O
3 Substrate is converted to products
4 Products are released
Glucose
Fructose

Mechanism of action

- Cofactors prepare the enzyme's active centre or catalytic site for catalysis.
- Cofactors provide additional reactive groups to the enzymes and thereby aid in catalysis.



Discovery and History of Enzymes

1833: **Payne and Persoz** found that that an alcohol precipitate of a malt extract contained a substance that converted starch into sugar. This was the first discovery of an enzyme and they named it diastase.

1850: **Louis Pasteur** observed that ferment of sugar into alcool by yeast is catalysed by ferments (later named enzymes), which are always associated with the yeast cells.

1876: **W.F. Kuhne** coined the term enzyme (Greek, which means ‘in yeast’) since the fermenting ability was associated with the yeast.

1894: **Emil Fischer** performed some classical studies on carbohydrate metabolizing enzymes in which he demonstrated the specificity shown by an enzyme for its substrate. On the basis of his experiments, Fischer proposed the **lock and key hypothesis** to describe the interaction of enzyme with substrate.

1897: **Edward Buchner** succeded in extracting the set of enzymes from the yeast cells in active form and demonstrated for the first time the conversion of sugar into alcohol in vitro.

1926: **J.B. Sumner** (Cornell University, USA) isolated, purified and also successfully crystallized the enzyme urease from jack beans. He found that the urease crystals are purely made of proteins and hence reported that enzymes are nothing but proteins. But his conclusions were opposed vehemently by the well known German biochemist **Richard Willstater**, who insisted that enzymes are nothing but low molecular weight organic compounds and the proteins crystals were found in the urease preparation could be impurities.

1930: **John Northrop and his colleagues from Rockfeller University, USA** crystallized pepsin and trypsin and found that they were also proteins crystals. Received Nobel Prize in 1935.

1958: The induced-fit model was proposed by **Daniel Koshland**. His theory asserts that when the active site on the enzymes makes contact with the proper substrate, the enzyme molds itself to the shape of the molecule.

1964: **R.B. Merrifield** and his group paved the way for laboratory synthesis of enzymes for the first time (tailor made synthetic enzymes called **Synzymes.** The first enzyme which was assembled on a solid phase matrix was the Ribonuclease, which contains 124 amino acids.

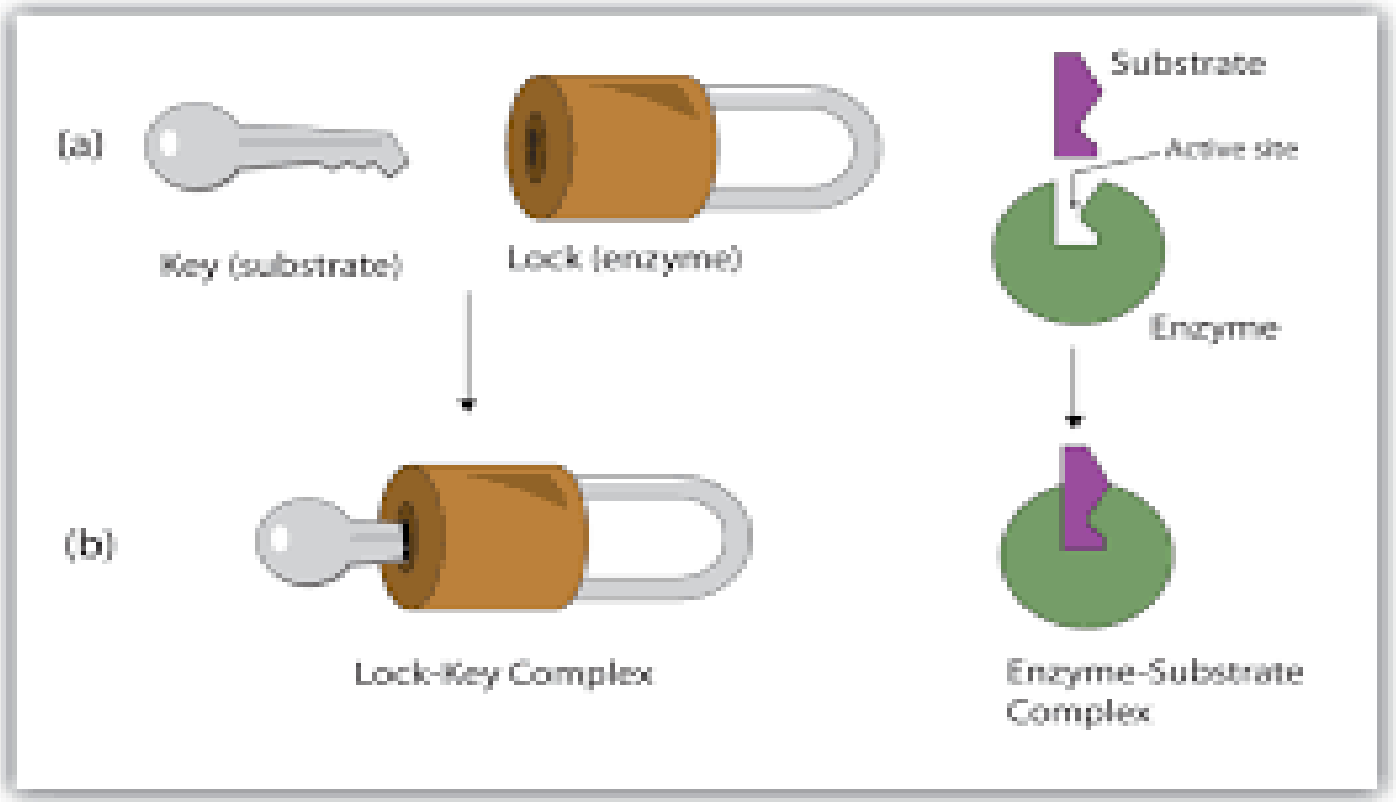
1965: Lysozyme was the first enzyme for which the X-ray structure was determined at high resolution by **David Phillips**.

1962 & 1967: **Arber and Geller** groups discovered restriction enzymes and ligases. Paved the way for the new branch of biology –Biotechnology.

1986: The belief that '**All enzymes are proteins but all proteins are not enzymes'** was shattered by **Alexander Rich and Thomas Cech's** group discovered that certain RNA molecules also exhibited catalytic properties like enzymes. Those self-splicing 'Ribonucleic acid enzymes are called **Ribozymes**.

1996: Site- directed mutagenesis technique developed by **M. Smith** for precisely manipulating the genes of any enzyme even at one nucleotide level and study its effect on the properties of the new mutant enzyme.

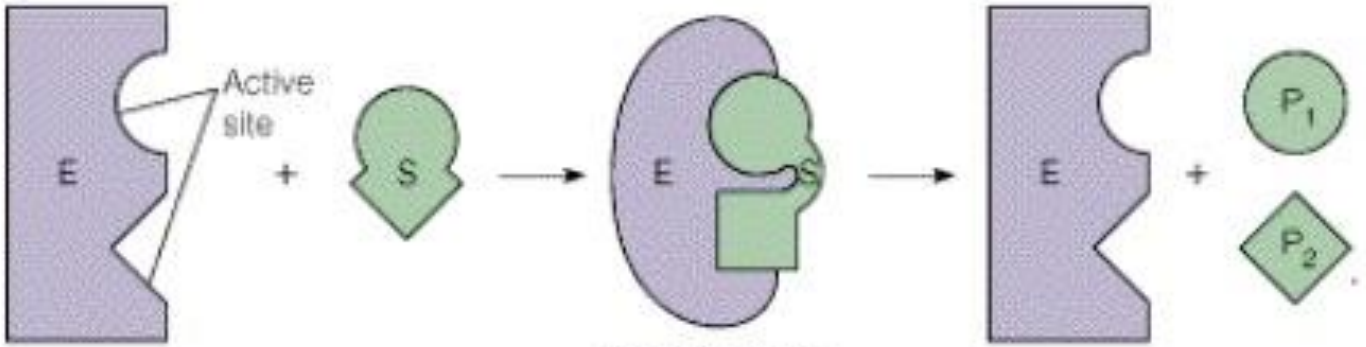
The lock and key hypothesis states that the substrate fits perfectly into the enzyme, like a lock and a key would.
This is in contrast with the induced fit hypothesis, which states that both the substrate and the enzyme will deform a little to take on a shape that allows the enzyme to bind the substrate.



Induced fit hypothesis

✓proposed in 1958 by Daniel E. Koshland, Jr.: the binding of substrate induces a conformational change in the active site of the enzyme.

✓In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state

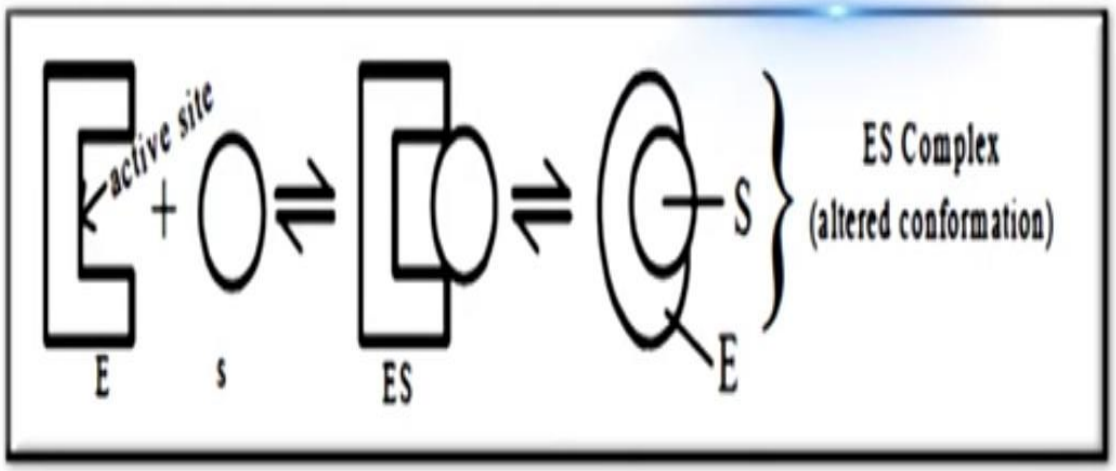


Induced fit model

Active site of enzymes

- An active site is that part of an enzyme that directly binds to a substrate and carries a reaction.

- Particular amino acids residue is present in the active sites that leads to catalytic action, and promotes the formation or degradation of bonds. These are called as 'catalytic' or 'active' amino acids or 'catalytic residues'.



Factors that influence enzyme activity

- Enzyme activity is influenced by several factors such as:
  - pH
  - Temperature
  - Substrate concentration
  - Metal ions
  - Inhibitors
  - Enzyme concentration

Effect of pH

- Each enzyme has a particular pH where its activity is maximum. This pH is known as **optimal pH.**

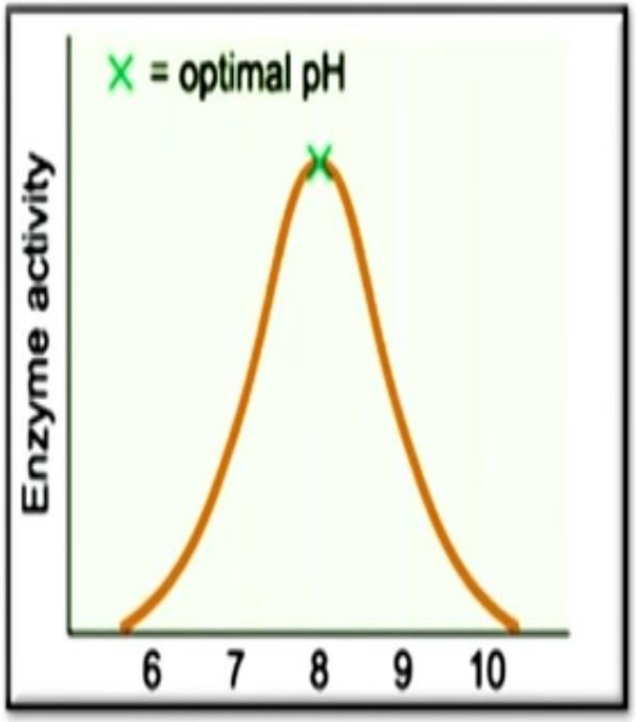


Figure 3 The pH activity relationship

Effect of Temperature

- Each enzyme also has a particular temperature at which is activity is maximum. This temperature is often referred to as the **optimum temperature.**
- The optimum temperature of most enzymes is found to be 37°C.

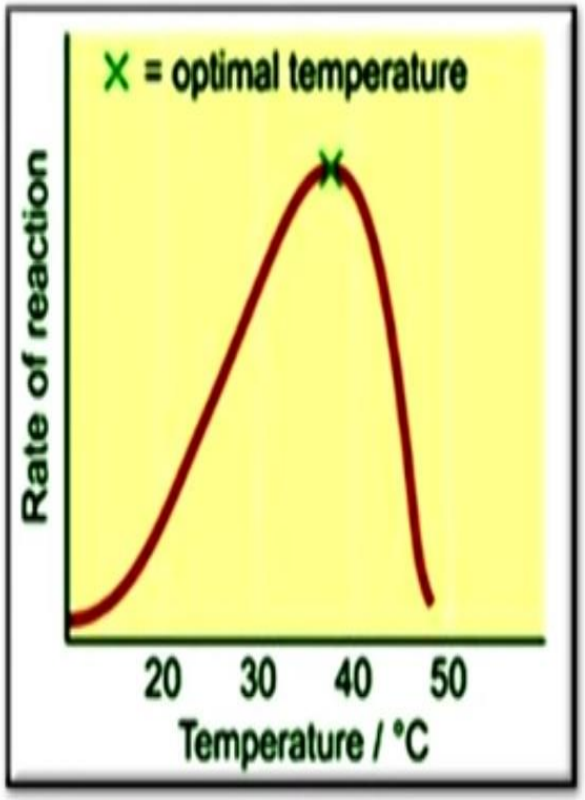


Figure 4 The temperature activity relationship

Effect of concentration of the substrate

- Rate of the reaction increases proportionally with increase in the concentration of substrate.
- At a particular substrate concentration, all active sites are saturated with substrate molecules. Further increase in concentration of substrate therefore does not lead to any increase the reaction rate

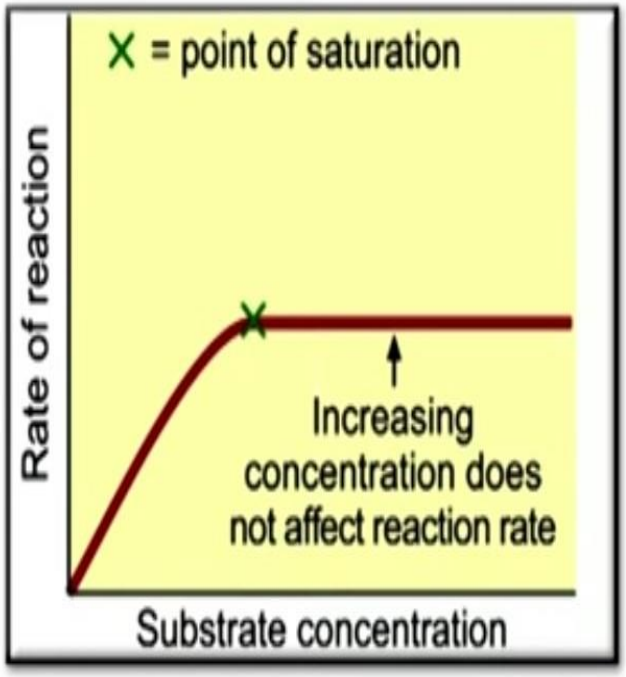


Figure 5 Substrate concentration - activity relationship

Effect of concentration of the enzyme

- Rate of an enzyme catalyzed reaction is directly proportional to the concentration of enzyme.
- At a fixed substrate concentration, all the substrate molecules are utilised completely. Even as the enzyme concentration is increased, there is no change in the reaction rate.

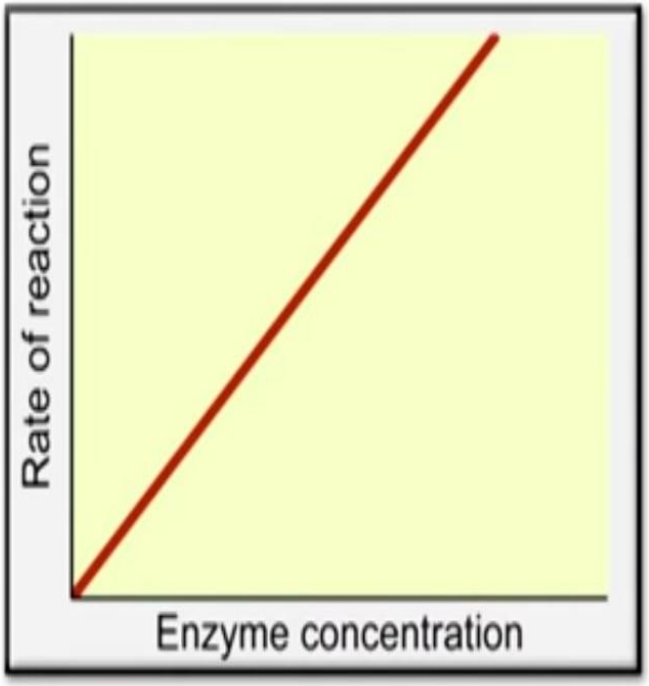


Figure 6 Enzyme concentration - activity relationship

- Enzymes which require metal ions for their activity or enzymes which contain metal ions in their structure are therefore known as **metalloenzymes.**

- Metal ions can be divalent , like Mg^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} or monovalent such as Na^{+} and K^{+}.

- Cl^{-} ions are required for activity by amylases, Zn^{2+} ions are required by carbonic anhydrases for them to be catalytically active

Isoenzymes

- There are some enzymes that may exist in two or more forms.
- While these may differ in terms of physical, chemical as well as electrophoretic attributes, they have the same catalytic activity
- Examples of isoenzymes are lactate dehydrogenase which can exist in 5 different iso enzymic forms and catalyse the same reaction of conversion of lactate to pyruvate.

AN OVERVIEW OF METABOLISM

INTRODUCTION

Metabolism is defined as the chemical processes by which cells produce the substances and energy needed to sustain life. It is subdivided into:

Anabolism - the phase of metabolism in which complex molecules, such as the proteins and fats that make up body tissue, are formed from simpler ones.

Catabolism - the metabolic breakdown of complex molecules into simpler ones, often resulting in a release of energy.

The process of metabolism within the body changes as a result of surgery or acute illness and so is an important area of knowledge for **anaesthetists and intensivists.**

A key metabolic process within the body is the liberation of energy by the breakdown of substances ingested as food. The major constituents of a normal diet are **Carbohydrates, Protein and Fat.**

OVERVIEW OF METABOLISM

Figure 1 is an overview of the pathways by which the three major energy sources (fat, carbohydrate and protein) can be used to produce ATP, the main unit of energy within the body. Glucose is predominantly metabolized via glycolysis, free fatty acids via β-oxidation and proteins via deamination. The common endpoint for all three pathways is acetyl coenzyme A (acetyl coA), which can be used as a substrate for very efficient production of ATP via the citric acid cycle (also known as the Krebs or tricarboxylic acid cycle), when oxygen is available (aerobic metabolism). Acetyl coA is also the start point by which glycolysis can be reversed (gluconeogensis) - glucose is regenerated and used by specific glucose-requiring organs (the brain and heart) during fasting. All of these processes are explained in more detail below. It is important to understand the concept that different metabolic processes are going on in different organs at different times, dependent on the activities and requirements for energy of those tissues, and those of the body as a whole. For example, the liver may be breaking down glycogen to release glucose for the brain (glycogenolysis), whilst the fat is being broken

down by β-oxidation for production of ATP via the Krebs cycle and production of glucose via gluconeogenesis. ATP cannot be transferred around the body, only produced and used within cells, so the body is dependent on efficient transfer of the metabolites and substrates shown in **Figure 1** around the body, in order to meet the needs of individual organs under different conditions.

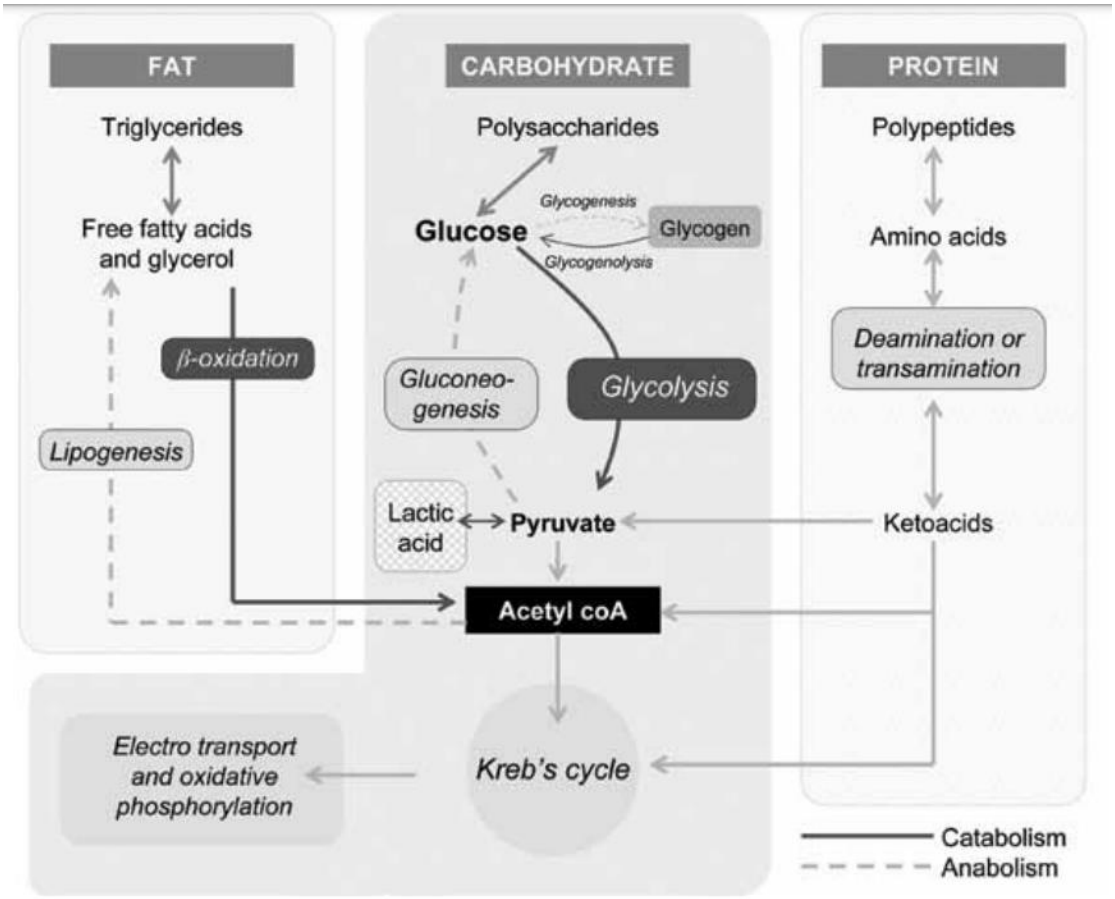


Figure 1. *A highly simplified overview of fat, carbohydrate and protein metabolism*

The body has various mechanisms that it can use to adjust to the availability of various substrates:

• They can be transformed into other types of substrate, or

• They can be transported around the body and used as needed by other organs to make ATP, which can then be used within the cells of that organ.

TYPES OF MOLECULES

Carbohydrate

Carbohydrate molecules contain carbon, hydrogen and oxygen atoms, usually in the proportion 1:2:1, and must contain both aldehyde and ketone groups.

Figure 2. *A - an aldehyde, B - a ketone*

Carbohydrates in their most basic form are called monosaccharides, such as glucose. Monosaccharide units may be joined into chains to become polysaccharides, such as starch.

Fats

Fats are ingested as triglycerides and cholesterol. Triglycerides are esters of three fatty acids chains bonded to a glycerol molecule.

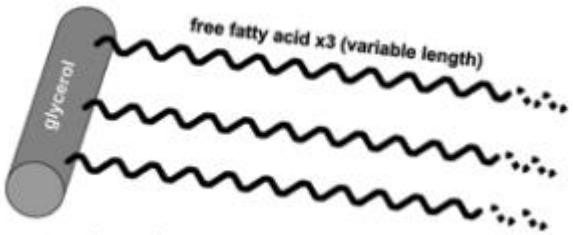


Figure 3. *A triglyceride*

Proteins

Proteins are chains of amino acids, molecules containing an amine group (nitrogen-containing, -NH2), a carboxylic acid group (-COOH) and a specific side chain (R in Figure 4).

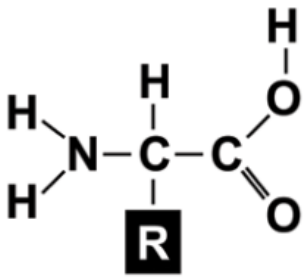
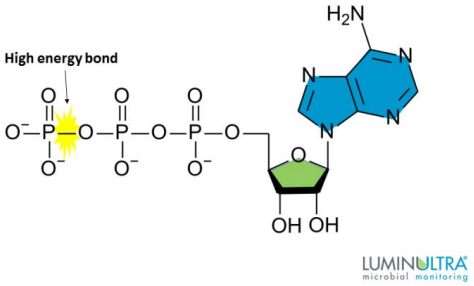


Figure 4. *An amino acid*

Energy

Energy is required for the body to perform activities or 'work'. This work can be external (skeletal muscle) or internal (cardiac muscle, biochemical processes). Under normal conditions it is obtained by breakdown of dietary carbohydrate, protein and fat. The average energy requirement for a 70kg man is 2500kcal per day. The basic unit for supply of energy for processes within the body is adenosine triphosphate (ATP), a molecule that has high energy bonds with its attached phosphate groups. The breakdown of adenosine triphosphate to adenosine diphosphate (ADP) releases energy.

ATP can then be regenerated from ADP using either:



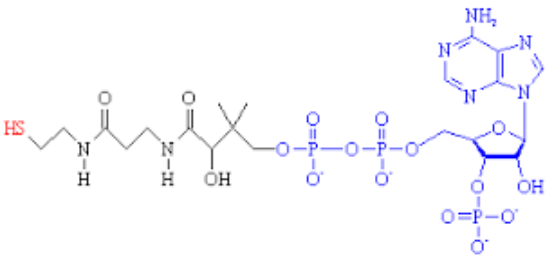
• energy generated within the cytoplasm by **anaerobic respiration** (no oxygen is required),

• **aerobic respiration** in mitochondria (oxygen requiring and far more productive of ATP), or

• by **direct interaction** with **Creatine phosphate**.

Remember that ATP and ADP are intracellular molecules that provide energy **in situ** within a cell, but cannot be transferred around the body from one organ to another. Movement of energy sources around the body is achieved by the pathways for breakdown (catabolism) and build up (anabolism) of the substances shown in Figure 1.

Acetyl coenzyme A



Acetyl coA is the central converting substance that links together the metabolism of fat, carbohydrate and protein and so is known as the universal intermediate**.**

Glycolysis

KEY CONCEPT QUESTIONS IN GLYCOLYSIS

1- How do substrate availability and enzyme activity levels control glycolytic flux?

2- Why is muscle lactate dehydrogenase activity required for short bursts of intense exercise?

……..

Glycolysis (Gk. glykys = sweet, lysis = splitting), also called glycolytic pathway or Embden-Meyerhof-Parnas (EMP) pathway, **is the sequence of reactions that metabolises one molecule of glucose to two molecules of pyruvate with the concomitant net production of two molecules of ATP.**

Glycolysis is almost an universal central pathway of glucose catabolism, and the complete pathway of glycolysis was elucidated by 1940, *largely through the pioneering contributions of G. Embden, O. Meyerhof, J. Parnas, C. Neuberg, O. Warburg, G. Cori, and C. Cori.*

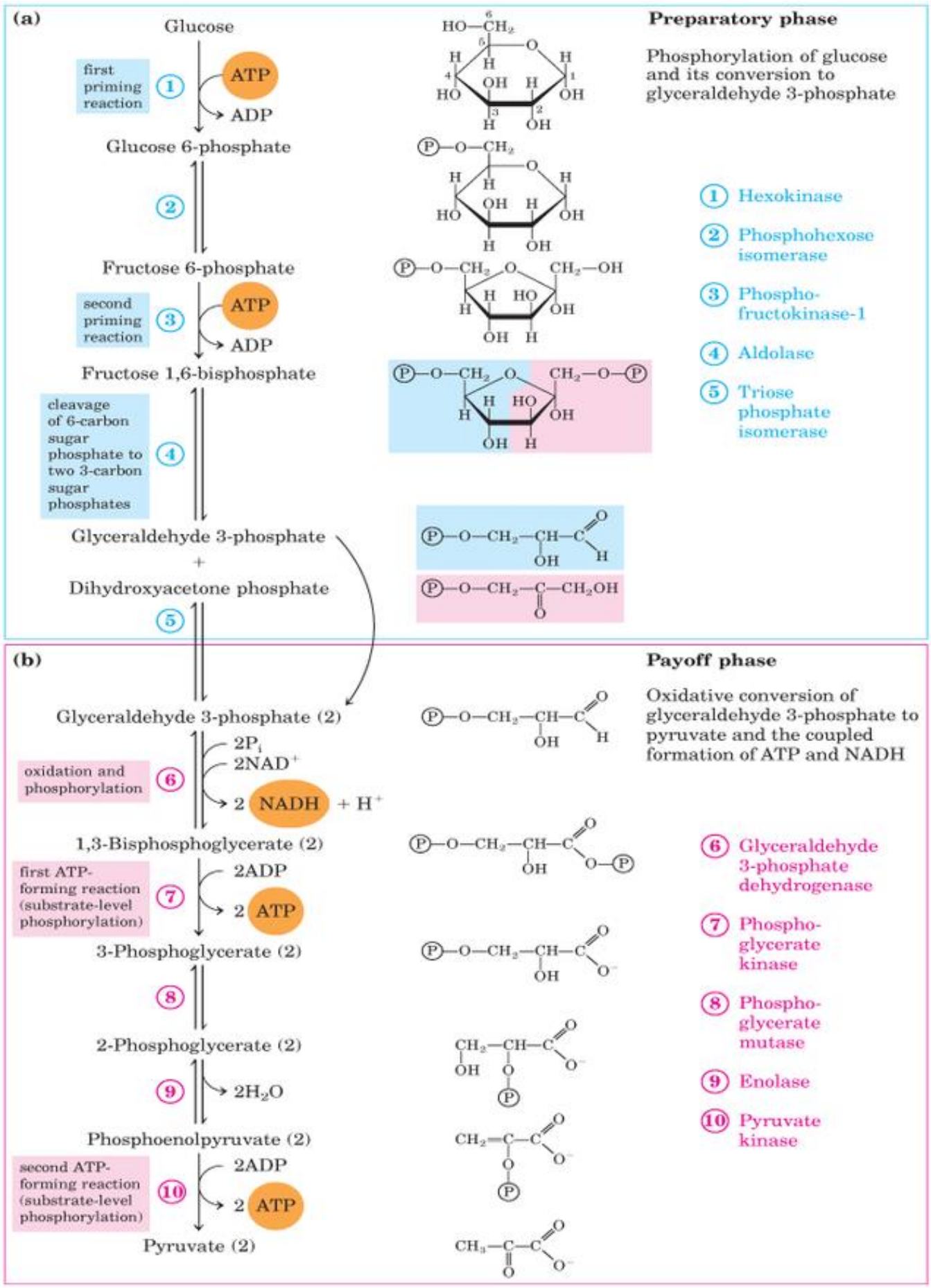
However, glycolysis occurs in all major groups of microorganisms and functions in the presence or absence of oxygen. It is located in the **cytoplasmic matrix** of the cells of an organism.

The whole process of glycolysis (i.e., the breakdown of the 6-carbon glucose molecule into two molecules of the 3-carbon pyruvate) occurs in ten steps (Fig.1).

The first five-steps constitute the preparatory phase while the rest live-steps represent the payoff phase (oxidation phase).

In **preparatory phase** there is phosphorylation of **glucose** and its conversion to **glyceraldehyde 3-phosphate** at the **expense** of two molecules of **ATP**.

Oxidative conversion of **glyceraldehyde 3-phosphate** to **pyruvate** and the coupled formation of **ATP** and **NADH** is the feature of **payoff phase**.


(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase
(b)
Glyceraldehyde 3-phosphate (2)
oxidation and phosphorylation
6
2Pi
2NAD+
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

The whole of glycolysis can be represented by the following simple equation:

Glucose + 2ADP + 2P_i + 2NAD^+ ⟶ 2 pyruvate + 2ATP + 2NADH + 2H^+

REACTIONS OF GLYCOLYSIS IN DETAIL:

1. Phase-1- Preparatory Phase

STEP-1: Glucose to glucose-6-phosphate

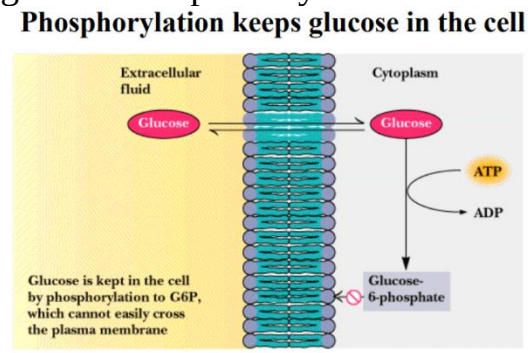
- Phosphorylation of glucose by **HEXOKINASE**

KINASE Enzymes that catalyze the transfer of a phosphoryl group from ATP to an acceptor substrate

-Type of **TRANSFERASE** enzyme

-Regulated but not the committed step

- Glucose-6-phosphate can form glycogen or other pathways



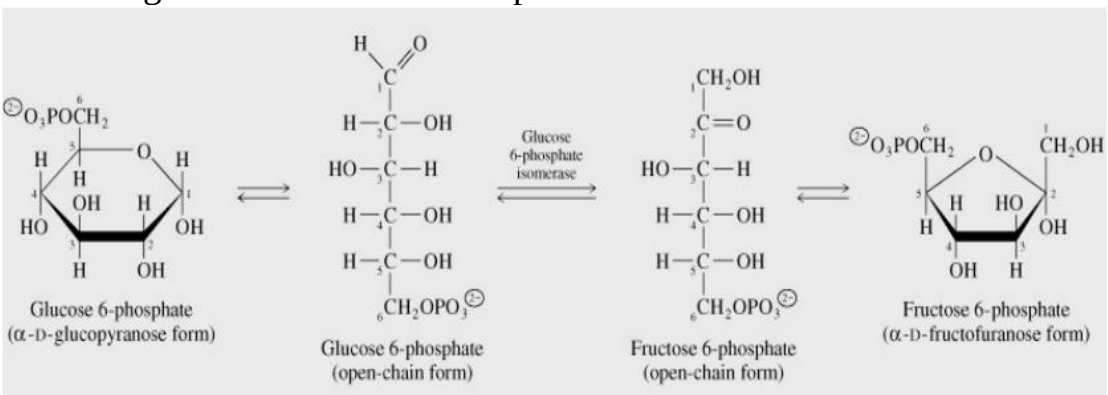
STEP-2: Glucose-6-phosphate to fructose-6-phosphate

ENZYME: Phosphoglucose Isomerase

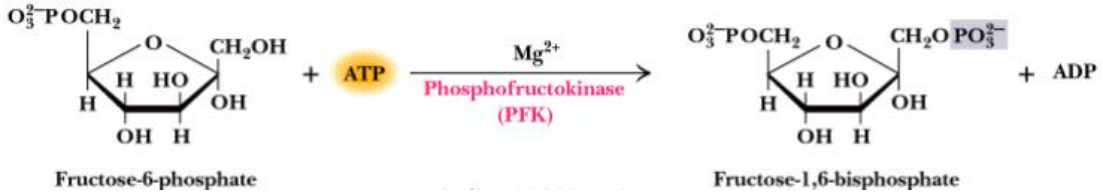
Type of **ISOMERASE** – Rearrangement of functional groups to form the isomer; ΔG – near zero; concentration of reactants and products affect direction.

Convert Glucose-6-phosphate to fructose-6-phosphate

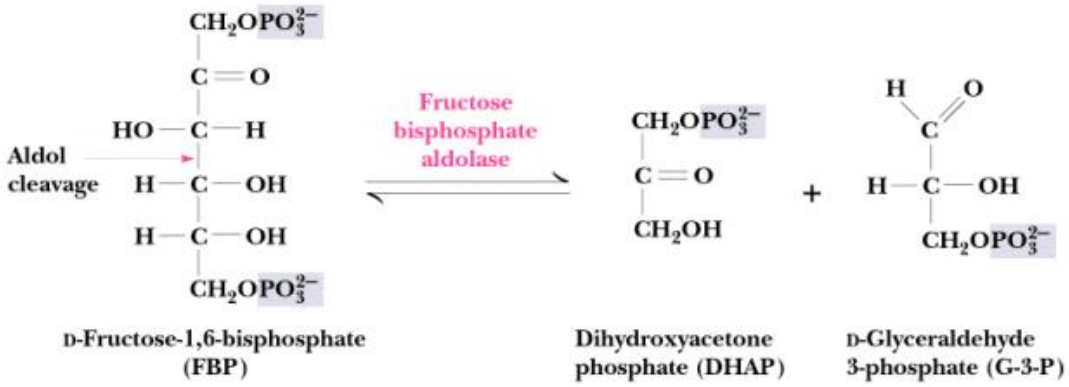
- Not a regulated or committed step.



STEP-3: Fructose-6-phosphate to fructose-1,6-bisphosphate
- ENZYME: Phosphofructokinase
KINASE – same as first step; TRANSFERASE reaction
-2nd Investment of energy – one more ATP used.
KEY CONTROL STEP – IRREVERSIBLE!!
-Committed step
- Note HIGHLY negative ΔG°' – means not reversible.

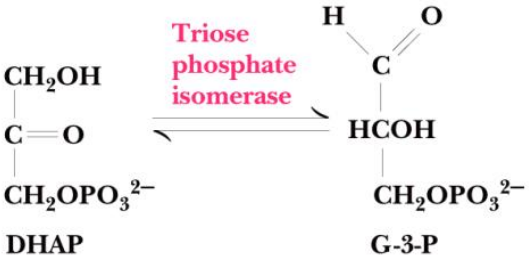


STEP 4:
Fructose-1,6-bisphosphate ⟶ glyceraldehyde-3-phosphate & dihydroxyacetone phosphate



- ENZYME: Aldolase
-Non-hydrolytic Cleavage reaction (type of lyase)
-Cleaves glucose molecule into 2 molecules.
- Not a regulatory step.

STEP 5: Dihydroxyacetone phosphate to glyceraldehyde-3-phosphate -
ENZYME: Triose phosphate isomerase
-Isomerization – rearrangement reaction
-Isomerase enzyme.
- Not a regulatory step.

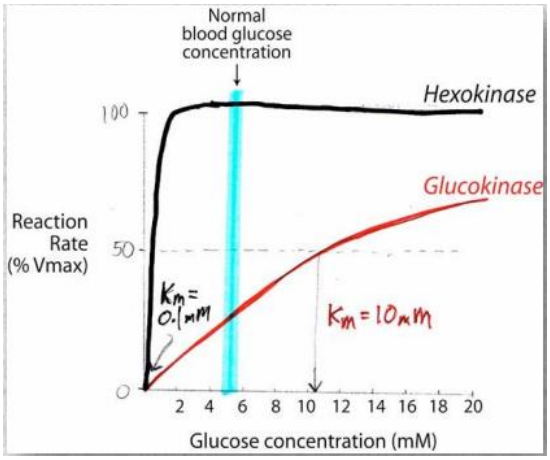


Sum: Glucose + 2 ATP = 2 glyceraldehyde-3-phosphate +2 ADP +2 Pi

-Irreversible reactions in metabolic pathways are called rate-limiting steps because the level of enzyme activity can be low even when substrate levels are high.

-Rate-limiting enzymes in metabolic pathways serve as regulated "valves" that are opened or closed in response to cellular conditions.

-hexokinase I, which has a high affinity for substrate(Km for glucose is ~0.1mM), is expressed in all tissues, phosphorylates a variety of hexose sugars, and is inhibited by the product of the reaction, glucose-6-P.



-This difference in tissue expression and glucose affinity between hexokinase and glucokinase plays an important role in controlling blood glucose levels, which ultimately controls rates of glycolytic flux in all cells by limiting substrate availability.

-The role of glucokinase in liver cells is to trap the extra glucose that is available from the diet so that it can be stored as glycogen for an energy source later.

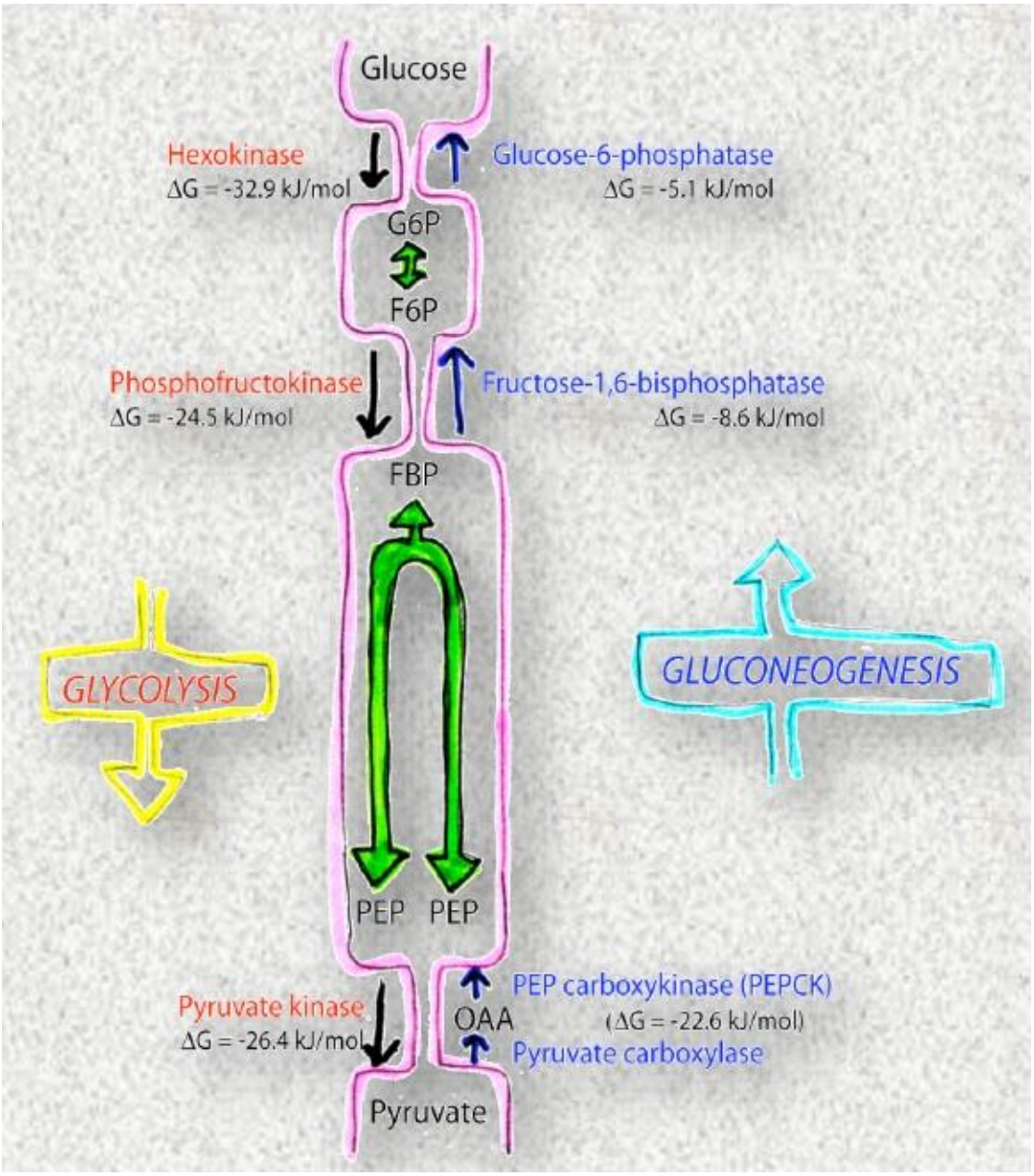
-By being active in liver cells only when glucose concentrations exceed normal limits (>5mM), glucokinase ensures that the liver is the major sink for dietary glucose, and at the same time, is able to efficiently remove glucose from the blood to help restore normal blood glucose concentrations.

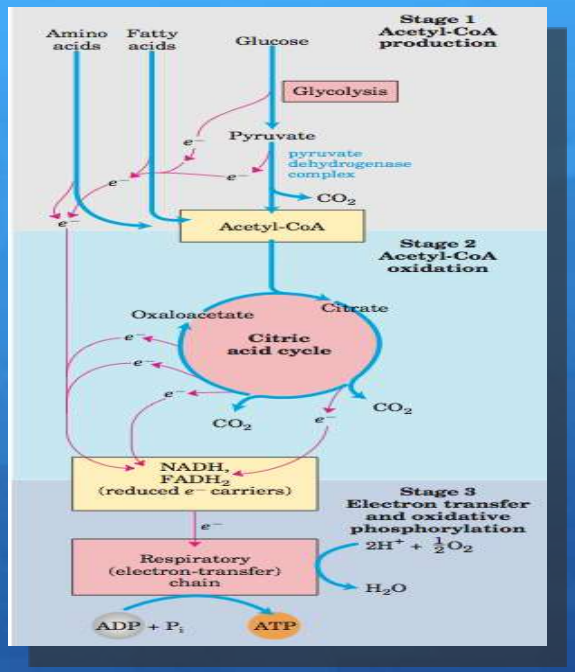
-Another important function of glucokinase is to act as a glucose sensor in pancreatic β cells where glucokinase enzyme levels are activated by increased glucose import mediated by glucose transporter proteins (GLUT proteins).

-when the concentration of glucose in the blood is elevated, glucose import into pancreatic β cells leads to higher glucokinase enzyme levels resulting in increased flux through glycolysis and net ATP synthesis.

This increase in ATP levels causes inhibition of ATP-sensitive K^+ channels, membrane depolarization, and activation of voltage gated Ca^{+2} channels.

-Elevated levels of intracellular Ca^{+2} triggers fusion of insulin containing vesicles with the plasma membrane and subsequent release of insulin into the blood.





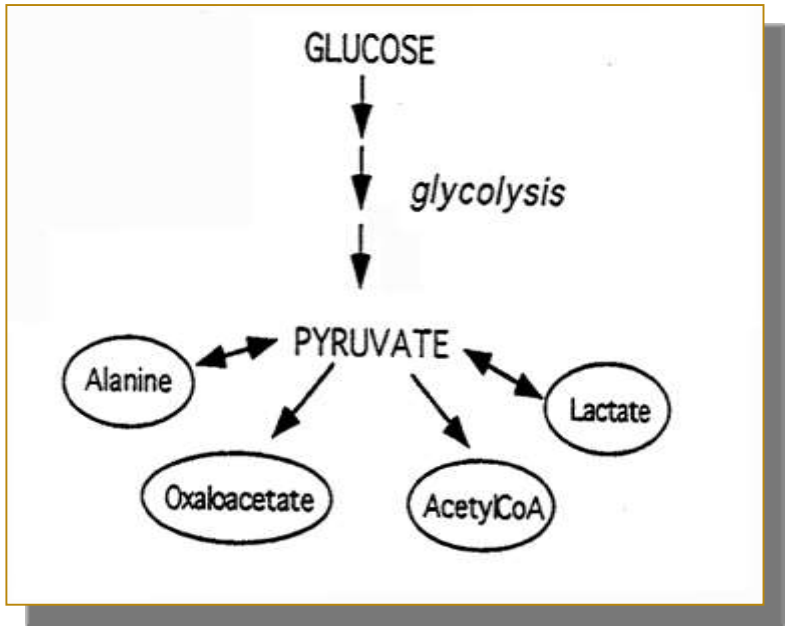
Lect.6

Pyruvate Metabolism

M.Sc. Dhurgham Aziz
Organic Chemistry

Introduction:

- In animals, pyruvate has a few main fates. Pyruvate can be converted to alanine, oxaloacetate, either as part of gluconeogenesis or for other biosynthetic purposes, or it can be converted to acetyl-CoA.
- **In animals, the conversion of pyruvate to acetyl-CoA is irreversible,** and produces a compound that has fewer physiological uses.



Continue….

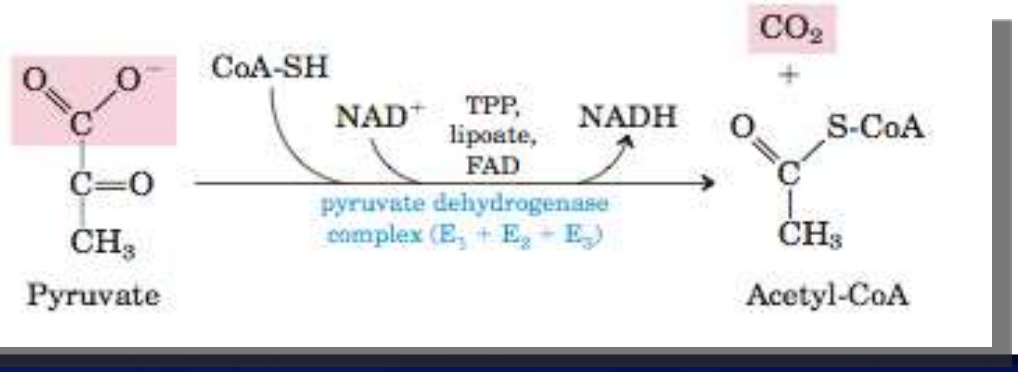
- Acetyl-CoA is used for lipid synthesis or for a few other, relatively minor, pathways,or as the substrate for the TCA cycle.
- In animals, acetyl-CoA cannot be used to synthesize amino acids or carbohydrates. This means that the conversion of pyruvate to acetyl-CoA is an important step, and must be tightly controlled.
- On the other hand, the conversion of pyruvate to acetyl-CoA is a necessary step.

Pyruvate import into mitochondrion

- Under aerobic conditions, pyruvate passes by a special transporter into mitochondria.
- Pyruvate is actually pumped into the mitochondria.
- So it is possible for the pyruvate concentration inside the mitochondria to be higher than outside.
- The energy for the pump comes from a proton gradient, in which the proton concentration outside the mitochondria is higher than it is inside.

Reactions of the pyruvate dehydrogenase complex

- ✓ The first step in the oxidation of pyruvate is an oxidative decarboxylation reaction.
- ✓ This reaction is carried out by a very large enzyme complex, the **pyruvate dehydrogenase complex,** which is located in the mitochondrial matrix.
- ✓ The reaction catalyzed by the pyruvate dehydrogenase complex is irreversible, and is tightly regulated.



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- ✓ However, in humans, the complex contains well over one hundred subunits.
- ✓ The complex is comprised of **three separate enzymes** involved in the actual catalytic process, and uses a total of **five different cofactors.**
- ✓ The large size of the complex allows the complicated reaction to proceed without dissociation of the reaction intermediates, and also allows regulation of the complex.
- ✓ The pyruvate dehydrogenase complex is closely related to the:

1- α-ketoglutarate dehydrogenase complex (an TCA cycle enzyme).

2-α -ketoacid dehydrogenase complex (in the metabolism of leucine, valine, and isoleucine).

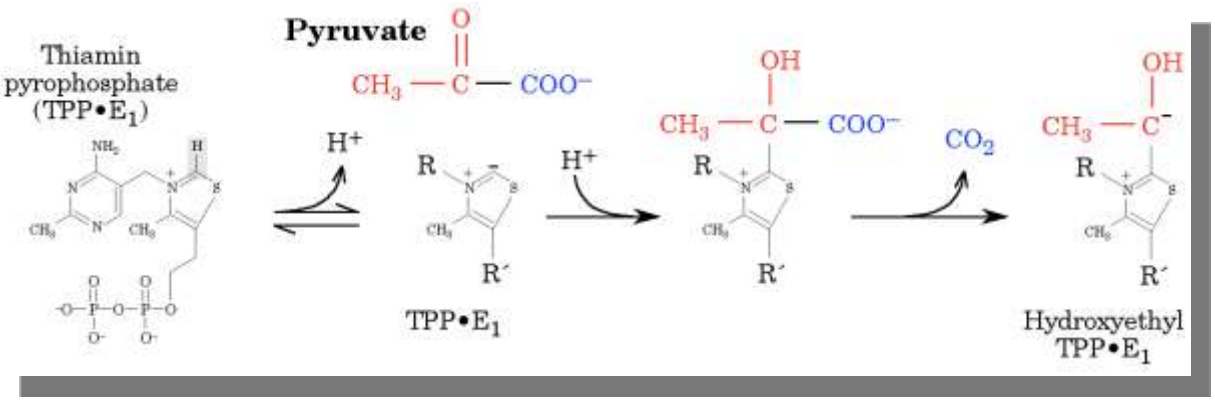
Pyruvate Dehydrogenase Complex

- ✓ The PDH complex contains 3 enzymes which catalyzes the reaction in 3 steps :

E1 = **Pyruvate dehydrogenase**,

E2 = **Dihydrolipoyl transacetylase**

E3 = **Dihydrolipoyl dehydrogenase**



Pyruvate Dehydrogenase Complex (Cont.)

- ✓ The complex requires 5 different coenzymes or prosthetic groups:

1-Thiamine pyrophosphate (TPP),

2-Flavin adenine dinucleotide (FAD),

3- Coenzyme A (CoA)

4- Nicotinamide adenine dinucleotide (NAD),

5-Lipoic acid

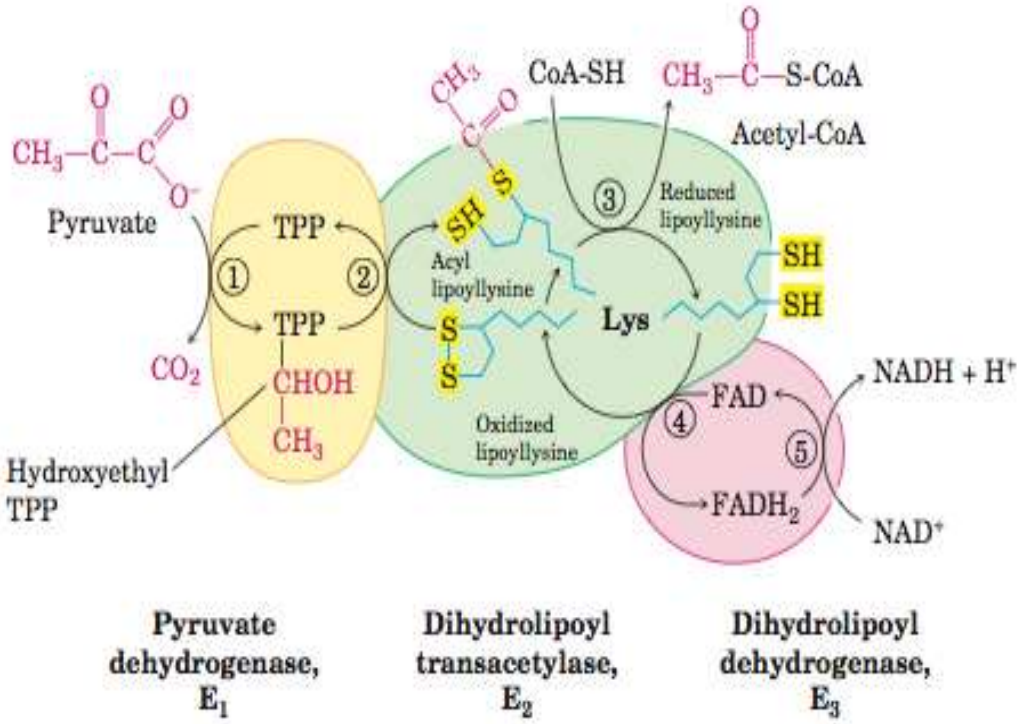


FIGURE 16–6 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex. The fate of pyruvate is traced in red. In step ① pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1), undergoing decarboxylation to the hydroxyethyl derivative (see Fig. 14–13). Pyruvate dehydrogenase also carries out step ②, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group. Step ③ is a transesterification in which the —SH group of CoA replaces the —SH group of E_2 to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group. In step ④ dihydrolipoyl dehydrogenase (E_3) promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E_2 to the FAD prosthetic group of E_3, restoring the oxidized form of the lipoyllysyl group of E_2. In step ⑤ the reduced $FADH_2$ of E_3 transfers a hydride ion to NAD^+, forming NADH. The enzyme complex is now ready for another catalytic cycle. (Subunit colors correspond to those in Fig. 16–5b.)

Continue

TABLE 17.1 Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

Table 17-1
Biochemistry, Sixth Edition

Regulation of PDH complex

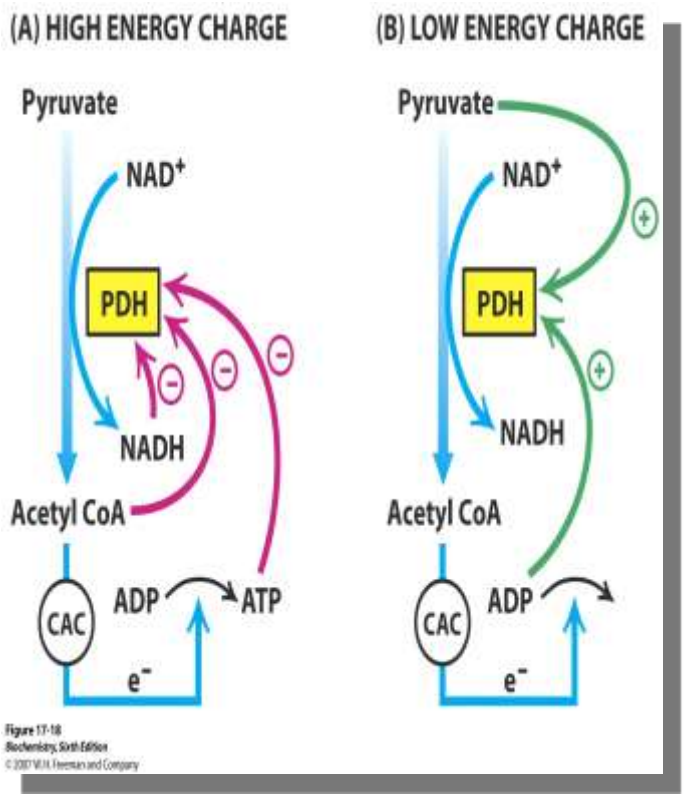
- ✓ PDH complex is regulated in 3 ways:

1- Allosteric inhibition

By products **: Acetyl CoA** and **NADH**

By high **ATP**

2- Allosteric activation by **AMP**



Regulation of PDH complex (Cont.)

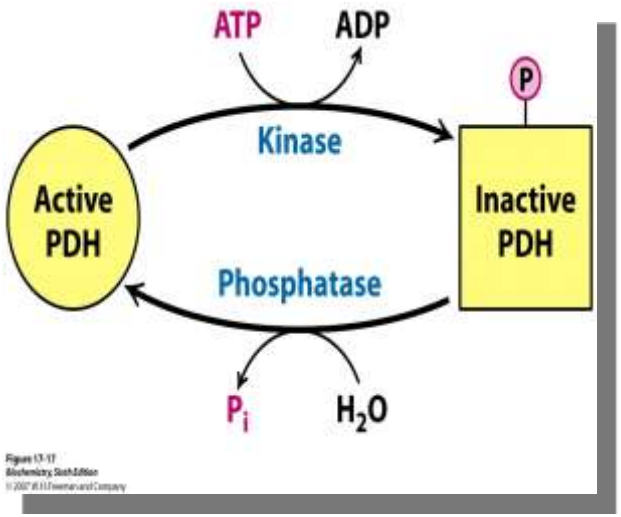
3- Covalent modification through phosphorylation and dephosphorylation of E_1 subunit :

- ✓ **Phoshorylated (inactive)**

Protein kinase converts active to inactive

- ✓ **Dephoshorylated (active)**

Phosphatase converts inactive to active



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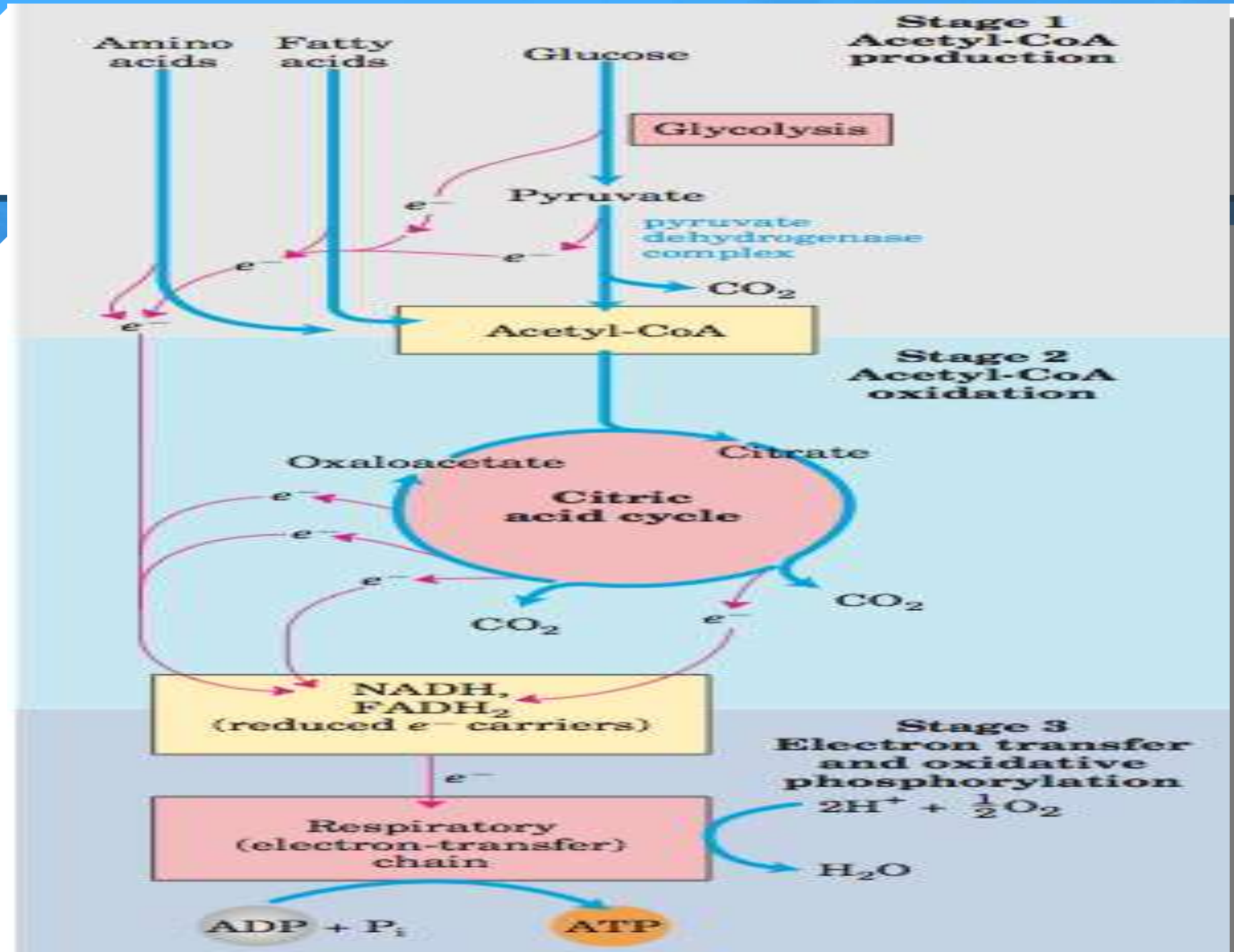

Amino acids
Fatty acids
Glucose
Stage 1
Acetyl-CoA production
Glycolysis
Pyruvate
pyruvate dehydrogenase complex
CO_2
Acetyl-CoA
Stage 2
Acetyl-CoA oxidation
Oxaloacetate
Citrate
Citric acid cycle
CO_2
CO_2
NADH, $FADH_2$ (reduced e^- carriers)
Stage 3
Electron transfer and oxidative phosphorylation
$2H^+ + \frac{1}{2}O_2$
Respiratory (electron-transfer) chain
H_2O
ADP + P_i
ATP

Krebs cycle

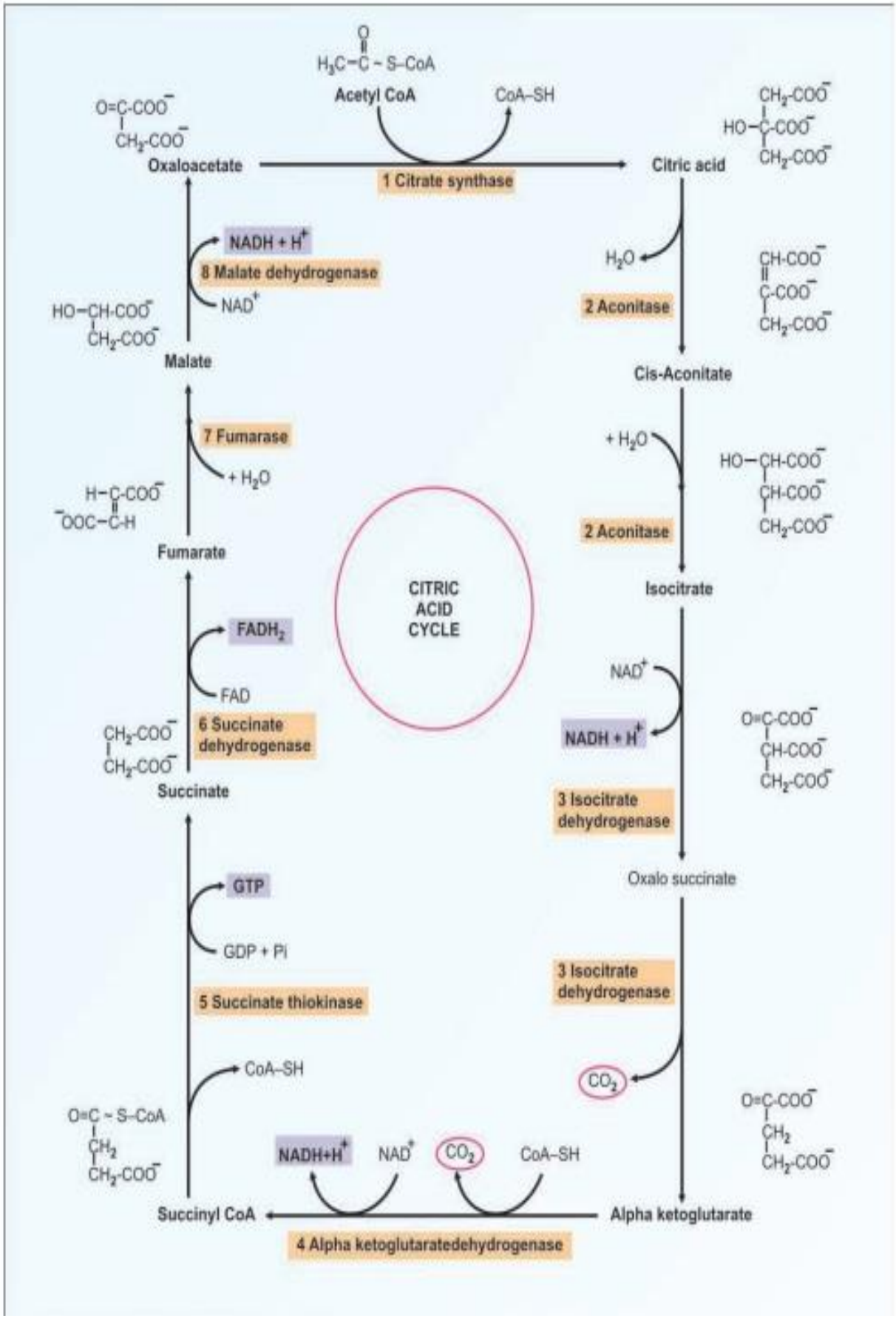
The Tricarboxylic Acid Cycle ([TCA cycle] also called the citric acid cycle or the Krebs cycle) plays several roles in metabolism. It is the final pathway where the oxidative catabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to CO_2.

This oxidation provides energy for the production of the majority of adenosine triphosphate (ATP) in most animals, including humans. The TCA cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport, which oxidize the reduced coenzymes (NADH and $FADH_2$) produced by the cycle.

The TCA cycle is an aerobic pathway, because O_2 is required as the final electron acceptor. Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic reactions.

The TCA cycle also supplies intermediates for a number of important synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of some amino acids, and it provides building blocks for the synthesis of some amino acids and heme.

Therefore, this cycle should not be viewed as a closed circle but, instead, as a traffic circle with compounds entering and leaving as required.


Acetyl CoA
CoA–SH
Oxaloacetate
Citric acid
1 Citrate synthase
NADH + H+
8 Malate dehydrogenase
NAD+
H2O
2 Aconitase
Malate
Cis-Aconitate
7 Fumarase
+ H2O
+ H2O
2 Aconitase
Fumarate
Isocitrate
CITRIC ACID CYCLE
FADH2
FAD
NAD+
NADH + H+
6 Succinate dehydrogenase
Succinate
3 Isocitrate dehydrogenase
Oxalo succinate
GTP
GDP + Pi
3 Isocitrate dehydrogenase
5 Succinate thiokinase
CoA–SH
CO2
NADH+H+
NAD+
CO2
CoA–SH
Succinyl CoA
Alpha ketoglutarate
4 Alpha ketoglutaratedehydrogenase

Tricarboxylic acid cycle: functions

The tricarboxylic acid cycle is often described as the “hub of intermediary metabolism.” It has both catabolic and anabolic functions—it is **amphibolic**.

As a catabolic pathway, it initiates the “terminal oxidation” of energy substrates. Many catabolic pathways lead to intermediates of the tricarboxylic acid cycle, or supply metabolites such as **pyruvate** and **acetyl-CoA** that can enter the cycle, where their C atoms are oxidized to CO_2. The reducing equivalents obtained in this way are then used for oxidative phosphorylation—i. e., to aerobically synthesize ATP.

The tricarboxylic acid cycle also supplies important precursors for anabolic pathways. Intermediates in the cycle are converted into:

• Glucose (gluconeogenesis; precursors: oxaloacetate and malate—

• Porphyrins (precursor: succinyl-CoA—

• Amino acids (precursors: 2-oxoglutarate, oxaloacetate—

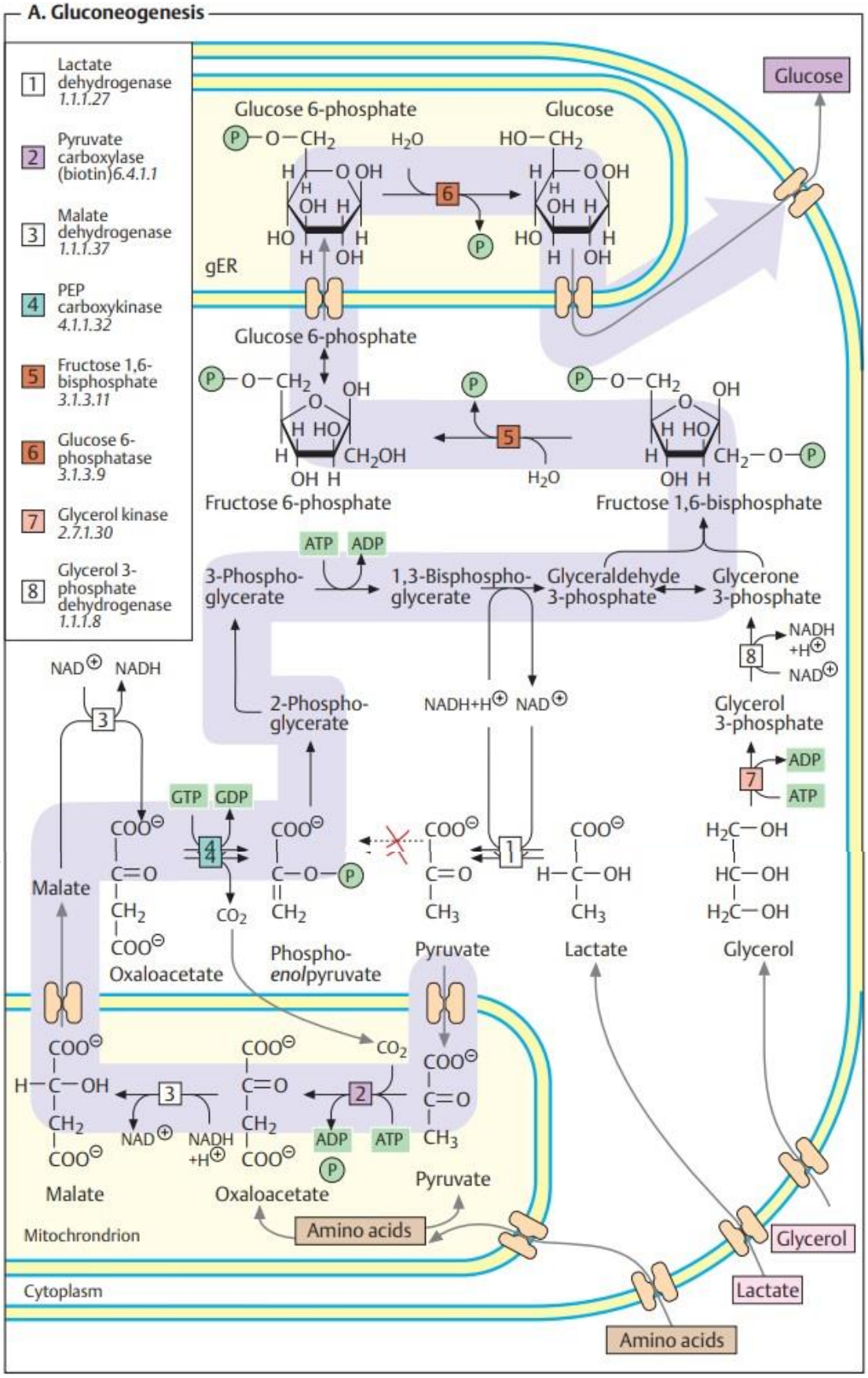
• Fatty acids and isoprenoids (precursor: citrate—

The intermediates of the tricarboxylic acid cycle are present in the mitochondria only in very small quantities. After the oxidation of acetyl-CoA to CO_2, they are constantly regenerated, and their concentrations therefore remain constant, averaged over time. Anabolic pathways, which remove intermediates of the cycle (e. g., gluconeogenesis) would quickly use up the small quantities present in the mitochondria if metabolites did not reenter the cycle at other sites to replace the compounds consumed. Processes that replenish the cycle in this way are called anaplerotic reactions. The degradation of most amino acids is anaplerotic, because it produces either intermediates of the cycle or pyruvate (glucogenic amino acids;.

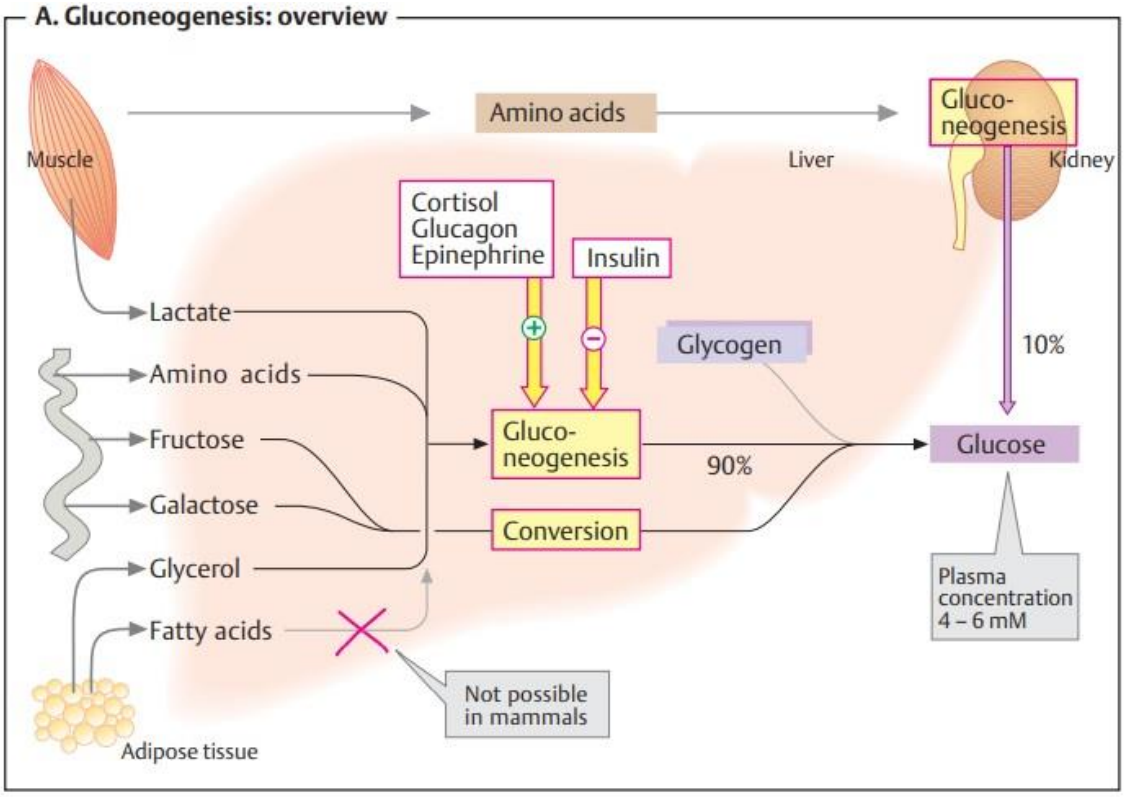
Gluconeogenesis is in fact largely sustained by the degradation of amino acids. A particularly important anaplerotic step in animal metabolism leads from pyruvate to oxaloacetic acid. This ATPdependent reaction is catalyzed by pyruvate carboxylase. It allows pyruvate yielding amino acids and lactate to be used for gluconeogenesis.

GLUCONEOGENESIS

Some tissues, such as brain and erythrocytes, depends on a constant supply of glucose. If the amount of carbohydrate taken up in food is not sufficient, the blood sugar level can be maintained for a limited time by degradation of hepatic glycogen.



If these reserves are also exhausted, de-novo synthesis of glucose (gluconeogenesis) begins. The liver is also mainly responsible for this, but the tubular cells of the kidney also show a high level of gluconeogenetic activity.



The main precursors for gluconeogenesis are amino acids derived from muscle proteins.

Another important precursor is lactate, which is formed in erythrocytes and muscle proteins when there is oxygen deficiency. Glycerol produced from the degradation of fats can also be used for gluconeogenesis.

However, the conversion of fatty acids into glucose is not possible in animal metabolism. The human organism can synthesize several hundred grams of glucose per day by gluconeogenesis.

Apart from the liver, the kidneys are the only organs capable of producing glucose by neosynthesis. The main substrate for gluconeogenesis in the cells of the proximal tubule is glutamine. In addition, other amino acids and also lactate, glycerol, and fructose can be used as precursors. As in the liver, the key enzymes for gluconeogenesis

are induced by cortisol. Since the kidneys also have a high level of glucose consumption, they only release very little glucose into the blood.

Many of the reaction steps involved in gluconeogenesis are catalyzed by the same enzymes that are used in glycolysis . Other enzymes are specific to gluconeogenesis and are only synthesized, under the influence of cortisol and glucagon when needed. Glycolysis takes place exclusively when needed in the cytoplasm, but gluconeogenesis also involves the mitochondria and the endoplasmic reticulum (ER). Gluconeogenesis consumes 4 ATP (3 ATP + 1 GTP) per glucose—i. e., twice as many as glycolysis produces.

1- Lactate as a precursor for gluconeogenesis is mainly derived from muscle, and erythrocytes. LDH oxidizes lactate to pyruvate, with $NADH+H^+$ formation.

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

3- The oxaloacetate formed in the mito chondrial matrix is initially reduced to ma late, which can leave the mitochondria via inner membrane transport systems.

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

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

bisphosphate. In between these reactions lies the isomerization of fructose 6-phosphate to glucose 6-phosphate.

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

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

Glycerol initially undergoes phosphorylation at C-3. The glycerol 3-phosphate formed is then oxidized by an NAD^+ -dependent dehydrogenase to form glycerone 3- phosphate, and thereby channeled into gluconeogenesis. An FAD-dependent mitochondrial enzyme is also able to catalyze this reaction (known as the “glycerophosphate shuttle”

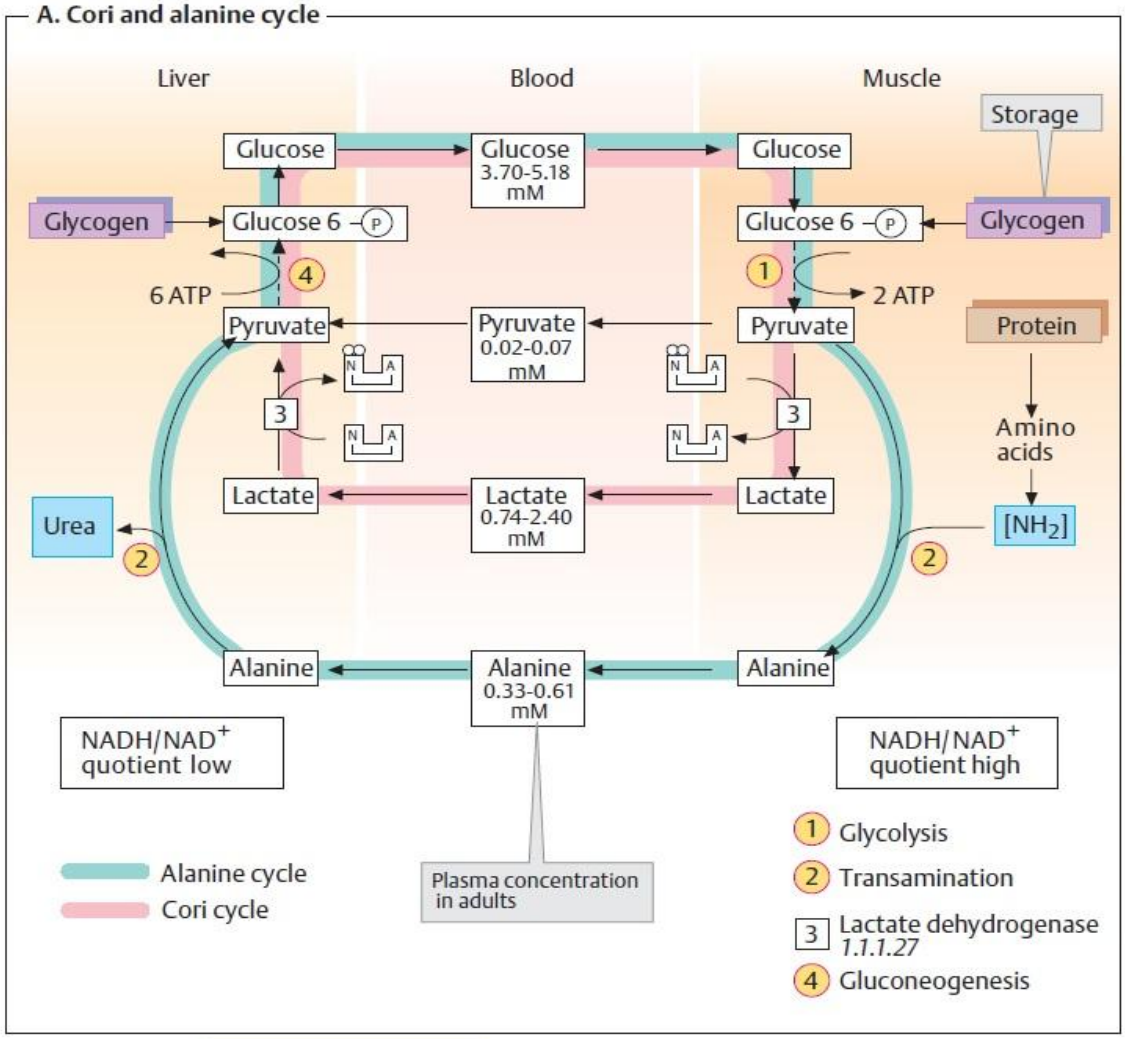
Muscle metabolism

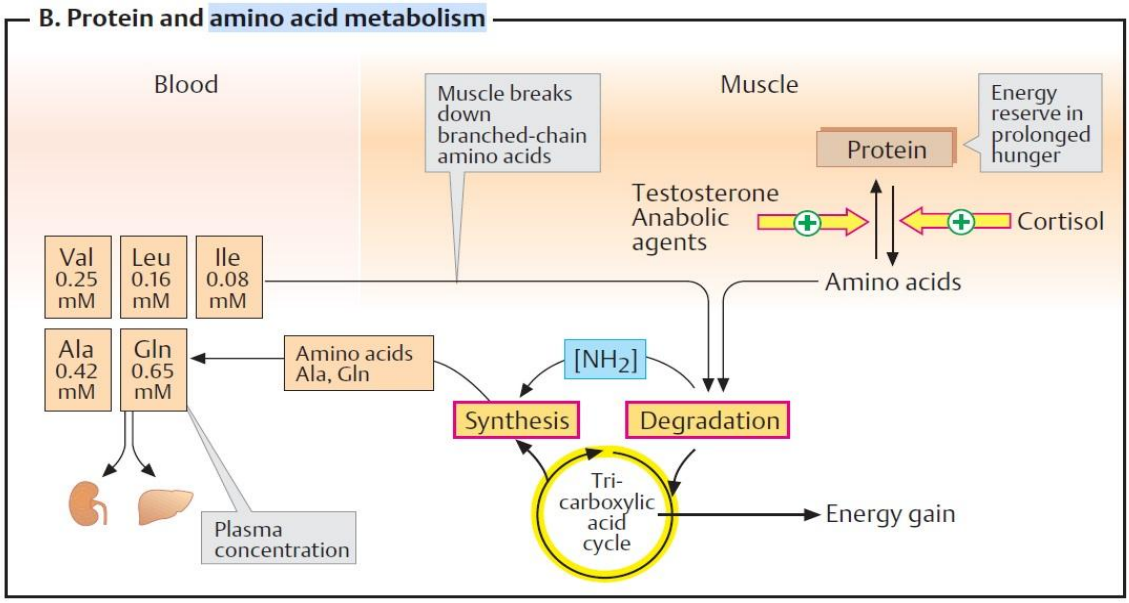
A. Cori and alanine cycle

White muscle fibers mainly obtain ATP from **anaerobic glycolysis**—i. e., they convert glucose into lactate. The **lactate** arising in muscle and, in smaller quantities, its precursor **pyruvate** are released into the blood and transported to the liver, where lactate and pyruvate are resynthesized into glucose again via *gluconeogenesis,* with ATP being consumed in the process . The glucose newly formed by the liver returns via the blood to the muscles, where it can be used as an energy source again. This circulation system is called the **Cori cycle**, after the researchers who first discovered it. There is also a very similar cycle for erythrocytes, which do not have mitochondria and therefore produce ATP by anaerobic glycolysis.

The muscles themselves are not capable of gluconeogenesis. Nor would this be useful, as gluconeogenesis requires much more **ATP** than is supplied by glycolysis. As $\mathbf{O_2}$ deficiencies do not arise in the liver even during intensive muscle work, there is always sufficient energy there available for gluconeogenesis. There is also a corresponding circulation systemfor the amino acid alanine. The alanine cycle in the liver not only provides alanine as a precursor for gluconeogenesis, but also transports to the liver the amino nitrogen arising in muscles during protein degradation.

In the liver, it is incorporated into urea for excretion. Most of the amino acids that arise in muscle during proteolysis are converted into glutamate and **2-oxo** acids by transamination. Again by transamination, glutamate and pyruvate give rise to alanine, which after glutamine is the second important form of transport for amino nitrogen in the blood. **In the liver**, alanine and 2-oxoglutarate are resynthesized into pyruvate and glutamate. Glutamate supplies the urea cycle, while pyruvate is available for gluconeogenesis.


A. Cori and alanine cycle
Liver
Blood
Muscle
Storage
Glucose
Glucose 3.70-5.18 mM
Glucose
Glycogen
Glucose 6 – P
Glucose 6 – P
Glycogen
4
6 ATP
1
2 ATP
Pyruvate
Pyruvate 0.02-0.07 mM
Pyruvate
Protein
N A
3
N A
3
Amino acids
Lactate
Lactate 0.74-2.40 mM
Lactate
Urea
[NH2]
2
2
Alanine
Alanine 0.33-0.61 mM
Alanine
NADH/NAD+ quotient low
NADH/NAD+ quotient high
Alanine cycle
Cori cycle
Plasma concentration in adults
1 Glycolysis
2 Transamination
3 Lactate dehydrogenase 1.1.1.27
4 Gluconeogenesis


B. Protein and amino acid metabolism
Blood
Muscle breaks down branched-chain amino acids
Muscle
Energy reserve in prolonged hunger
Protein
Testosterone
Anabolic agents
Cortisol
Amino acids
Val 0.25 mM
Leu 0.16 mM
Ile 0.08 mM
Ala 0.42 mM
Gln 0.65 mM
Amino acids Ala, Gln
[NH2]
Synthesis
Degradation
Tri-carboxylic acid cycle
Energy gain
Plasma concentration

B. Protein and amino acid metabolism

The skeletal muscle is the most important site for degradation of the branched-chain amino acids (**Val, Leu, Ile**;), but other amino acids are also broken down in the muscles.

Alanine and glutamine are resynthesized from the components and released into the blood. They transport the nitrogen that arises during amino acid breakdown to the liver (**alanine cycle**;) and to the kidneys.

During periods of hunger, muscle proteins serve as an energy reserve for the body. They are broken down into amino acids, which are transported to the liver.
In the liver, the carbon skeletons of the amino acids are converted into intermediates in the tricarboxylic acid cycle or into acetoacetyl-CoA. These amphibolic metabolites are then available to the energy metabolism and for gluconeogenesis. After prolonged starvation, the brain switches to using ketone bodies in order to save muscle protein.
The synthesis and degradation of muscle proteins are regulated by hormones. Cortisol leads to muscle degradation, while testosterone stimulates protein formation. Synthetic anabolics with a testosterone-like effect have repeatedly been used for doping purposes or for intensive muscle-building.

Smoothmuscle differs from skeletal muscle in various ways. Smooth muscles—which are found, for example, in blood vessel walls and in the walls of the intestines—do not contain any muscle fibers. In smooth-muscle cells, which are usually spindle-shaped, the contractile proteins are arranged in a less regular pattern than in striated muscle. Contraction in this type of muscle is usually not stimulated by nerve impulses, but occurs in a largely spontaneous way. Ca2+ (in the form of Ca^{2+}-calmodulin;) also activates contraction in smooth muscle; in this case, however, it does not affect troponin, but activates a protein kinase that phosphorylates the light chains in myosin and thereby increases myosin's ATPase activity. Hormones such as epinephrine and angiotensin II are able to influence vascular tonicity in this way, for example.

Bone and teeth

The family of connective-tissue cells includes fibroblasts, chondrocytes (cartilage cells), and osteoblasts (bone-forming cells). They are specialized to secrete extracellular proteins, particularly collagens, and mineral substances, which they use to build up the extracellular matrix. By contrast, osteoclasts dissolve bone matter again by secreting H+ and collagenases.

A. Bone

Bone is an extremely dense, specialized form of connective tissue. In addition to its supportive function, it serves to store calcium and phosphate ions. In addition, blood cells are formed in the bone marrow. The most important mineral component of bone is apatite, a form of crystalline calcium phosphate. Apatites are complexes of cationic Ca2+ matched by HPO42–, CO32–, OH–, or F– as anions. Depending on the counter-ion, apatite can occur in the forms carbonate apatite Ca10(PO4)6CO3, as hydroxyapatite Ca10(PO4)6 (OH)2, or fluoroapatite Ca10(PO4)6F2. In addition, alkaline earth carbonates also occur in bone. In adults, more than 1 kg calcium is stored in bone. Osteoblast and osteoclast activity is constantly incorporating Ca2+ into bone and removing it again. There are various hormones that regulate these processes: calcitonin increases deposition of Ca2+ in the bone matrix, while parathyroid hormone (PTH) promotes the mobilization of Ca2+, and calcitriol improves mineralization (for details, see p. 342). The most important organic components of bone are collagens (mainly type I; see p. 344) and proteoglycans (see p. 346). These form the extracellular matrix into which the apatite crystals are deposited (biomineralization). Various proteins are involved in this not yet fully understood process of bone formation, including collagens and phosphatases. Alkaline phosphatase is found in osteoblasts and acid phosphatase in osteoclasts. Both of these enzymes serve as marker enzymes for bone cells.

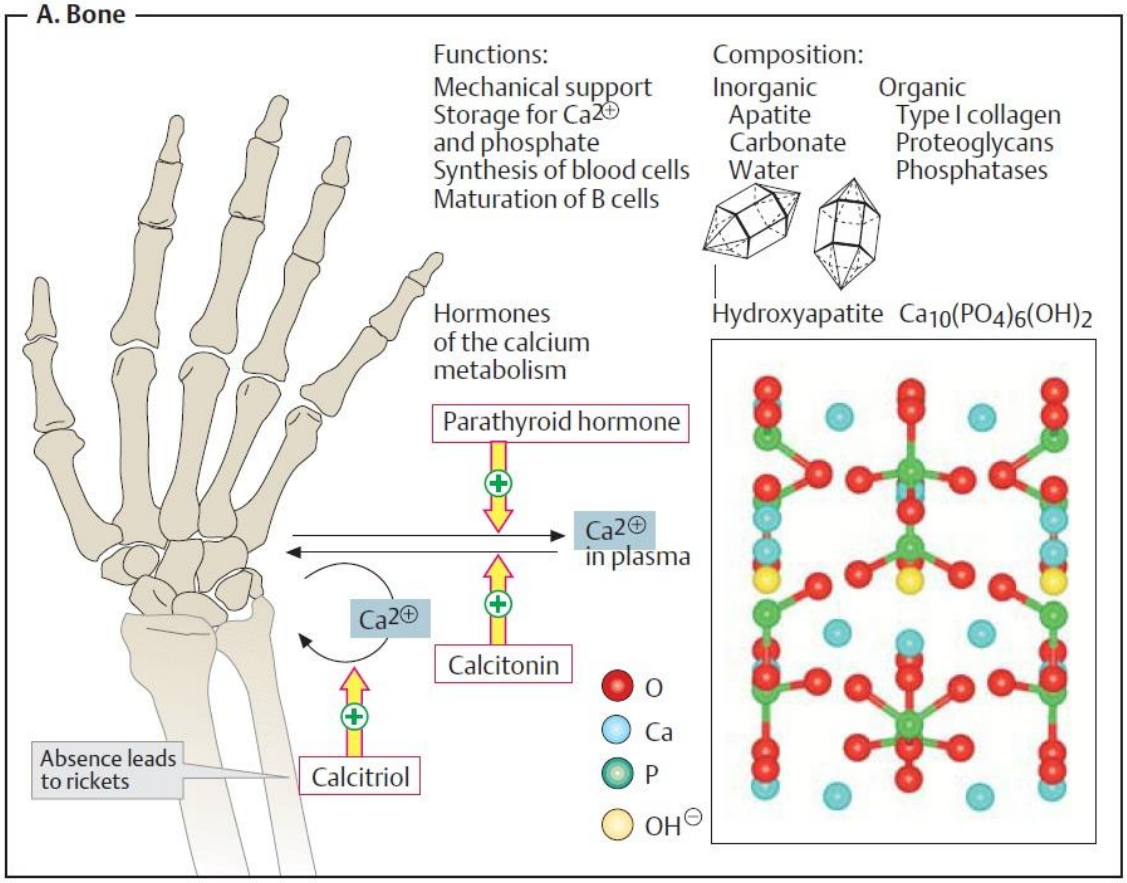
B. Teeth

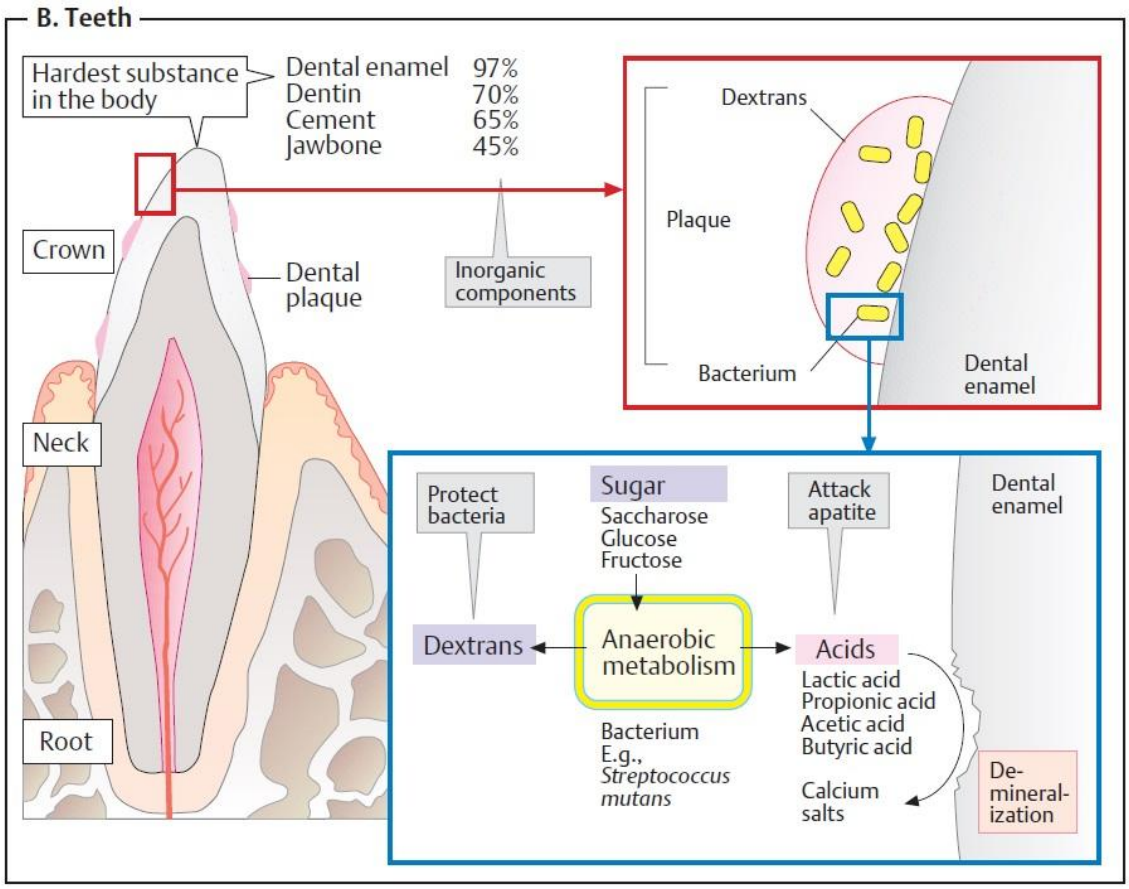
The illustration shows a longitudinal section through an incisor, one of the 32 permanent teeth in humans. The majority of the tooth consists of dentine. The crown of the tooth extends beyond the gums, and it is covered in enamel. By contrast, the root of the tooth is coated in dental cement. Cement, dentin, and enamel are bone-like substances. The high proportion of inorganic matter they contain (about 97% in the dental enamel) gives them their characteristic hardness. The organic components of cement, dentin, and enamelmainly consist of collagens and proteoglycans; their most important mineral component is apatite, as in bone (see above).

A widespread form of dental disease, caries, is caused by acids that dissolve the mineral part of the teeth by neutralizing the negatively charged counter-ions in apatite (see A). Acids occur in food, or are produced by microorganisms that live on the surfaces of the teeth (e. g., Streptococcus mutans). The main product of anaerobic degradation of sugars by these organisms is lactic acid.

Other products of bacterial carbohydrate metabolism include extracellular dextrans (see p. 40)—insoluble polymers of glucose that help bacteria to protect themselves from their environment. Bacteria and dextrans are components of dental plaque, which forms on inadequately cleaned teeth. When Ca2+ salts and other minerals are deposited in plaque as well, tartar is formed.

The most important form of protection against caries involves avoiding sweet substances (foods containing saccharose, glucose, and fructose). Small children in particular should not have very sweet drinks freely available to them. Regular removal of plaque by cleaning the teeth and hardening of the dental enamel by fluoridization are also important. Fluoride has a protective effect because fluoroapatite (see A) is particularly resistant to acids.



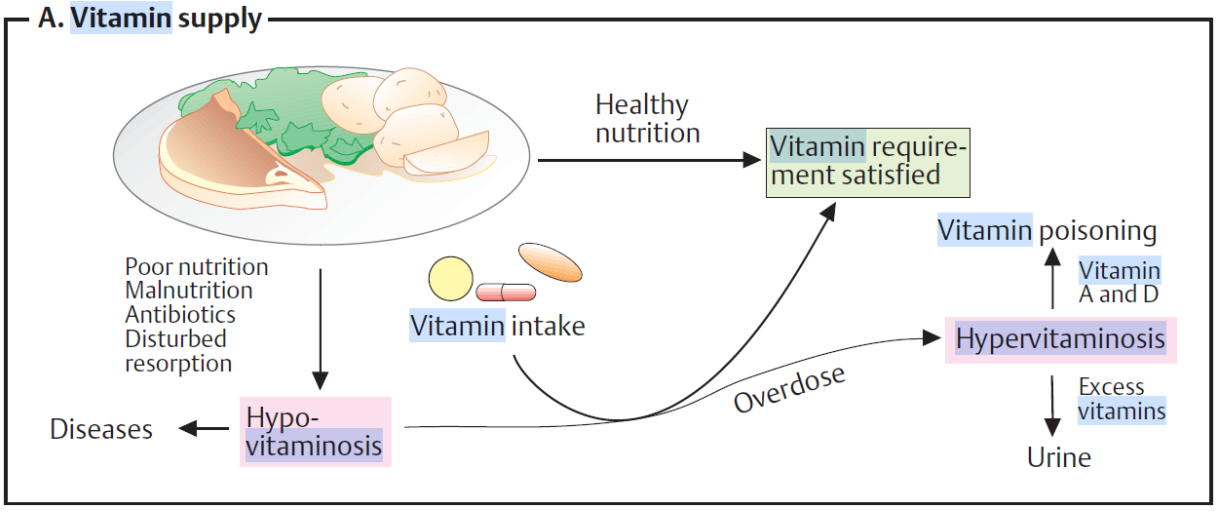

B. Teeth
Hardest substance in the body
Dental enamel 97%
Dentin 70%
Cement 65%
Jawbone 45%
Crown
Dental plaque
Inorganic components
Neck
Root
Dextrans
Plaque
Bacterium
Dental enamel
Protect bacteria
Sugar
Saccharose
Glucose
Fructose
Attack apatite
Dental enamel
Dextrans
Anaerobic metabolism
Acids
Lactic acid
Propionic acid
Acetic acid
Butyric acid
Bacterium
E.g.,
Streptococcus mutans
Calcium salts
De-mineral-ization

Vitamins

Vitamins are essential organic compounds that the animal organism is not capable of forming itself, although it requires them in small amounts form metabolism. Most vitamins are **precursors of coenzymes**; in some cases, they are also precursors of **hormones** or act as **antioxidants**. Vitamin requirements vary from species to species and are influenced by age, sex, and physiological conditions such as pregnancy, breast-feeding, physical exercise, and nutrition.

A. Vitamin supply _

A healthy diet usually covers average daily vitamin requirements. By contrast, malnutrition, malnourishment (e. g., an unbalanced diet in older people, malnourishment in alcoholics, ready meals), or resorption disturbances lead to an inadequate supply of vitamins from which **hypovitaminosis,** or in extreme cases avitaminosis, can result. Medical treatments that kill the intestinal flora—e. g., antibiotics— can also lead to vitamin deficiencies (K, B_{12}, H) due to the absence of bacterial vitamin synthesis. Since only a few vitamins can be stored (A, D, E, B_{12}), a lack of vitamins quickly leads to **deficiency diseases.** These often affect the skin, blood cells, and nervous system. The causes of vitamin deficiencies can be treated by improving nutrition and by administering vitamins in tablet form. An overdose of vitamins only leads to **hypervitaminoses**, with toxic symptoms, in the case of vitamins A and D. Normally, excess vitamins are rapidly excreted with the urine.



B. Lipid-soluble vitamins _

Vitamins are classified as either lipid-soluble or water-soluble. The lipid-soluble vitamins include vitamins A, D, E, and K, all of which belong to the isoprenoids.

The ability to synthesize particular isoprenoids is limited to a few species of plants and animals. For example, rubber is only formed by a few plant species, including the rubber tree (*Hevea brasiliensis*). Several isoprenoids that are required by animals for metabolism, but cannot be produced by them independently, are vitamins; this group includes *vitamins A, D, E,* and *K*. Due to its structure and function, vitamin D is now usually classified as a steroid hormone.

Vitamin A (retinol)

Is the parent substance of the *retinoids,* which include *retinal* and *retinoic acid*. The retinoids also can be synthesized by cleavage from the provitamin β-carotene. Retinoids are found in meat-containing diets, whereas β-carotene occurs in fruits and vegetables (particularly carrots). **Retinal** is involved in visual processes as the pigment of the chromoprotein *rhodopsin*. **Retinoic acid**, like the steroid hormones, influences the transcription of genes in the cell nucleus. It acts as a differentiation factor in growth and development processes. Vitamin A deficiency can result in *night blindness*, *visual impairment,* and *growth disturbances.*

Two types of photoreceptor cell are found in the human retina—*rods* and *cones*. Rods are sensitive to low levels of light, while the cones are responsible for color vision at higher light intensities.

Signaling substances and many proteins are involved in visual processes. Initially, a **light-induced** *cis–trans* **isomerization** of the pigment retinal triggers a conformational change in the membrane protein *rhodopsin*. Via the G protein *transducin*, which is associated with rhodopsin, an enzyme is activated that breaks down the second messenger *cGMP*. Finally, the cGMP deficiency leads to *hyperpolarization* of the light-sensitive cell, which is registered by subsequent neurons as *reduced neurotransmitter release.*

A. Photoreceptor _

The cell illustrated opposite, a **rod**, has a structure divided by membrane discs into which the 7-helix receptor **rhodopsin** is integrated.

In contrast to other receptors in the 7-helix class, rhodopsin is a light-sensitive *chromoprotein*. Its protein part, **opsin**, contains the aldehyde **retinal**—an isoprenoid which is bound to the ε-amino group of a lysine residue as an *aldimine*.

The light absorption of rhodopsin is in the visible range, with a maximum at about 500 nm. The absorption properties of the visual pigment are thus optimally adjusted to the spectral distribution of sunlight. Absorption of a photon triggers isomerization from the 11-*cis* form of retinal to all *trans*-retinal (top right). Within milliseconds, this *photochemical process* leads to an allosteric conformational change in rhodopsin.

The active conformation (**rhodopsin***) binds and activates the G protein **transducin**. The *signal cascade* (**B**) that now follows causes the rod cells to release less neurotransmitter (glutamate) at their synapses. The adjoining bipolar neurons register this change and transmit it to the brain as a signal for light. There are several different rhodopsins in the **cones**. All of them contain retinal molecules as light-sensitive components, the absorption properties of which are modulated by the different proportions of opsin they contain in such a way that colors can also be perceived.

B. Signal cascade _

Dark (bottom left). Rod cells that are not exposed to light contain relatively high concentrations (70 μM) of the cyclic nucleotide **cGMP**, which is synthesized by a *guanylate cyclase*. The cGMP binds to an ion channel in the rod membrane (bottom left) and thus keeps it open. The inflow of cations (Na^+, Ca^{2+}) depolarizes the membrane and leads to release of the neurotransmitter glutamate at the synapse.

Light (bottom right). When the G protein transducin binds to light-activated rhodopsin*, it leads to the GDP that is bound to the transducin being exchanged for GTP. In transducin* that has been activated in this way, the GTP-containing α-subunit breaks off from the rest of the molecule and in turn activates a membrane *cGMP phosphodiesterase*. This hydrolyzes cGMP to GMP and thus reduces the level of free cGMP

within milliseconds. As a consequence, the cGMP bound at the ion channel dissociates off and the channel closes. As cations are constantly being pumped out of the cell, the membrane potential falls and **hyperpolarization** of the cell occurs, which interrupts glutamate release.

Regeneration.

After exposure to light, several processes restore the initial conditions:

1. The α-subunit of transducin* inactivates itself by GTP hydrolysis and thus terminates the activation of cGMP esterase.

2. The reduced Ca^{2+} concentration causes activation of guanylate cyclase, which increases the cGMP level until the cation channels reopen.

3. An isomerase (Glutamate decarboxylase) transfers all-*trans* –retinal to the 11-*cis* -form, in which it is available for the next cycle. A dehydrogenase (4-Aminobutyrate transaminase) can also allow retinal to be supplied from vitamin A (retinol).

Vitamin D (calciol, cholecalciferol)

Is the precursor of the hormone *calcitriol* (1α,25-dihydroxycholecalciferol. Together with two other hormones (parathyrin and calcitonin), calcitriol regulates the calcium metabolism.

The effects of the steroid hormone **calcitriol** in bone are complex. On the one hand, it promotes bone formation by stimulating osteoblast differentiation (top). This is particularly important in small children, in whom calcitriol deficiency can lead to mineralization disturbances (*rickets*). On the other hand, calcitriol increases blood Ca^{2+} levels through increased Ca^{2+} mobilization from bone. An overdose of vitamin D (cholecalciferol), the precursor of calcitriol, can therefore have unfavorable effects on the skeleton similar to those of vitamin deficiency (*hypervitaminosis*).

Calciol can be synthesized in the skin from 7-dehydrocholesterol, an endogenous steroid, by a photochemical reaction. Vitamin D deficiencies only occur when the skin receives insufficient exposure to ultraviolet light and vitamin D is lacking in the diet. Deficiency is observed in the

form of *rickets* in children and *osteomalacia* in adults. In both cases, bone mineralization is disturbed.

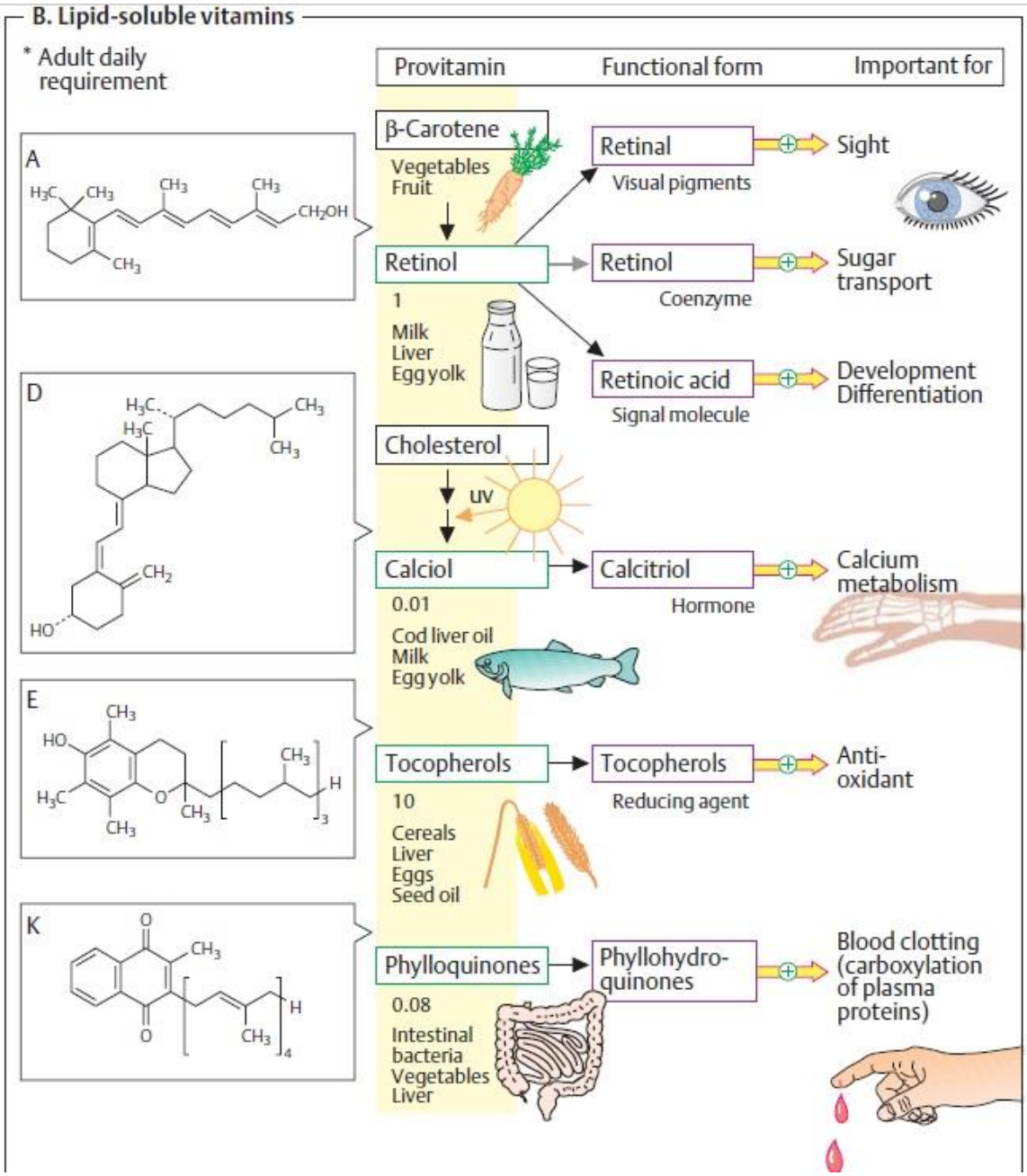
Vitamin E (tocopherol) and related compounds

only occur in plants (e. g., wheat germ). They contain what is known as a *chroman ring.* In the lipid phase, vitamin E is mainly located in biological membranes, where as an *antioxidant* it protects unsaturated lipids against **Reactive oxygen species** (ROS) and other radicals.

To protect them against ROS and other radicals, all cells contain **antioxidants**. These are *reducing agents* that react easily with oxidative substances and thus protect more important molecules from oxidation. Biological antioxidants include vitamins C and E, coenzyme Q, and several carotenoids. Bilirubin, which is formed during heme degradation, also serves for protection against oxidation.

Vitamin K (phylloquinone) and similar substances

With modified side chains are involved in carboxylating glutamate residues of coagulation factors in the liver. The form that acts as a cofactor for carboxylase is derived from the vitamin by enzymatic reduction. Vitamin K antagonists (e. g., coumarin derivatives) inhibit this reduction and consequently carboxylation as well. This fact is used to inhibit blood coagulation in *prophylactic treatment against thrombosis*. Vitamin K deficiency occurs only rarely, as the vitamin is formed by bacteria of the intestinal flora.


B. Lipid-soluble vitamins
* Adult daily requirement
Provitamin
Functional form
Important for
A
D
E
K
β-Carotene
Vegetables
Fruit
Retinal
Visual pigments
Sight
Retinol
1
Milk
Liver
Egg yolk
Retinol
Coenzyme
Sugar transport
Retinoic acid
Signal molecule
Development Differentiation
Cholesterol
uv
Calciol
0.01
Cod liver oil
Milk
Egg yolk
Calcitriol
Hormone
Calcium metabolism
Tocopherols
10
Cereals
Liver
Eggs
Seed oil
Tocopherols
Reducing agent
Anti-oxidant
Phylloquinones
0.08
Intestinal bacteria
Vegetables
Liver
Phyllohydro-quinones
Blood clotting (carboxylation of plasma proteins)

Water-soluble vitamins I

The B group of vitamins covers water-soluble vitamins, all of which serve as precursors for coenzymes. Their numbering sequence is not continuous, as many substances that were originally regarded as vitamins were not later confirmed as having vitamin characteristics.

A. Water-soluble vitamins I ◑

Vitamin B_1 (thiamine) contains two heterocyclic rings—a *pyrimidine ring* (a six-membered aromatic ring with two Ns) and a *thiazole ring* (a five-membered aromatic ring with N and S), which are joined by a methylene group. The active form of vitamin B_1 is **thiamine diphosphate** (TPP), which contributes as a coenzyme to the transfer of hydroxyalkyl residues (active aldehyde groups). The most important reactions of this type are oxidative decarboxylation of 2-oxoacids (see p. 134) and the transketolase reaction in the pentose phosphate pathway (see p. 152). Thiamine was the first vitamin to be discovered, around 100 years ago. Vitamin B_1 deficiency leads to *beriberi,* a disease with symptoms that include neurological disturbances, cardiac insuf ciency, and muscular atrophy.

Vitamin B_2 is a complex of several vitamins: riboflavin, folate, nicotinate, and pantothenic acid.

Riboflavin (from the Latin *flavus,* yellow) serves in the metabolism as a component of the redox coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD; see p. 104). As prosthetic groups, **FMN** and **FAD** are cofactors for various oxidoreductases (see p. 32). No specific disease due to a deficiency of this vitamin is known.

Folate, the anion of folic acid, is made up of three different components—a *pteridine derivative, 4-aminobenzoate,* and one or more *glutamate* residues. After reduction to tetrahydrofolate (**THF**), folate serves as a coenzyme in the C_1 metabolism (see p. 418). Folate deficiency is relatively common, and leads to disturbances in nucleotide biosynthesis and thus cell proliferation. As the precursors for blood cells divide particularly rapidly, disturbances of the blood picture can occur, with increased amounts of abnormal precursors for megalocytes (*megaloblastic anemia*). Later, general damage ensues as phospholipid synthesis and the amino acid metabolism are affected.

In contrast to animals, microorganisms are able to synthesize folate from their own components. The growth of microorganisms can therefore be inhibited by *sulfonamides,* which competitively inhibit the incorporation of 4-aminobenzoate into folate (see p. 254). Since folate is not synthesized in the animal organism, sulfonamides have no effect on animal metabolism.

Nicotinate and **nicotinamide,** together referred to as "niacin," are required for biosynthesis of the coenzymes nicotinamide adenine dinucleotide (**NAD^+**) and nicotinamide adenine dinucleotide phosphate (**$NADP^+$**). These both serve in energy and nutrient metabolism as carriers of *hydride ions* (see pp. 32, 104). The animal organism is able to convert *tryptophan* into nicotinate, but only with a poor yield. Vitamin deficiency therefore only occurs when nicotinate, nicotinamide, and tryptophan are all simultaneously are lacking in the diet. It manifests in the form of skin damage (*pellagra*), digestive disturbances, and depression.

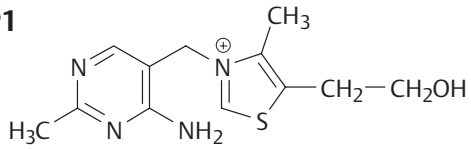
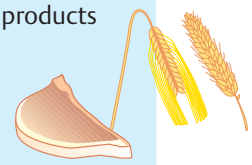
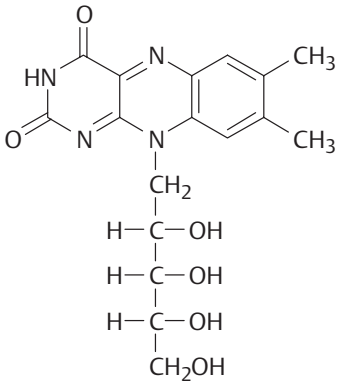
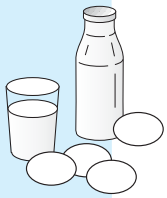
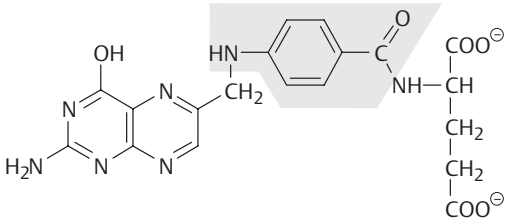
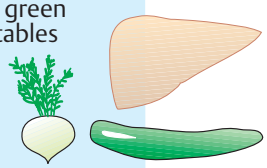
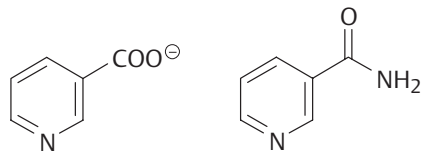
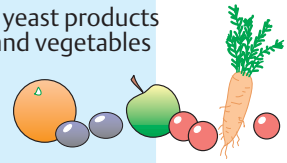
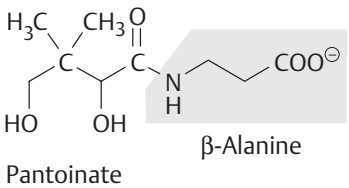
Pantothenic acid is an acid amide consisting of β-alanine and 2,4-dihydroxy-3,3′-dimethylbutyrate (pantoic acid). It is a precursor of *coenzyme A,* which is required for activation of acyl residues in the lipid metabolism (see pp. 12, 106). *Acyl carrier protein* (ACP; see p. 168) also contains pantothenic acid as part of its prosthetic group. Due to the widespread availability of pantothenic acid in food (Greek *pantothen* = "from everywhere"), deficiency diseases are rare.

Further information

The requirement for vitamins in humans and other animals is the result of mutations in the enzymes involved in biosynthetic coenzymes. As intermediates of coenzyme biosynthesis are available in suf cient amounts in the diet of heterotrophic animals (see p. 112), the lack of endogenous synthesis did not have unfavorable effects for them. Microorganisms and plants whose nutrition is mainly autotrophic have to produce all of these compounds themselves in order to survive.

A. Water-soluble vitamins I

* Adult daily requirement

Vitamin	Active form: coenzyme	Function in metabolism
B_1 	Thiamine 1.5 mg * Grain Yeast products Pork 	TPP Thiamine diphosphate	Transfer of hydroxy-alkyl residues
B_2 	Riboflavin 1.8 mg* Milk Eggs 	FMN FAD	Hydrogen transfer
4-Aminobenzoate residue  Pteridine derivative Glu	Folate 0.2 mg* Fresh green vegetables Liver 	THF Tetrahydro-folate	C_1-metabolism
 Nicotinate Nicotinamide	Nicotinate Nicotinamide 20 mg* (or 1.2 g tryptophan) Meat, yeast products Fruit and vegetables 	NADP NAD	Hydride transfer
 Pantoinate β-Alanine	Pantothenate 7 mg* Widely distributed	CoA	Activation of carboxy-lic acids

Water-soluble vitamins II

A. Water-soluble vitamins II ◑

Vitamin B_6 consists of three substituted pyridines—**pyridoxal**, **pyridoxol**, and **pyridoxamine.** The illustration shows the structure of pyridoxal, which carries an aldehyde group (–CHO) at C-4. Pyridoxol is the corresponding alcohol ($–CH_2OH$), and pyridoxamine the amine ($–CH_2NH_2$).

The active form of vitamin B_6, **pyridoxal phosphate,** is the most important coenzyme in the amino acid metabolism (see p. 106). Almost all conversion reactions involving amino acids require pyridoxal phosphate, including transaminations, decarboxylations, dehydrogenations, etc. *Glycogen phosphorylase,* the enzyme for glycogen degradation, also contains pyridoxal phosphate as a cofactor. Vitamin B_6 deficiency is rare.

Vitamin B_{12} (cobalamine) is one of the most complex low-molecular-weight substances occurring in nature. The core of the molecule consists of a tetrapyrrol system (*corrin*), with cobalt as the central atom (see p. 108). The vitamin is exclusively synthesized by microorganisms. It is abundant in liver, meat, eggs, and milk, but not in plant products. As the intestinal flora synthesize vitamin $B_{12,}$ strict vegetarians usually also have an adequate supply of the vitamin.

Cobalamine can only be resorbed in the small intestine when the gastric mucosa secretes what is known as *intrinsic factor*—a glycoprotein that binds cobalamine (the *extrinsic factor*) and thereby protects it from degradation. In the blood, the vitamin is bound to a special protein known as *transcobalamin.* The liver is able to store vitamin B_{12} in amounts suf cient to last for several months. Vitamin B_{12} deficiency is usually due to an absence of intrinsic factor and the resulting resorption disturbance. This leads to a disturbance in blood formation known as *pernicious anemia.*

In animal metabolism, derivatives of cobalamine are mainly involved in rearrangement reactions. For example, they act as coenzymes in the conversion of methylmalonyl-CoA to succinyl-CoA (see p. 166), and in the formation of methionine from homocysteine (see p. 418). In prokaryotes, cobalamine derivatives also play a part in the reduction of ribonucleotides.

Vitamin C is L-**ascorbic acid** (chemically: 2-oxogulonolactone). The two hydroxyl groups have acidic properties. By releasing a proton, ascorbic acid therefore turns into its anion, **ascorbate**. Humans, apes, and guinea pigs require vitamin C because they lack the enzyme L-*gulonolactone oxidase (1.1.3.8),* which catalyzes the final step in the conversion of glucose into ascorbate.

Vitamin C is particularly abundant in fresh fruit and vegetables. Many soft drinks and foodstuffs also have synthetic ascorbic acid added to them as an antioxidant and flavor enhancer. Boiling slowly destroys vitamin C. In the body, ascorbic acid serves as a reducing agent in variations reactions (usually hydroxylations). Among the processes involved are *collagen synthesis, tyrosine degradation, catecholamine synthesis,* and *bile acid biosynthesis.* The daily requirement for ascorbic acid is about 60 mg, a comparatively large amount for a vitamin. Even higher doses of the vitamin have a protective effect against infections. However, the biochemical basis for this effect has not yet been explained. Vitamin C deficiency only occurs rarely nowadays; it becomes evident after a few months in the form of *scurvy,* with connective-tissue damage, bleeding, and tooth loss.

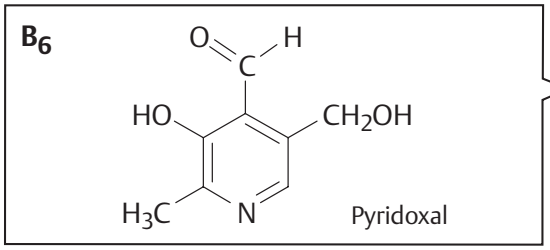
Vitamin H (**biotin)** is present in liver, egg yolk, and other foods; it is also synthesized by the intestinal flora. In the body, biotin is covalently attached via a lysine side chain to enzymes that catalyze carboxylation reactions. Biotin-dependent carboxylases include *pyruvate carboxylase* (see p. 154) and *acetyl-CoA carboxylase* (see p. 162). CO_2 binds, using up ATP, to one of the two N atoms of biotin, from which it is transferred to the acceptor (see p. 108).

Biotin binds with high af nity ($K_d = 10^{-15}$ M) and specificity to *avidin*, a protein found in egg white. Since boiling denatures avidin, biotin deficiency only occurs when egg whites are eaten raw.

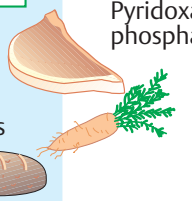
A. Water-soluble vitamins II

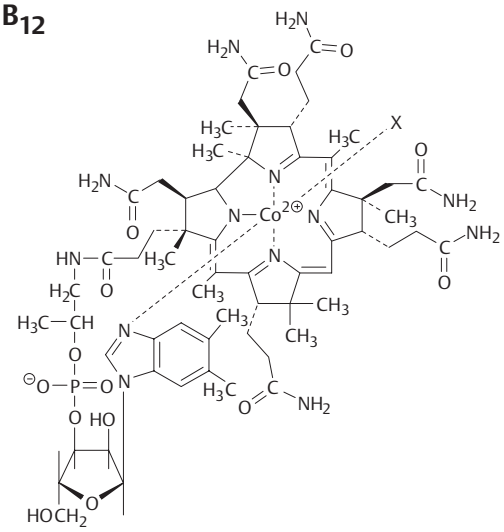
* Adult daily requirement

Vitamin	Active form: coenzyme	Function in metabolism
B_6	Pyridoxal Pyridoxol Pyridoxamine	PLP Pyridoxal phosphate	Activation of amino acids
B_{12}	Cobalamine	5-Deoxy-adenosyl cobalamine	Isomerization e.g.
C	Ascorbic acid	Ascorbate	Stabilization of enzyme systems, coenzyme, antioxidant
H	Biotin	B Biotin	Transfer of carboxyl groups

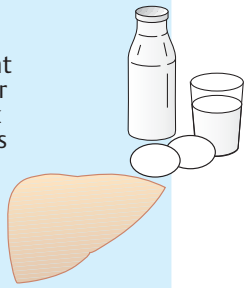


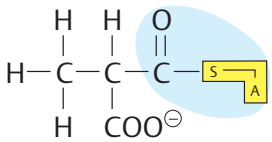
2 mg*
Meat
Vegetables
Grain products



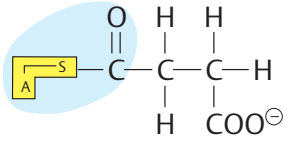


0.002 mg*
Meat
Liver
Milk
Eggs

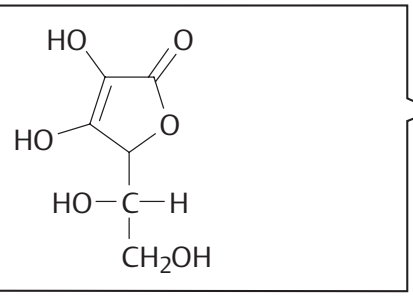




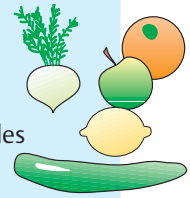
Methylmalonyl CoA

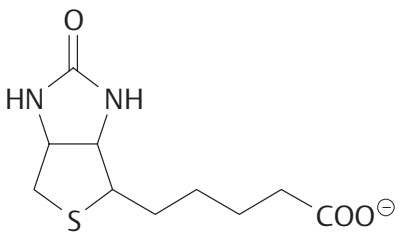


Succinyl CoA

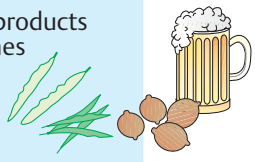


60 mg*
Fruit
Vegetables





0.1 mg*
Yeast products
Legumes
Nuts



Lipid metabolism

Lipids constitute a heterogeneous group of compounds of biochemical importance. Lipids may be defined as compounds which are relatively insoluble in water, but freely soluble in nonpolar organic solvents like benzene, chloroform, ether, hot alcohol, acetone, etc.,

Functions of Lipids:

1. Storage form of energy (triglycerides)
2. Structural components of biomembranes (phospholipids and cholesterol)
3. Metabolic regulators (steroid hormones and prostaglandins)
4. Act as surfactants, detergents and emulsifying agents (amphipathic lipids)
5. Act as electric insulators in neurons
6. Provide insulation against changes in external temperature (subcutaneous fat)
7. Give shape and contour to the body
8. Protect internal organs by providing a cushioning effect (pads of fat)
9. Help in absorption of fat soluble vitamins (A, D, E and K)
10. Improve taste and palatability to food.

Clinical Applications:

1. Excessive fat deposits cause obesity.
2. Abnormality in cholesterol and lipoprotein metabolism leads to atherosclerosis and cardiovascular diseases.
3. In diabetes mellitus, the metabolisms of fatty acids and lipoproteins are deranged, leading to ketosis.

CLASSIFICATION OF LIPIDS

Based on the chemical nature, lipids are classified:

I. Simple Lipids
They are esters of fatty acids with glycerol or other higher alcohols. They are subclassified as:

a) Triacylglycerol or Triglycerides or neutral fat.
b) Waxe

II. Compound Lipids

They are fatty acids esterified with alcohol; but in addition they contain other groups. Depending on these extra groups, they are subclassified as:

A) Phospholipids, Containing Phosphoric Acid

B) Non-phosphorylated Lipids such as Glycosphingolipids (carbohydrate)

III. Derived Lipids

They are compounds which are derived from lipids or precursors of lipids, e.g. fatty acids, steroids, prostaglandins, leukotrienes, terpenes, dolichols, etc. For details of cholesterol and steroids.

IV. Lipids Complexed to Other Compounds Proteolipids and lipoproteins.

Fatty acids:

Fatty acids are straight-chain hydrocarbons with a terminal carboxyl group. They are frequently identified by the number of carbon atomes and number of douple bonds, as diunsaturated linolic acid. The location of the douple bound in the n- or omega (ω) numbering system designates the number of carbon atoms from the terminal methyl, thus linolic acid designated as 18:2n-6 and called an ω-6 fatty acid.

Fatty acids exist mostly as ester of glycerol in both triglycerides and some phospholipids and as ester of high –molecular –weight alcohols. The fatty acidds of triglycereides are mostly C16 or C18, and in phospholipids, they are C18 to C22.

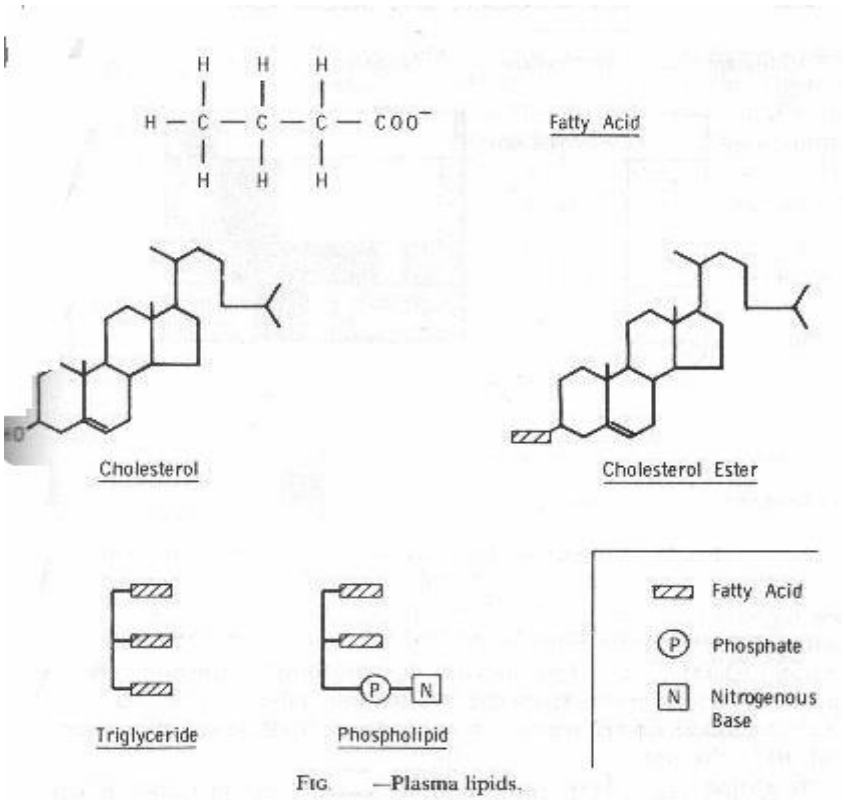
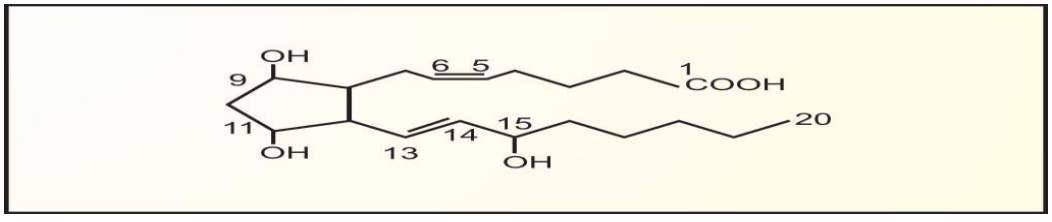


FIG. —Plasma lipids.

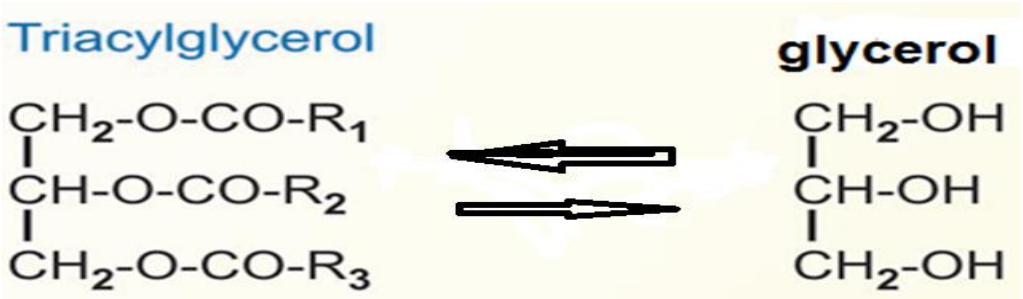
Prostaglandins

Prostaglndies (PGs) were originally isolated from prostate tissue and hence the name. But they are present in almost all tissues. They are the most potent biologically active substances; as low as one nanogram/ml of PG will cause smooth muscle contraction. The diverse physiological roles of prostaglandins confer on them the status of local hormones. Chemical Structure: All prostaglandins are considered to be derived from the 20 C cyclic saturated fatty acid, The five carbon ring is saturated. All naturally occurring PGs have an alpha oriented OH group at C15. Classification of Prostaglandins: According to the attachment of different substituent groups to the ring, PGs are named with capital letters such as A, B, E and F. In the same series, depending on number of double bonds on the side chains they are denoted by a subscript after the capital letter, *e.g.* PGE 1 , PGE 2 , PGE 3 , etc. Series 2 have 2 double bonds at 13–14 (trans) and 5–6 (cis). Structure of PGF 2 is shown in Figure below:



Triglycerides

Most of the fatty acids in the body are components of triglyceride and are stored in the depots (adipose tissue) as fat. Adipose cells convert fatty acids into triglyceroide by esterification with glycerol-3-phosphate, compounds that arises from glucose metabolism. cells must contain glucose for triglyceride formation. glucose is absent during periods of fasting, starvation, or uncontrolled diabetes mellitus, and in these condition, hydrolysids of triglycerides, and withdrawal of their fatty acids from the depots predominate. Excess carbohydrate ingested during a meal may be stored tempoarily as triglycerides after conversion of glucose to fattty acids. The hormone insulin promotes the synthesis of triglycerides by adipose cells, whereas its deficiency accelerated triglyceride hydrolysis. The first step in the catabolism of triglycerides begins with their hydrolysis. The fatty acids apperear in the plasma as nonesterfied (free) fatty acids bounded to albumin as a carrier.



Phosphosholipids

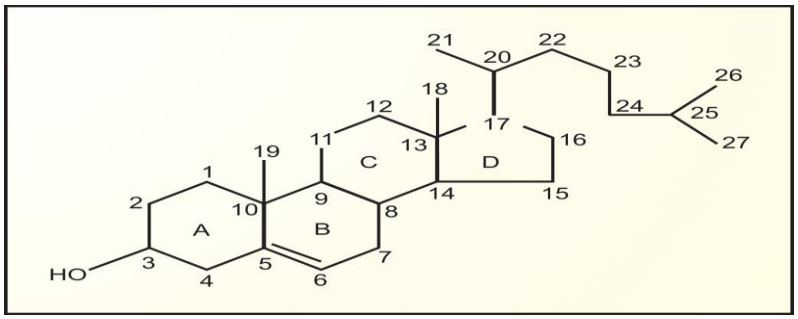
The principal phospholids are composed of triglyceride esterified with phosphoric acid, which in turn, is bound as an ester to a nitrogen containing base (choline, ethanolamine) or to serine, and inositol are some times collectively referred to as cephalins. Phospholipds are essential compounds of cell membrane becouse of their ability align themselves between water and lipids phase. Phosphoethanolamine, a constituent of blood platelts, is a necessary participant in the clooting process. Phospholips in lipoprotiens also supply the fatty acids necessary for the esterification of cholesterol. The phospholipids play a role in mitochondrial metabolism, in blood cogulation, and lipid transport as part of lipoprotins, and are important structural components of membranes.

Sphongolipids

The sphingolipids are all compounds containing the long chain, dihydroxyamino alcohol sphingosine. All the sphingolipids bind a fatty acid in amide linkage to the amino group and are also known as ceramides, becouse they are cerebral lipids containg an amide group.

Cholesterol

Cholesterol, the principal body sterol, is a complex alcohol formed of four fused rings and a side chain, pure cholesterol is a solid at body temperature. The major sites of synthesis of cholesterol are liver, adrenal cortex, testis, ovaries and intestine.



Structure of cholesterol

Approximately 70 % of plasma cholesterol exists in an acyl ester form. The esterification takes place almost exclusively in high density lipoprotein (HDL) complex. Most of the cholsterol in the body is syntheszed from acetyl CoA, but we also ingest some when we eat meat, dairy products, or eggsplants do not contain cholesterol, although they do have closely related sterols. Cholesterol is catabolized in hepatic cells by oxidation to bile acids (cholic and chenodeoxycholic acids that conjugate with glycine or taurine before secreation into bile. These bile acids and conjugates are emulsifying agents that are essential for the

digestion and absorption of fats. Some of cholesterol is also secreated as such into the bile. Both the bile acids and biliary cholesterol are reabsorbed to some extent in the intestine by an entrohepatic circulation. *Thus*, the liver is the site of cholresterol disposal or degradation, as well as its major sit of synthesis.

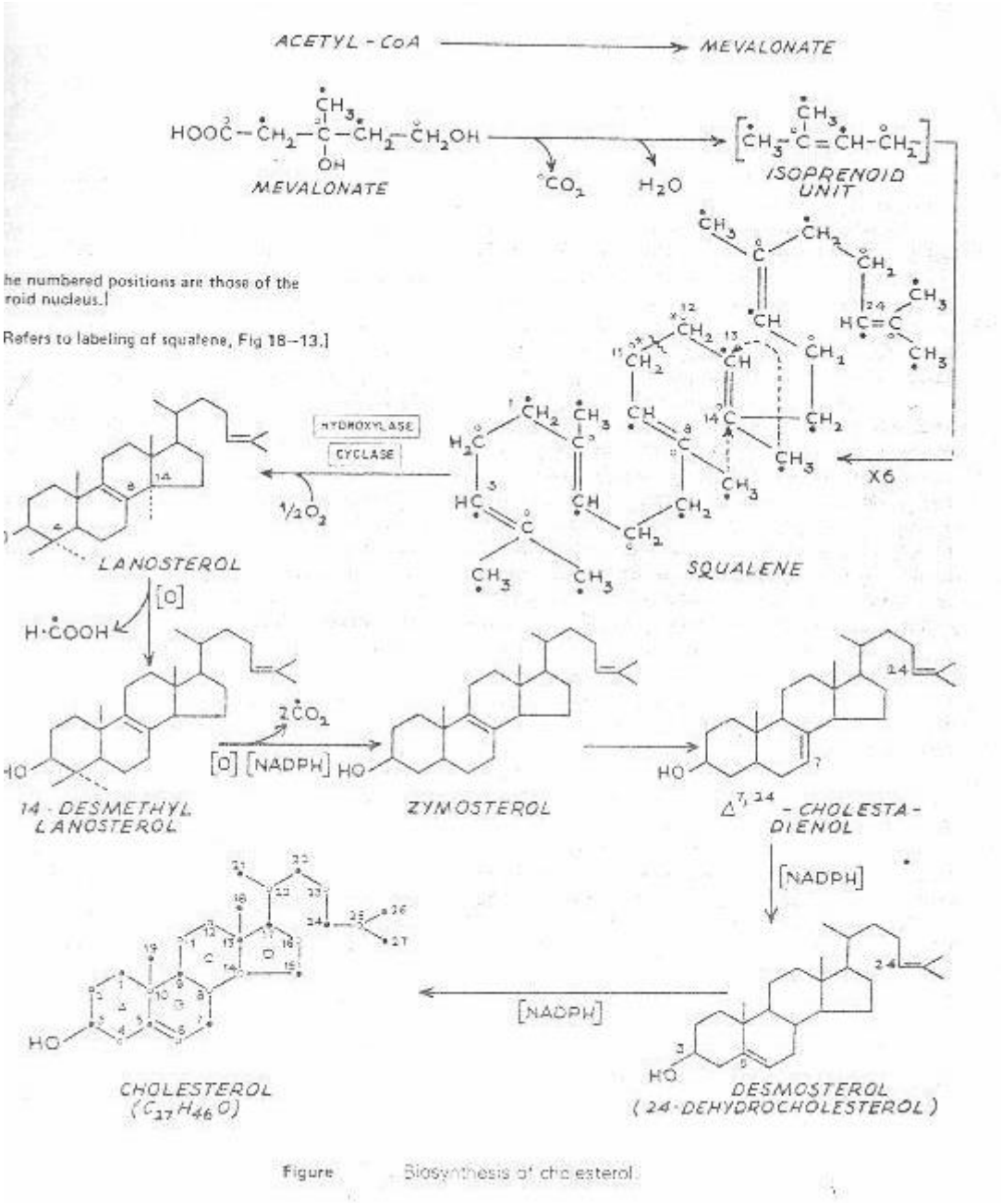


Figure Biosynthesis of cholesterol

A negative feed back mechanism controls to a limited extent the rate of synthesis of cholesterol. When the diet is high in cholesterol, the increased amoun of cholesterol brought to the liver decrease the receptors – mediate hepatic intake of cholesterol and inhibits the rate – limiting enzyme (β-hydroxy- β-methylglutaryl CoA reductase) essential for te synthesis of mevalonic acid, step in the synthesis of cholesterol. *Furthermore*, the reabsorption of bile acids and cholesterol in the enteroheatic circulation is decreased, so more cholesterol is excreated in the form of bile acids and free cholesterol.

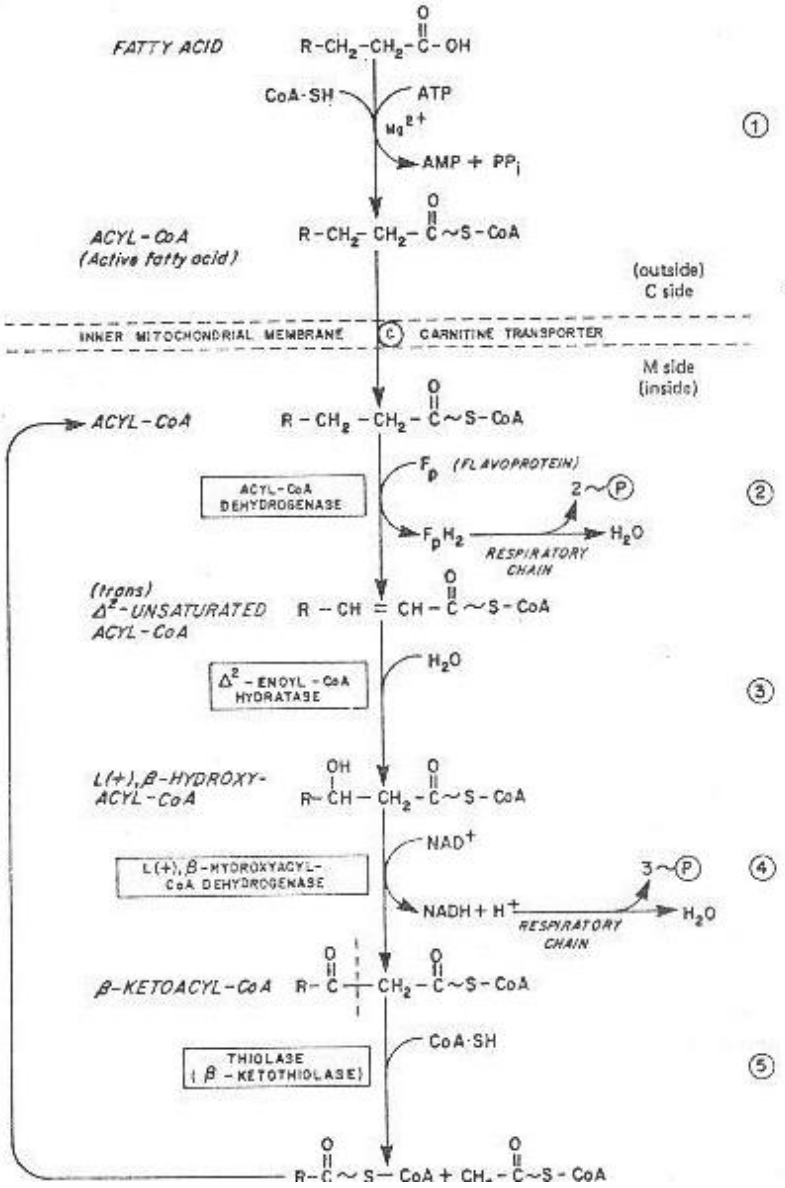
Serum cholesterol concentration can rise to high levels in some pathological states. An elevated cholesterol concentration has been implicatede as one of sever risk factors leading to coronary artery disease (atherosclerosis or myocardial infraction); *thus* the measurment of serum cholesterol is a fairly common lab. procedure.

Significance and Functions of Cholesterol:

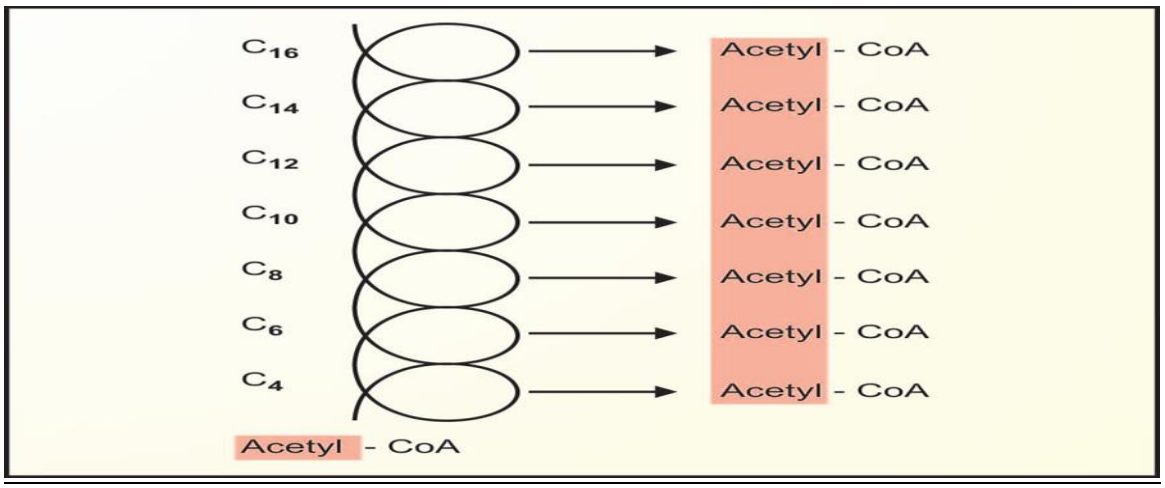
1. Heart diseases: The level of cholesterol in blood is related to the development of atherosclerosis. Abnormality of cholesterol metabolism may lead to cardiovascular accidents and heart attacks.
2. Cell membranes: Cholesterol is a component of membranes and has a modulating effect on the fluid state of the membrane.
3. Nerve conduction: Cholesterol is a poor conductor of electricity, and is used to insulate nerve fibers.
4. Bile acids and bile salts: The 24 carbon bile acids are derived from cholesterol. Bile salts are important for fat absorption.
5. Steroid hormones: 21 carbon glucocorticoids, 19 carbon androgens and 18 carbon estrogens are synthesized from cholesterol.
6. Vitamin D: It is synthesized from cholesterol.

β oxidation

The major pathway for the catabolism of the saturated fatty acid is a mitochonderial pathway called β-oxidation, which was proposed by Knoop. In this process, oxidation of fatty acids occures at β-carbon atom and two carbon fragments are successively removed from the carboxyl end of fatty acel-CO A. this in result in the elemination of two terminal carbon atoms as acetyl CoA, thereby leaving fattyacyl CoA that has two carbons less than the original fatty acid.



The β-oxidation

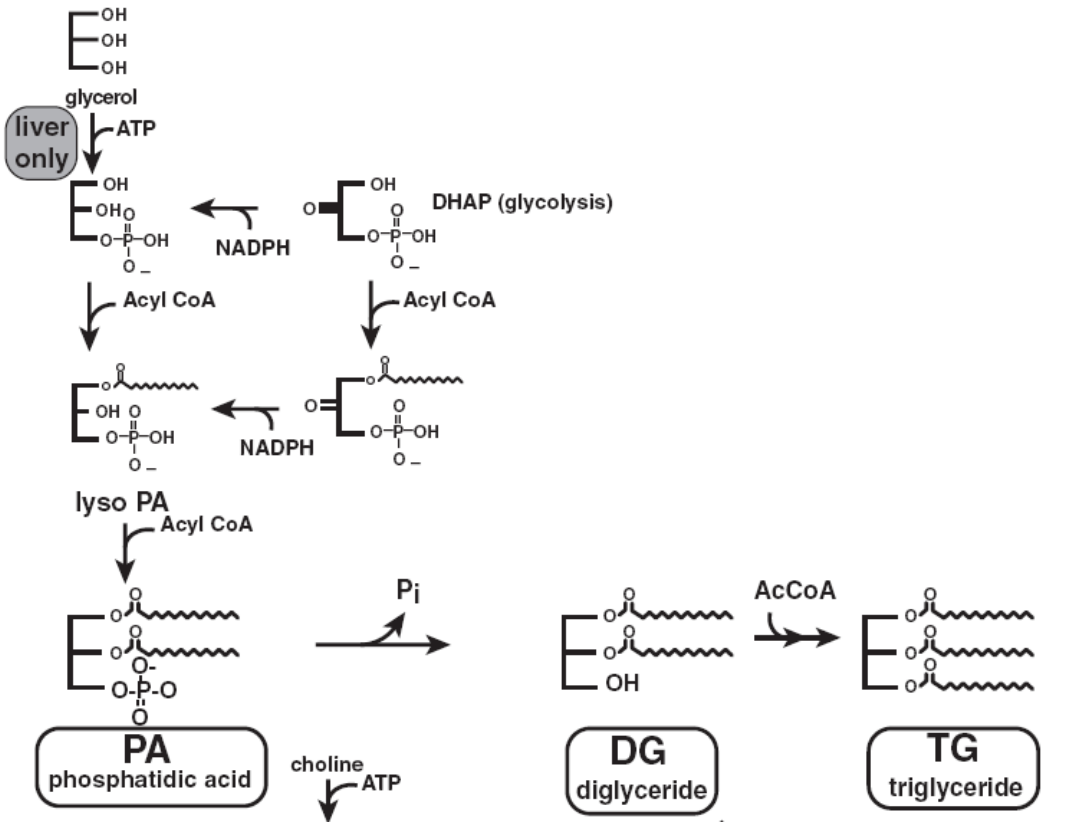


Summary of beta-oxidation of palmitic acid (16 C). It undergoes 7 cycles, which give rise to 8 molecules of acetyl CoA

TRIGLYCERIDE AND PHOSPHOLIPID SYNTHESIS

Glycerol phosphate comes from glycerol (*not in adipose*) or from dihydroxyacetone phosphate (in *liver* and *adipose*).

Nitrogen-containing phospholipids are made from diglyceride. Other phospholipids are made from phosphatidic acid.



Exogenous and endogenous pathways

The body lipids are derived from two sources that require separate metabolic pathways. The first source is fats, oils, and tissue lipids in the diet. After ingestion, the dietary lipids are hydrolyzed in the intestine and absorbed and transported to various tissues. The rout is the exogenous pathway, dealing with lipids from outside. The liver, however, readily synthesized saturated and monounsaturated fatty acids from Acetyl CoA and converts them to triglycerides that atre distrbuted to tissues. Cholesterol is also synthesis in liver from acetyl CoA units. The internal synthesis and distribution of lipids is the endogenous pathway. Both pathways require a means for soluilization and transportation of water insoluble lipids throught the body stream. Lipoprotiens are the particles that transport and distribute the lipids.

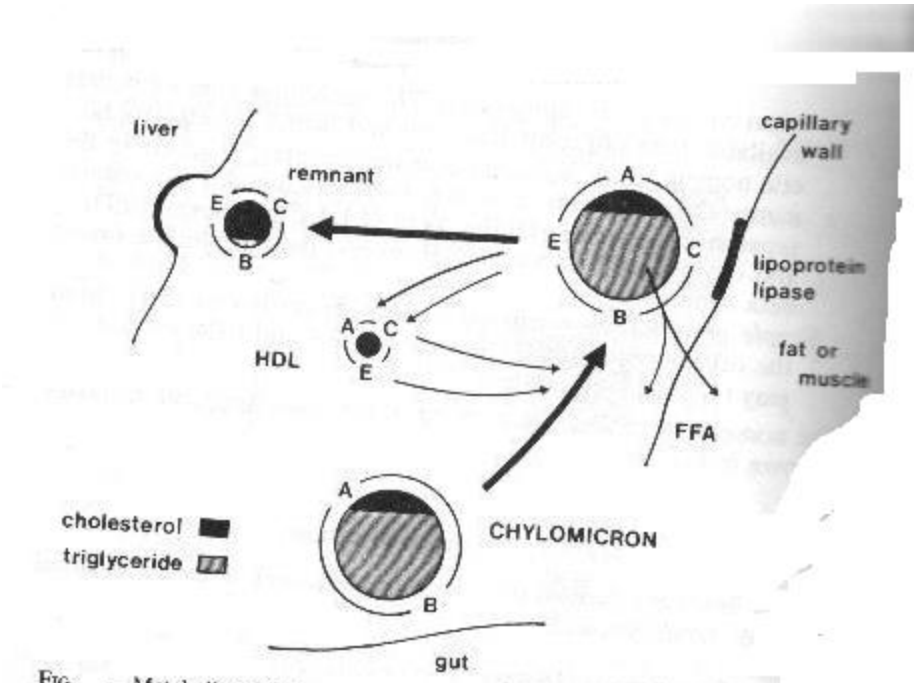
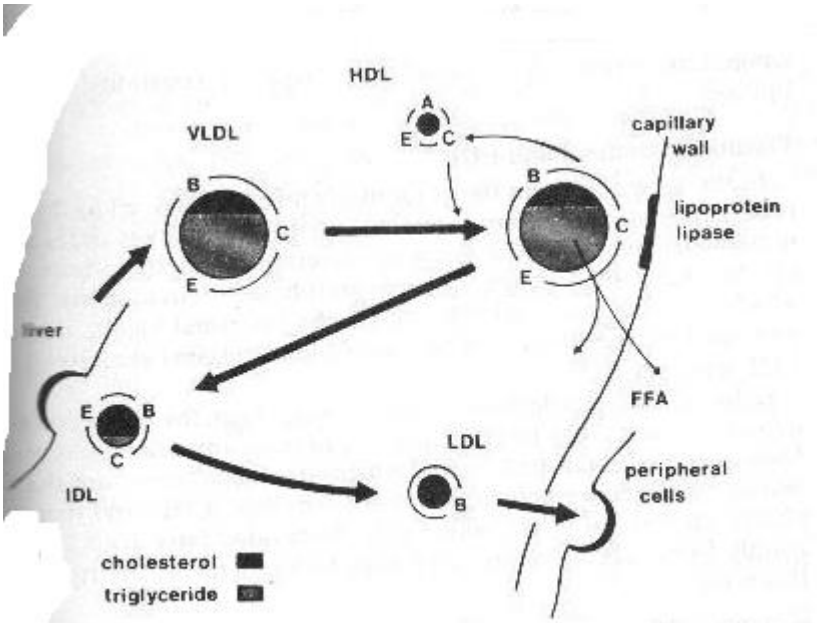


FIG. —Metabolism of exogenous lipids. Lipoprotein core containing cholesterol and triglycerides, surrounded by phospholipids and apoproteins (clear area).



—Metabolism of endogenous lipids. Lipoprotein core containing cholesterol and triglycerides, surrounded by phospholipids and apoproteins (clear area).

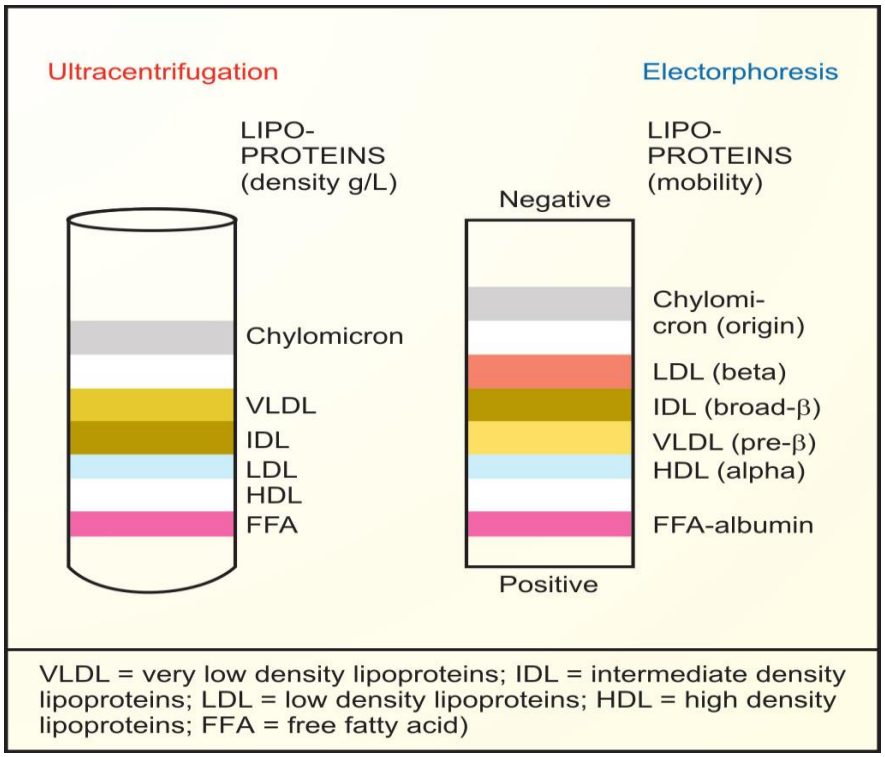
Lipoproteins and apoplipoproteins:

Lipoprotiens are lipids – filled particles that have an outer membrane consisting of monolayer of special protiens called apolipoprotiens intrespersed with the polar lipids (phospholipids and nonesterified cholesterol) the polar lipids are aligned with their charged heads facing

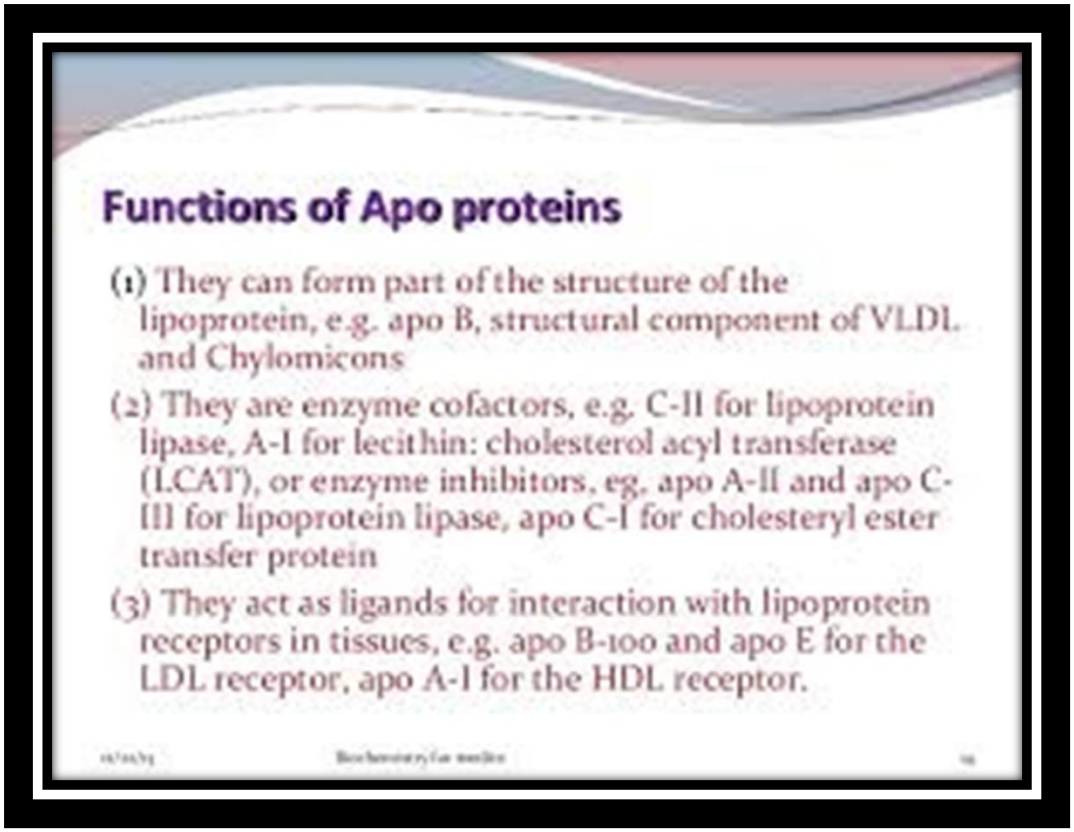
outward and the hydrophobic tails pointing inward. The outer membrane sourrouns a central core of neutral lipids (triglycerides and cholesterol esters).

Classes of lipoprotiens

The five different classes of lipoprotiens have distinctive physical properties structures. Each class of lipoproteins has a specific set of apolipoproteins in the membrane and different of lipids in the core. The most commonly used names of lipoproteins clases are derived frome their relative densities upon ultracenterfugation. The structure and function of lipoproteins are described in more details in a subsequent section.



VLDL = very low density lipoproteins; IDL = intermediate density lipoproteins; LDL = low density lipoproteins; HDL = high density lipoproteins; FFA = free fatty acid)



1. chylomicrons

chylomicrons are the largest and least dense of all lipoproteins . they arise in the intestine and transport ingested triglycerides to adipose tissue and they muscle cells.

1. very low density lipoprotein (VLDL)

VLDL is a lipoproteins made in the liver and is designed primarly to transport triglycerides synthesized by the liver to muscle and adipose cells.

3. intermediate density lipoprotein (IDL)

IDL is a transitory remnant of VLDL , circulating in plasma after about half ofr VLDL triglyceride have been transferred to adipose tissue or muscle cells. Most of the IDL undergose further delipidation, transfers to HDL all its apolipoproteins except ApoB, and thus beccoumes LDL. A small percentage of IDL binds to liver cells, where it is degraded.

4. Low density lipoprotin (LDL)

LDL, rich in cholesterol, arise in plasma from IDL, LDL delivers cholesterol either to liver for bile acid formation or to other tissues for use as a structural components of new cells membrane, as a precursor of steroid hormones, or for storage as cholesterol esters.

5. High density liopoprotien (HDL)

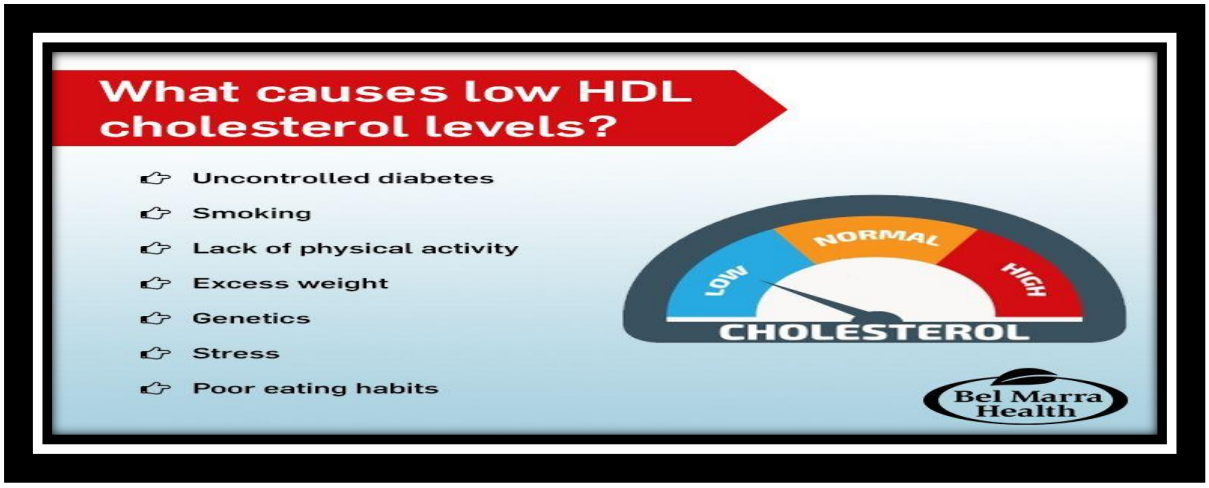
HDL has a complicated life cycle and undergose growth and change after its initial formation. HDL particles are made both by liver and intestinal mucosa cells. A newly formed (nascent) HDL particle forms a complex with some lipoproteins, LCAT (lecithin cholesterol acyl transferase) esterifies cholesterol by transferring to it a fatty acids from lecithin. HDL also trasfer some apolipoproteins baack and froth to other lipoproteins at various stages in their life cycles.

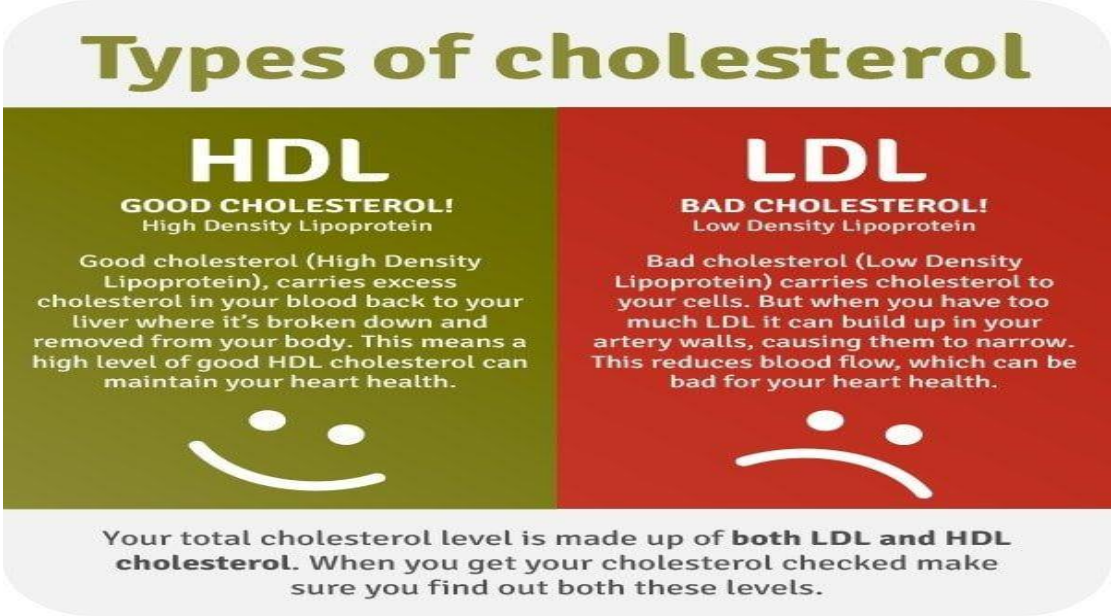
Function of HDL

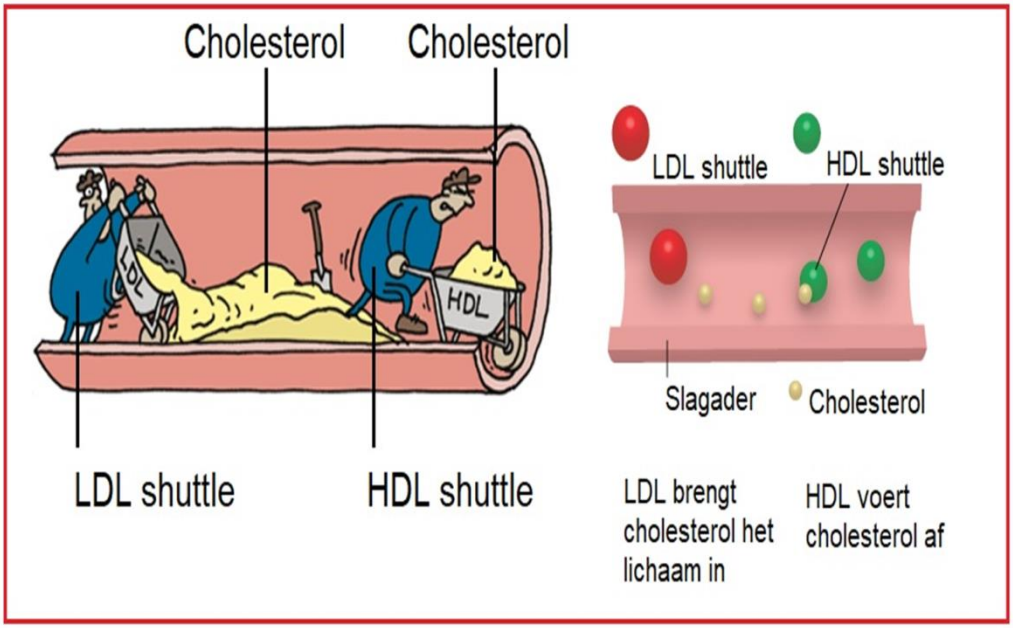
i. HDL is the main transport form of cholesterol from peripheral tissue to liver, which is later excreted through bile. This is called reverse cholesterol transport by HDL.

ii. The only excretory route of cholesterol from the body is the bile.

iii. Excretion of cholesterol needs prior esterification with poly unsaturated fatty acids. Thus poly unsaturated fatty acids will help in lowering of cholesterol in the body, and so poly unsaturated fatty acids is anti-atherogenic.



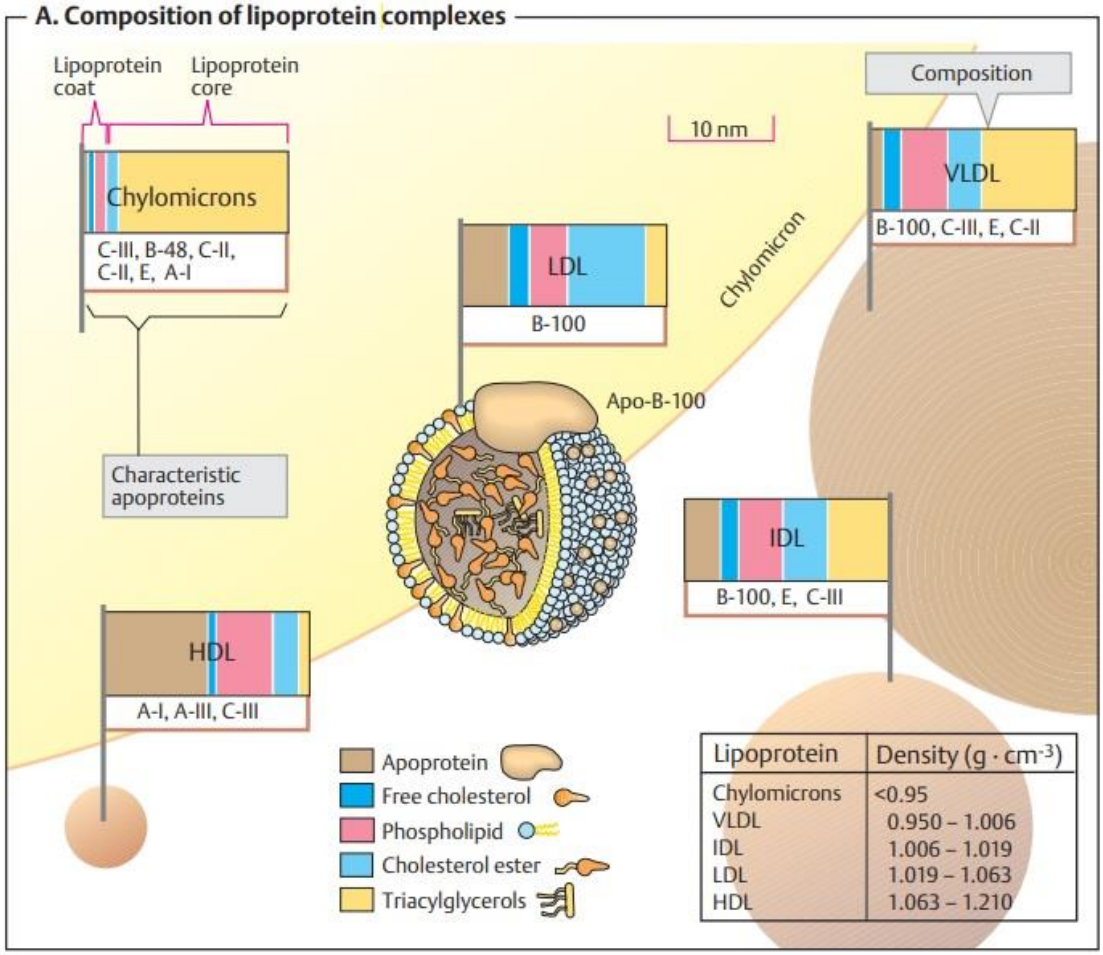

Types of cholesterol
HDL
GOOD CHOLESTEROL!
High Density Lipoprotein
Good cholesterol (High Density Lipoprotein), carries excess cholesterol in your blood back to your liver where it's broken down and removed from your body. This means a high level of good HDL cholesterol can maintain your heart health.
LDL
BAD CHOLESTEROL!
Low Density Lipoprotein
Bad cholesterol (Low Density Lipoprotein) carries cholesterol to your cells. But when you have too much LDL it can build up in your artery walls, causing them to narrow. This reduces blood flow, which can be bad for your heart health.
Your total cholesterol level is made up of both LDL and HDL cholesterol. When you get your cholesterol checked make sure you find out both these levels.


Cholesterol
Cholesterol
LDL
HDL
LDL shuttle
HDL shuttle
LDL shuttle
HDL shuttle
Slagader
Cholesterol
LDL brengt cholesterol het lichaam in
HDL voert cholesterol af

LIPOPROTEIN METABOLISM

Most lipids are barely soluble in water, and many have amphipathic properties. In the blood, free triacylglycerols would coalesce into drops that could cause fat embolisms. By contrast, amphipathic lipids would be de□posited in the blood cells' membranes and would dissolve them. Special precautions are therefore needed for lipid transport in the blood. While long-chain fatty acids are bound to albumin and short-chain ones are dissolved in the plasma , other lipids are transported in lipoprotein complexes, of which there several types in the blood plasma, with different sizes and composition.

The lipoprotein system evolved to solve the problem of transporting fats around the body in the aqueous environment of the plasma. A lipoprotein is a complex spherical structure which has a hydrophobic core wrapped in a hydrophilic coating (Fig. 1). The core contains triglyceride and cholesterol ester, while the surface contains phospholipid, free cholesterol and proteins the Apolipoprotein.



Types of lipoprotein

Lipoproteins are classified into five groups. According to the decreasing size and increasing density, these are: **chylomicrons**, **VLDLs** (very-low-density lipoproteins), **IDLs** (inter□mediate-density lipoproteins), **LDLs** (low□density lipoproteins), and **HDLs** (high-density lipoproteins). The proportions of **apoproteins** range from 1% in chylomicrons to over 50% in HDLs. These proteins serve less for solubility purposes, but rather function as recognition molecules for the membrane receptors and enzymes that are involved in lipid exchange.

Cholesterol is an essential component of all cell membranes and is the precursor for steroid hormone and bile acid biosynthesis. Triglyceride is central to the storage and transport of energy within the body.

Metabolism:

Lipoprotein metabolism can be thought of as two cycles, one exogenous and one endogenous, both centered on the liver. These cycles are interconnected.

Two key enzyme systems are involved in lipoprotein metabolism, e.g

• Lipoprotein lipase (LPL) releases free fatty acids and glycerol from Chylomicrons and VLDL into the tissues.

• Lecithin- cholesterol acyltransferase (LCAT): The LCAT enzyme helps transport cholesterol out of the blood and tissues by a process called cholesterol esterification.

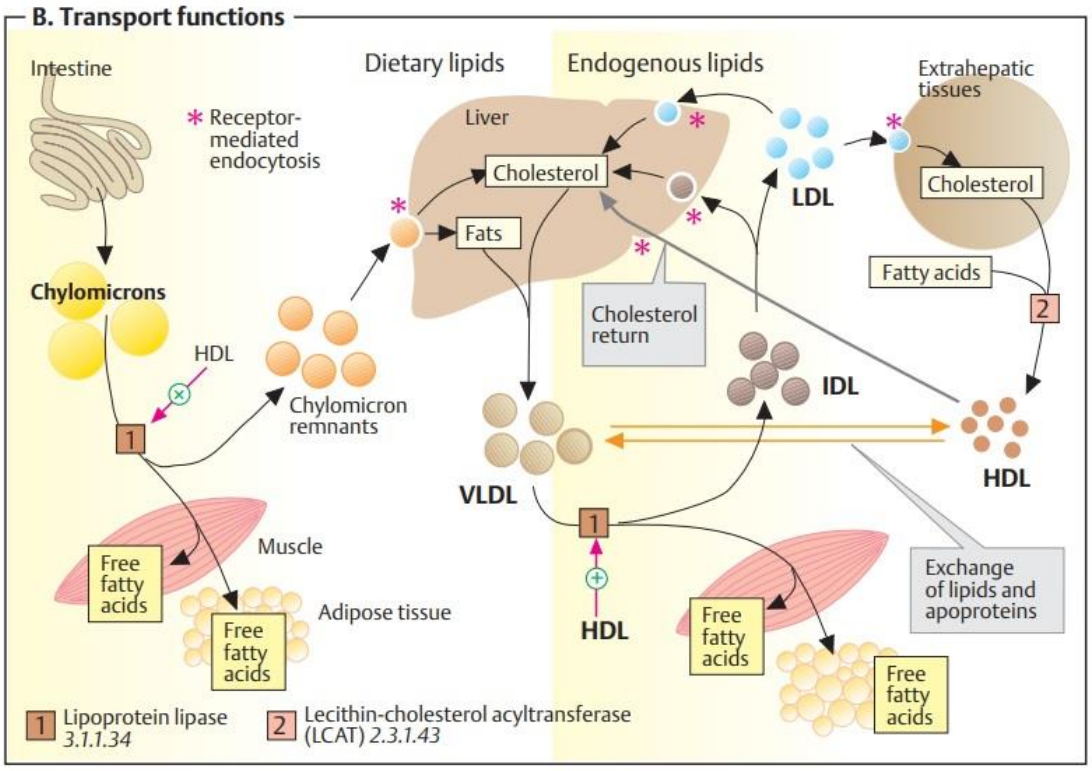
A. The exogenous lipid cycle:

Dietary lipid is absorbed in the small intestine and incorporated into Chylomicrons which are secreted into the lymphatics and reach the bloodstream via the thoracic duct. In the circulation, triglyceride is gradually removed from these lipoproteins by the action of lipoprotein lipase. This enzyme is present in the capillaries of a number of tissues, predominantly adipose tissue and skeletal muscle. As it loses triglyceride, the chylomicron becomes smaller and deflated, with folds of redundant surface material. These remnants are removed by the liver.

The cholesterol may be utilized by the liver to form cell membrane components or bile acids, or may be excreted in the bile. The liver provides the only route by which cholesterol leaves the body in significant amounts.

B. The endogenous lipid cycle:

The liver synthesizes VLDL particles which undergo the same form of delipidation as Chylomicrons by the action of lipoprotein lipase. This results in the formation of an intermediate density lipoprotein (IDL) which becomes low density lipoprotein (LDL) when further delipidated. LDL may be removed from the circulation by the high affinity LDL receptor or by other scavenger routes which are thought to be important at high LDL levels and the main way in which cholesterol is incorporated into plaques. HDL particles are derived from both liver and gut, they act as cholesteryl ester shuttles, removing the sterol from the peripheral tissues and returning it to the liver. The HDL is taken up either directly by the liver, or indirectly by being transferred to other circulating lipoproteins, which then return it to the liver. This process is thought to, be anti-atherogenic, and an elevated HDL-cholesterol level has been shown to confer a decreased risk of coronary heart disease on an individual.



Diabetes Mellitus Lect.8

Objective :

-Introduction of Diabetes mellitus.

- Types Diabetes mellitus.

I-TYPE 1 DIABETE

A-Diagnosis of type 1 diabetes

B- Metabolic changes in type 1 diabetes

1-Hyperglycemia and ketoacidosis

2. Hypertriacylglycerolemia

C. Treatment of type 1 diabetes

1. Standard treatment versus intensive treatment

2. Hypoglycemia in type 1 diabetes

3. Contraindications for tight control

Introduction of Diabetes mellitus.

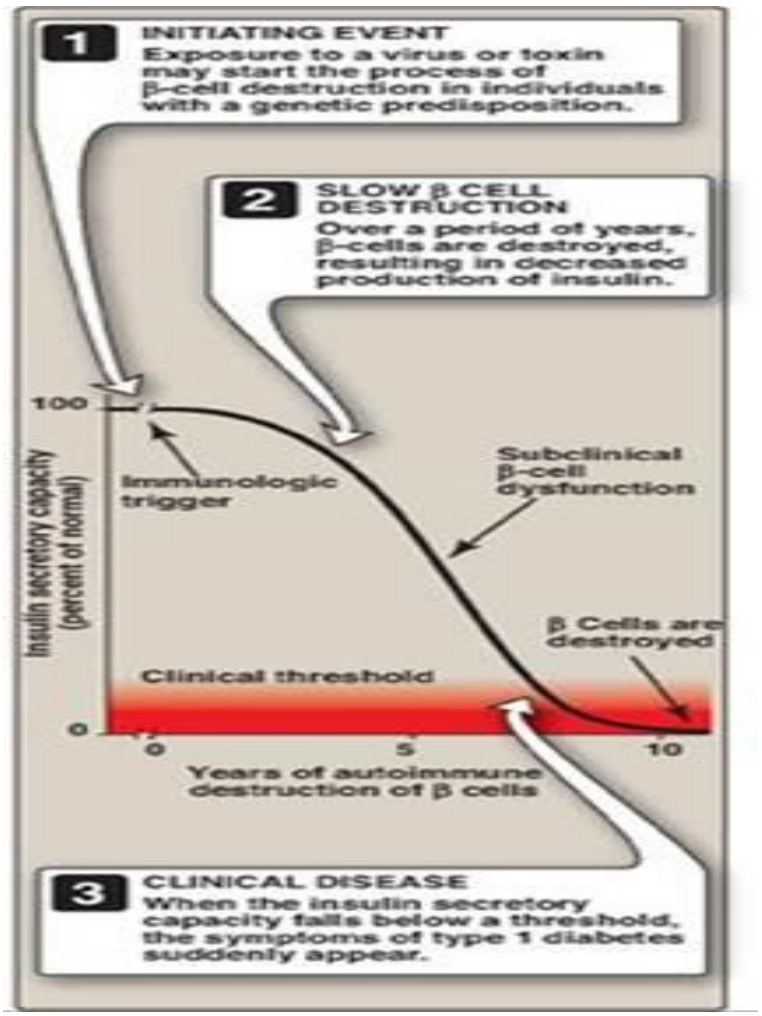
Diabetes mellitus (diabetes) is not one disease, but rather is a heterogeneous group of multifactorial, polygenic syndromes characterized by an elevated fasting blood glucose (FBG) caused by a relative or absolute deficiency in insulin. Nearly 26 million people in the United States (about 8% of the population) have diabetes. Of this number, approximately 7 million are as yet undiagnosed. Diabetes is the leading cause of adult blindness and amputation and a major cause of renal failure, nerve damage, heart attacks, and strokes.

Most cases of diabetes mellitus can be separated into two groups, **type 1 ([T1D]** formerly called insulin-dependent diabetes mellitus) and **type 2 ([T2D]** formerly called noninsulin-dependent diabetes mellitus). The incidence and prevalence of T2D is increasing because of the aging of the population and the increasing prevalence of obesity and sedentary lifestyles. The increase in children with T2D is particularly disturbing.

Types Diabetes mellitus

I-TYPE 1 DIABETES

The disease is characterized by an absolute deficiency of insulin caused by an autoimmune attack on the β cells of the pancreas. in T1D, the islets of Langerhans become infiltrated with activated T lymphocytes, leading to a condition called insulitis. Over a period of years, this autoimmune attack on the β cells leads to gradual depletion of the β-cell population. However, symptoms appear abruptly when 80%–90% of the β cells have been destroyed. At this point, the pancreas fails to respond adequately to ingestion of glucose, and insulin therapy is required to restore metabolic control and prevent life-threatening ketoacidosis.



Figure(1):Insulin secretory capacity during the onset of type 1 diabetes.

[Note: Rate of autoimmune

destruction of β cells may be faster or slower than shown.]

A-Diagnosis of type 1 diabetes

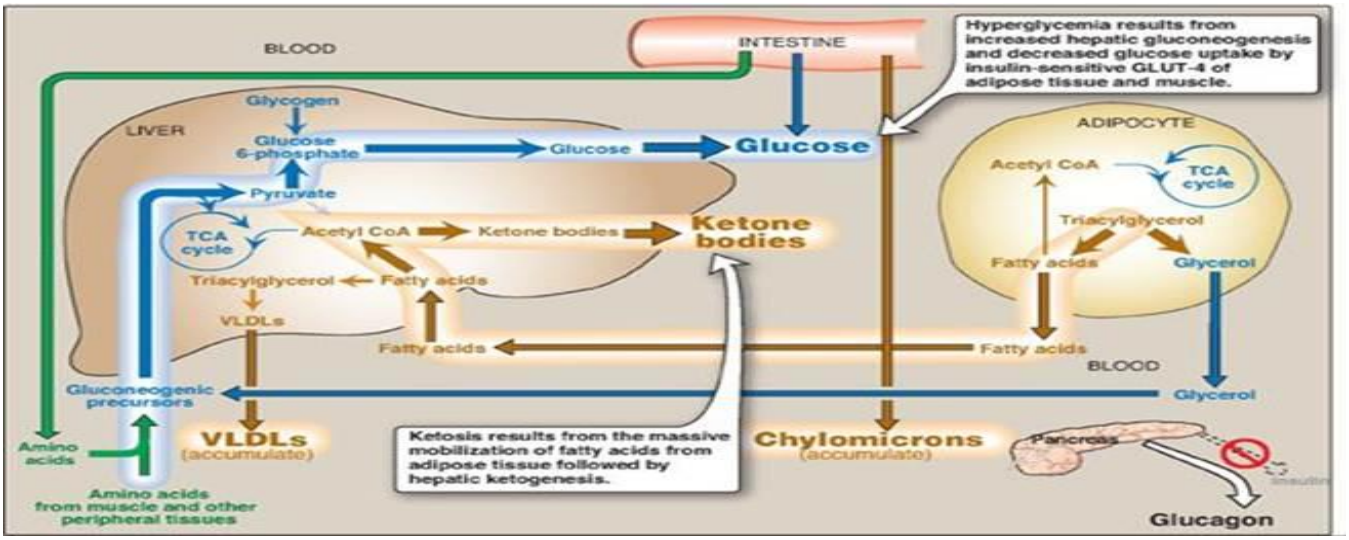
The onset of T1D is typically during childhood or puberty, and symptoms develop suddenly. Patients with T1D can usually be recognized by the abrupt appearance of polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (excessive hunger), often triggered by physiologic stress such as an illness. These symptoms are usually accompanied by fatigue and weight loss. The diagnosis is confirmed by glycosylated hemoglobin concentration ≥ 6.5 mg/dl (normal is less than 5.7), or an FBG ≥ 126 mg/d. Fasting is defined as no caloric intake for at least 8 hours. Diagnosis can

also be made on the basis of a non-fasting (random) blood glucose level greater than 200 mg/dl in an individual with symptoms of hyperglycemia.

When blood glucose is greater than 180 mg/dl, the ability of the kidneys to reclaim glucose is impaired. This results in glucose "spilling" into the urine. The loss of glucose is accompanied by the loss of water, resulting in the characteristic polyuria (with dehydration) and polydipsia of diabetes.

B- Metabolic changes in type 1 diabetes

The metabolic abnormalities of T1D mellitus result from a deficiency of insulin that profoundly affects metabolism in three tissues: liver, muscle, and adipose.(see figure 2)



1-Hyperglycemia and ketoacidosis: Elevated levels of blood glucose and ketone bodies are the hallmarks of untreated T1D . Diabetic ketoacidosis (DKA), a type of metabolic acidosis, occurs in 25%–40% of those newly diagnosed with T1D and may recur if the patient becomes ill (most commonly with an infection) or does not comply with therapy. DKA is treated by replacing fluid and electrolytes and administering short-acting insulin to gradually correct hyperglycemia without precipitating hypoglycemia.

2. Hypertriacylglycerolemia: Not all of the FAs flooding the liver can be disposed of through oxidation or ketone body synthesis. These excess fatty acids are converted to triacylglycerol (TAG), which is packaged and secreted in very-low-density lipoproteins [VLDLs]. Chylomicrons are synthesized from dietary lipids by the intestinal mucosal cells following a meal. Because lipoprotein degradation catalyzed by lipoprotein lipase in the capillary beds of adipose tissue is low in diabetics (synthesis of the enzyme is

decreased when insulin levels are low), the plasma chylomicron and VLDL levels are elevated, resulting in Hypertriacylglycerolemia. (see figure 2)

C. Treatment of type 1 diabetes

Individuals with T1D must rely on **exogenous insulin delivered subcutaneously** either by **periodic injection** or **continuous pump-assisted infusion to control hyperglycemia and ketoacidosis.** Two therapeutic regimens are currently in use, standard and intensive insulin treatment.

1. Standard treatment versus intensive treatment: Standard treatment typically consists of one or two daily injections of **recombinant human insulin**. Mean blood glucose levels obtained are typically in the 225–275 mg/dl range, with a glycosylated hemoglobin (HbA1c) level of 8%–9% of the total hemoglobin In contrast to standard therapy, intensive treatment seeks to more closely normalize blood glucose **through more frequent monitoring** and **subsequent injections of insulin**, typically three or more times a day. Nonetheless, patients on intensive therapy show a 50% or more reduction in the long-term microvascular complications of diabetes (that is, retinopathy, nephropathy, and neuropathy) compared with patients receiving standard care. This confirms that the complications of diabetes are related to an elevation of plasma glucose.

2. Hypoglycemia in type 1 diabetes: One of the therapeutic goals in cases of diabetes is to **decrease blood glucose levels** in an effort to minimize the development of long-term complications of the disease. However, an appropriate dosage of insulin is difficult to achieve. Hypoglycemia caused by excess insulin is the most common complication of insulin therapy, occurring in over 90% of patients. **Recall that in normal individuals, hypoglycemia triggers a compensatory secretion of counter regulatory hormones, most notably glucagon and epinephrine, which promote hepatic production of glucose.** However, patients with T1D also develop a deficiency of glucagon secretion. This defect occurs early in the disease and is almost universally present 4 years after diagnosis. The combined deficiency of glucagon and epinephrine secretion creates a symptom-free condition sometimes called "hypoglycemia unawareness." Thus, patients with long-standing T1D are particularly vulnerable to hypoglycemia.

3. Contraindications for tight control: Children are not put on a program of tight control of blood glucose before age 8 years **because** of the risk that episodes

of hypoglycemia may adversely affect brain development. **Elderly** people typically do not go on tight control because hypoglycemia can cause strokes and heart attacks in this population. Also, the major goal of tight control is to prevent complications many years later. Tight control, then, is most worthwhile for otherwise healthy people who can expect to live at least 10 more years.

Urea cycle

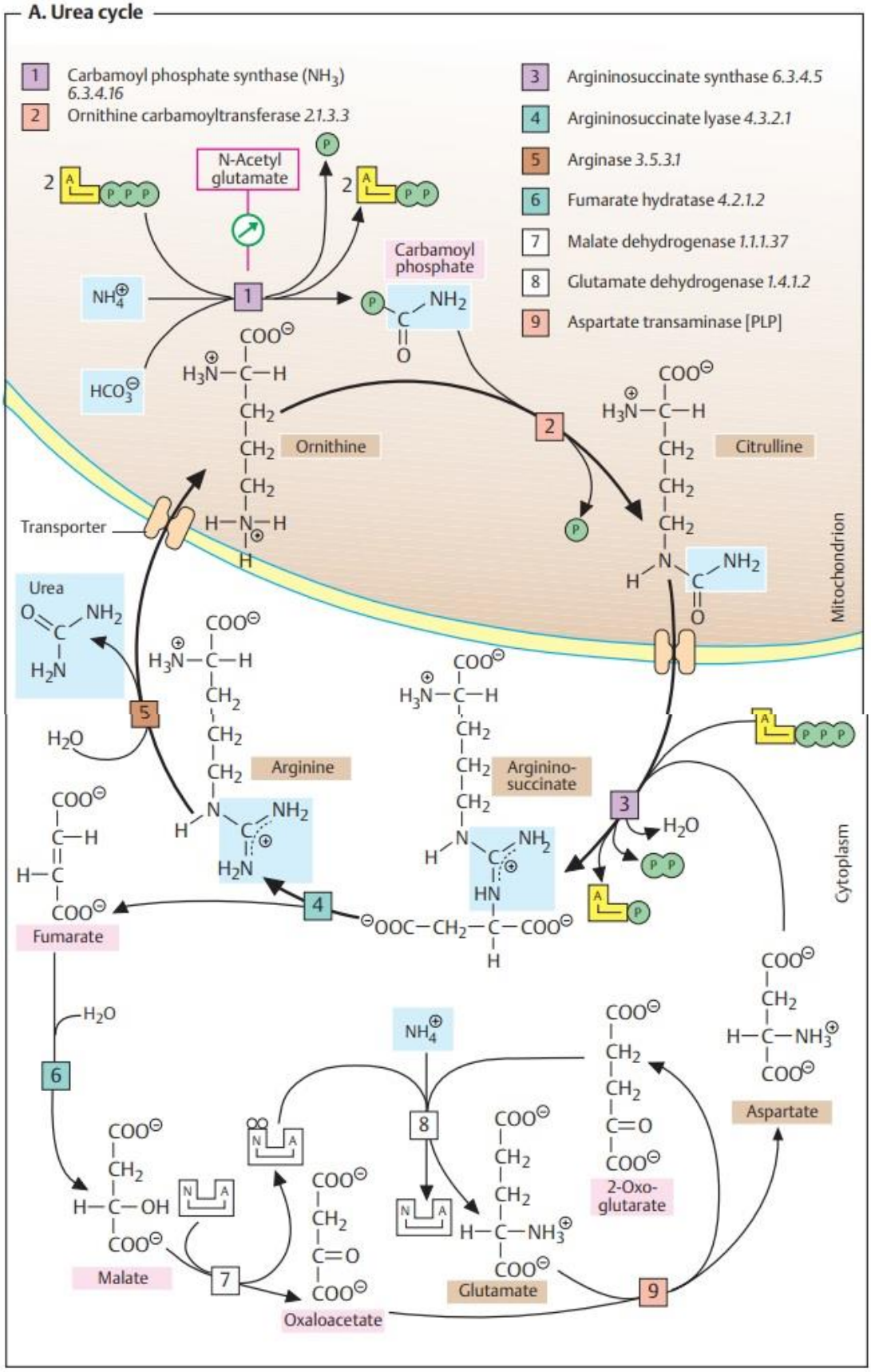
Amino acids are mainly broken down in the liver. Ammonia is released either directly or indirectly in the process. The degradation of nucleobases also provides significant amounts of ammonia. Ammonia (NH_3) is a relatively strong base, and at physiological pH values it is mainly present in the form of the ammonium ion NH_4^+.

NH_3 and NH_4^+ are toxic, and at higher concentrations cause brain damage in particular. Ammonia therefore has to be effectively inactivated and excreted. This can be carried out in various ways. Aquatic animals can excrete NH_4^+ directly.

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

The reasons for the neurotoxic effects of ammonia have not yet been explained. It may disturb the metabolism of glutamate and its precursor glutamine in the brain.

Urea ($H_2N–CO–NH_2$) is the diamide of carbonic acid. In contrast to ammonia, it is neutral and therefore relatively non-toxic. The reason for the lack of basicity is the molecule's mesomeric characteristics. The free electron pairs of the two nitrogen atoms are delocalized over the whole structure, and are therefore no longer able to bind protons. As a small, uncharged molecule, urea is able to cross biological membranes easily. In addition, it is easily transported in the blood and excreted in the urine. Urea is produced only in the liver, in a cyclic sequence of reactions (the urea cycle) that starts in the mitochondria and continues in the cytoplasm. The two nitrogen atoms are derived from NH_4^+ (the second has previously been incorporated into aspartate; see below). The keto group comes from hydrogen carbonate (HCO_3^-), or CO_2 that is in equilibrium with HCO_3^-.



[1] In the first step, carbamoyl phosphate is formed in the mitochondria from hydrogen carbonate (HCO_3^-) and NH_4^+ , with two ATP molecules being consumed. In this compound, the carbamoyl residue (–O–CO–NH_2)

is at a high chemical potential. In hepatic mitochondria, enzyme makes up about 20% of the matrix proteins.

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

[3] The second NH_2 group of the later urea molecule is provided by aspartate, which condenses with citrulline into arginine succinate. ATP is cleaved into AMP and diphosphate (PPi) for this endergonic reaction. To shift the equilibrium of the reaction to the side of the product, diphosphate is removed from the equilibrium by hydrolysis.

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

[5] In the final step, isourea is released from the guanidinium group of the arginine by hydrolysis (not shown), and is immediately rearranged into urea. In addition, ornithine is regenerated and returns via the ornithine transporter into the mitochondria, where it becomes available for the cycle once again.

The fumarate produced in step [4] is converted via malate to oxaloacetate [6, 7], from which aspartate is formed again by transamination [9].

The glutamate required for reaction [9] is derived from the glutamate dehydrogenase reaction [8], which fixes the second NH_4^+ in an organic bond. Reactions [6] and [7] also occur in the tricarboxylic acid cycle. However, in urea formation they take place in the cytoplasm, where the appropriate isoenzymes are available.

The rate of urea formation is mainly controlled by reaction [1]. N-acetyl glutamate, as an allosteric effector, activates carbamoylphosphate synthase.

In turn, the concentration of acetyl glutamate depends on arginine and ATP levels, as well as other factors.