PHARMACEUTICAL BIOCHEMISTRY

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„Az élettudományi-klinikai felsőoktatás gyakorlatorientált és hallgatóbarát korszerűsítése a vidéki képzőhelyek nemzetközi versenyképességének erősítésére”
TÁMOP-4.1.1.C-13/1/KONV-2014-0001

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Manuscript completed: October 2015

Publisher: University of Pécs

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ISBN

Length: 189 oldal

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1. INTRODUCTION

Biochemistry **evolved from** the development of organic chemistry, enzymology and bioenergetics and equally notable, from molecular biology. Formerly, we saw noted how molecular biology interacts with the structure and function of the living cell, based on the working of the subcellular compartments. We examined how the cell typically answers or responds to external stimuli. Considering the synthetic reactions involved, the topics of molecular biology evolved into the regulated synthesis of the biological macromolecules: DNA, RNA and proteins. Biochemistry **examines** how a cell reacts to metabolic changes, how the building block of the macromolecules (nucleotides, sugars, amino acids, lipids) are synthetized, how the different molecules from the food are used and absorbed for energy supply. We will study how the metabolic processes are **regulated** within a cell, in a specific tissue (liver, muscle, heart, adipocytes) and in the whole body. These regulation processes respond to changes in the composition and timing of food intake, as well as different physiological (overnight starvation, sport activities, pregnancy and lactation) and pathophysiological (liver, renal diseases, inflammations, chronic diseases) situations.

The ultimate **energy source** for all organisms on Earth is natural sunlight. Plant and some microorganisms use this energy directly. Humans take food originated from plants and animals. The organic molecules of food are burned in the cell, releasing CO_2 and generating reducing equivalents (primarily in the form of NADH). Reducing equivalents are accepted by oxygen, the energy produced can be used for **ATP synthesis**, the basic energy providing molecule of the cell. Energy in the cell is stored in two forms: *chemical bonds* (e.g. ATP) and *(electro)chemical gradients* (more on this later). The stored **energy is used** mainly for biosynthetic reactions, active transport, muscle contraction, heat generation, maintaining intracellular conditions, which are different from the extracellular milieu.

In the biochemical **metabolic pathways** several reactions follow one another in a regulated manner. Each pathway has starting substrate(s) and final product(s). The **catabolic** pathways are degradative reactions, they produce small molecules from large ones while energy is conserved either directly in the form of ATP/GTP, or indirectly by generating NADH/$FADH_2$. **Anabolic** pathways are synthetic processes: large, complex macromolecules can be synthetized from simple precursors. Many of these steps are reductive using NADPH. All of these pathways are strictly **regulated** via enzymes and/or hormones according to the need and the metabolic supply of the cell.

Biochemical reactions have a distinct role in **diagnosis** of inherited metabolic diseases including other pathophysiological conditions (malfunction of liver, kidney, heart and muscle tissues, metabolic disorders, such as diabetes mellitus). Certain inherited enzyme deficiencies may be cured by eliminating special molecules from the diet.

The **chemical forces** which are important in biochemistry are very similar to the ones studied in molecular biology: covalent bonds (single and double, created via enzymatic reactions), non-covalent bonds (electrostatic interactions, hydrogen bonds, van der Waals interactions). The value of the presence of water including the hydrophobic interactions is similar to molecular biological interactions.

The structurally and functionally essential **macromolecule**s are: carbohydrates, proteins, nucleic acids (DNA and RNA) and lipids. Their metabolisms, including the synthesis of the building blocks, will be among the major topics of your biochemical studies.

In biochemistry, there are central **key molecules** (pyruvate, acetyl-CoA f.e.) which are components of additional pathways. In this measure, they provide the potential for regulation. In diverse organisms, the metabolic pathways demonstrate similar features, which help to understand mechanisms in the human body. Species specific molecules, biochemical steps or regulatory procedures make the picture complete.

Some *unusual features* of biochemical studies: equations are not always complete; metal ions involved in complexes are many times omitted; often abbreviations are used; arrows may indicate uptake or release of molecules; biochemical reactions are identified by the name of the catalyzing enzyme.

2. MAJOR REGULATORY PATHWAYS OF METABOLISM

The major biochemical pathways are either **anabolic or catabolic**. The energy requiring and energy supplying pathways are connected with ATP, the most important energy storing molecule of cells. The different pathways contain some common, **key molecules** and they operate with the same **type of reactions**.

A **metabolic pathway** is referred to as a chain of reactions, which follow one another. Usually the product of one reaction is the substrate of the next one. The major **types of pathways** are: linear, branched, interlinked with another one and cyclic (see examples in the lecture).

Committed step of a reaction chain: following this step, the pathways continue to produce the final product. Notably, this is often irreversible and a well-regulated reaction.

Some **basic types of regulations** are: *feedback* regulation – *negative*: the end product is blocking the committed step of the pathway; *positive*: the end or side product activates the enzyme of the committed step. *Feed forward* regulation: accumulated precursor molecules activate a specific pathway. If there is an **enzymatic block** (genetic reasons and/or drug inhibition) in a pathway, the metabolites in front of the blockade collide, while the product after the block is considered missing. Occasionally, unusual side products may appear (the cell is attempting to eliminate the materials in excess).

2.1. ENERGY PRODUCTION AND REGULATION

In **our diet** fats, polysaccharides and proteins are the major components. They are digested into small molecules (fatty acid, glycerol, glucose and other monosaccharides, amino acids), and are broken down within the cell at different catabolic pathways (beta oxidation and/or glycolysis). A common molecule produced during this break down is acetyl-CoA, which can enter the citric acid cycle to completely burn. As we noted before, all these steps promote ATP synthesis.

In addition to ATP, there are other molecules with specific groups, the hydrolysis of which releases “high” energy. Among these **high-energy molecules** are 1,3-bisphosphoglycerate (1,3 BPG, glycolytic intermediate), phosphoenolpyruvate (PEP, glycolysis) , creatine phosphate (CP, energy storing molecule of the muscle cells), ATP and GTP. Note: phosphate esters, such as glucose-6-phosphate, do NOT contain high energy bonds. The above mentioned high-energy bond containing molecules can be used for **coupled reactions**. In cases like these, a thermodynamically unfavorable reaction is joined (coupled) to an energetically favorable (spontaneous) one. The energy released in the latter reaction is enough to drive the other reaction forward.

Energy charge

The hydrolysis of phosphoanhydride linkages of ATP provides the availability of immense energy. The ratio of these bonds to the total adenine nucleotide pool provides the energy charge, which is normally between 0.8 and 0.95. The energy charge of the cell is a significant component of **metabolic regulation** and energy generating pathways are inhibited by high energy charge.

2.2. COMMON MOLECULES IN METABOLIC PATHWAYS

Activated group carriers

There are several biochemical molecules, which are components of additional pathways and feature the same function in each: **transfer of groups** in different reactions. By this measure, ATP can be considered as a phosphoryl group transfer molecule.

NAD and FAD are electron carriers during oxidative processes. The reactive part of AD is the nicotinamide ring, a derivative of the *vitamin niacin*. It is able to accept a hydride ion. Similarly, FAD is an electron acceptor molecule in dehydrogenation reactions, typically in those where a double bond is created. The active part of FAD is its isoalloxazine ring, once again, a *vitamin (riboflavin)* derivative. FMN (flavin mononucleotide, without AMP) is a similar electron carrier molecule.

NADPH contains an addition phosphate in the 2'-OH group compared to NADH, and this small difference provides a considerable functional change: NADPH is involved mainly in the reductive stapes of biosynthetic pathways.

Coenzyme A (CoA) is the activated carrier of acyl groups (acyl-CoA, acetyl-CoA). It is composed of a nucleotide, a vitamin-derivative (*pantothenate*) and a reactive part with a terminal SH group.
All of the above listed carrier molecules are independently stable, without a catalyzer. Additionally, a small number of these specific molecules act in a wide variety of reactions which is an effective and economical solution.

2.3. MAJOR METABOLIC REGULATORY MECHANISMS

Concentrations

The amount of an enzyme is the subject of transcriptional, translational regulation, and is dependent upon the half-life of the enzyme. Additionally, the concentrations of the substrates and cofactors have an effect on the metabolic rate.

Enzyme activity regulations

Two major types of it are the *allosteric and covalent* modifications (see more about enzyme activity regulation in the Enzymology). The *energy charge* of the cell is one characteristic controlling factor in energy-generating and energy-using mechanisms.

Hormones

Hormones provide regulatory machinery for metabolic control among tissues, signaling the needs of the whole body. Many times they act via intracellular signaling pathways, the result of which may be reversible modifications of central enzymes of metabolic pathways.

Compartmentalization

Many biochemical pathways are separated into different cellular organelles, especially into mitochondria. Distinctively, it provides a possibility for regulation by *separating pools* of molecules (acetyl-CoA in mitochondria enters citric acid cycle, in cytosol, it may be used for fatty acid synthesis) and by *effecting transport* of molecules to compartments.

2.4. BASIC CHEMICAL REACTIONS OF METABOLISM

The immense number of single steps of biochemical pathways is best defined as a variety featuring six basic chemical reaction types. These are the following:

Oxidation-reduction with electron transfer

Ligation reactions with the energy of ATP hydrolysis to create covalent bonds

Isomerisation within a molecule

Transferring functional groups between molecules

Hydrolysis

Addition or removal of groups involving double bonds.

3. DIET AND VITAMINS

The **energy needs** of the human being is dependent on a number of factors: age, sex, physiological and pathophysiological conditions and the level of physical activity, just a few to mention. Nevertheless, there are accepted values for energy usage after the statistical evaluation of a group of healthy volunteers.

DEE (daily energy expenditure) is the sum of the **BMR (basal metabolic rate),** the **DIT (diet induced thermogenesis)** and the energy requirement of the physical activity. The **BMR** is generally lower in women, decreases with age, higher during a fever, influenced by the body surface and affected by hormones (thyroxine, GH, epinephrine, cortisol, sex hormones). BMR can be calculated as 24 kcal/day/kg body weight. The energy requirements of activities are roughly as follows: 30% of BMR while studying, 60-70% of BMR for 2 hours moderate exercise/day. **DIT** is the energy used for absorption, digestion and storage of nutrients, in which at or about 10% include the up-taken of calories. Longer starvation results in the considerable reduction of the **BMR**.

In normal conditions, metabolic homeostasis should be achieved in an organism. The amount of available nutrients and the fuel requirement of the organs should be balanced. This is provided via the circulation including the proper blood level of nutrients, via the regulation of the central nervous system (CNS) either directly or through hormones. The caloric intake is used for energy requiring metabolic processes and the surplus is stored in the form of glycogen and triacyl-glycerol (TAG).

The **major components of the diet** consist of carbohydrates, fats and proteins. Following digestion and absorption, the small building blocks of the macromolecules enter the blood stream, will be taken up by the cells and used for energy generation, synthesis of biomolecules or storage. The energy demanding processes are the basic physiological functions, physical activities, and perhaps some other conditions, like pregnancy, lactation or illness.

The diet contains not only the molecules which provide energy for the body, but also the building monomers for biosynthesis of the structural and functional molecules. Some **essential components** should be taken up with the food, like essential fatty acids (FA), essential amino acids (AA), vitamins, minerals and water. The metabolic waste products and the xenobiotic compounds are released from the body via excretion with urine and feces.

As we noted before, the **fuel molecules** are generating electrons and some key molecules, like acetyl-CoA. The latter one enters TCA, producing further electrons and CO_2. Electrons are utilized by the mitochondrial electron transport chain for ATP synthesis.

The **caloric content of the fuels** are the following:

- carbohydrate .....4 kcal/g
- fat .....9 kcal/g
- protein .....4 kcal/g
- alcohol.....7 kcal/g.

The **daily energy need** is about 2000-3000 kcal. In order to shed and intentionally lose weight, the maximum caloric intake reduction can be 1000 kcal less/day. This means 2 lbs/week (about 0.9 kg) weight loss. At the onset of dieting, there is an increased weight loss due to the breakdown of glycogen, which is a hydrated molecule.

3.1. MACROMOLECULES OF THE DIET

The **carbohydrate** content of the diet is primarily starch which comes from plants, and mono-, disaccharides. There is no essential need of carbohydrate, largely due to every necessary type of monosaccharide can be synthetized in the cell via gluconeogenesis and sugar interconversion reactions.

The **proteins** of the diet are digested into amino acids and either are broken down (CO_2, water and ammonium) or are incorporated into the cell's own proteins. Distinctively, amino acids are the sources of some important derivatives as they provide nitrogen for synthetic reactions (nucleotides and heme). The protein requirement of an adult is 50-60 g/day, which ideally contains the essential amino acids.

Lipids in the diet should contain the essential fatty acids (plant or fish oil origin) as they are precursors of derivatives which are involved in (patho)physiological processes (prostaglandins, leukotrienes, thromboxans). Other lipids will be used to synthetize membrane components (sphyngolipids, phospholipids), which are not only structural but also functional elements (signal transduction, for example). The majority of fatty acids will be stored in the form of TAG in adipose tissue. This is the most reduced biological molecule yet it generates the largest energy due to oxidation.

Alcohol is an energy providing molecule.

Stored energy of the body (in % of total amount):

- glycogen (polar molecule, binds considerable volume of water)
  - muscle.....0.4% (used for muscle contractions)
  - liver.....0.2% (blood glucose level regulation)
- protein .....14.4% (functional molecules, used only in starvation and diseases as energy source)
- TAG.....85%

3.2. VITAMINS

Vitamins are needed for the body in small amounts (mg - µg). When a specific vitamin is missing or is in low concentration only, characteristic signs manifest themselves which help the diagnosis of vitamin insufficiency. The **two major groups** of vitamins are the water soluble and fat soluble (A, D, E, K) vitamins. They can be modified in the body, and used as cofactors, prosthetic groups of enzymatic reactions. In other instances, they behave like hormones. High dosage of fat soluble vitamins are definitely harmful, however, they can be stored and as a result, cause toxic effects. Additionally, extra high levels of water soluble vitamins are not recommended.

Fat soluble vitamins

Vitamin A

Active forms of **vitamin A** are retinol, retinal and retinoic acid within the cell. They are derivatives of carotenoids. A modified retinol (retinyl phosphate) is involved in glycoprotein synthesis which is the base of the protective effect of vitamin A in mucus secretion. Retinal is an essential components of the visual cycle. Retinoic acid acts like a hormone taking part of regulation of growth and differentiation.

Vitamin D

Synthesis of **vitamin D** starts from cholesterol, requires the UV fraction of sunlight and contains two hydroxylation reactions: 1-OH derivative is produced in the liver while the 25-OH modification is carried out in the kidney. The active 1,25-dihydroxycholecalciferol is also identified as calcitriol (see reaction pathway in the lecture).

The main physiological function of vitamin D is the control of calcium and phosphorus homeostasis (together with parathormone). Vitamin D acts as a hormone: regulating cell proliferation, immune system, insulin secretion.

Vitamin K

The different forms of **vitamin K** belong to the quinones. The biochemical function of this vitamin is to catalyze the γ-carboxylation of specific glutamate residues in proteins which are involved in blood coagulation and bone structure. Due to this chemical modification, these proteins are able to bind Ca^{2+}, so they can actively participate in blood clotting. Dicumarol and related-drugs inhibit the reductase which converts vitamin K into its active form, thus they *inhibit blood clot* formation.

Vitamin E

Vitamin E (tocopherol) is an antioxidant molecule, protecting the membrane structures, other vitamins, fatty acids from oxidation.

Water soluble vitamins

In summary, only the **basic biochemical features** of the water soluble vitamins are illustrated: name, biochemically important derivatives and reactions where they act as coenzymes. The remainder of the information in reference to vitamins is found in the lecture and seminar material.

Vitamins involved in *energy production*

Vitamin B_1 – thiamin - thiamine pyrophosphate (TPP) - PDC, αKG-DH, transketolase, transaldolase

Vitamin B_2- riboflavin – FAD, FMN – electron transfer (succinate DH and many more)

Niacin, niacinamide – NAD, NADP – electron transfer (αKG-DH, malate DH and many more)

Vitamin B_6 – pyridoxal and related molecules – PLP – amino acid metabolism, transaminase reactions, neurotransmitter synthesis, heme synthesis

Pantothenic acid – component of CoA (FA metabolism, citric acid cycle…)

Biotin - carboxylase reactions (pyruvate, acetyl-CoA, propionyl-CoA)

Vitamin C: no coenzyme is produced - antioxidant, involved in the synthesis of collagen (OH-derivatives of AAs), carnitine, norepinephrine, promotes iron absorption.

Vitamins involved in *hemopoiesis* (deficiency will cause megaloblastic anemias)

Folic acid – tetrahydrofolate (THF) – one carbon carrier (synthesis of serine, glycine, purines, dTMP, methionine synthesis together with vitamin B_{12})

Vitamin B_{12} – (cyano)cobalamine – methionine synthesis, methylmalonyl-CoA mutase (degradation of odd-carbon chain fatty acids).

Choline is an essential biomolecule – acetyl-choline and phospholipid synthesis.

Carnitine is conditionally essential (FA oxidation in mitochondria).

Biochemically important minerals

Magnesium – ATP is complexed with Mg-ions in many enzymatic reactions.

Cuprum – metal cofactor to a number of enzymes: ceruloplasmin – iron metabolism, cytochrome c oxidase – electron transport chain, norepinephrine synthesis, collagen crosslinking, superoxide dismutase, desaturation of long chain fatty acids.

Selenium – selenoproteins are found among enzymes (glutathione peroxidase, thioredoxin reductase).

4. MACROMOLECULES IN BIOCHEMISTRY

5. BASICS OF THERMODYNAMICS

The subject was studied extensively in Physical Chemistry. Now, let's recap the biochemically important terms and relationships without a detailed description of the equations.

In biochemical reactions, the **substrates and products** are considered as components of the collision theory. The reactions are described with rates: **forward, reverse and the rate constant**, the latter one has a relationship to the concentrations of the components and is also is influenced by the temperature and ionic strength of the reactants. The **unit** of the reaction rate is concentration/time.

Equilibrium has been detected when the forward and reverse reactions are equal. **Steady state** is the real biological situation of an equilibrium which involves substrate, intermediate and product. Steady state is reached when the concentration of the *intermediate* becomes constant, and is not the same as the equilibrium.

Basic laws of thermodynamics are true for living organisms. Within the cell, by breaking down and by oxidizing macromolecules, energy is provided for the cell's needs. A cell is considered as an open system which is in connection to material and as an energy exchange with its surroundings. The uptake of **energy generating molecules should be constant** for synthesis of complex biomolecules, movements, and transport processes to keep up the disequilibrium of the cell within its environment. **The energy is used** for work and heat exchange by a system (labeled + if heat is added to the system or work is done by the system).

In a living organism, **different types of energy exists**: thermal, chemical, kinetic and potential. In a cell, a specific form of energy can be *converted to another form* (by the formation of a chemical bond, energy is released, which is used to make another bond, released as heat or stored in the form of potential energy) – **first law of thermodynamics**. The internal energy (H – enthalpy) of a system by itself is not informative, rather the **change of the enthalpy** at the beginning and the end of a biochemical process (ΔH). If ΔH is negative, this means the reaction is *exothermic* and thus, energy is released into the surroundings. Not all of the energy change can be used for work, as a part of it will change and alter the **entropy (S) of the system**. Entropy is the disorder of a system. When ΔS is positive, there is less order at the end of the reaction - which is characteristic for a spontaneous reaction (**second law of thermodynamics**).

The actual energy used for work has been described by the **Gibbs free energy (G). ΔG** is the difference of the free energy at the initial and final state. An energetically favorable reaction has a negative ΔG, while at equilibrium the ΔG is zero. The equation **$\Delta G = \Delta H - T\Delta S$** demonstrates, regardless of the sizes and signs of enthalpy and entropy change, as long as free energy change is negative, the reaction is **favorable**. As a result, ΔG determines whether a reaction may happen at all. It is important a favorable reaction may provide enough energy to make an unfavorable reaction happen (coupling of reactions biochemically).

At standard conditions reactions can be carried out and **standard free energy change (ΔG^o)** can be determined. The actual free energy change is related to the standard free energy change according to the equation **$\Delta G = \Delta G^o + RT \ln[Y]/[X]$,** so it is obvious that the starting concentrations of the reactants will determine the direction of the reaction. At equilibrium ΔG is zero, so $\Delta G^0 = -RT \ln[Y]/[X]$, at equilibrium the relative concentrations of the reactants will provide the standard free energy.

The equilibrium constant (K_{eq}) is dependent on the ratio of the rate constants of the forward and reverse reactions. This means the equilibrium constant is related to the standard free energy change of the reaction. It is important to note, small changes in the free energy will lead to a relatively large difference in the equilibrium constant. The standard free energy change provides the information whether a reaction is energetically favorable, while the ratio of the products and reactants demonstrate how far the reaction will proceed at equilibrium.

In living systems, none of the above terms describes correctly the rate (speed) of a biochemical reaction, because it is determined by the catalyzing enzymes (see chapters of enzymology).

6. ENZYMOLOGY

6.1. INTRODUCTION, GROUPS OF ENZYMES

Enzymes are essential for nearly all known biochemical reactions. They are mainly proteins, some are RNAs or RNPs. The major **advantages** of the presence of enzymes are their capability to increase the rate (speed) of the reactions, their specificity to recognize only a small number of substrates, and the possibility in the regulation of their activity, and through this, the regulation of the metabolism of the whole organism. Enzymes are changed only temporarily during the reactions. Following the release of the product, the enzyme is now ready for the next catalyzing step. Their catalytic power is proved by calculating the reaction rates with and without the enzyme present.

The **major function** of the enzyme is the specific binding of a substrate in the active center of the enzyme, providing the ideal environment for the specific reaction to occur, and then to release the product. Energetically this means, that within a catalyzed reaction the equilibrium is reached in a shorter period of time than without an enzyme but K_{eq} is not been changed or altered. In the event of an irreversible reaction, the amount of the starting material is below the detection level compared to the final product level.

Enzymatic actions are the target of a variety of specific pharmaceutical *drugs*. In many cases, there are isozymes present, which means different forms are expressed in different tissues or subcellular organelles which are regulated differently. Selective drugs often target or aim towards isozymes specifically due to diminished side effects.

According to international regulations, enzymes are **classified** into six major groups:

1. oxidoreductases
   They catalyze electron movement between a donor and an acceptor molecule. Among them are the mono-oxygenases and the dioxygenases.

2. transferases
   Transferases carry out group transfer between molecules. In kinases transfer phosphate groups, the donor is mainly ATP. Other types include aminotrasnferases, acetyl transferases, glycosyl and transferases.

3. hydrolases
   Hydrolyses catalyze mostly irreversible reactions. They break covalent bonds with the addition of a OH group and a proton.

4. lyases
   Mostly they catalyze carbon-carbon bond breakage (aldolase, decarboxylase, thiolase).

5. isomerases
   Isomerases move groups or double bonds within one molecule (epimerases, mutases).

6. ligases
   They join atoms (C with C, S, O, N) during synthesis with the use of energy (hydrolysis of ATP – synthetases).

Examples of each type of reaction can be seen in the lecture material.

6.2. ACTION OF ENZYMES

To change a substrate into a product, the enzyme has to bind the substrate specifically at its active site. In this specific, the enzyme **lowers the energy of activation** which is necessary for the formation of the transition state. **Transition state** is the highest energy level intermediate between a substrate and the product, when the rearrangements of the chemical bonds and interactions begin to develop but have not reached the final stage of the product. Energetically, the starting and the final energy level is the same with and without the enzyme, as indicated by the K_{eq} of the reaction. The reduction in the activation energy is directly proportional to the rate (velocity) of the reaction. The tightest binding occurs between the enzyme and the transition state molecule (see appropriate diagrams in the lecture).

Specificity of the enzyme has been described in **two models**: the lock-and-key model presumes rigid structures (enzyme and substrate) which fit completely to one another at the active site of the enzyme. The induced-fit demonstrates increased flexibility in both molecules and they change their conformations when in close proximity to one another.

Enzymes operate with a variety of **mechanisms**. Even in a single reaction, more mechanisms may occur. The most frequent types of reactions are the general acid and general base reactions, in which proton movement generates a nucleophile (negatively charged group) which can attack a positive center. This type of ionic interaction is the major component of creating partially charged groups in making carbon-carbon bonds, decarboxylation reactions (α-ketoacids with coenzymes), oxidation reactions (alcohol to aldehyde or creating C-C double bond), proteases, kinases (transfer of phosphate group from ATP in the presence of Mg-ion). Due to proton movement during reactions, most of the enzymes feature a pH optimum, in which they perform at the highest rate. Other types of catalytic mechanism may occur such as the substrate becomes involved in covalent binding with the enzyme (chymotrypsin – see more in Digestion and absorption of macromolecules), some kinases).

Active site of an enzyme

The active site or active center of an enzyme is responsible for carrying out the actual biochemical reaction and stabilizing the transition state. The side chains of those amino acids which are participating in the biochemical reaction create the **catalytic group**. Most of the time, polar amino acids are involved as they are able to take part in proton movement mechanisms. Amino acids distanced from one another in the primary structure of the protein often provide the common catalytic group. The active site is very small compared to the entire enzyme but the larger part of the enzyme molecule is also necessary for the activity: it provides the proper three dimensional structure of the molecule involving the active center. Without the entire molecule, the enzyme is not ideally suitable to acquire such a high specificity such as, distinguishing stereoisomers of molecules.

Coenzymes and cofactors

Some of the enzymes do not have the proper amino acid side chains in their active centers to carry out the reactions by themselves. They can use cofactors or coenzymes (organic or inorganic molecules) as helper components in the biochemical processes. Cofactors are primarily **metal ions**. Coenzymes may be involved in activation/transfer processes or oxidation/reduction reactions. Many of the coenzymes are the **derivatives of vitamins** (see: Diet and vitamins). They can act as substrates and change at the reaction, often meaning they will have to be regenerated. The intracellular concentrations of the coenzymes are relatively stable. The protein part without the coenzyme is called apoenzyme, with the coenzyme it is a **holoenzyme**. A group of these organic molecules are bound to the apoenzyme covalently, so they are called **prosthetic groups** (flavins: FMN, FAD; heme; biotin). Coenzymes are relatively free (NAD, coenzyme A).

NAD and NADP are the derivatives of niacin. The group functionality is the nicotinamide ring, which acquires reduction once a hydride ion is accepted from the substrate while a proton is released. Following this reaction, the reduced coenzyme leaves the enzyme and then may participate in yet another reaction. The ratio of the oxidized and reduced NAD(P) is important information about the cell's oxido-reduction state.

Riboflavin is the precursor of flavin mononucleotide (**FMN**) and flavin adenine dinukleotid (**FAD**). The active part of the molecules is the isoalloxazine ring which is able to accept or donate two electrons in oxidation/reduction reactions. Flavin coenzymes are rather prosthetic groups bound tightly to the apoenzyme.

In addition to **ATP** serving as the main energy providing molecule of the cell, it also is involved in reactions as the co-substrate. In a kinase reaction, a phosphate group is transferred to the substrate while ATP is transformed into ADP. Notably, ATP is an allosteric effector modulating several crucial enzyme activities, such as, signaling the energy supply of the cell.

Metalloenzymes have a transitional metal which is essential for either binding the substrate or for stabilizing intermediates during the reaction. They may also serve in providing both the proper and higher structure of the protein enzyme. In other circumstances, following substrate binding, they generate conformational changes of the enzyme active site.

Iron-sulfur proteins are involved in electron transfer processes. Cytochromes contain heme prosthetic groups. Heme, similar to iron and copper, may participate in the actual chemical reactions.

Enzyme kinetics

As in thermodynamics, enzyme kinetics was studied in the physical Chemistry courses. Here we summarize the basics which are essential to understanding the performance mechanisms of the enzymes without going into mathematical details.

The **rate of a biochemical reaction** can be described with the amount of substrate (S) disappearing or the amount of product (P) appearing in a unit of time (sec). The velocity of the reaction (the speed of the product concentration increase) is proportional to the S concentration ([S]) with the *k* rate constant. The **rate constant** is dependent on the different physicochemical conditions of the reaction and related to the activation energy. Larger *k* means the substrate disappears in a shorter period of time. By reaching **equilibrium** of a reversible reaction, there is no net change in [S] or [P]. The ratio of the forward and reverse rate constants provide the equilibrium constant (K_{eq}) and it is equal to the ratio of [P] and [S].

The **Michaelis-Menten model** of enzyme kinetics describes the relationship of catalytic rate, concentration of substrate and enzyme. Once constant enzyme concentration is achieved, increasing substrate concentration will cause an increase of the velocity of the reaction until all the enzymes are saturated. The substrate and the enzyme provide a complex (ES) which has two fates: dissociation back into S and E, or E will make and release a product (P) (at the early stage of the reaction there is no or only a small amount of product, so the product will not change back to S). This process, held to a steady state, means the formation and the breakdown of the ES complex are equal. The ratio of the three rate constants will provide K_M and the Michaelis is constant. If we suspect more S is present in the reaction, then the enzyme, at maximal rate (V_{max}) and all subsequent enzymes will be bound by S.

From all these assumptions and simplifications, the **Michaelis-Menten equation** is drafted:

$$V_0 = V_{max}\,[S]/([S]+K_M).$$

If we substitute [S] into K_M, we understand, the actual velocity will be half of V_{max}, and therefore, K_M is the substrate concentration once the reaction reaches half of the maximum velocity. **K_M can be determined** by plotting the reaction velocity versus [S] or in a double reciprocal plot (see details in lecture material). K_M can be simply considered as the **affinity of the substrate and the enzyme**: the smaller is the K_M the larger is the affinity, and as such, lower substrate concentration will be ½ V_{max}. K_M is an important characteristic of an enzyme in relation to a specific substrate. Many times there are differences among isozymes in K_M resulting in different function or regulation of the isoforms.

During the maximum rate of a reaction, all the enzymes are occupied, meaning the rate constant can be defined as a catalytic constant (k_{cat}). This provides the term of the **"turn-over" number**: the number of substrate molecules turned into product at a unit of time (1/sec). In cellular conditions, typically, not all the enzyme molecules are occupied so the actual enzymatic rate is below the catalytic rate.
One of the best characteristic of an enzyme is its **catalytic efficiency**, given by the k_{cat}/K_M ratio. In the case when production of P is faster than the dissociation of ES complex, the limit of the reaction is the ES complex formation. Within the cell, the upper limit of this step is the speed of diffusion. Those enzymes which work in this range show the phenomenon of **kinetic perfection** (superoxide dismutase, acetyl-cholinesterase). To reach this rate, enzymes use the channeling between one another and the creation of electrostatic forces at the active site.
The above calculations describe various situations of a one-substrate reaction. In the event **of two-substrates,** there are two distinct possibilities: sequential (ordered or random) and double displacement reactions (see examples of both in the lecture).

Inhibitors of enzymes

There are natural and synthetic molecules with specific enzyme inhibitory activity. Some of the natural compounds act during enzyme activity regulation. Many pharmaceutical drugs have enzyme inhibitory activities. Additionally, inhibitors can help locate the specific residues of the molecule which are involved in the formation of the active site.

Enzyme inhibition can be defined as being **irreversible** once the inhibitor is tightly bound, either covalently or non-covalently (Penicillin and Aspirin). **Reversible** inhibitors may all be competitive, uncompetitive and non-competitive.

Competitive enzyme inhibitors recognize the substrate binding site whereby they bind to the enzyme alone and thus prevent the binding of the substrate. The rate of the catalysis will be reduced and the proportion of effective ES complexes will decrease. Many times the inhibitor resembles the structure of the substrate (methotrexate). With increasing concentration of the substrate the inhibitory effect of the competitive inhibitor may be eliminated as both molecules try to bind to the same site of the enzyme (they are competing for binding). Many pharmaceutical drugs act as competitive inhibitors (Ibuprofen and Statins).

Uncompetitive inhibitors bind to the enzyme-substrate complex, because the enzyme should take a conformational change to provide binding site for this inhibitor. In a "triple" (ESI: enzyme-substrate-inhibitor) complex, there is no possibility for product formation, and so V_{max} will be reduced.

Noncompetitive inhibitors are able to bind to free enzyme and to the ES complex. They act mainly by reducing the concentration of the functional enzyme. The apparent V_{max} will be lower. A special type is the mixed inhibition when substrate binding is inhibited and the enzyme "turn-over" number is reduced.

These reversible inhibition types can be easily distinguishable on double-reciprocal plot formats. Competitive inhibitors increases K_M but V_{max} will not change. In uncompetitive inhibitions, the slope of the curve will not change, K_M and V_{max} decreases at the same rate. In noncompetitive inhibition, K_M is stable, V_{max} will be reduced (see the appropriate figure in the lecture).

Irreversible inhibitors

Three types of distinguished characteristics: group specific reagents, reactive substrate analogs and suicide inhibitors.

Group-specific reagents bind to one or more amino acid side chains of the enzyme active center (serine of chymotrypsin, acetyl cholinesterase). Many times they are not specific for an enzyme, rather they recognize certain reactive side chains.

Affinity labels (or reactive substrate analogs) are more specific by recognizing and covalently binding the active site of an enzyme.

Suicide inhibitors (or mechanism–based inhibitors) are binding to the active site of the enzyme and following the biochemical reaction, initiates the inhibitor producing a very reactive derivative which covalently modifies and inactivates the enzyme (MAO inhibitor Deprenyl).

Transition state analogs are among the most potent pharmaceutical drugs. They resemble the structure of the molecule in the transition state and as such, are considerably, very specific. Another advantage is they can be used as antigens for producing antibodies with catalytic activity (abzymes).

Regulation of enzyme activity

Enzymatic activity regulation occurs at different levels with various speeds. The result of them will be an adequate enzyme activity depending on the tissue and body needs. There are five possibilities for regulation: allosteric, presence of isozymes, covalent modifications, activation by proteolysis and controlling the amount of the enzyme.

Allosteric regulation

In allosterically regulated enzymes, in addition to the active center, there is a regulatory site for binding of the regulator molecule known as an **effector or modulator** which can be an activator or an inhibitor. In biosynthetic pathways, often a **feed-back inhibition** can be seen when the end product is inhibiting during an early step (the committed step) of the biosynthetic pathway. In energy generating pathways, a **feed forward** activation may occur when a large amount of starting substrate actives the pathway.

In the event of a **homotropic** interaction, the substrate is the effector which is binding to a regulatory site and may cause an activation effect. In **heterotropic** interactions, another molecule other than the substrate, is the regulator (activator or inhibitor). The effectors can change the K_M of the reaction (K class) or the V_{max} of it (V class).

Many times an allosterically regulated enzyme has more subunits and the catalytic and regulatory sites may be found on separate subunits. Frequently, the **multi-subunit enzymes** are considerably cooperative.

In monomeric enzymes, the binding of an allosteric activator will change the conformation of the enzyme, meaning the binding of the substrate will be easier. In contrary, an inhibitor molecule will initiate a type of structural change of the enzyme and as a result, the interaction with substrate will be repressed.

A good **example** for an allosterically regulated enzyme is the **aspartate transcarbamoylase (ATCase**) of the pyrimidine synthetic pathway with an end product of cytidine triphosphate (CTP). With increasing concentration of CTP present ATCase has decreasing activity. CTP should have another interacting site with ATCase than the substrates (carbamoyl phosphate and aspartate) because it has

such a distinctive structure. **CTP is a feed-back inhibitor** of ATCase. When we plot the velocity of ATCase versus aspartate (substrate) concentration, we see a sigmoidal curve, however, it is not the usual hyperbolic one. To explain this phenomenon, we have to examine the structure of ATCase in detail. Apparently, the subunits (catalytic and regulatory) can take on two special three dimensional conformations: T (tense) and R (relaxed) state. In the T state, the enzyme can readily bind CTP and not easily to the substrate. With CTP binding, this conformation is considerably more stable. In the R state, the enzyme is willing to bind the substrates. The **sigmoidal curve** of ATCase is the apparent ratio of the T and R state enzymes (see more in the lecture).

When substrate is binding, ATCase velocity is increasing rapidly by a small elevation of substrate concentration. This physiologically important fact is produced by the **cooperativity** of the subunits which means the active sites of a multisubunit enzyme are not working independently. According to the **concerted model**, all subunits of an enzyme are in either the R or the T state (ATCase). The other model of cooperativity is the **sequential mechanism**, when substrate binding to one enzyme subunit will change the conformation of the same and a neighboring subunit, but not all the subunits at the same time. Many allosteric enzymes demonstrate cooperativity with the mixture of the two types of mechanisms.

Another allosteric regulator of ATCase is the **ATP** level. Apparently ATP competes with CTP in the binding of ATCase. During high ATP concentration, the inhibitory effect of CTP is less obvious. The reason for this is purines and pyrimidines should be present in the cell close to equal amount for proper nucleic acid biosynthesis. Also, high ATP level indicates there is enough energy for RNA and DNA synthesis in the cell.

Another way for the regulation of enzyme activity is the presence of **isozymes (isoenzymes).** They are located in different tissues or subcellular organelles or they are active at distinct developmental phases. They catalyze the same biochemical reaction, but they are coded by various genes. They are regulated unequally and the kinetic parameters of them are uncommon. They can be separated according to their biochemical properties which can be a diagnostic tool in certain cases. The existence of isozymes provides a possibility for delicate regulation, as at given conditions the various tissues can provide more answers depending on the need of the whole organism.

A good example for isozymes is the **lactate dehydrogenase (LDH**). It contains two types of polypeptide chains in the form of a tetramer. There are five ways for subunit gathering of the H (heart type) and M (muscle type) protein chains: H4, H3M1, H2M2, H1M3 and M4. At the two extremities the enzymes behave oppositely to a certain extent: H4 isoenzyme uses lactate as substrate and produces pyruvate which can be taken up by the citric acid cycle and burn aerobically in the heart muscle cells. This enzyme is inhibited by pyruvate. The M4 form in skeletal muscle is generating lactate from pyruvate anaerobically which can enter the Cori cycle.

Covalent modification of enzymes

The reversible covalent modifications happen frequently posttranslationally (see molecular biology studies). Remember, histone acetylation by which modification gene activation, can occur (the histone acetylase enzyme may be modified by phosphorylation). One type of irreversible modification is the covalent attachment of lipids to proteins for membrane targeting. By ubiquitination, the proteins can be labeled for degradation. The most frequent covalent modification is the phosphorylation and dephosphorylation among proteins including enzymes. An allosterically regulated enzyme may become phosphorylated, too.

Phosphorylation of proteins intracellularly is carried out **by kinases** which are found in a large numbers in every cell. They are tools of tissue and time specific regulation. In the kinase reactions, usually ATP is the phosphoric group donor. According to the acceptor amino acid side chain, we distinguish serine/threonine and tyrosine kinases. Some kinases are dedicated (substrates are just a few related

proteins) other kinases are multifunctional (with more substrates). To find the proper amino acid for phosphorylation, it has to be part of a consensus sequence and the conformation of the protein is also essential. The reverse reaction, the hydrolysis of the phosphorus group, is carried out by phosphatases.

The biochemical **relevance of phosphorylation** is the following:

- it cannot happen spontaneously just by enzymatic reactions which means it is regulated,
- phosphorylation creates additional negative charges, so new bonds, including a new structure, may be created, and the enzymatic reaction can be very fast or relatively slow depending on the circumstances by phosphorylation of key molecules, in which a cascade may be started and
- ATP is used as the group donor. Notably, the cell's energy status is related to the metabolic regulation.

A good example of the relation of the **allosteric and covalent regulations** is the activation process of protein kinase A (PKA). This is a central kinase which is activated by hormonal effects via the elevation of the cAMP concentration. PKA contains two regulatory and two catalytic subunits. In the inactive state of the enzymes, a part of the regulatory subunit binds to the active site of the catalytic subunit as a pseudosubstrate. Once cAMP interacts with the regulatory subunit, it will dissociate itself of the catalytic polypeptide which can bind the real substrate.

Activation by proteolytic cleavage

This type of enzyme activation is irreversible and may occur extracellularly, too. Proenzymes (zymogens) are cleaved specifically for activate an inactive enzyme without the energy of ATP. Examples of this type of activation include digestive enzymes, components of the blood clotting system, hormones (insulin), collagen from procollagen, procollagenase for collagen break down (development, remodeling) and apoptosis.

Digestive enzymes

Digestive enzymes are synthetized as zymogens by the pancreas and stored in vesicles. After hormonal or nerve impulses, they are secreted into the gut lumen. Chymotrypsinogen is activated by a single cut by trypsine, then the activated chymostrypsin carries out additional cleavages. The result will be three polypeptides joined by disulfide bridges and now feature a new conformation which is able to bind the substrates. Due to the protein content of the diet, it must be digested in coordination with the different digestive enzymes, and, the activation of the numerous zymogens must occur in a short period of time. Trypsin is the major activator and the first molecules of trypsin are activated by enteropeptidase, an enzyme of the enterocytes. Deactivation of the digestive enzymes happen via and in the role of very specific and tight binding inhibitor molecules (pancreatic trypsin inhibitor and alpha1 antitrypsin). Alpha1 antitrypsin (A1AT) is a rather elastase inhibitor. The genetic mutation of A1AT will result in an increased elastase activity which will lead to connective tissue destruction especially in the lungs (emphysema). This situation is worsened by cigarette smoking as it will cause the oxidation of a special methionine in A1AT, thus inactivating the inhibitor.

Additionally, the factors of the **blood clotting** system are activated by protein cleavage (see details in Blood clotting).

7. CARBOHYDRATES

Carbohydrates (sugars and polymers made of sugars) are the most abundant biomolecules on Earth.

- Certain carbohydrates (sugar and starch) are a dietary staple in most parts of the world and serve as the central energy source for the cell.
- Carbohydrate polymers (also called glycans) serve as structural and protective elements in the cell walls of bacteria, plants and in the connective tissue of animals.
- In animals, the complex polymers known as glycosaminoglycans (chain of unmodified and modified sugars) lubricate and cushion joints; help form skin, hair and feathers, help regulate blood clotting and assist the immune system and help control cell-to-cell adhesion and interaction.
- Complex carbohydrate polymers covalently attached to proteins or lipids act as signals that determine the intracellular location or metabolic fate of these hybrid molecules, called *glycoconjugates*.

7.1. GENERAL FEATURES AND NOMENCLATURE

Carbohydrates are **polyhydroxy aldehydes or ketones**, or substances that yield such compounds during hydrolysis. Many carbohydrates have the empirical formula $(CH_2O)_n$; some also contain nitrogen, phosphorus or sulfur.

There are three major size classes of carbohydrates: monosaccharides, oligosaccharides and polysaccharides. **Monosaccharide**, or simple sugar is a single polyhydroxy aldehyde or ketone that does not hydrolyze.

Oligosaccharides consist of short chain of monosaccharide units, joined by characteristic linkage called glycosidic bonds, that can be hydrolyzed to monosaccharide units. The most abundant are the disaccharides, with two monosacharide units. All common monosaccharides and disaccharides have names ending with the suffix ˝-ose˝. In cells, most oligosaccharides consisting of three or more units do not occur as free entities but are joined to nonsugar molecules (lipids or proteins) in glycoconjugates.

The **polysaccharides** are sugar polimers containing more than 20 monosaccharide units; some have hundreds or thousands of units. Some, such as cellulose, are linear chains; others (glycogen) are branched.

7.2. SIMPLE SUGARS: MONOSACCHARIDES

Monosaccharides are colorless, crystalline solids that are freely soluble in water, but insoluble in nonpolar solvents. Most have a sweet taste.

An **aldose** is a sugar derived from an aldehyde and a **ketose** is derived from a ketone.

A triose is a carbohydrate with three carbon atoms; a tetrose has four carbons; a pentose has five carbons; and a hexose has six carbons. The simplest monosaccharides are the two three-carbon trioses glyceraldehyde and aldotriose, dihydroxyacetone and ketotriose (lecture fig.).

7.3. STEREOCHEMISTRY

A very important feature of sugars is the **large number of isomers** possible within the overall molecular formula, even for simple pentoses and hexoses.

Stereoisomers are molecules with the same molecular formula, but they differ in the way that the constituent atoms are oriented in space.

Enantiomers are molecules containing a center of assymmetry, usually a carbon atom, and are mirror images of one another.

- They have the same physical properties, except they rotate polarized light in different directions.
- By convention, a + sign indicates rotation of the polarization to the right, while a - sign indicates rotation to the left.
- Since their physical properties are the same, it is often very difficult to physically separate enantiomers from one another.

Diastereomers are stereoisomers that are not mirror images.

Their physical properties will then differ, and this serves to enable their physical separation. A pair of stereoisomers that are not enantiomers must then be diasteromers.

All the monosaccharides, except dihydroxyacetone, contain one or more asymmetric **(chiral)carbon atoms** and thus occur in optically active isomeric forms. In general, a molecule with *n* chiral centers can have 2^n stereoisomers. The simplest aldose, glyceraldehyde, contains one chiral center, and therefore has two different optical isomers, or enantiomers. Lecture figure illustrates these two forms using a Fischer projection.

By convention, the C1 carbon is the aldehyde carbon, and its drawn at the top of a vertical line of carbon atoms. The chiral carbon is C2¸ the OH group is drawn to the right of the chiral carbon for what is denoted as the D isomer, the L isomer has this group drawn to the left side of this carbon.

In glyceraldehyde, the D isomer rotates plane of polarized light to the right; the L isomer rotates it to the left. In other carbohydrates, those in which the configuration at the reference carbon (the most distant chiral center from the carbonyl carbon) is the same as that of the D-glyceraldehide are designated **D isomers**, and those with the same configuration as L-glyceraldehide are **L isomers**.

The D,L convention for stereoisomers can also be applied to other simple chiral compounds, such as amino acids. However, while many D monosaccharides do, indeed rotate plane-polarized light to the right, this behavior is not universal. There the D, L symbols indicates just the stereochemistry.

Most of the sugars of living organism are **D isomers**, however some sugars occur naturally in their L form; examples are L-arabinose and the L isomers of some sugar derivatives that are common components in glycoconjugates.

Lecture figure shows the structure of the D isomers of all the aldoses and ketoses having five to six carbon atoms. The carbons are numbered at the beginning at the end of the chain nearest to the carbonyl group. Each of the eight D aldohexoses, which differ in the stereochemistry at C-2, C-3, or C-4 has it own name. The four-and five-carbon ketoses are designated by inserting ″ul″ into the name of a corresponding aldose. The ketohexoses are named otherwise. D-(+)-glucose is sometimes referred to as *dextrose*, while *levulose* is D-(-)-fructose.

The sugars that differ only in the configuration around one carbon atom are called **epimers**: D-glucose and D-mannose, which differ only in the stereochemistry at C-2 are epimers, as are D-glucose and D-galactose (which differs at C-4) (Lecture fig.).

7.4. CYCLIZATION

The aldopentoses and the hexoses tend to **cyclize** in aqueous solution, forming a ring with five or six members. This ring contains an oxygen atom as one of its members. Thus, sugars with a five membered ring resemble the compound furan, while those which a six-membered ring resemble pyran. This leads to designating the sugars in such conformations as **furanoses** and **pyranoses** (Lecture fig.).

As a pyranose, glucose form a **hemiacetal** (the product of 1:1 reaction of an aldehyde with an alcohol), which is relatively stable, due to its cyclic structure. This reaction creates a new chiral center at C1 and leads to two new optically-active forms, called **anomers.** The anomers are designated as **α-D-glucopyranose** and **β-D-glucopyranose** (Lecture fig.). The designation α indicates that the hydroxyl group at the anomeric center is, in the Fischer projection, on the same side as the hydroxyl attached at the farthest chiral center, whereas β indicates that these hydroxyl groups are on the opposite sides.

However, the six member pyranose ring is not planar, but tends to assume either of two chair conformations. Bonds to substituents and hydrogen atoms on the ring carbons may be either axial (the α anomer), projecting parallel to the vertical axis through the ring or equatorial (the β anomer), projecting roughly perpendicular to this axis.

The α and β anomers of D glucose interconvert in aqueous solution by a process called **mutarotations**.

Fructose can cyclize to give either a furanose ring or a pyranose, through the formation of a **hemiketal** (the product of an alcohol reacting with the carbonyl of a ketone). For either the pyranose or the furanose, two anomers are possible, α - and β-D-fructofuranose and α- and β-D-fructopyranose (Lecture fig.). The more common anomer of this sugar in combined forms or derivatives is β-D-fructofuranose.

Ribose and deoxyribose can also cyclize to form a furanose, with two anomers (Lecture fig.).

7.5. REACTIVITY AND COMMON DERIVATIVES

The hemiacetals and hemiketals formed in cyclizing pentoses and hexoses are in equilibrium with the open-chain form of these sugars. This equilibrium leaves small amounts of “free” aldehyde or ketone available for reaction.

They can react with oxidizing agents to give a positive result in classical tests for “reducing sugars”. **Oxidation** of the carbonyl (aldehyde) carbon of glucose to the carboxyl level produces **gluconic acid**; other aldoses yield other **aldonic acid**.

Conversely, the carbonyl group on aldoses and ketoses may be **reduced** to give the corresponding alcohol. Lecture figure demonstrates two important examples of this class of compounds. **D-sorbitol** is also called D-glucitol. In diabetes mellitus, sorbitol accumulates in the lens of the eye and is associated with the formation of cataracts. The mechanism of cataract formation may possibly involve redox reactions in the formation of sorbitol from glucose and associated oxidative cross-linking of proteins through disulfide bridges.

Enzymes can oxidize monosaccharides to give a number of different products. Oxidation of the carbon at the other end of the carbon chain-C-6 of glucose, galactose or mannose-forms the corresponding **uronic acid**: glucoronic, galacturonic or mannuronic acid. Two important uronic acids are derived from glucose are **glucorunic** and **iduronic acid**, which are epimers (Lecture fig.).

Glycosides are formed from hemiacetals or hemiketals (known as reducing sugars) by reaction with an alcohol. The product is a **full acetal** or **full ketal** (Lecture fig.). Glycosides are not reducing sugars. If the sugar is specifically identified, we may have a glucoside (from glucose), a galactoside (from galactose), etc. Glycosides are fairly stable, and are not readily cleaved by the hydroxide ion. However, the bond linking the alcohol and sugar can be cleaved by enzymes called **glycosidases**. Glycosides are widely distributed in plants and animals.

Sugars can react with phosphoric acid to form **phosphate esters** (Lecture fig.). These compounds are quite important in the metabolism of carbohydrates. Sugar-phosphate esters are relatively stable at neutral pH and possess a negative charge. One effect of sugar phosphorylation within the cell is to trap the sugar inside the cell; most cells do not have plasma membrane and are transporters for phosphorylated sugars. Phosphorylation also activates sugars for subsequent chemical transformation.

Natural polysaccharides may contain sugars modified by **sulfation** or **attachment of an amino group** (Lecture fig.). Two common amino sugars are β-D-glucosamine and β-D-galactosamine. These amino sugars can be further modified by **acetylation** or other alterations. The **N-acetylglucosamine** is part of many structural polymers, including the bacterial cell wall. Bacterial cell walls also contain a derivative of glucosamine, N-acetylmuramic acid, in which lactic acid is ether-linked to the oxygen at C-3 of N-acetylglucosamine. In addition to this hexose derivatives, one acidic sugar deserve mention: **N-acetylneuraminic acid** (a sialic acid), a derivative of N-acetylmannosamine, is a component of many glycoproteins and glycolipids in animals. Also, in the natural polysaccharides, sulfate groups may be attached to sugars in a variety of ways.

7.6. DISACCHARIDES

Disaccharides (such as maltose, lactose, and sucrose) consist of two monosaccharides joined covalently by an **O-glycosidic bond**, which is formed when a hydroxyl group of one sugar reacts with the anomeric carbon of the other. This reaction represents the formation of an acetal from a hemiacetal and an alcohol, and the resulting compound is called a **glycoside**. Glycosidic bonds are readily hydrolysed by acid but resist cleavage by base. Thus, disaccharides can be hydrolysed to yield their free momosaccharide component by boiling with dilute acids, or by glycosidase enzymes.

When the anomeric carbon is involved in a glycosidic bond, the sugar residue cannot take the linear form and therefore becomes a **nonreducing sugar**. In describing disaccharides or polysaccharides, the end of a chain with a free anomeric carbon (one not involved in a glycosidic bond) is commonly called the **reducing end**.

Sucrose (table sugar) is a disaccharide of **glucose** and **fructose** (Lecture fig.). It is formed by plants but not by animals. The sucrose contains no free anomeric carbon atoms; the anomeric carbons of both monosaccharide units are involved in the glycosidic bond. Sucrose is therefore a **nonreducing sugar**. By convention, the name describes a compound written with its nonreducing end to the left, therefore the sucrose is a glucoside and a fructoside. Sucrose is a major intermediate product of photosynthesis; in many plants it is the principal form in which sugar is transported from the leaves to other parts of the plant body. It is commercially prepared from sugar cane or sugar beets.

Lactose is present in milk at approximately 5% concentration and is commercially prepared as a dairy by-product. A disaccharide of **galactose and glucose** (Lecture fig.), is itself a **reducing sugar**, with the glucose attached to one of the oxygens of the galactose unit. This makes lactose technically a galactoside, not a glycoside; it is the glucose unit with its ˝free˝ hemiacetal that gives the disaccharide its character as a reducing sugar. Formally lactose is 4-β-D-galactopyranosyl-D-glucose.

The disaccharide **maltose** (Lecture fig.) contains two D-glucose residue joined by a glycosidic linkage between C-1 (the anomeric carbon) of one glucose residue and C-4 of the other. The breakdown of starch yields this disaccharide. Because the disaccharide retains a free anomeric carbon (C-1 of the glucose residue on the right), maltose is a reducing sugar. The configuration of the anomeric carbon atom in the glycosidic linkage is α.The glucose residue with the free anomeric carbon is capable of existing an α -and β-pyranose form.

Trehalose, Glc(α1↔1α)Glc, a disaccharide of D-glucose, that like sucrose, is a nonreducing sugar and is a major constituent of the circulating fluid (hemolimph of insects) serving as an energy-storage compound. Fungi also contain trehalose and are used as a commercial source of this sugar.

7.7. POLYSACCHARIDES

Polysaccharides (glycans) differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bonds linking the units, and in the degree of branching. **Homopolysaccharides** contain only a single monomeric species; **heteropolysaccharides,** and contains two or more different kinds.

Some homopolysaccharides are stored forms of **fuel** (starch and glycogen) while others (cellulose and chitin for example) serve as **structural elements** in plant cell walls and animal exoskeletons. Heteropolysaccharides provide **extracellular support** for different types of organisms.

Unlike proteins, polysaccharides generally **do not have a defining molecular weight**. For polysaccharide synthesis there is no template; rather the program for polysaccharide synthesis is intrinsic to the enzymes that catalyze the polymerization of monomeric units, and there is no specific stopping point in the synthetic process.

The most important **storage polysaccharides** are starch in plant cells and glycogen in animal cells. Both polysaccharides occur intracellularly as large clusters of granules. Starch and glycogen molecules are heavily hydrated, because they have many exposed hydroxyl groups available to hydrogen bond with water.

Starch, a plant polysaccharide, is a **water-soluble polymer of glucose**, with two main fractions, amylose and amylopectin.

Amylose is a linear polymer of around 200 units of glucose with **α(1→4)** linkages.

Amylopectin is also a glucose polymer, but with at least 1000 glucose units per molecule. Most of the glucose residues are joined by **α(1→4)** linkages but there are branches (occurring every 24 to 30 residues), formed using **α (1→6) linkages**. In the starch, granules strands of amylopectin form double-helical structures with each other or with amylose strands (Lecture figure). Glucose residues at the nonreducing ends of the outer branches are removed enzymatically during the mobilization of starch for energy production.

Glycogen (Lecture fig.) is an **animal polymer of glucose**, similar to amylopectin, but with **more branching** (on average, every 8 to 12 residues). In vertebrates, glycogen is found primarily in the liver and skeletal muscle. In these cells glycogen is found in large granules, which themselves consist of clusters of smaller granules composed of single, highly branched glycogen molecule with a huge molecular weight. Each branch ends with a nonreducing ends, the only reducing end of the molecule buried inside the granule. Such glycogen granules also contain in a tightly bound form, the enzymes responsible for the synthesis and degradation of glycogen.

Cellulose, (Lecture fig.) a fibrous, tough **water insoluble** glucose polimer, is found in the cell walls of plants, particularly in stalks, stems, trunks, and all the woody portions of the plant body. Cellulose constitutes much of the mass of wood, and cotton is almost pure cellulose.

Like amylose, the cellulose molecule is a **linear, unbranched homopolysaccharide**, consisting of 10,000 to15,000 D-glucose units, but the glucose residues in cellulose are linked by **(β1→4) glycosidic bonds**, in contrast to the (α 1→4) bonds in amylose.

It is not digestible by humans or by most animals because they lack an enzyme to hydrolyse the . (β1→4) linkage. Ruminant animals and insects such as termite carry in their gut certain strain of bacteria, that secrete cellulase, which hydrolyses the (β1→4) linkage, enabling these animal hosts to digest cellulose. Wood-rot fungi and bacteria also produce cellulase.

As part of the cell wall, the polymer chain of cellulose lie side-by-side, forming bundles; these bundles are twisted together to create strands and finally visible fibers (lecture figure). Wood contains **lignin** (cross linked C9 aromatic units, related in structure and biosynthesis to the aromatic amino acids; see in lecture figure) embedded in cellulose fibers; the lignin gives the wood its very high strength.

7.8. OTHER NATURAL POLYSACCHARIDES OF INTERESTS

Dextrans are bacterial and yeast polysaccharides made up of (α 1→6) linked poly-D-glucose, all have (α 1→3) branches, and some also have (α 1→2) or (α 1→4) branches. Dental plaque, formed by bacteria growing on the surface of teeth, is rich in dextrans. Synthetic dextrans are used in several commercial products (for example Sephadex) that serve as fractionation of proteins by size exclusion chromatography. The dextrans in these products are chemically cross-linked to form insoluble materials of various porosities.

Chitin is a linear homopolysaccharide composed of N-acetylglucosamine residues in (β1→4) linkage (Lecture fig.) The only chemical difference from cellulose is the replacement of the hydroxyl group at C-2 with an acetylated amino group. Chitin forms extended fibers similar to those in cellulose, and like cellulose, cannot be digested by vertebrates. Chitin is a principal component of the hard exoskeleton of several species of arthropods-insects, lobsters and crabs, and is probably the second most abundant polysaccharide, next to cellulose, found in nature.

Certain marine red algae have cell walls that contain **agar**; a mixture of sulfated and nonsulfated heteropolysaccharides, consisting of D-galactose and L-galactose derivative ether linked between C-3 and C-6. Agar is also used to form a surface for the growth of bacterial colonies. Another commercial use of agar is for capsules, in which some vitamins and drugs are packed; the dried agar material dissolves readily in the stomach and is metabolically inert. **Agarose** is derived from agar as the mostly unsulfated fraction. Once dissolved in hot water and then cooled, it forms a gel with suitable strength for lab use in electroforesis; it is also used as food additive to thicken liquid suspensions.

7.9. CARBOHYDRATES IN THE EXTRACELLULAR SPACE

The extracellular space in the tissues of multicellular animals is filled with a gel-like material, the extracellular matrix (ECM), which holds the cells together and provides a porous pathway for the diffusion of nutrients and oxygen to individual cells.

The reticular ECM that surrounds fibroblast and other connective tissue cells is composed of an interlocking meshwork of **heteropolysaccharides** and fibrous proteins such as fibrillar **collagens, elastin**, and **fibronectin**. Basement membrane is a specialized ECM that underlies epithelial cells; it consists of specialized collagens, laminin, and heteropolysaccharides.

These heteropolysaccharides, the **glycosaminoglycans**, are a family of linear polymers composed of **repeating disaccharide units**. They are unique to animals and bacteria and are not found in plants. One of the two monosaccharides is always either N-acetylglucosamine or N-acetylgalactosamine, the other is in most cases, a uronic acid, usually D-glucuronic or L-iduronic acid. Some glycosaminoglycans contain esterified sulfate groups. The combination of sulfate groups and the carboxylate groups gives glycosaminoglycans a very **high density of negative charge**. To minimize the repulsive forces among the neighboring charged groups, these molecules assume an extended conformation in solution, forming a rod-like helix. Additionally, the charges will help to attract water molecules, so the chains are **heavily hydrated**. The **specific patterns of sulfated and nonsulfated sugar** residues in glycosaminoglycans provides for specific recognition by a variety of protein ligands that bind electrostatically to these molecules.

The sulfated glycosaminoglycans are attached to extracellular proteins to form **proteoglycans (mucopolysaccharides**). Proteoglycans are more carbohydrate than protein, so their properties are mainly determined by the carbohydrate portion of the molecule.

Several types of glycosaminoglycans exist including chondroitin sulfate, hyaluronate, heparan sulfate, and keratan sulfate (Lecture fig.). They are found outside the cell, on the cell surface, or as part of the extracellular matrix. They perform various roles, serving as mechanical support and cushioning joints, as cellular signals involved in cell proliferation and cell migration, and as inhibitors of certain key enzymes.

Hyaluronic acid (HA is also known as hyaluronate) is a long polymer of alternating N-acetylglucosamine and glucuronic acid residues. It serves as a lubricant in joints in the form of synovial fluid; HA is also a principal constituent of the vitreous humor within the eye and it helps to form cartilage. Unlike other GAGs, this polymer is neither sulfated nor attached to a protein core. It is secreted directly to the extracellular matrix. The degradation of HA that occurs after tissue injury, releases smaller chains, which can participate in cell proliferation, migration and differentiation; these degradation products also help to recruit leukocytes to the site of injury. Intra articular injections of purified HA have been used therapeutically in cases of osteoarchitis.

Chondroitin sulfate (CS) is a relatively short polymer, consisting of alternating residues of glucuronic acid and galactose N-acetyl 4-sulfonate. It lends mechanical support and flexibility to tissue; contributes to the tensile strength of cartilage, tendom, ligaments, and the walls of the aorta. **Dermatan sulfate** (DS) is a closely related GAG, which is composed of iduronic acid and N-acetylgalactosamine. It contributes to the pliability of skin and is also present in blood vessels and heart valves. On membranes, CS and DS are involved in interactions with receptors for growth factors, where they may serve as cofactors for various growth factors.

Keratan sulfate (KS) has no uronic acid and their sulfate content is variable. It is formed from alternating units of galactose and sulfated N-acetylglucosamine. It is found in proteoglycans in three forms, two of which is branched. KS is present in cornea, cartilage, bone, and a variety of horny structures formed of dead cells: horn, hair, hoofs, nails, and claws, where it serves primarily as a mechanical/structural role.

Heparan sulfate (HS) is a sulfated polysaccharide, found as a component of cell surface proteoglycans in mast cells, and on the surface of endothelial cells lining blood vessels. It is composed of repeating units of N-acetyl glucosamine and uronic acids (either glucuronic or iduronic acids). Sulfation can be found at several positions on these residues; additionally, the acetyl group on N-acetyl glucosamine may be replaced by a sulfate group. Following an injury to tissue, oligosaccharides derived from this GAG are released and subsequently help to mediate the inflammatory response. Some of the released oligomers promote activity by growth factors, chemokines, and cytokines; others are associated with recruitment of leukocytes to the site of injury.

A very important activity is the action of a particular pentasaccharide sequence (Lecture fig.) as an anticoagulant. This pentasaccharide contains a rare 3-O-sulfated glucosamine (Lecture fig.). The fraction of heparan sulfate containing oligomers with this pentasaccharide sequence is designated as **heparin**. The pentasaccharide binds to and activates the enzyme antitrombin III. This enzyme is responsible for inhibiting thrombin, a protease involved in blood clot formation; thus inhibition of thrombin, in turn, blocks blood clotting. Note that heparin is much smaller than the heparan sulfate and is not linked to a protein core. Heparin is also more highly sulfated than the average random pentasaccharide sequence in heparan sulfates. A synthetic version of the key pentasaccharide is used clinically as an anticoagulant.

7.10. GLYCOCONJUGATES: PROTEOGLYCANS, GLYCOPROTEINS, AND GLYCOLIPIDS

Proteoglycans

Proteoglycans are macromolecules of the cell surface or extracellular matrix in which one or more sulfated glycosaminoglycan chains are joined covalently to a membrane protein or secreted protein. Mammalian cells can produce 40 types of proteoglycans. These molecules acts as tissue organizers, and they influence various cellular activities, such as growth factor activation and adhesion. In the proteoglycan structure, a typical tetrasaccharide linker connects the gycosaminoglycan to a serine residue in the core protein (Lecture fig.).

Many proteoglycan are secreted into the extracellular matrix, but some are integral membrane proteins. For example, there are two major families of membrane heparan sulfate proteoglycans: syndecans and glycipans. **Syndecans** have a single transmembrane domain and an extracellular domain bearing three to five chains of heparan sulfate and in some cases chondroitin sulfate. **Glycipans** are attached to the membrane by a lipid anchor, a derivative of the membrane lipid phosphatidylinositol (Lecture fig.). A protease in the ECM that cuts close to the membrane surface release syndecan ectodomains (those domains outside the plasma membrane), and a phospholipase that breaks the connection to the membrane lipid releases glycipans. Numerous chondroitin sulfate and dermatan sulfate proteoglycans also exist, some as membrane-bound entities, others as secreted products in the ECM.

The glycosaminogylcan chains of proteoglycans can bind to a variety of extracellular ligand through electrostatic interactions and thereby modulate the ligand interaction with specific receptors of the cell surface (Lecture fig.)

Some proteoglycans can form **proteoglycan aggregates**, enormous supramolecular assemblies of many core proteins all bound to a single molecule of hyaluronan (Fig.).

In the ECM, interwoven with these enormous extracellular proteoglycans, are fibrous matrix proteins such as collagen, elastin, and fibronectin, forming a cross-linked meshwork that gives the whole matrix strength and resilience.

The **biosynthesis** of mammalian proteoglycans and the associated GAGs start with the attachment of a xylose residue to the side chain of a serine on the core protein; two galactose residues and a glucoronic acid residue are then attached to the xylose (Lecture fig.). The pathways leading to the different GAG diverge at this point, with different enzymes catalyzing the joining of the sugar residues and more enzymes modifying the residues after they are joined. Chain initiation takes place in the endoplasmic reticulum, and chain elongation and modification occurs in the Golgi complex. The proteoglycans move to the cell surface and to the ECM, carried by vesicles that bud off the Golgi

complex. The biosynthetic path is not applicable to hyaluronic acid, however, because this GAG is not attached to a protein but is instead extruded directly to the ECM.

Sulfation reaction takes place in both the endoplasmic reticulum and the Golgi complex. The donor of the sulfate group is a compound called PAPS, which is derived from ATP and sulfate (Lecture fig.).

Glycolipids

Glycolipids, which consist of sugars attached to lipids, are found in biomembranes. The lipids are sphingolipids, a general class of membrane lipids, which are composed of one molecule of the long-chain aminoalcohol sphingosine, one molecule of a long chain fatty acid and a polar head group. Glycosphingolipids are a subclass of sphingolipids in which sugars are attached to a ceramide molecule (the N-acyl derivative of sphingosine). A single sugar may be attached, or there may be longer chains, some with branches. The combination of ceramide and a single sugar residue is called a cerebroside; a globoside is ceramide with a chain of a couple of sugars; and a ganglioside is ceramide with a rather complex sugar chain attached, involving one or more N-acetylneuraminic acid (sialic acid) at the termini (lecture fig.). Gangliosides are prevalent in the membranes of neurons.

The sugar moiety(ies) of a glycolipid project outward from the surface of the cell´s membrane, and these groups are frequently involved in cell-cell recognition.

Hexosidases are enzymes that break down cerebrosides and gangliosides, removing one sugar residue at a time. These enzymes are found in the lysosomal compartments of the cell. Defects in these enzymes can lead to serious pathologies (lipidosis), such as Tay-Sachs disease. Lecture Table lists several of these glycolipid storage diseases.

Glycoproteins

Many cellular proteins are modified through the attachment of one or more carbohydrate chains, these are glycoproteins.

Unlike proteoglycans, glycoproteins are **mostly protein**, with attached carbohydrate chains. Their properties mostly reflect those of the protein, not the carbohydrate.

The attached **carbohydrate chains** are generally **shorter**, contain **more branching**, and have **more diversity in sugar sequence** than the chains found in proteoglycans. The carbohydrate is attached at its anomeric carbon through a glycosidic link to the –OH of a Ser or Thr residue (O-linked), or through an N-glycosyl link to the amide nitrogen of an Asn residue (N-linked).

In mammals, most cell surface proteins are glycosylated. Glycoproteins may also be found as secreted proteins (in mucins, in hormones such as thyroid-stimulating hormone and erythropoietin, in antibodies, and in lactalbumin). Soluble proteins in the cell may also be glycosylated; for example, ribonuclease B differs from ribonuclease A only in the attachment of sugar chain to a particular asparagin in this enzyme.

Extracellular matrix also contains a number of **multiadhesive glycoproteins** that have multiple domains, each with specific binding sites for other macromolecules and for receptors on the surface of the cells. These glycoproteins contribute to organizing the matrix and helping the cells to attach to it. **Fibronectin**, for example, is a soluble multiadhesive matrix glycoprotein. Fibronectins help regulate the shape of the cells and organize the cytoskeleton They are also essential for migration and cellular differentiation of many cell types during embryogenesis, and important characteristic in healing wounds and blood clotting. **Laminin**, a multiadhesive glycoprotein family, is a prevalent constituent of all basal laminae.

Following protein synthesis, polypeptide chains in eukaryotes **enter the lumen of the endoplasmic reticulum** and later **pass through the Golgi** complex. During this passage, sugars may be attached to a nitrogen in the side chain of Asn residue of the polypeptide or to oxygens in the side chain of Ser or Thr residues. **N-links** are formed in the **endoplasmic reticulum** and in the **Golgi complex**, while **O-links** are formed only in the **Golgi complex**. Complex carbohydrate chains destined to be attached to Asn are first assembled using a special lipid, **dolichol phosphate**, embedded in the membrane of the endoplasmic reticulum. The assembled chain is then transferred to the target protein. Simpler carbohydrate chains start with attachment of a single sugar residue to the target proteins, and the chain is elongated by specialized enzymes. After processing in the endoplasmic reticulum, the glycoproteins are **further modified in the Golgi** complex. Finally, these glycoproteins are sorted in the Golgi complex, to direct them to their proper cellular locations.

Lectins, found in all organisms, are proteins that specifically bind carbohydrate chains. Lectins serve in a wide variety of cell-cell recognition, signaling, and adhesion processes and in intracellular targeting of newly synthesized proteins.

- One class of lectins, C-type lectins, requires calcium ions to help form protein carbohydrate complexes.
- Some microbial pathogens have lectins that mediate bacterial adhesion to host cell or the entry of toxin into cells.
- Viruses enter cells by using the protein hemagglutinin to bind to sialic acid residues on glycoproteins embedded in the cell membrane. The ″H″ part of the designation of strains of influenza virus (e.g. theH1N1 strain of the influenza virus, commonly referred to as swine flu) refers to hemagglutinin; several different, but closely related forms of this viral protein exist. The ″N″ part of the nomenclature for the influenza virus refers to neuraminidase (sialidase), another viral protein of which there are several variants. Neuraminidase is an enzyme that cleaves the glycosidic bond joining the sialic acid residue to the embedded protein after the virus enters cell, an action that frees the virus for unpackaging and replication.
- Selectins (lectins) in the plasma membrane of certain cells mediate cell-cell interactions, such as those of neutrophils with the endothelial cells of the capillary wall at an infection site.
- Lectins also act intracellularly. The mannose 6-phosphate receptor/lectin of the trans-Golgi complex binds to the oligosaccharide of lysosomal enzymes, targeting them for transfer into the lysosome.

7.11. CLINICAL APPLICATIONS

Clotting and heparin

- Heparin is a fraction of heparan sulfate. It is basically a **repeating disaccharide** of glucosamine and iduronate, and therefore isv**heavily sulfa**ted.
- A pentameric sequence within this glycosaminoglycan has great affinity for **antithrombin III**, a plasma protein that inhibits proteases involved in forming blood clots.
- Complexation of heparin with antihrombin **enhances the activity of antithrombin** greatly, so heparin-and the pentasaccharide in particular- is used in **anticoagulant therapy**.
- Heparin is usually prepared from intestinal mucosa, which is rich in heparan sulfate proteoglycans.

Hemoglobin glycosylation and diabetes

- Glucose as a reducing sugar can be **covalently and nonenzymatically** linked to a protein through the side chain amino group of a lysine residue or the terminal $–NH_2$ of the protein chain.

- The extent of glycosylation of a common protein, such as hemoglobin (Hb) in circulating erythrocytes, depends on the **concentration of free glucose**.
- Glucose levels in blood are unusually high in the disease diabetes mellitus, so that Hb becomes glycosylated. Glycosylated hemoglobin is denoted as **HbA1**; **the major sugar derivative** comes from the reaction of glucose with the amino terminal group on the β subunit of Hb. This product, which is denoted as **HbA1c**, can be detected and quantified using suitable clinical assays.
- The lifetime of erythrocyte is 110 to 120 days, so the amount of glycosylated Hb is a record of the average level of glucose in the blood over the previous couple of months. A high level of HbA_{1c} increases high blood glucose levels, signifying that the blood glucose level is not well controlled; conversely, one check for satisfactory control of blood glucose in diabetes mellitus is the reduction in detected HbA_{1c}.

Blood group antigens

- The membrane of red blood cells contain glycolipids that carry oligosaccharides recognized by the immune system.
- The lipid is a derivative of sphingosine.
- The oligosaccharide is composed of a glucose linked directly to the lipid, followed by a galactose residue, an N-acetylgalactoseamine residue, and then a galactose residue. There is usually a terminal L-fucose residue in a 1→2 linkage.
- The exact sequence of sugars, and the manner in which they are linked and branched by glycosyltransferase enzymes, determines the blood group type (Lecture fig.).
    - One type of glycosyltransferase attaches a branching N-acetylgalactosamine to the galactose residue toward the end of the chain, giving the ″A″ blood type.
    - Another type attaches galactose instead, giving the ″B″ blood type.
    - In the ″0″ blood type, neither enzyme is active, and the chain is not branched.
    - A rare blood type is produced by a deficiency in the enzyme that attaches the L-fucose; this is the ″I″ blood type.
    - Other rare blood types are caused by linking the fucose in a 1→4 manner rather than a 1→2 linkage.
- The immune system can produce antibodies against these various short oligosaccharides. People with A-type blood will have antibodies against the B-type oligosaccharides; conversely, people with B-type blood will produce antibodies against the A-type antigen. The antibodies can cause blood cells to clump together, leading to serious circulatory problems. For this reason, it is essential in blood transfusions to match the type of the blood donor to that of the blood recipient. People with the O-type antigens are called universal donors, in recognition of the fact that people with A- or B-type blood lack antibodies to the O antigen. Note, however, that people with O-type blood must receive that type in a transfusion, and not A- or B-type blood, because the A and B antigens would be recognized and attacked by the immune system.

Action of penicillin

A major component of bacterial cell walls is **peptidoglycan**, which is made of **short peptide chains** joined to a **long heteropolysaccharide** of alternating N-acetylglucosamine and N-acetylmuramic acid residues (Lecture fig.).

The peptide chains are unusual in that they often contain a large proportion of **D-amino acids**, instead of the usual L-amino acids. These short **peptide chains act as cross-links** between polysaccharide chains.

The **cross-linking** reaction is catalyzed by peptidoglycan **transpeptidase**. It is this enzyme that is the **target of the antibiotic penicillin**. Without a strong, intact cell wall, the bacteria cannot survive, and penicillin weakens the cell wall by inhibiting the cross-linking reaction.

8. GLYCOLYSIS

Glucose plays a central role in the cellular metabolism. It is relatively rich in potential energy and it serves as a precursor for metabolic intermediates for biosynthetic reactions. Glycolysis is an almost universal central pathway of anaerob glucose catabolism. It takes place in the cytosol because the plasma membrane generally lacks transporters for phosphorylated sugars and so the intermediates cannot leave this compartment. Glycolysis could be divided to two parts: the breakdown of the six-carbon glucose to two three carbon compounds is the preparatory phase; conversion of glyceraldehyde 3-phosphate to pyruvate yielding ATP is the payoff phase.

1. Glucose is first phosphorylated at the hydroxyl group on C-6 forming glucose 6-phosphate, with ATP as the phosphoryl donor. This reaction, which is irreversible under intracellular conditions, is catalyzed by hexokinase. Hexokinase is not specific for glucose it catalyzes the phosphorylation of other common hexoses. The liver specific isozyme of hexokinase is the hexokinase IV (also called glucokinase), which is specific for glucose as a substrate and it is not inhibited allostericly by the product glucose 6-phosphate.
2. The enzyme phophohexose isomerase catalyzes the reversible isomerization of glucose 6-phosphate (aldose) to fructose 6-phosphate (ketose).
3. The committed step of glycolysis is the transfer of a phosphoryl group from ATP to fructose 6-phosphate to yield fructose 1,6-bisphosphate catalyzed by the phophofructokinase-1 (PFK-1). This reaction is irreversible under cellular conditions.
4. Fructose 1,6-bisphosphate is cleaved to yield two different triose phophates: glyceraldehyde 3-phosphate (aldose) and dihydroxyacetone phosphate (ketose). The step, catalyzed by aldolase is reversible and acts in the opposite direction during the process of gluconeogenesis.
5. Dihydroxyacetone phosphate is reversibly converted to glyceraldehyde 3-phosphate by the triose phosphate isomerase and the two glyceraldehyde 3-phosphate molecules are directly degraded in the subsequent steps of glycolysis.
6. The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate by glyceraldehyde 3-phosphate dehydrogenase. The free energy of oxidation of the aldehyde group is conserved by formation of the acid anhydride with phosphoric acid while NAD is reduced to NADH. The active site of the enzyme contains an –SH group (Cys residue) and it can be inhibited by monoiodoacetate. Arsenate toxicity is based on this reaction as well: arsenate is structurally similar to phosphate and glyceraldehyde 3-phosphate dehydrogenase can use it as substrate. The unstable product is then spontaneously degraded and the glycolysis goes on until pyruvate, however the net ATP production will be 0 at that case.
7. The enzyme phosphoglycerate kinase transfers the high-energy phosphoryl group from 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate. This reversible reaction is a substrate-level phosphorylation and the first ATP-producing step in glycolysis.
8. Phosphoglycerate mutase catalyzes a reversible shift of phosphoryl group between C-2 and C-3 of glycerate forming 2-phosphoglycerate.
9. Enolase promotes reversible removal of a molecule of water from 2-phosphoglycerate to yield phosphoenolpyruvate (PEP). The reaction converts a compound with a relatively low phosphoryl group transfer potential to one with high potential. Enolase could be inhibited by fluoride, a method used during clinical blood sugar determination.
10. The last step in glycolysis is the transfer of the phosphoryl group from PEP to ADP, catalyzed by pyruvate kinase. In this irreversible phosphate-level phosphorylation step pyruvate and ATP are formed completing the glycolytic sequence.

In the overall glycolytic process, one molecule of glucose is converted to two molecules of pyruvate and energetically 2 NADH and 4 ATP molecules are formed. Since two ATP are necessary for the

kinase reactions in the preparatory phase the net energy production is the following: glucose + 2NAD + 2ADP –> 2 pyruvate + 2NADH + 2ATP.

Hexoses other than glucose (e.g. fructose and galactose) can undergo glycolysis after conversion to a phosphorylated derivative.

In the muscles and kidney fructose is phosphorylated by hexokinase forming fructose 6-phosphate. In the liver, fructose enters by a different pathway. The liver enzyme fructokinase catalyzes the phosphorylation of fructose at c-1 rather than C-6 yielding fructose 1-phosphate. The fructose 1-phosphate is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by fructose 1-phosphate aldolase. Dihydroxyacetone phosphate is converted to glyceraldehyde 3-phosphate by the glycolytic enzyme triose phosphate isomerase. Glyceraldehyde is phosphorylated by ATP and triose kinase to glyceraldehyde 3-phosphate. Thus both products of fructose 1-phosphate hydrolysis enter the glycolytic pathway as glyceraldehyde 3-phosphate.

Galactose, a product of the hydrolysis of lactose is first phosphorylated in the liver by galactokinase, at the expense of ATP, yielding galactose 1-phosphate. Galactose 1-phosphate is further converted to UDP-galactose by UDP-glucose:galactose 1-phosphate uridylyltransferase. UDP-glucose 4-epimerase forms UDP-glucose from UDP-galactose. Finally the UDP-glucose is converted to glucose 6-phosphate through glucose 1-phosphate. A defect in any of the three enzymes in this pathway (galactokinase, uridylyltransferase, epimerase) causes galactosemia in humans. In galactosemia high galactose concentrations are found in blood and urine. Affected individuals develop cataracts, caused by deposition of the galactose metabolite galactitol in the lens.

8.1. REGULATION

Glycolysis and the glucose producing gluconeogenesis are tightly and reciprocally regulated in coordination with other energy-yielding pathways. When glycolysis is stimulated gluconeogenesis is inhibited and vice versa. The regulation of glycolysis is based on the three irreversible steps. The first (hexokinase) was already discussed. The most important regulatory step is the PFK-1 reaction which is inhibited allosterically by ATP and citrate and activated by AMP and ADP. The hormonal regulation of PFK-1 is based on the amount of the potent allosteric regulator molecule: fructose 2,6-bisphosphate. Upon low blood sugar level, glucagon hormone is secreted stimulating the cAMP-PKA signaling pathway leading to the activation of the bifunctional PFK-2/fructose 2,6-bisphosphatase enzyme’s phosphatase activity. This leads to the breakdown of fructose 2,6-bisphophate inhibiting glycolysis and stimulating gluconeogenesis. Insulin has the opposite effect, stimulating the activity of a phosphoprotein phosphatase (PP) that catalyzes removal of the phosphoryl group from the bifunctional protein PFK-2/FBPase-2, activating its PFK-2 activity, increasing the level of fructose 2,6-bisphosphate, stimulating glycolysis and inhibiting gluconeogenesis. The third regulatory step in glycolysis is the last one the pyruvate kinase step. High concentrations of ATP, acetyl-CoA, long-chain fatty acids, and alanin allosterically inhibit, while fructose 1,6-bisphosphate activates all isozymes of pyruvate kinase. The liver isozyme is subjected to further regulation by phosphorylation: the glucagon-cAMP-PKA pathway phosphorylates and inactivates it. Insulin through activation of PP could dephosphorylate and activate the liver specific pyruvate kinase isoform.

9. GLUCONEOGENESIS

Gluconeogenesis is the biosynthesis of glucose from pyruvate and related three- and four-carbon compounds (lactate, glucoplastic amino acids, glycerol and propionic acid). Gluconeogenesis and glycolysis are not identical pathways running in opposite directions, although they do share reversible steps. However, three reactions of glycolysis are irreversible and cannot be used in gluconeogenesis. In gluconeogenesis, these steps are bypassed by a separate set of enzymes: pyruvate kinase is bypassed by two reactions, the other two irreversible kinase steps (hexokinase, PFK-1) are bypassed by phosphatase enzymes.

1. Pyruvate carboxylase, a mitochondrial enzyme that requires ATP and the coenzyme biotin, converts the pyruvate to oxaloacetate.
2. The oxaloacetate is then converted to PEP by phosphoenolpyruvate carboxykinase. The enzyme has a mitochondrial and a cytosolic isozyme and requires GTP as the phosphoryl group donor. When lactate is the glucogenic precursor cytosolic NADH is generated in the lactate dehydrogenase reaction. In this case oxaloacetate is converted to PEP by the mitochondrial PEP carboxykinase and the PEP is transported out of the mitochondrion to continue on the gluconeogenic path. When cytosolic NADH/NAD ration is low oxaloacetate is first converted to malate in the mitochondrial matrix by malate dehydrogenase, at the expense of NADH. Malate leaves the mitochondrion through a specific transporter and in the cytosol it is reoxidized to oxaloacetate, with the production of cytosolic NADH. The oxaloacetate is then converted to PEP by cytosolic PEP carboxykinase. The transport of malate from the mitochondrion to the cytosol and its reconversion there to oxaloacetate effectively moves reducing equivalents to the cytosol, where they will be used later in the glyceraldehyde 3-phosphate dehydrogenase reaction. The two paths from pyruvate to PEP provide an important balance between NADH produced and consumed in the cytosol during gluconeogenesis.
3. The generation of fructose 6-phosphate from fructose 1,6-bisphosphate is catalyzed by fructose 1,6-bisphophatase, which promotes the essentially irreversible hydrolysis of the C-1 phosphate (no ATP production!). This enzyme is regulated by fructose 2,6-bisphosphate as we saw it earlier.
4. The third bypass is the reversal of the hexokinase reaction producing glucose from glucose 6-phosphate by the glucose 6-phosphatase enzyme (no ATP production!). This enzyme is found on the lumenal side of the endoplasmic reticulum of hepatocytes (and with lesser extinct in renal cells and epithelial cells). Glucose 6-phosphate is transported to ER lumen by a special transporter (T1) and the products are transported back to the cytosol by T2 (glucose) and T3 (phosphate) transporters. Since glucose 6-phosphatase is a liver specific enzyme this tissue has a significant importance in glucose production and maintenance of blood glucose level.

For each molecule of glucose formed from 2 pyruvates requires four high-energy phosphate groups from ATP and two from GTP. In addition, two molecules of NADH are required for the reduction of two molecules of 1,3-bisphosphoglycerate.

The source of carbon skeleton for gluconeogenesis, apart from pyruvate, could be the lactate through the Cori-cycle and alanin through the alanin-cycle. The first one transports carbon skeleton to the liver mainly from the erythrocytes and from the muscles; the latter one from the muscles. Some or all of the carbon atoms of most amino acids (except lysine and leucine) are ultimately catabolized to pyruvate or to intermediate of citric acid cycle. Such amino acids can undergo net conversion to glucose and are sad to be glucogenic. No net conversion of fatty acids to glucose occurs in mammals since acetyl-CoA cannot be converted to glucose. However, glycerol, produced by the breakdown of fats (triacylglycerols) can be used for gluconeogenesis. Phosphorylation of glycerol by glycerol kinase, followed by oxidation of the central carbon, yields dihydroxyacetone phosphate, an intermediate in gluconeogenesis

in liver. Moreover, the oxidation of odd number fatty acids yields propionyl-CoA which can further metabolized in three steps to succinyl-CoA, a citric acid cycle intermediate.

Glycolysis and gluconeogenesis are reciprocally regulated through the fructose 2,6-bisphosphate as we saw it in the previous chapter.

10. GLYCOGEN METABOLISM

Glucose 6-phosphate has a central role in the carbohydrate metabolism. Its function in the glycolysis and gluconeogenesis was already discussed. Later we will describe the pentose phosphate pathway, where glucose 6-phosphate is the starting compound. Now, we turn our attention to the glycogen metabolism, another process in glucose 6-phosphate is involved.

Glycogen is the stored form of glucose in vertebrates. Glycogen is found primarily in the liver and skeletal muscle. Mobilization of energy from glycogen stores is a rapid process and it works under anaerobic conditions as well, however it contains less energy than the same amount of stored fat. Glycogen is the polymeric form of glucose using 1,4-glycosidic bonds inside the chain and 1,6-bonds in the branch points. From the branch points new chains are started forming a large particle which consists of up to 50 000 glucose residues with about 2 000 nonreducing ends. The large number of nonreducing ends is necessary for the fast metabolism of glycogen and it increases the solubility of the macromolecule. There is only one reducing end (the reducing OH- group of the first glucose unit) in a glycogen molecule bound by glycogenin (see later). There are different functions and different regulation of glycogen stored in different tissues: the glycogen in muscle is there to provide a quick source of energy. Liver glycogen serves as a reservoir of glucose for other tissues and to regulate blood sugar levels.

10.1. SYNTHESIS

1-2. The starting point for synthesis of glycogen is glucose 6-phosphate which is converted to glucose 1-phosphate in the phosphoglucomutase reaction. The product of this reaction is converted to UDP-glucose by the action of UDP-glucose pyrophosphorylase. The reaction is essentially irreversible because pyrophosphate is rapidly hydrolyzed by inorganic pyrophosphatase. Tagging of glucose with UDP is a way to set a pool of hexoses inside the cell for one purpose (here glycogen synthesis) and since UDP is an excellent leaving group it facilitates the attachment of glucose to the glycogen chain during the synthesis.

3. UDP-glucose is the donor of glucose residues in the reaction catalyzed by glycogen synthase, which promotes the transfer of the glucose to a nonreducing end of a glycogen chain containing at least 8 glucose residues so far. This is the committed step of glycogen synthesis.
4. Branch points are formed by the glycogen-branching enzyme, also called transglycosylase, or glycosyl transferase. This enzyme catalyzes transfer of a terminal fragment of 7 glucose residues from the nonreducing end of a glycogen branch having at least 11 residues to the C-6 hydroxyl group of a glucose at a more interior position of the same or another glycogen chain, thus creating a new branch.
5. Glycogen synthase cannot initiate a new glycogen chain de novo. Glycogenin is the protein which catalyzes the first steps of the synthesis producing an 8 glucose unit containing *primer* with its intrinsic glycosyl-transferase activity. Glygogenin remains in the middle of the glycogen particle, covalently attached to the single reducing end of the glycogen olecule.

10.2. DEGRADATION

1. Glycogen phosphorylase as the main enzyme in glycogen degradation catalyzes the reaction in which a glucose residue at a nonreducing end of glycogen undergoes attack by inorganic phosphate producing glucose 1-phosphate. Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction. The enzyme acts on the nonreducing ends of glycogen until it reaches a point four glucose residues away from a branch point.

2. Further degradation can occur only after the debranching enzyme, which catalyzes two successive reactions that transfer branches. First, the transferase activity of the enzyme shifts a block of three glucose residues from the branch to a nearby nonreducing end. The single glucose residue remaining at the branch point in 1,6 linkage, is then released as free glucose by the enzyme's glucosidase activity. This is the only step during the degradation of glycogen where free glucose instead of glucose 1-phosphate is produced.
3. Glucose 1-phosphate, the end product of glycogen phosphorylase reaction, is converted to glucose 6-phosphate by phosphoglucomutase in a reversible reaction. The glucose 6-phosphate formed from glycogen in skeletal muscle can enter glycolysis; in liver it is further converted to glucose by glucose 6-phosphatase and released into blood.

10.3. REGULATION

Glycogen metabolism is under coordinated hormonal and allosteric regulation. The key enzymes in this process are the glycogen phosphorylase and the glycogen synthase. Glucagon and epinephrine could stimulate the glycogen degradation and inhibit glycogen synthesis; insulin has an opposite effect: it stimulates glycogen synthesis and inhibits breakdown.

Glycogen phosphorylase is activated by phosphorylation and inactivated by dephosphorylation. In response to glucagon (liver) or epinephrine (muscle) cAMP concentration is increased activating PKA (cAMP-dependent protein kinase A). PKA activates phosphorylase kinase, which then activates glycogen phosphorylase. In muscle, there are two allosteric control mechanisms beside the covalent regulation. Ca^{++} ,the signal for muscle contraction, activates phosphorylase kinase stimulating the glycogen breakdown. AMP, which accumulates in contracting muscle as a result of ATP breakdown, activates glycogen phosphorylase, speeding the release of glucose 1-phosphate. This activation cascade has a large amplification effect on the initial signal, which can reach a 10 000 fold amplification resulting a release of 10 000 glucose molecules into the blood after a single hormone-receptor interaction. Glycogen phosphorylase of liver acts as a glucose sensor, since it is regulated allosterically by glucose. Glucose enters hepatocytes and binds to an inhibitory allosteric site on glycogen phosphorylase resulting a conformational change which leads to the dephosphorylation of the enzyme by phosphoprotein phosphatase (PP1). Insulin also acts indirectly to stimulate PP1 and slow glycogen breakdown. Phosphorylation and dephosphorylation of glycogen phosphorylase by glucagon and insulin, respectively is a typical example of opposite hormonal regulation of one key enzyme in a metabolic process.

Glycogen synthesis is mainly under insulin regulation. Insulin binding to its receptor activates a tyrosine kinase in the receptor. In one hand, it phosphorylates the insulin-sensitive kinase which activates PP1. PP1 then dephosphorylates glycogen synthase, activating it and dephosphorylates glycogen phosphorylase, inactivating it. On the other hand, insulin receptor activates protein kinase B (PKB) through phosphatidylinositol 3-kinase and a protein kinase called PDK-1. PKB phosphorylates glycogen synthase kinase 3 (GSK3) inactivating it. The inactivation of GSK3 means activation of glycogen synthase since GSK3 is an inhibitory kinase of its target glycogen synthase. PKA could also phosphorylate and inactivate glycogen synthase, thus PKA stimulates glycogen degradation through glycogen phosphorylase and inhibits the glycogen synthesis through glycogen synthase. Beside the hormonal regulation, glycogen synthase is under the stimulatory regulation of glucose and glucose 6-phosphate.

Glycogen targeting protein (G_M) in the muscle can be phosphorylated at two different sites in response to insulin and epinephrine. Insulin-stimulated phosphorylation of G_M activates PP1, which dephosphorylates phosphorylase kinase, glycogen phosphorylase and glycogen synthase. Epinephrine-stimulated double phosphorylation of G_M by PKA, causes dissociation of PP1 from the abovementioned enzymes. In that way, insulin inhibits glycogen breakdown and stimulates glycogen synthesis, and epinephrine has the opposite effects.

Glycogen storage diseases:

- von Gierke disease: lack of glucose 6-phosphatase – accumulation of glycogen because the phosphorylated glucose cannot leave the hepatocytes
- McArdle disease: lack of muscle glycogen phosphorylase – there is no glycogen degradation. Lack of ATP for muscle contraction.
- Andersen disease: lack of branching enzyme – there are no branch points in the glycogen chain, no glycogen granules, impossible to maintain the blood sugar level – lethal disease
- Pompe disease: lack of 1,4-glucosidase – accumulation of glycogen in the lysosomes

11. PENTOSE PHOSPHATE PATHWAY

Beside the glycolytic breakdown, an alternative fate of glucose 6-phosphate is the direct oxidation of glucose 6-phosphate to pentose phosphates by the pentose phosphate pathway. In this oxidative pathway **NADPH** and **ribose 5-phosphate** are produced in the cytosol and the pathway has two phases: oxidative and nonoxidative.

11.1. OXIDATIVE PHASE

1. The first reaction, the committed step of the pathway, is the irreversible oxidation of glucose 6-phosphate by **glucose 6-phosphate dehydrogenase** to form **6-phosphoglucono-δ-lactone**. NADP is the electron acceptor forming **NADPH**.
2. The lactone is hydrolyzed to **6-phosphogluconate** by a specific **lactonase**.
3. The next step is an **oxidative decarboxylation** in which **ribulose 5-phosphate** and **NADPH** are produced by the enzyme **6-phosphogluconate dehydrogenase**. The net result of the oxidative phase is the production of **2NADPH** and **CO_2**.

Ribulose 5-phosphate is a branch point: phosphopentose **isomerase** converts it to its aldose isomer, **ribose 5-phosphate**, a precursor for nucleotide synthesis; or ribulose 5-phosphate **epimerase** converts it to **xylulose 5-phosphate** as the first step of the nonoxidative phase.

11.2. NONOXIDATIVE PHASE

In a series of rearrangements of the carbon skeletons, six pentoses (ribulose 5-phosphate) are converted to five hexoses (glucose 6-phosphate). Two enzymes unique to the pentose phosphate pathway act in these interconversions of sugars: **transketolase** and **transaldolase**. Transketolase catalyzes the transfer of a two-carbon fragment from a ketose donor to an aldose acceptor, the cofactor is **TPP**. The ketose becomes aldose and the aldose becomes ketose. In the pentose phosphate pathway, the first transketolase converts xylulose 5-phosphate (C5) and ribose 5-phosphate (C5) to glyceraldehyde 3-phosphate (C3) and sedoheptulose 7-phosphate (C7). Then in the second transketolase reaction xylulose 5-phosphate (C5) and erythrose 4-phosphate (C4) are converted to glyceraldehyde 3-phosphate (C3) and fructose 6-phosphate (C6). Transaldolase catalyzes a reaction similar to the transketolase but three instead of two carbons are transferred: sedoheptulose 7-phosphate (C7) and glyceraldehyde 3-phosphate (C3) are converted to erythrose 4-phosphate (C4) and fructose 6-phosphate (C6). As a consequence of further enzymatic steps, involving glycolytic and gluconeogenic enzymes, glucose 6-phosphate is formed. Overall, **six pentose** phosphates have been converted to **five hexose** phosphates completing the cycle.

11.3. REGULATION

Regulation of the pentose phosphate pathway is based on the **NADP/NADPH** ratio. Glucose 6-phosphate dehydrogenase is regulated by availability of the substrate NADP. As NADPH is utilized in reductive synthetic pathways, the increasing concentration of NADP allosterically stimulates the pathway, to replenish NADPH. When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction, NADPH concentration rises and inhibits glucose 6-phosphate dehydrogenase directing the glucose 6-phosphate to the glycolysis.

The role of **NADPH** and **glutathione** in protecting cells against reactive oxygen species was already discussed. Glutathione however has another important role in **phase II. detoxification**. Conjugation of glutathione with different xenobiotics helps in the elimination process as we will see later. To detoxification, NADPH is also necessary since it provides the reducing power for **cytochrome P450**

monooxygenase system. Finally, NADPH provides electrons for biosynthetic reactions such as fatty acid, cholesterol or steroid hormone synthesis.

Ribose 5-phosphate is a precursor for nucleotide and nucleic acid synthesis. Both for purine and pyrimidine nucleotides **5-phosphoribosyl 1-pyrophosphate (PRPP)** is the precursor molecule which is produced from ribose 5-phosphate by the **PRPP synthetase** reaction.

The flow of glucose depends on the need for NADPH, ribose 5-phosphate and ATP.

1. **Ribose 5-phosphate is required.** In rapidly dividing cells nucleotide (DNA, RNA) biosynthesis requires large amount of pentose phosphate and relatively low amount of NADPH. Glucose 6-phosphate dehydrogenase and so the oxidative phase is inhibited by NADPH, while the nonoxidative phase produces three molecules of ribose 5-phosphate from two molecules of fructose 6-phosphate and from one molecule of glyceraldehyde 3-phosphate.
2. **There is need for NADPH and ribose 5-phosphate.** Under these conditions the oxidative phase is active and the nonoxidative is inactive. The result is the formation of two NADPH and one ribose 5-phosphate.
3. **NADPH is required.** In six complete pentose phosphate pathways (oxiadative and nonoxidative phases) glucose 6-phosphate is completely oxidized to six CO_2 with the concomitant generation of 12 NADPH. In this situation ribose 5-phosphate produced by the pathway is recycled into glucose 6-phosphate by the enzymes of the nonoxidative phase.
4. **NADPH and ATP are required.** Ribose 5-phosphate formed by the oxidative branch can be converted into pyruvate. Fructose 6-phosphate and glyceraldehyde 3-phosphate enter the glycolysis rather than the nonoxidative branch. In this mode, ATP and NADPH are generated, and five of the six carbons of glucose 6-phopshate emerge in pyruvate. Pyruvate can be further oxidized to generate more ATP by the pyruvate dehydrogenase complex and in the citric acid cycle.

Glucose 6-phosphate dehydrogenase deficiency affects about 400 million people worldwide. One of the symptoms is hemolytic anemia, usually appears with damaged hemoglobin molecules in the red blood cells, the so called **Heinz bodies**. Red blood cells are highly sensitive for the defect of the pentose phosphate pathway because in these cells NADPH is extremely important for the proper function of glutathione system. In G6PD-deficient individuals, the NADPH production is diminished and ROS elimination is inhibited. This deficiency however has an important advantage: resistance to **malaria**. The parasite Plasmodium falciparum is very sensitive to oxidative damage and is killed by a level of oxidative stress that is tolerable to a G6PD-deficient human host. Another pentose phosphate pathway-related genetic defect is the **Wernicke-Korsakoff syndrome** a disorder caused by a severe deficiency of thiamine (TPP). The syndrome can be exacerbated by a mutation in the gene for transketolase resulting a lowered affinity for TPP. The syndrome is more common among people with alcoholism and with chronic malnutrition.

12. METABOLISM OF COMPLEX POLYSACCHARIDES

Glycosylation of molecules may occur with protein posttranslationally (**glycoproteins**) and with lipids (**glycolipids**). The **polysaccharide complex** may contain a variety of subunits expressing divergent types of bonding. These complex polysaccharides contain *unusual sugar* units as well as *specific derivatives* of them (see lecture material). These often give rise to a *large number of variations* with specific physiological **functions**: cell surface receptors, cell-cell interactions, attachment of pathogens, endocytosis, signal transduction – just a few to mention. In the biosynthesis of these polysaccharides, specific **glycosyltransferases** are involved which use nucleotide derivatives of sugar units as substrates. In the break down processes of the polysaccharides, **glycosidases** takes place which specifically hydrolyze the different types of glycosidic bonds.

Divergent sugars may be synthetized from glucose. Every monosaccharide enters the **interconverting reactions** with phosphorylation by ATP. Via isomerization, phosphorylation, oxidoreduction, decarboxylation and transamidation (glutamine), a variety of sugar derivative molecules are synthetized. They can be linked to several **nucleotides** (UTP, GTP, TTP) which means they are activated for further synthetic reactions as well as "tagged" for other routes than energy providing reactions. Each of these synthetic pathways has a negative feed-back control of its specific end product.

Nucleotide coupled sugars are the products of pyrophosphorylase enzymes (such as, UDP-glucose pyrophosphorylase). The pyrophosphate generated in the reaction is hydrolysed rapidly by pyrophosphates thus making the reaction energetically favorable. The formation of *glucuronic acids*, one of the important intermediates, are carried out by UDP-glucose dehydrogenase (see lecture material). Many exogen (drugs, toxins) and endogen (bilirubin) substances are conjugated with glucuronic acid for elimination.

One type of polysaccharides is the **glycosaminoglycans** (GAGs) which are built up by repeated disaccharide units, many of whom are carrying an immense number of negative charged groups (sulfate and carboxyl) (see more in: Structure of carbohydrates). **Proteoglycans** are complex molecules in which chains of GAGs are attached to a protein core where the polypeptide part is the relatively smaller.

The **synthesis of proteoglycans** is illustrated with the example of chondroitin sulfate synthesis. It starts with the polypeptide synthesis to which activated (UDP-bound) monosaccaharides are joined one after another. After a few sugar units, the characteristic disaccharide units are added repeatedly meanwhile the necessary sulfations take place with the help of PAPS (3'-phosphoadenosine 5'-phosphosulfate) as sulfate donor and sulfotransferase enzymes.

Glycoproteins are the products of the posttranslational protein modification systems in the endoplasmic reticulum (ER) and the Golgi apparatus. These polysaccharide units are shorter than in the case of proteoglycans. They can be attached to the proteins with *N*- (to asparagine) or *O*- (serine, threonine, 5-hydroxylysine) glycosyl bonds in a simple (*O*-linked) or complex type. In the *N*-linked polysaccaharides there are two major subtypes: high mannose and complex. Both of them possess a core pentasaccharide unit and further modifications occur in the ER and the Golgi. *O*-linked modifications only take place in the ER.

The **synthesis** of the *N*-linked polysaccharide complex starts with *dolichol phosphate,* which is located in the ER membrane. After the core oligosaccharide unit is ready, the entire complex flips over into the ER lumen. Additional sugar units are added as dolichol phosphate activated derivatives. The oligosaccharide complex is then transferred to a special asparagine of the receiving polypeptide. Sugar units are the subjects of changes as the glycoprotein is *maturating* as well as of *quality control* before release from the ER (see more details in molecular biology and in the lecture). There a couple of *antibiotics* which specifically interact with oligosaccharide synthesis.

There are known enzyme deficiencies of the complex polysaccharide synthesis with a wide variety of clinical signs.

Glycolipids, glycoproteins and proteoglycans are **degraded** by some common enzymes (glycosidases, deacetylases, sulfatases). The absences of specific lysosomal enzymes are responsible for the decreased break down of these glycosylated molecules which will result in lysosomal **accumulation** of the original or partially degraded substrates. One group of these **storage diseases** is the mucopolysaccharidoses, caused by the defect of proteoglycan break down. Notably a rare condition is the I-cell disease, in which the lysosomes contain no functional hydrolyzing enzymes. The reason for this is due to an enzymatic block in the addition of mannose 6-phosphate to lysosomal enzymes as a targeting signal. Enzymes are synthetized and found in blood and urine, but they are not able to enter the lysosomes.

13. FATE OF PYRUVATE

1. Pyruvate formed in glycolysis could be further converted under anaerobic conditions to ethanol and CO_2 (alcoholic fermentation). The enzyme pyruvate decarboxylase converts pyruvate to acetaldehyde, followed by alcohol dehydrogenase reaction yielding ethanol. In the first reaction decarboxylation needs TPP as a cofactor, in the second NADH is oxidized to NAD. This letter step gives the significance of the process since NAD regeneration is necessary for maintaining glycolysis.

2. Pyruvate could be further converted under anaerobic conditions to lactate as well by the enzyme lactate dehydrogenase (lactic acid fermentation). The reduction of two molecules of pyruvate to two of lactate regenerates two molecules of NAD. The lactate formed by active skeletal muscles or by erythrocytes can be recycled; it is carried in the blood to the liver, where it is converted to glucose in the gluconeogenesis. This cycle is known as Cori cycle. The enzyme lactate dehydrogenase consists of four subunits. These subunits are encoded in two different genes (M and H) so there are five different lactate dehydrogenase isoforms with five different subunit composition, with different tissue distribution and affinity to the substrate (pyruvate).

3. Similarly to the Cori cycle, the carbon skeleton of pyruvate could be transported from muscles to the liver in the form of alanin. In alanin cycle, pyruvate is first converted to alanin by transaminase in muscles which is then carried in the blood to the liver, where it is converted back to pyruvate and ammonium. Pyruvate enters the gluconeogenesis forming glucose; ammonium is converted further to urea in the urea cycle.

4. Under aerobic conditions pyruvate, formed in glycolysis, is transported to mitochondrial matrix through a pyruvate transporter. The mitochondrial enzyme pyruvate dehydrogenase complex (PDC) converts pyruvate to acetyl-CoA. This reaction is an oxidative decarboxylation because NADH is formed (oxidation) and CO_2 is produced (decarboxylation). Five cofactors participate in the reaction mechanism: coenzyme A, NAD, FAD, TPP, lipoate. The PDC consists of three distinct enzymes: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), dihydrolipoyl dehydrogenase (E3).

Reaction mechanism:

E1: TPP is the prosthetic group of the enzyme. Pyruvate reacts with TPP, undergoing decarboxylation yielding hydroxyethyl thiamine pyrophosphate. This first step is the slowest and therefore limits the rate of the overall reaction.

E2: lipoate is the prosthetic group of the enzyme which transfers acetyl group from hydroxyethyl TPP to CoA to yield acetyl-CoA. Oxidation of the substrate is accompanied with the reduction of the lipoyl group.

E3: promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E2 to the FAD, and the reduced $FADH_2$ transfers hydride ion to NAD forming NADH.

The reaction catalyzed by PDC is irreversible. There is no bypass mechanism to convert acetyl-CoA to pyruvate.

13.1. REGULATION OF PDC

PDC is under tight allosteric and covalent regulation. Members of the complex are regulated separately. E1 is regulated by AMP/ATP ratio (cellular energy balance); E2 by CoA/acetyl-CoA ratio (substrate/product-based regulation); E3 by NAD/NADH ratio (cellular redox state). Covalent regulation is

based on the phosphorylation (inactivation)-dephosphorylation (activation) of E1. The first process is catalyzed by the enzyme PDH kinase which is under allosteric regulation too: NADH and acetyl-CoA could activate; pyruvate and ADP could inactivate it. Dephosphorylation of PDH is catalyzed by PDH phosphatase which is regulated by Ca^{++} as a hormone-mediated second messenger. Taken together, at the presence of molecules indicating low energy level in the cell PDC is activated, and ample of energy is a signal to inhibit the activation of PDC and the related cellular processes such as the citric acid cycle.

14. CITRIC ACID CYCLE

Oxidation of acetyl-CoA to CO_2 takes place in the **mitochondria**. During the process there are **two CO_2** one **GTP** and **four reduced coenzyme** (three NADH and one FADH) are produced. Intermediates of the citric acid cycle are siphoned off as biosynthetic precursors. The cycle is a hub in metabolism, with degradative pathways leading in and anabolic pathways leading out, and it is closely regulated in coordination with other pathways.

Albert Szent-Györgyi has been played an important role in the discovery of the citric acid cycle. His major contribution was the description of the role of fumaric acid in biological combustion processes. Later Hans Krebs described the entire cycle, which is also called after him Krebs cycle.

1. The first step of the cycle is the condensation of **acetyl-CoA** with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**. The CoA is cleaved hydrolytically and the reaction is essentially **irreversible**.
2. The enzyme **aconitase** catalyzes the reversible transformation of citrate to **isocitrate**. Aconitase contains an iron-sulfur center, which acts in the catalytic addition or removal of H_2O.
3. **Isocitrate dehydrogenase** catalyzes **oxidative decarboxylation** of isocitrate to form α-**ketoglutarate**. The leaving carbon atom is released as **CO_2** and a reduced **NADH** is also produced.
4. The next step is another **oxidative decarboxylation**, in which α-ketoglutarate is converted to **succinyl-CoA** and **CO_2** by the action of **α-ketoglutarate dehydrogenase complex**. NAD serves as an electron acceptor and **NADH** is produced. This reaction is virtually identical to the pyruvate dehydrogenase complex reaction discussed earlier in both structure (three enzymes: E1, E2, E3) and function (the same 5 cofactors: **NAD, FAD, CoA, TPP, lipoate**).
5. Succinyl-CoA has a high energy thioester bond. Energy released in the breakage of this bond by **succinyl-CoA synthetase** is used to the synthesis of **GTP** (equivalent to ATP). This succinate producing reversible reaction is another example for the **substrate-level phosphorylation**.
6. The succinate is oxidized to **fumarate** by the **succinate dehydrogenase**, which enzyme is tightly bound to the mitochondrial inner membrane. The enzyme contains three different iron-sulfur clusters and one molecule of FAD which is reduced during the reaction yielding **FADH**. Since succinate dehydrogenase is a part of the mitochondrial respiratory chain (Complex II) the electrons from FADH are passed to **ubiquinone** and then further to the final electron acceptor.
7. The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase**. This enzyme is highly stereospecific: L-malate is always the product.
8. In the last reaction of the cycle **NAD-linked malate dehydrogenase** catalyzes the oxidation of malate to **oxaloacetate**. The concentration of oxaloacetate in the mitochondria is extremely low pulling the malate dehydrogenase reaction toward the formation of oxaloacetate. Low mitochondrial **[NADH]:[NAD]** ratio is also necessary to this reaction. Without the oxidation of NADH by the respiratory chain the citric acid cycle would stop because of the accumulated NAD in the mitochondria.

14.1. REGULATION

The flow of carbon atoms from pyruvate into and through the citric acid cycle is under tight regulation which is based on the intracellular energy balance. The sites of the regulation are the **irreversible** reactions: **PDC, citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex** and the concentration of oxaloacetate. The major controls of the cycle are the

[NADH]:[NAD] and the **[ATP]:[ADP]** ratios. When these ratios are high the irreversible steps are inhibited When these ratios decrease, allosteric activation occurs. Feedback inhibition by **succinyl-CoA**, **citrate** and **ATP** also slows the cycle. In muscle, Ca^{++} signals contraction and stimulates the PDC, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex reactions. The concentration of **oxaloacetate** is also an important regulatory point since this metabolite has the lowest concentration in the mitochondria. Reactions, which could replenish the citric acid cycle intermediates, most importantly oxaloacetate, are the **anaplerotic** reactions.

The most important **anaplerotic** reaction in mammalian liver and kidney is the reversible carboxylation of pyruvate by CO_2 to form oxaloacetate, catalyzed by **pyruvate carboxylase**. The enzyme needs **biotin** as a cofactor and **ATP**. Oxaloacetate produced in this reaction is used in the gluconeogenesis as well beside the citric acid cycle. Another oxaloacetate yielding anaplerotic reaction is the **phosphoenolpyruvate carboxykinase** (or carboxylase in plants, yeasts and bacteria) where GTP is the source of energy. **Malic enzyme** catalyzes the reversible conversion of pyruvate to malate and the malate could further converted to oxaloacetate. In the opposite direction the reaction produces **NADPH**, important reducing equivalent for biosynthetic pathways and for maintaining the antioxidant capacity of glutathione. **Transaminase** reactions could also serve as source of ketoacids for the citric acid cycle: glutamate -> α-ketoglutarate; aspartate -> oxaloacetate. And finally, the **oxidative deamination of glutamate** to α-ketoglutarate by **glutamate dehydrogenase** is an anaplerotic reaction too.

15. TRANSPORT PROCESSES LINKED TO THE CITRIC ACID CYCLE

Since the mitochondrial inner membrane is impermeable for most of the molecules, special transport processes are necessary for proper transport between the cytosol and the mitochondrial matrix.

1. Transport systems of the inner mitochondrial membrane carry ADP and P_i into the matrix and newly synthesized ATP into the cytosol. The ADP/ATP exchange is processed by the **adenine nucleotide translocase** (antiporter); the phosphate is transported to the matrix together with **proton** (H^+) by the **phosphate translocase** (symporter).

2. Transport of cytosolic NADH into the mitochondria I. **Glycerol 3-phosphate shuttle**:

   This shuttle operates in **skeletal muscle** and the **brain**. In the cytosol, dihydroxyacetone phosphate accepts two reducing equivalents from **NADH** (generated in the glycolysis) in a reaction catalyzed by **cytosolic glycerol 3-phosphate dehydrogenase**. A **mitochondrial isozyme** of glycerol 3-phosphate dehydrogenase, bound to the outer face of the inner membrane, then transfers two reducing equivalents from glycerol 3-phosphate through **FAD** in the intermembrane space to **ubiquinone (Q)**. Since this shuttle delivers the reducing equivalents from NADH to Complex III and not to Complex I, missing one proton pump in the respiratory chain, it provides only enough energy to synthesize **1.5 ATP** molecules per pair of electrons. This is the **P/O ratio** which means the ratio of phosphorylation (ATP synthesis) to oxidation (NADH -> NAD).

3. Transport of cytosolic NADH into the mitochondria II. **Malate-aspartate shuttle**:

   This shuttle for transporting reducing equivalents from cytosolic NADH into the mitochondrial matrix is used in **liver**, **kidney**, and **heart**. First NADH in the cytosol passes two electrons to oxaloacetate, producing malate by the **cytosolic malate dehydrogenase**. Malate crosses the inner membrane via the malate - α-ketoglutarate transporter. In the matrix, malate passes two electrons to NAD, and the resulting **NADH** is oxidized by the respiratory chain (Complex I). Oxaloacetate formed by the **mitochondrial malate dehydrogenase** cannot pass directly back into the cytosol. Oxaloacetate is first **transaminated** by aspartate aminotransferase to aspartate and aspartate can leave via the glutamate-aspartate transporter. Oxaloacetate is regenerated in the cytosol by aspartate aminotransferase, completing the cycle. Substrates necessary for transamination reactions (α-ketoglutarate, glutamate) are circulating via the two transporters. Electrons of NADH moved in by this shuttle enter the respiratory chain at Complex I and yield a **P/O ratio of 2.5**.

   Using either shuttle mechanism, only the reducing equivalents are transported through the mitochondrial inner membrane and not the NADH itself! The cytosolic and the mitochondrial NAD pools are separated.

4. **Activated fatty acids** with 14 or more carbons, cannot pass directly through the mitochondrial membrane, they need a special shuttle, the **acyl-carnitine/carnitine shuttle**. First, fatty acyl-CoA is attached to the hydroxyl group of **carnitine** to form **fatty acyl-carnitine**. It moves into the matrix through the transporter in the inner membrane. In the matrix, the acyl group is transferred to mitochondrial coenzyme A, freeing carnitine to return to the cytosol through the same transporter. This shuttle links two separate pools of coenzyme A, one in the cytosol, the other in the mitochondria.

5. **Acetyl-CoA** used in fatty acid synthesis is formed in mitochondria from pyruvate oxidation and from catabolism of the carbon skeletons of amino acids. The mitochondrial inner membrane is impermeable to acetyl-CoA, so acetyl groups pass out of the mitochondrion as **citrate** on the **citrate transporter**. In the cytosol, citrate cleavage by **citrate lyase** re-

generates acetyl-CoA and oxaloacetate in an ATP-dependent reaction. Oxaloacetate cannot return to the matrix directly instead, cytosolic malate dehydrogenase reduces it to malate, which can return to the mitochondria through the malate - α-ketoglutarate transporter. However, most of the malate produced in the cytosol is used to generate cytosolic **NADPH** through the activity of **malic enzyme**. The pyruvate produed by the malic enzyme is transported to the mitochondria by the pyruvate transporter, and converted back into oxaloacetate by pyruvate carboxylase completing the cycle. The fate of the citrate is based on the energy needs of the cell. High **ATP** concentration inhibits the enzyme **isocitrate dehydrogenase**, and so the citric acid cycle, leading to the shuttle of the acetyl groups out of the mitochondria supporting the storage of energy in the form fatty acids.

16. OXIDATIVE PHOSPHORYLATION

All oxidative steps in the catabolic pathways converge at the final stage of cellular respiration, in which the reduced coenzymes are oxidized back serving energy for the synthesis of **ATP**. The final electron acceptor is the **O_2** which is reduced to **H_2O**. In eukaryotes, **oxidative phosphorylation** occurs in the inner membrane of mitochondria. The electron-transfer chain consists of membrane bound large enzyme **complexes** (Complex I-V) and mobile elements in the membrane (ubiquinone, also called Q) and in the intermembrane space (cytochrome c). Iron containing electron-carrying molecules are also involved in the transport of electrons: **(1) cytochromes** are proteins with iron-containing heme prosthetic groups; **(2) iron-sulfur proteins** in which iron is present in iron-sulfur centers; **(3)** some complexes contain **Fe-Cu** in their electron-transfer center. Reducing equivalents from NADH and FADH are passed through the respiratory complexes and the energy of electron flow is conserved by the concomitant pumping of protons across the membrane, producing an **electrochemical gradient, the proton motive force**. This force is the drive of the ATP synthesis in the mitochondria and since oxidation of reduced coenzymes is coupled to the phosphorylation of ADP to ATP, the process is called **oxidative phosphorylation**.

1. **Complex I (NADH dehydrogenase; NADH:ubiquinone oxidoreductase)**

   Complex I catalyzes two coupled processes: **(1) $NADH^+ + H^+ + Q \rightarrow NAD^+ + QH_2$; (2) $4H^+_N \rightarrow 4H^+_P$** , where subscripts indicate the location of the protons: P for the positive side (the intermembrane space) and N for the negative side (the matrix). The overall reaction is: **$NADH^+ + 5H^+_N + Q \rightarrow NAD^+ + QH_2 + 4H^+_P$** . Electrons from mitochondrial NADH pass through Complex I, using an FMN-containing flavoprotein and at least six iron-sulfur centers, to ubiquinone. During electron transfer four protons are pumped from the matrix to the intermembrane space. **Amytal, rotenone, and piericidin A** inhibit electron flow from the Fe-S centers of Complex I to ubiquinone.

2. **Complex II (succinate dehydrogenase)**

   Succinate dehydrogenase is the only one membrane-bound enzyme of the citric acid cycle. Electrons move from succinate to FAD, then through the three Fe-S centers to ubiquinone. Since **Complex II hasn't got proton pump activity**, electron flow through this complex does not contribute to the electrochemical gradient.

   Ubiquinone is the point of entry for electrons derived from **Complex I**, **Complex II**, fatty acid oxidation, and from reactions in the cytosol. Acyl-CoA dehydrogenase (from fatty acid oxidation) transfers electrons to **electron-transferring flavoprotein (ETF)**, from which they pass to Q. The **glycerol 3-phosphate** shuttle donates electrons from cytosolic NADH-producing reactions to ubiquinone.

3. **Complex III (ubiquinone:cytochrome c oxidoreductase)**

   Complex III contains cytochromes (b and c) and iron-sulfur proteins and transports electrons from ubiquinol (QH_2) to **cytochrome c** with the vectorial transport of protons from the matrix to the intermembrane space. Complete oxidation of QH_2 needs the reduction of two cytochrome c and during these two cycles four protons are transported through the membrane. Cytochrome c is a soluble protein of the intermembrane space and it can move freely from Complex III to Complex IV to donate the electron. Specific inhibitors of Complex III are **antimycin A** and **myxothiazol**. The overall reaction catalyzed by Complex III is: **$QH_2 + 2$ cit c (oxidized) $+ 2H^+_N \rightarrow Q + 2$ cit c (reduced) $+ 4H^+_P$** .

4. Complex IV (cytochrome oxidase)

In the final step, Complex IV carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O. Electron transfer through Complex IV is from cytochrome c to the Cu center, to heme a, to the heme a_3 – Cu center, and finally to O_2. For every four electrons passing through this complex, the enzyme transports four protons from the matrix to the intermembrane space. This four-electron reduction of O_2 involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates (reactive oxygen species) that would damage cellular components. The overall reaction catalyzed by Complex IV is: **4 cyt c (red.) + $8H^+_N$ + O_2 -> 4 cyt c (ox.) + 4 H^+_P + $2H_2O$**. Specific inhibitors of this complex are **CN** and **CO**.

The transfer of two electrons from NADH through the respiratory chain to molecular oxygen can be written as: **NADH + $11H^+_N$ + $1/2O_2$ -> NAD^+ + H_2O + $10H^+_P$** . The energy stored in the proton gradient is the proton-motive force, the ultimate driving force for ATP synthesis.

5. Complex V (F_oF_1 ATP synthase)

Mitochondrial ATP synthase is an **F-type ATPase** consisting of two components. **F_1** is a peripheral membrane protein in the inner surface of the inner mitochondrial membrane and it has 9 subunits ($\alpha_3\beta_3\gamma\delta\varepsilon$). Each of the β subunits has one catalytic site for **ATP synthesis**. **F_o** is an **oligomycin-sensitive** (that is why *o* denoting oligomycin and not zero) protein, which is integral to the membrane forming a **proton channel**. F_o subunit consists of one *a* and two *b* subunits and a *c* ring. The mechanism of ATP synthesis was described as a **rotational catalysis** in which the three active sites of F_1 take turns catalyzing ATP synthesis. The three β subunits of F_1 have three different conformations: (i) binds ADP and P_i; (ii) binds ATP; (iii) empty. One round of catalysis (360°) means the synthesis of one ATP molecule in three consecutive steps (120°). The conformational changes, and so the rotation, are driven by the passage of protons through the F_o portion of ATP synthase. The overall reaction equation is: **ADP + Pi + nH^+_P -> ATP + H_2O + nH^+_N.**

ATP synthesis is measurable in intact mitochondria, as is the decrease in O_2. The number of ATP synthesized per O consumed gives the **P/O ratio**. The most widely accepted experimental value for P/O ratio is 2.5 when mitochondrial NADH is the electron donor and 1.5 when succinate. At the case of cytosolic NADH the ratio depends on the shuttles: 2.5 for malate-aspartate and 1.5 for glycerol 3-phosphate shuttle.

16.1. REGULATION

Oxidative phosphorylation is not under classical allosteric or covalent regulation. The rate of oxidation and ATP synthesis is regulated by the energy demand of the cell. In short, ATP is formed only as fast as it is used in energy-requiring cellular activities. The target of this regulation is the F_oF_1 ATP synthase, however inhibition of Complex V has an overall effect on the respiratory chain because oxidation (respiratory chain) and phosphorylation (ATP synthesis) are **coupled**. Separation of these two processes leads to **uncoupled mitochondria**, in which respiration occurs without ATP production. The classical proton ionophore **2,4-dinitrophenol (DNP)** is an uncoupling agent that can shuttle protons across mitochondrial inner membrane, dissipating the proton gradient. Instead of producing ATP, the energy is lost as heat. Most newborn mammals, including humans, have a type of adipose tissue called **brown adipose tissue** with mitochondria containing a special protein in their inner membrane: **thermogenin** or **uncoupling protein (UCP)**. UCP provides a path for protons back to the matrix without passing through the F_oF_1 ATP synthase. As a result, the energy of oxidation is not conserved as ATP but dissipated as heat, which contributes to maintaining the body temperature. Beside newborns, hibernating animals use the same system to generate heat during their long dormancy.

Several steps in the path of oxygen reduction in mitochondria have the potential to produce reactive free radicals. Complete reduction of O_2 to H_2O needs four electrons in the cytochrome oxidase reaction. This step is the major site for reactive oxygen species production. The passage of electrons to ubiquinone from Complex I and II; and the passage of electrons from QH_2 to Complex III are also potential reactions to generate free radicals. **Superoxide, hydrogen peroxide** and the most reactive **hydroxyl radical** formed in these reactions, can react and damage enzymes, membrane lipids and nucleic acids. Moreover, hydrogen peroxide can participate further reactions (Fenton, Haber-Weiss) producing more hydroxyl radicals. Reactive oxygen species are generated not only by the mitochondrial respiratory chain but also by **NADPH oxidases** which are membrane-bound enzyme complexes in neutrophils. Free radicals produced by NADPH oxidases play an important role in the immune response killing bacteria during respiratory burst. Regulation of redox-sensitive signaling pathways is also depends on reactive oxygen species produced by the mitochondria and NADPH oxidases.

To prevent oxidative damage, cells have several enzymatic and non-enzymatic systems to eliminate free radicals. **Superoxide dismutase** produces hydrogen peroxide from superoxide: $\mathbf{2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2}$. The cytoplasmic isoform of the enzyme contains **Cu** or **Zn** ions, the mitochondrial one contains **Mn** ion. Hydrogen peroxide is further reduced by **catalase ($\mathbf{2H_2O_2 \rightarrow 2H_2O + O_2}$)**, an enzyme found mainly in the liver, kidney and blood mononuclear cells. Another option to eliminate H_2O_2 is the **glutathione peroxidase** reaction: $\mathbf{2GSH + H_2O_2 \rightarrow GSSG + 2H_2O}$. Reduced glutathione (**GSH**) is oxidized in the reaction to **GSSG**, which is re-reduced by **glutathione reductase** using electrons from the **NADPH** generated by the pentose phosphate pathway. Defect of key enzymes of the pentose phosphate pathway (e.g. glucose 6-phosphate dehydrogenase) leads to inappropriate NADPH production and increased oxidative stress level. Non-enzymatic systems based on antioxidant molecules and vitamins such as vitamin C, vitamin E, bilirubin, vitamin A, uric acid and several natural antioxidant molecules.

17. LIPIDS

17.1. CLASSES AND CHEMICAL PROPERTIES OF LIPIDS

Biological lipids are a chemically diverse group of compounds, the common and defining feature of which is their insolubility in water. For convenience, lipids may be categorized into **five different classes** (Lecture Fig.):, based on their structure:

- **Fatty acids** are distinguished by their terminal carboxylic acid moiety and long saturated or unsaturated carbon chains.
- Glyceryl esters are built from fatty acids esterified to the trihydric glycerol molecule; **phosphoglycerides** (or glycerophospholipids) are formed from fatty acids and glycerol 3-phosphate, and **triglycerides** are formed from a glycerol esterified with three faty acids.
- **Sphingolipids** are built up from fatty acids and sphingosine, a long chain alcohol carrying an amine group.
- **Sterols** have a characteristic structure of four fused rings (the steroid nucleus).
- **Terpenes** are formed by the head –to-tail condensation of isoprene units (2-methyl-1,3-butadiene). Terpenes include vitamins A, E, and K; biological pigments such as β-carotene, and many essential oils derived from plants.

17.2. BIOLOGICAL ROLES OF LIPIDS

- They serve as building blocks of phospholipids and glycolipids, which are components of biomembranes;
- They act as hormones and second messengers inside cells, for communication and regulation;
- Lipids are also fuel molecules and serve as a way for the cell to store energy;
- They act as “molecular recognition” features, in the acylation of proteins, for targeting of proteins to membranes and organelles.
- Not to be overlooked is the service of certain unsaturated lipids as protective anti-oxidants (e.g., vitamin E), as pigments and odorants, detergents and membrane cofactors.

17.3. FATTY ACIDS

Fatty acids are carboxylic acids with hydrocarbon chains ranging from 4 to 36 carbons long (C4 to C36). In some fatty acids, this chain is unbranched and fully **saturated** (contains no double bonds-saturated fatty acids), in others the chain contains one or more double bonds (**mono-or poly-unsaturated fatty acids**) (Lecture Fig.).

The most commonly occuring fatty acids have even numbers of carbon atoms in an unbranched chain of 12 to 24 carbons). In most monounsaturated fatty acids the double bond is between C-9 and C-10 (Δ9), and the other double bonds of

polyunsaturated fatty acids are generally Δ12 andΔ15. (Arachidonic acid is an exception to this generalization) The double bonds of polyunsaturated fatty acids are almost never conjugated, but are separated by a methylene group, and the double bonds are in the cis configuration. (Trans fats are derived from polyunsaturated natural fats (soy oil, etc.) by an industrial hydrogenation process leaving some double-bonds in the trans geometry).

17.4. NOMENCLATURE

- **Δ-numbering** convention: starting from the carboxylate end as carbon #1.
- **n or ω numbering**: numbering starts from the other end.
- **Greek letter** convention: carbons 2 and 3 (in the Δ-numbering convention) usually are denoted as α and β, respectively. The terminal methyl group is usually denoted as ω.
- There are also multiple conventions on denoting the **location of the double bonds**.

1. cis-Δ9 -cis double bond between carbon 9 and 10 (e.g. palmitoleic acid),
2. 18:3(9,12,15)-C18 FA with three double bonds at position 9,12, and 15 (e.g. linolenic acid)
3. ω9 or ω-9-the distance from the terminal methyl group is given

17.5. Ω-3 (OMEGA-3) FATTY ACIDS

It is a family of **polyunsaturated fatty acid** (PUFA) with special importance in human nutrition. Omega-3 fatty acids are major constituents of fish oil, and are thought to be the compounds responsible for these cardio-protective activities. These fatty acids actually originate from phytoplankton and are passed up the food chain until they reach humans.

Two common omega-3 fatty acids are eicosapentaenoic acid (**EPA**; 20:5(Δ5,8,11,14,17)), which has 20 carbons in the chain and 5 double bonds, and docosahexaenoic acid (**DHA**; 22:6(Δ4,7,10,13,16,19)), which has 22 carbons and 6 double bonds (Lecture fig.).

17.6. ESSENTIAL FATTY ACIDS

Essential: the human body is unable to synthesize it, yet it serves as a precursor to other biomolecules made by the body

- **Linoleic acid** (18:2(Δ9,12)) is essential: it must be provided in the diet. It is a precursor to other eicosanoids (lecture fig.).
- **Arachidonic acid** is considered by some authorities to be an essential fatty acid, although it is synthesized from linoleic acid and thus not itself absolutely required in the diet. Arachidonic acid is a precursor to leukotrienes, prostaglandins, and thromboxanes.
- **α-linolenic acid** (18:3(Δ9,12,15)) is also essential, and the precursor of other omega-3 fatty acids (EPA, DHA) important in cellular functions.

Some of the most important fatty acids found in mammalian tissue are **acetic acid**; which is related to the common metabolic intermediate acetyl-CoA; **palmitic,, stearic, and oleic acids**, that are prevalent in phospholipids and triglycerides, and are common in biomembranes and in adipose tissue deposits of fat (lecture fig.). **Linoleic, linolenic, and arachidonic acids** are minor constituents of phospholipids, so are mainly associated with biomembranes.

Lecture Table shows a number of dietary sources-both animal and plant- of fatty acids.

The animal fats tend to have a higher proportion of saturated and long chain fatty acids when compared with plant oils, the one exception being coconut oil).

17.7. OTHER IMPORTANT PROPERTIES OF FATTY ACIDS

- Fatty acids are **carboxylic acids** (pKa about 4.8); ionized under physiological conditions.
- They have **long nonpolar "tails"** that reduce their solubility in water (the longer the fatty acil chain and the fewer the double bonds, the lower is the solubility in water).

  - Most fatty acids are **slightly soluble in water**, but at acid pH the aqueous solubility is negligible.
- Having one or more **double bonds** in the tail will **lower the melting point** of the free fatty acid.
  - This is due to steric interference in the packing of tails by the bends or kinks in the tail caused by the cis geometry of the double bonds (lecture fig.). This has implications for the temperature-dependence of the fluidity of biomembranes.
  - Unsaturated fatty acids (as phospholipids) can modulate the close packing of tails in the lipid bilayer of the membrane, thereby altering the local fluidity or viscosity. This in turn can change the activity of membrane-embedded enzymes and transport systems.

In vertebrates, the free fatty acids (unesterified fatty acids, with a free carboxylate group) circulate in the blood bound noncovalently to a protein carrier, serum **albumin**. However, fatty acids are present in blood plasma mostly as carboxylic acid derivatives such as esters or amides. Lacking the charged carboxylate group, these fatty acid derivatives are even less soluble in water than are free fatty acids (they are transported in **lipoproteins**).

17.8. TRIACYLGLYCEROLS (TRIGLYCERIDES, TRIACYLGLYCERIDES OR NEUTRAL FATS)

Triacylglycerols are composed of **three fatty acids each in ester linkage with a single glycerol**. They can contain the same kind of fatty acid in all the three positions (called simple triacylglycerols), but most naturally occuring triacylglycerols are mixed; they contain two or three different fatty acids.

Because the polar hydroxyls of glycerol and the polar carboxylates of the fatty acids are bound in ester linkage, triacylglycerols are **nonpolar, hydrophobic molecules**, essentially **insoluble in water**. Lipids have **lower specific gravities than water**, which explains why mixtures of oil and water have two phases (oil floats on the aquous phase).

17.9. FAT AS A FUEL

Triglycerides are stored as oil droplets in adipose tissue or in the seeds of plants (lecture fig.) Adipocytes and germinating seeds contain lipases, enzymes catalyzing the hydrolysis of stored triacylglicerols, releasing fatty acids for export to sites where they are required as fuel.

There are **two significant advantages** to using triacylglycerols as stored fuels, rather than polysaccharides such as glycogen and starch.

- First the carbon atoms of fatty acids are more reduced than those of sugars, and oxidation of triacylglycerols yields more than twice as much energy (about 38kJ/g versus about 17kJ/g for glycogen and starch), as the oxidation of carbohydrates.
- Secondly, due to tryacylglicerols which are characteristically hydrophobic and therefore non-hydrated, meaning the organism does not bear the extra weight of water and or hydration often associated with stored polysaccharides.

Moderately obese people with 15 to 20 kg of triglyceride deposited in their adipocytes potentially can meet their energy needs for months by drawing on their fat stores. In contrast, the human body can store less than a day´s energy supply in the form of glycogen.

Carbohydrates such as glucose and glycogen do offer certain advantages as quick source of metabolic energy, one of which is their ready solubility in water.

Fatty acid oxidation provides at least half the oxidative energy in major organs and tissues.

Monoglycerides (monoacylglycerols) and **diglycerides** have one or two fatty acids, respectively, and are attached to glycerol (Fig.). Monoglycerides have some slight solubility in water; triglycerides usually do not. Monoglycerides or diglycerides may appear with the acyl groups at positions 1,2 or 3 of the glycerol backbone.

17.10. Phospholipids, Sphingolipids and Eicosanoids

See in complex lipid metabolism chapter

17.11. Cholesterol

17.12. Structure and Chemical Properties of Cholesterol

- Cholesterol has a characteristic structure with **four fused rings (steroid nucleus**). The numbering and ring lettering system is presented in lecture fig.). The steroid nucleus is almost planar and is relatively rigid; the fused ring does not permit rotation about C-C bond.
- Cholesterol is **amphipathic**, with a polar head group (the hydroxyl group at C3) and a nonpolar hydrocarbon body (the steroid nucleus and the hydrocarbon side chain at C17), about as long as a C16-carbon fatty acid in its extended form.
- The presence of only one polar functional group best defines cholesterol as being nonpolar, featuring **low aqueous solubility and high lipid solubility**.
- Cholesterol is the major sterol in animal tissues, and similar sterols are found in other eukaryotes: **stigmasterol** in plants and **ergosterol** in fungi. Bacteria cannot synthesize sterols, but a few bacteria can incorporate exogenous sterols into their membranes.
- Cholesterol is synthesized from simple five-carbon isoprene subunits.
- Cholesterol **can be esterified with fatty acids** (e.g., with palmitic or linoleic acid), resulting in greater hydrophobicity.
- The normal range for serum cholesterol is 3.9 to 6.2 mmol/L, or 150 to 240 mg/dL and is dependent on sex, age, diet and emotional stress.
  - Approximately 70% of plasma cholesterol is esterified.
  - This high value is due to much of the cholesterol being bound by plasma lipoproteins.

17.13. Biological Roles of Cholesterol

- Cholesterol is an **important membrane constituent** that serves to stiffen neighboring acyl groups and reduce their flexibility, thus overall reducing membrane fluidity.
  - This can be important for the functioning of membrane-embedded proteins (cross-membrane transporters and channels, enzymes, and various receptors) and for cellular processes such as cell division or phagocytosis.
- Cholesterol and sphingolipids can associate to form recognizable domains (termed ″lipid rafts″) in the outer leaflet of the lipid bilayer of a biomembrane.
- Cholesterol is a **precursor to** digestive and solubilizing agents (**bile acids**, such as cholic acid, or conjugates with glycine or taurine) (lecture fig.).
- Cholesterol is a **precursor to the steroid hormones**, such as the gonadal hormones estradiol and testosterone, or the adrenocortical hormone hydrocortisone (cortisol) (Fig.).
- **Vitamin precursor**(vitamin D3 or cholecalciferol) (lecture fig.).

18. MEMBRANES AND TRANSPORT PROCESSES

Membranes are composed of **lipids and proteins**. The ratio of the two major components depends on tissue and subcellular location of the membrane. The basic lipids in the membranes are glycerophospholipids, sphingolipids and cholesterol. Their exact proportion is not identical within the membrane of the cell and the subcellular organelles. Lipid components may carry out special **movements** (rotation, lateral diffusion) in the membrane. Also, the proteins of the membrane are able to laterally diffuse. Proteins can be integral membrane proteins with one or more transmembrane (TM) segments, or can be attached to the membrane covalently or via non-covalent (electrostatic, hydrophobic) interactions. The composition of the inner and out leaflets differs from one another.

Membranes have highly **selective permeability** since they have to protect the intracellular environment and must provide the transport mechanisms for the building blocks and energy providing molecules for the cell, including the excretion of harmful waste products. Due to the hydrophobic interior of the membranes, they are permeable to hydrophobic, small neutral molecules (gases, lipophilic molecules), and slightly to water and impermeable to large, polar molecules and ions (transmembrane protein transport: in molecular biology studies).

Membrane transport proteins can be divided into **two basic groups**: channels (and pores) and carriers (or transporters).

Main characteristics of the **channels**: selectivity, gating mechanism (ligand, voltage, stretch or temperature activated) and they carry out facilitated diffusion (down concentration gradient).

Carriers or transporters: primary active (with the energy of ATP or illumination they generate and maintain the concentration or electrochemical gradients – pumps), secondary active transporters (use the gradient created by the pumps, and carry compounds against concentration gradients), and passive transporters (facilitated diffusion). With respect to the direction, they can be: uniporters, symporters (cotransporters) and or antiporters (exchangers).

The **mechanism** in which a compound can pass the membrane via channel or transporter is not the same: after opening a channel to allow the ion or other component pass through, while simultaneously the transporter is binding the molecule and following conformational change, release it on the other side of the membrane.

18.1. DIFFUSION THROUGH MEMBRANE

Distinctively, only inert, small and/or lipophilic molecules can diffuse passively through a membrane. This reaction is directly proportional to the concentration of the material which can only diminish the concentration gradient. Diffusion depends on a couple of physicochemical properties: solubility and diffusion coefficient (shape, size) of the compound.

18.2. TRANSPORTERS

The transported molecule moves only a small distance within the membrane via four steps of the transport: recognition (binding) of the molecule, transport through the membrane, release of the molecule on the other side of the membrane and recovery of the transporter. The transport of charged molecules can be electrogenic (leads to membrane potential) or neutral. Transporters are similar to enzymes which are specific for the cargo, they can be inhibited, they increase the rate of reaching the equilibrium, but not due to change, they are saturable. The ATP driven primary transporters are: P-type (SERCA, Na^+-K^+ ATPase), V-type and F-type. The secondary active transporters are mainly electro-chemical potential (SMF: sodium motive force, PMF: proton motive force) driven transporters and,

notably, they can act as symporters or antiporters. The passive transporters carry out facilitated transport down the concentration gradient (uniport).

An example of transporters is the **GLUT family**, of which, the members move glucose by facilitated transport. The various types of the family are located in tissues including the intestine, pancreas, liver heart, muscle and exhibit distinct regulation. Among the secondary active transporters, the sodium/glucose cotransporter (symporter) is noted, including many other symporters of the enterocytes and cells of the kidney, in the role of transporting sugars and amino acids. The neurotransmitters released into the synaptic cleft are taken up by sodium coupled symporters, too, which are targets of a number of drugs. By inhibiting them, the concentration of the neurotransmitters can be increased. In polarized cells (enterocytes), more types of transporters act on the apical and basal surfaces (see details in the lecture). Numerous antiporter systems help to maintain the intracellular milieu; these are either Na^+-driven exchangers (H^+, Cl^- -HCO_3^-) or independent exchangers, like Cl^- -HCO_3^- exchanger to keep the pH normal.

18.3. ATP-DRIVEN PUMPS

ATP-driven pumps belong to the primary active transporters which use the energy of ATP hydrolysis to transport substances against concentration gradient. These pumps create chemical or electrochemical gradients. The pumps can be saturated, inhibited and they are specific. The transporter protein may become transiently phosphorylated. **Subfamilies** for inorganic ion transport: P-type (becoming phosphorylated and dephosphorylated), V-type (proton pump, acidification of vacuoles) and F-type (works in an opposite way: transports protons with ATP synthesis in mitochondrion). Among P-type ATPases, we mention Ca^{2+} and Na^+-K^+ pumps (Na^+-K^+ pump (or ATPase).

This pump, which is found in every cell throughout the animal kingdom, generates and maintains the high intracellular K-ion concentration. It is an **electrogenic** pump, whereas 2 potassium-ions move into the cell while 3 sodium-ions exit. The operation of this pump helps the membrane potential to further develop. The pump is working fast and using a vast amount of energy. It has an N (nucleotide binding), P (phosphorylation) and A (activator) **domains**. *Cardiac glycosides* inhibit this pump and create increased heart muscle contractility.

18.4. CA^{2+} PUMP

In the cytosol, the Ca-ion concentration is low. When an action potential causes Ca^{2+} release from the ER store for muscle contraction, this elevated Ca-ion concentration should also then be reduced. Two Ca^{2+} pumps (**SERCA**: in the ER and **PMCA**: in the plasma membrane) and a Na^+-Ca^{2+} exchanger (antiporter) are responsible for re-establishing low cytosolic Ca-ion concentration. The working mechanism of SERCA: in the presence of Ca-ions SERCA is then becoming phosphorylated at a special Asp residue. The resulted phosphoprotein binds Ca^{2+} stronger and generates a conformational change in the transporter which will cause the release of the Ca-ions on the other side of the membrane.

A **V-type of transporter** is the vacuolar ATPase. Its function is to pump protons into acidic organelles (endosomes and lysosomes). It contains two large domains, a cytosolic with ATP binding and catalytic (hydrolysis) site and a membrane bound domain which is responsible for the proton translocation from the cytosol into the organelles. As we noted before, a number of exchangers are helping to maintain cytosolic pH, too.

18.5. ABC TRANSPORTERS

A large superfamily with many members earned its name because they contain conserved ATP binding domains (“cassettes”). They bind many different types of solute molecules and two ATPs. Following ATP binding, the transporter suffers a conformational change so the bound small molecule will dissociate itself off on the other side of the membrane. Following the hydrolysis of the ATPs, the transporter returns to its original conformation. Most of the ABC transporters facilitate substance (peptides, toxic components and drugs) **movement out of the cell** yet only in one direction. A special group of these transporters is the MDR (multidrug resistance) family. The glycoprotein members of this family are responsible for removing a number of drugs, including cytostatic compounds from the cells.

18.6. DRUG TRANSPORT PROTEINS

Drug transport proteins are grouped into influx and efflux transporters. The former group has members of the SLC (solute carrier) family, which operate in the facilitated diffusion or as secondary active transporters. Efflux transporters (ABC transporters) remove drugs from the cell. Other types of division of the transporters is the absorptive (from gut lumen through the cell into the blood) and the secretary (into bile, urine, gut lumen) transporters.

18.7. CHANNELS

Several special channels include the gap junction between cells and the nuclear pore complexes on the nuclear membranes for protein; however, RNP transport is not discussed here.

In this chapter, we describe the narrow, very **selective and gated** channels of the plasma membrane which are responsible for rapid and controlled ion movement by facilitated diffusion. The structure of these channels can be alpha-type or beta-type depending on the secondary structure of the TM domain of the protein. Today, the examples we consider include the auaporins, the voltage gated ion channels and the ligand gated (nicotinic) acetylcholine (ACh) channel. The channels can be **blocked** by specific inhibitors. The gating mechanisms are mainly, through the change of the transmembrane potential (voltage gated) or by binding of specific ligands (extracellular like many neurotransmitters or intracellular like messenger molecules: cAMP, Ca^{2+}, IP_3). A special group of these ion channels are the G-protein linked receptor coupled channels which were formerly discussed in molecular biology (muscarinic Ach, neurotransmitters, sensory receptors, hormones). Both ligand gated and voltage gated channel have different **positions** including the following, open (active), closed (basal) and inactivated when the channel seems to be open by blocked with and inactivating mechanism (see ball and chain theory for quick closing of the channel). The conformational change and opening of the channel is initiated by either the ligand binding (ligand gated) or the movement of a special TM segment with positive charges (voltage gated).

Special channels are defined as the aquaporins. They increase the rate of diffusion of water molecules (epithelial cell in kidney, endocrine tissues). They have to be very selective for water so the cell will not lose or gain any ions through them. This is solved by a narrow opening which will not permit hydrated ions through the opening. Also, specific amino acid side chains orient the dipole water molecules in the proper positioning.

18.8. ION CHANNELS

Ion channels are very selective for ions providing a **selectivity filter** at the narrow path of their TM domains. Ion flux increases to a certain level with ion concentration elevation, but the selectivity filter provides the limit for the transporting rate. Channels will open for a specific stimulus, but just for a brief

period of time as ion movement down concentration gradient is very fast. After a long time, impulses within the channels are inactivated (**desensitized**) and the stimulus has to be removed for the possibility of further activation. Ion channels are indispensable in electrically excitable muscle and nerve cells.

18.9. K^+ CHANNELS

There many types of K^+ channels according to **gating mechanisms**: K^+ leak channels are responsible for maintaining of the membrane potential, while voltage dependent, ligand gated, ATP sensitive and Ca^{2+}-activated channels all have specific functions. The structure of the channel depends on the type of gating, but the selectivity filters are similar. Though the filter is very narrow and the ions are binding tightly, their movement is rapid due to the repelling force between them. Some well-known diseases (cardiac and neurological signs) are caused by genetic mutations of the coding regions of potassium-ion channels. Acetyl choline ligand gated channels are permeable for sodium and potassium ions after opening. High conductance channels are sensitive for Ca^{2+} concentrations, too (smooth muscle cells). The ATP sensitive K^+ channels are directly in contact with the cell's metabolic status (cardiomyocytes, insulin secretion).

Na^+ channels can be gated several ways by voltage and ligands as well. The voltage gated channels are targets of numerous *drugs* (anaesthtetic, antiarrythmic, antiepileptic drugs). Na^+ channels are essential for action potential generation.

Epithelial Na^+ channels are regulated hormonally. They are involved in secreting saliva and through sodium reabsorption in the urinary tract. The latter channels are targets of antidiuretic drugs.

Sodium ion transport is complex, include primarily active transporters, secondary active transporters and ion channels all involved.

18.10. CA^{2+} CHANNELS

Ca^{2+} channels of the plasma membrane are gated by extracellular ligands, voltage and intracellular calcium ion concentration. They are *targets of drugs* against hypertension, cardiac arrhythmias, epilepsy and anxiety disorders. At neuronal synapses, the action potential of the presynaptic membrane will open voltage-gated Ca^{2+} channels. The increased calcium ion concentration will result in the fusion of **neurotransmitter** containing vesicles with the presynaptic membrane. Following the membrane fusion, neurotransmitters will be released into the synaptic cleft where they bind to and open ligand gated ion channels and generates **action potential** in the postsynaptic membrane (excitatory neurotransmitters – open Na^+ and sometimes Ca^{2+} channels). In *muscle cells*, Ca^{2+} channels are behaving differently. Action potential is generated in excitatory cells' membranes by organized opening and inactivation of ion channels (sodium and potassium). In the case of muscle cells, calcium ion channels also play an important role. Voltage gated, ligand gated channels including exchangers, can be involved in creating an action potential. To re-establish the original membrane potential, Na^+ -K^+ ATPase has to function. In muscle cells, after the action potential generated contraction, the intracellular calcium ion concentration has to be reduced to normal. Inhibitory neurotransmitters open K^+ channels or Cl^- channels.

19. CLINICAL IMPORTANCE OF LIPIDS

Lipids are energy storage molecules of the body as well as important structural (membranes), functional (signaling molecules, transport, digestion) and biosynthetic precursor (hormones, intracellular messengers, vitamins) compounds. Out of these large numbers of functions, we now focus our concentration on three areas of special pharmaceutical interest: metabolism of eicosanoids, lipopolysaccharides and lysosomal storage diseases.

Eicosanoids are 20-carbon fatty acids including (patho)physiologically important molecules such as *prostaglandins (PGs),* leukotrienes and thromboxanes. The precursor of these molecules is **arachidonic acid** (20:4) which is a derivative of the essential linoleic acid. Arachidonic acid has been located in cell membrane phospholipids from where it is released by *phospholipase* A_2. PGs have numerous physiological **activities**: some are involved in inflammation or platelet aggregation, others inhibit gastric acid secretion or are involved in reproduction. The exact functions of **leukotrienes** are not known: they play role in white blood cell functioning, smooth muscle cell contractions in the airways and bronchoconstriction. Some **thromboxanes** are helping platelet aggregation.

One of the enzymes in the pathway of synthesis of arachidonic acid derivatives is **prostaglandin H synthase,** featuring two enzymatic acitivities: *cyclo-oxygenase (COX)* and *hydroperoxidase*. The product PGH_2 is the source of other PGs, prostacyclins and thromboxanes. They act in the above described functions as local hormones. Leukotrienes are produced on a different pathway from arachidonic acid by the enzymes *lipoxygenases* which are mixed function oxidases located in several tissues.

In humans, there are two types of COX enzymes **COX1 and COX2.** The former one is working constantly and protects the gastric mucosa and kidney functions. COX2 activity produces PGs which are responsible for the signs of **inflammation**, pain and fever. One group of drugs called non-steroidal anti-inflammatory drugs (**NSAIDs**) has an effect on COX synthesis. An old and prominent representative of the group is **Aspirin** (acetylsalicylic acid) irreversibly inactivates COX by acetylating a crucial serine residue of the enzyme active site. Several other NSAIDs act by noncovalent inhibition of COX activity. These inhibitors are blocking both COX1 and COX2. There attempts to develop a **selective** COX2 inhibitor without side effects on gastric mucosa and platelet aggregation.

5-lipoxygenase is the enzyme which is involved in leukotriene biosynthesis from arachidonic acid. Drugs blocking 5-lipoxygenase or the leukotriene receptors are used to fight the symptoms of asthma bronchiale.

Gram negative bacteria carry **lipopolysaccahrides (LPS)** in their outer membrane. This consists of a lipid A core with polysaccharides attached to it. This complex molecule generates fever and inflammation in humans and may cause lethal toxic shock.

Lipidoses are **lysosomal storage diseases** generated by defective enzymes of the lipid degradation pathways. Clinical symptoms are caused by accumulated substances in the lysosomes. The enzymatic defects may be found in the cerebroside, sphingomyelin, cholesterol ester, ganglioside break down processes. Usually the liver, spleen, brain and kidneys are involved.

20. DEGRADATION OF LIPIDS – BETA OXIDATION OF FATTY ACIDS

In comparing the steps of fatty acid (FA) synthesis and break down we realize four repeated steps when the chain is elongated or shortened by two-carbon units and notably, nearly reverse the process.

During degradation, **acetyl CoA** units are released and the beta position carbon is oxidized. Acetyl CoA may enter the *citric acid cycle* or can be the source of *ketone body synthesis*. Fatty acids are stored in adipocytes in the form of **triacyl glycerol (TAG).** They are ideally prime sources of energy as the molecule is anhydrous (does not carry any charge) opposite to glycogen and reduced. In addition to providing energy for the body, fatty acids are involved in membrane structure, covalent modifications of membrane proteins and precursors of hormones.

Dietary lipids contain mainly TAG which is emulsified by the cholesterol derivative bile salts within the intestinal lumen and digested by the pancreatic lipase into FAs and monoacylglycerols. As a result, these absorbed components and specific proteins **chylomicrons** are then synthetized in the enterocytes. Chylomicrons reach the circulation via the lymphatic system. Cells of adipose tissue take up fatty acids and monoacylglycerols, and resynthesize **TAG for storage** (see more in the Digestion and transport of macromolecules).

Mobilization of stored TAG is induced by hormones (glucagon and epinephrine) via the intracellular **hormone sensitive lipases** (HSL). Their products consist of both fatty acids and glycerol. **Fatty acids are bound** to binding proteins intracellularly and to albumin in the blood. Once they reach other tissues (muscle) they are taken up with binding and transporting proteins. Fatty acid degradation takes place in the **mitochondrion**: however, fatty acids first have to be activated then transported into this organelle. **Glycerol** is carried to the liver, hepatocytes takes up and after phosphorylation by glycerol kinase and oxidation by glycerol phosphate dehydrogenase dihydroxyacetone phosphate is produced which is an intermediate of *glycolysis including gluconeogenesis* (it is possible to produce glycerol from dihydroxyacetone phosphate in the opposite way).

The **activation of fatty acids** occurs at the outer membrane of the mitochondrion by the acyl CoA synthetase in the presence of ATP and the release of a pyrophosphate. The latter one is hydrolyzed into orthophosphate, the energy of which is pulling the reaction in this direction. The product is a thioester (high energy) bond containing **acyl CoA**.

This activated acyl CoA is reacting with carnitine by **carnitine acyltransferase I,** an enzyme of the outer mitochondrial membrane. The acyl carnitine conjugate is carried into the matrix of the mitochondrion by a **translocase** located in the inner mitochondrial membrane. The acyl carnitine is transformed back to acyl CoA with the help of **carnitine acyl transferase II** of the inner membrane. Only long chain FAs require this special type of transport, medium chain (C_8-C_{10}) fatty acids can enter mitochondria without carnitine.

In the event of **carnitine deficiency,** patients exhibit muscle weakness especially after prolonged exercise and when fasting, hypoglycemia and hyperammonemia are apparent due to inadequate fatty acid degradation. Patients who suffer from carnitine deficiecny must ingest extra carnitine and avoid both fasting and long chain fatty acids in their diet.

Malonyl CoA is an early intermediate of fatty acid synthesis and **inhibits** carnitine acyl transferase I, and as a result, active fatty acid synthesis mitochondria cannot take up fatty acids for degradation thus futile cycles are inhibited (see more in Regulation of lipid metabolism).

The steps of one round of beta oxidation of an acyl CoA molecule are carried out by the **enzymes**: acyl CoA dehydrogenase (generating a $FADH_2$), enoyl CoA hydratase, 3-OH acyl CoA dehydrogenase (producing a NADH) and beta-ketothiolase. The **final products** of these reactions include an acetyl

CoA and a two-carbon shorter acyl CoA which is now ready for another set of similar reaction chains. The first enzyme in this row, acyl CoA dehydrogenase, has more isozymes including separate ones for long, medium and short chain fatty acids.

In summary, the **total energy yield** of the complete breakdown of a *palmitate (C16)* molecule taking into account the acetyl CoA enters the citric acid cycle, produces 10 ATP/cycle, one $FADH_2$ is the source of 1.5 ATP while NADH generates 2.5 ATP, the final amount will be 106 molecules of ATP (not forgetting the ATP used for the activation step).

The **overall reaction** of beta oxidation of palmitate is:

Palmitoyl CoA + 7 NAD^+ + 7 FAD + 7 CoA + 7 H_2O → 8 acetyl CoA + 7 NADH + 7 H^+ + 7 $FADH_2$.

Fatty acids containing **double bonds** require additional enzymes (isomerase and possibly reductase, depending on the position of the unsaturated bond) for breakdown. By degradation of **odd chain fatty acids,** in addition to acetyl CoA, propionyl CoA is produced. The latter one is converted into succinyl CoA by propionyl CoA carboxylase and methylmalonyl CoA mutase (one of the three reactions using cobalamin – *vitamin B_{12}* as coenzyme).

Several long chain fatty acids are becoming shortened in the **peroxisomes**. In this organelle, acyl CoA dehydrogenase generates $FADH_2$ which donates electrons directly to oxygen producing hydrogen peroxide. Peroxisomes are rich in the enzyme catalase which reacts with hydrogen peroxide. Fatty acids shortened in peroxisomes are used by mitochondria for further degradation.

21. KETONE BODIES

- The ketone bodies (**acetoacetate, β-D-hydroxybutyrate, and acetone**) are small water soluble forms of lipid based energy.
- They can arise under conditions where carbohydrate intake is restricted or absent.
- Ketone bodies result from the breakdown of lipids. They can be oxidized and serve as a (partial) replacement for glucose in cells that are metabolically active such as nerve cells.
- The liver is the primary organ generating ketone body.
- Both acetoacetate and β-D-hydroxybutyrate are formed in the mitochondria of the liver, from acetyl-CoA. Acetyl CoA enters the TCA cycle if there is sufficient oxaloacetate. If not, it is diverted to ketone bodies.
- Aceton is produced by spontaneous (nonenzymatic) decarboxylation of acetoacetate and by enzymatic reaction involving acetoacetate decarboxylase. Acetone, produced in smaller quantities than the other ketone bodies, is exhaled.
- Acetoacetate and β-D-hydroxybutyrate are transported by the blood to tissues other than the liver (extrahepatic tissues), where they are converted to acetyl-CoA and oxidized in the citric acid cycle, providing energy. Cardiac and skeletal muscle, as well as the renal cortex, can use ketone bodies directly, and the brain can adapt to use them as fuel during starvation. The liver lacks a key enzyme for the breakdown of ketone bodies, so they are not catabolized appreciably by the organ.
- The production and export of ketone bodies from the liver to extrahepatic tissues allows continued oxidation of fatty acids in the liver when acetyl-CoA is not being oxidized in the citric acid cycle.

21.1. KETOGENESIS

Ketogenesis is the biochemical process leading to the synthesis of ketone bodies.

1. The first step in the formation of acetoacetate, occurring in the liver (lecture fig.), is the enzymatic condensation of two molecules of acetyl CoA, catalyzed by thiolase.

2. The acetoacetyl-CoA then condenses with acetyl-CoA in a reaction catalyzed by the enzyme HMG-CoA synthase, to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA).

3. Next, it is cleaved to free acetoacetate and acetyl-CoA (by the enzyme HMG-CoA lyase).

4. The acetoacetate is reversibly reduced by D-β-hydroxybutyrate dehydrogenase, a mitochondrial enzyme, to D-β-hydroxybutirate (the enzyme is specific for the D stereoisomer)

5. In healthy people, acetone is formed in very small amounts from acetoacetate, which is easily decarboxylated, either spontaneously or by the action of acetoacetate decarboxylase.

Because individuals with untreated diabetes produce large quantities of acetoacetate, their blood contains significant amounts of acetone, which is toxic.

Acetone is volatile and imparts a characteristic odor to the breath, which is sometimes useful in diagnosing diabetes.

21.2. REGULATION OF KETONE BODY SYNTHESIS

- The HMG-CoA is an important intermediate in ketogenesis.Its synthesis is the rate-limiting step in ketogenesis, and the liver is the only organ containing large quantities of the enzyme catalyzing this step. Synthesis of this enzyme, the mitochondrial HMG-CoA synthetase is induced by fatty acids.
- HMG-CoA is also precursor to cholesterol, and the steps leading to ketone body generation are the same as those leading to cholesterol biosynthesis; however, the two process occur in different compartments.
- Therefore, the liver contains two HMG-CoA synthases, one cytosolic for cholesterol synthesis and an other mitochondrial rate limiting for ketone body synthesis.
- The latter undergoes a short-term covalent modification via succinylation/desuccinylation of pre-existing enzyme. This presents a rare method of covalent modification of an enzyme.

21.3. KETONE BODY CATABOLISM

In extrahepatic tissues,

1. D-β-hydroxybutirate is oxidized to acetoacetate by D-β-hydroxybutyrate dehydrogenase (Lecture fig.).

2. The acetoacetate is activated to its coenzyme A ester by transfer of CoA from succinyl-CoA, an intermediate of the citric acid cycle, in a reaction catalyzed by β-ketoacyl-CoA transferase, also called thiophorase.

3. The acetoacetyl-CoA is then cleaved by thiolase to yield two acetyl-CoA, which enter the citric acid cycle.

The first and the third reactions are the same as those in the production of ketone bodies; the middle reaction, however, is not. In fact, this second reaction catalyzes the rate-limiting step in the conversion process. Moreover, the thiophorase that catalyze this step is not expressed in liver. The liver is therefore a producer of ketone bodies for other tissues, but not a consumer.

The production and export of ketone bodies by the liver allows continued oxidation of fatty acids for energy production with only minimal oxidation of acetyl-CoA. When intermediates of the citric acid cycle (especially oxaloacetate) are being siphoned off for glucose synthesis by gluconeogenesis, for example, oxidation of cycle intermediates slow-and so does acetyl-CoA oxidation.

Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is engaged in acetyl-CoA, β-oxidation slows for want of the free coenzyme. The production and export of ketone bodies frees coenzyme A, allowing continued fatty acid oxidation.

21.4. KETOSIS, KETONURIA AND KETOACIDOSIS

The overproduction of ketone bodies, called **ketosis**, results in greatly increased concentration of ketone bodies in the blood (ketonemia), defined by a blood level between 0.3 and 7.0 mmol/L.

Conditions for Ketosis

- **Low carbohydrate intake** (lipids only; or complete starvation): In the absence of dietary carbohydrates, the body will metabolize fatty acids and amino acids. Glycerol and certain amino acids will generate precursors for gluconeogenesis, as the body attempts to maintain blood glucose levels for nerve cells, muscle, etc.

- **Diabetes**: Glucose is not taken up by tissues in the normal way, and the body acts as if it were starved for energy. Metabolism shifts in ways very similar to those seen under conditions of extended fasting or starvation.

Excess ketone bodies in the bloodstream will be filtered out by the kidneys and excreted in the urine. This condition is called **ketonuria**. The dissolved ketone bodies will exert an osmotic effect on the kidneys, causing the excretion of more urine than usual. This imbalance can lead to a severe disease state if **body stores** (and hence serum levels) **of ions** such a sodium, potassium, and phosphate become **depleted**; **dehydration** can be an issue as well.

Ketone bodies are organic acids and will acidify their environment.

If the concentration of these organic acids in the bloodstream exceeds the buffering capacity of intracellular and extracellular fluids, the **blood pH will drop** below pH 7.3. This is an **acidosis**; in particular, because of its origin this is a **ketoacidosis**.

Among other effects, the acidosis will reduce the oxygen-carrying capacity of erythrocytes, and so can be a very serious medical condition.

21.5. DIABETES MELLITUS

Diabetes mellitus arises from defects in insulin production or action.

- In type I diabetes, the β-cells of pancreas are diminished; no insulin is produced. This results in too much sugar in the blood (hyperglycemia) since insulin promotes transport of glucose into cells from the blood.
- In type II diabetes, insulin is produced and secreted, but some features of the insulin responsive system is defective.

In either case, the body acts as if it were starved for energy because glucose is not being taken up by the tissues. The ongoing demand for glucose causes low liver glycogen stores, which leads to protein and lipid breakdown for energy, with formation of ketone bodies.

Also, gluconeogenesis is increased. The body behaves as if it were starved for energy.

In diabetes mellitus the high blood glucose level leads to its excretion in urine.

- A side-effect is dehydration, due to ketone and sugar excretion.
- Another side-effect is non-enzymatic glycosylation of hemoglobin and other proteins. In fact, the stable glycosylated product hemoglobin A1c is used diagnostically for information about the severity of the hyperglycemia.

(Diabetes insipidus is a very different disorder- a pituitary disorder directly affecting kidney function; there is no unusual level of glucose in the urine (the urine is insipid and has no taste).

22. FATTY ACID BIOSYNTHESIS

- Fatty acid biosynthesis occurs in the **cytosol** and oxidation is compartmentalized inside mitochondria.
- The biosynthetic reactions are nearly the reverse of β-oxidation.
- The fatty acid chain is built up by **successive additions of two-carbon units**. The two-carbon units are derived **from acetyl-CoA**.
- There are **different cofactor** requirements:
  - The enzymes in biosynthesis use **$NADP^+$/NADPH** instead of NAD^+/NADH (this is characteristic of biosynthesis in general).
  - A special thiol (the acyl carrier protein, or **ACP**) replaces the CoA used in the oxidation reactions.

22.1. Overview of Fatty Acid Synthesis

- Two acetyl CoA units start the process. One is transferred to a thiol site on the fatty acid synthase (FAS).
- The other is first activated by carboxylation (by a speparate enzyme system) to form malonyl CoA; the malonyl CoA is then attached to a second thiol site, nearby on the FAS complex.
- These acyl moieties are combined in several steps to make a butyrate thioester attached at the second thiol site.
- Another malonyl unit is passed to the first thiol site, and the adjacent acyl groups are joined, with the product remaining attached at the second thiol site.
- This process cycles repeatedly to elongate the carbon chain, using several more molecules of malonyl CoA. Typically, the process will terminate when the chain reaches a length corresponding to 16 carbons

Initiating Fatty Acid Synthesis

- There are two basic steps: making malonyl CoA, and loading the FAS.

1. **Carboxylation of acetyl CoA**: Catalyzed by a separate enzyme (acetyl-CoA carboxylase (ACC) not the FAS), making malonyl CoA.
2. **Loading the FAS**: An acetyl CoA and a malonyl CoA are attached to thiols on the FAS.

Carboxylation of Acetyl-CoA by Acetyl-CoA Carboxylase (ACC)

ATP + HCO_3^- + acetyl-CoA → malonyl-CoA + ADP + p_i

- ACC catalyzes the formation of malonyl CoA from acetyl CoA in a two-step reaction driven by ATP hydrolysis. This is an irreversible step and can be considered the **committed step** in fatty acid biosynthesis.
- ACC uses **biotin as a cofactor** to acquire/donate carboxyl groups.
- The bacterial form of ACC is under study for development of antibiotics, as the bacterial forms are quite distinct from the eukaryotic forms.
- There are two **distinct forms** of mammalian **ACC**, ACC-α (or ACC-1) and ACC-β (ACC-2), with distinct genes, amino acid sequence and tissue-specific expression.
- Both ACC-α and ACC-β are regulated by phosphorylation and by the allosteric effector citrate.

Fatty Acid Synthase (FAS) Structure

- The eukaryotic form of FAS is a dimer of identical polypeptide chains.
- Each chain has multiple domains with different activities.
- Throughout the process of fatty acid synthesis, the intermediates remains covalently attached as thioesters to one of two thiol groups. One point of attachment is the –SH group of a Cys residue in one of the synthase domains (β-ketoacyl-ACP synthase, KS); the other is the –SH group of acyl carrier protein.
- Acyl-carrier protein (ACP) is the shuttle keeping the system together. It contains a phosphopanteteine prosthetic group, serving as a flexible arm.
- The phosphopanteteine arm and the compact arrangement of the active sites makes for efficient transfer of intermediates from one catalytic site to the next.
- The bacterial form of FAS is unlike this, inasmuch it is an aggregate of several different polypeptide chains, each with only a single enzymatic activity.

Loading the Fatty Acid Synthase

- First, the acetyl group of acetyl-CoA is transferred to ACP in a reaction catalyzed by the **malonyl/acetyl-CoA-ACP transferase (MAT)** domain of the multifunctional polypeptide.
- The acetyl group is then transferred to the Cys-SH group of the β-ketoacyl-ACP synthase (KS).
- The second reaction, transfer of the malonyl group from malonyl-CoA to the –SH group of ACP, is also catalyzed by malonyl/acetyl-CoA-ACP transferase (MAT).
- In the charged synthase complex, the acetyl-and malonyl groups are activated for the chain lengthening process.

Iterated Steps in FA Synthesis

- With the FAS loaded, synthesis can proceed. There are four steps (lecture fig.).
- The first step is a **condensation**, with decarboxylation of the malonyl moiety to generate a carbanion that attacks the neighboring acetyl unit. The reaction catalyzed by β-ketoacyl-ACP synthase (KS) and the product has a 3-keto group.
- Next, there is a **reduction** catalyzed by β-ketoacyl-ACP reductase (KR) that uses NADPH to reduce the 3-keto group to the corresponding alcohol.
- Next, there is a **dehydration** by hydroxyacyl-ACP dehydratase (DH) to give a carbon-carbon trans double bond .
- This is followed by a second **reduction** by the action of enoyl-ACP reductase (ER), again using NADPH, but now giving the saturated alkyl chain.

Termination Step

- The whole process cycles repeatedly until a sufficiently long alkyl chain is made.
- For humans, this will generally be a chain with sixteen carbons (a palmitoyl chain).
- Next, the **thioester link is hydrolyzed by the thioesterase (TE)**, releasing the free fatty acid and regenerating the FAS.

22.2. Palmitic Acid Synthesis

Acetyl-CoA + 7 Malonyl-CoA + 14 NADPH + 14 H^+ →
→ Palmitate + 14 $NADP^+$ +6 H_2O + 8 CoASH

8 Acetyl-CoA + 7 ATP + 14 NADPH + 14 H^+ →
→ Palmitate + 7 ADP + 7 P_i + 14 $NADP^+$ +6 H_2O + 8 CoASH

- One acetyl unit enters this pathway directly, while the rest (e.g., seven more for palmitic acid synthesis) come in as malonyl CoA. Thus, there is an energy cost in the carboxylation required, as well as in the redox reactions here.
- Note the energy costs here: the use of ATP and of NADPH. For the synthesis of a C-16 fatty acid, the process would use 7 ATP molecules (not 8) and 14 NADPH
- Note that only six net water molecules are produced, because one is used to hydrolyzed the thioester linking the palmitate product to the enyyme..
- The decarboxylation reaction and loss of CO_2 helps drive the condensation, in partial compensation for the ATP consumption.

Further notes

- Since fatty acids are elongated with C2 units, most fatty acids are **even chained.**
- Fatty acid synthase of the cytosol produces **max 16 C** long saturated fatty acids.
- Longer and/or unsaturated fatty acids are produced by enzyme systems of ER and mitochondria.
- Odd chain fatty acids are produced when AT enzyme accepts propionyl-CoA as a substrate by mistake.

22.3. CITRATE SHUTTLE

- The breakdown of carbohydrates or protein yields acetyl groups, but these are compartmentalized inside the mitochondrion.
- Fatty acids can be made from such acetyl groups; the problem is to **transfer the acetyl groups to the cytosol**, because the mitochondrial inner membrane is impermeable to acetyl-CoA..
- The citrate shuttle performs this function.
- Citrate is first made inside the mitochondrion from acetyl-CoA and oxaloacetate by the **citrate synthase**, then passes through the inner membrane on the **citrate transporter**. In the cytosol, citrate cleavage by **citrate lyase** regenerates acetyl-CoA and oxaloacetate in an **ATP dependent reaction**. Then the cytosolic malate dehidrogenase reduces the oxaloacetate to malate (there is no oxaloacetate transporter in the inner membrane), which can return to the mitochondrial matrix on the malate-α-ketoglutarate transporter in exchange for citrate. In the matrix, malate is reoxidized to oxaloacetate to complete the shuttle.
- In the cytosol, citrate can also serve as an indicator of the general energy state of the cell; it can be used as an allosteric regulator of certain enzymes.

22.4. SOURCE OF CYTOSOLIC NADPH

- In hepatocytes and adipocytes, cytosolic NADPH required for fatty acid synthesis is generated by the **pentose phosphate pathway** and by **malic enzyme**.
- Much of the malate produced in the cytosol (citrate shuttle) is used to generate cytosolic NADPH by the malic enzyme. The pyruvate produced is transported to the mitochondria by the pyruvate transporter, and converted back into oxaloacetate by pyruvate carboxylase in the matrix.
- The resulting cycle results in the consumption of two ATPs (by citrate lyase and pyruvate carboxylase) for every molecule of acetyl-CoA delivered to fatty acid synthesis.

22.5. Regulation of Fatty Acid Biosynthesis

- Short-term regulation involves hormonal signals and enzyme cascades. Control is exerted on both the FAS complex and on the enzyme acetyl CoA carboxylase. Short-term regulation (on a time scale of minutes) is sensitive to energy demands. The enzymes use citrate as an indicator of cellular energy supplies. Their state of phosphorylation, which depends on ATP levels, also provides sensitivity to energy levels.
- Long-term control of fatty acid metabolism (adaptive control) is exerted by relative rates of enzyme synthesis and degradation, principally of the fatty acid synthase itself as well as of acetyl CoA carboxylase.

22.6. Chain Elongation of Fatty Acids

- **Palmitate**, the principal product of the fatty acid synthase system in animal cells, **is the precursor** of other long chain fatty acids.
- Elongation occurs by sequential addition of two-carbon units, with **malonyl-CoA or acetyl-CoA** as **the source of the two-carbon units**.
- It is catalyzed by special synthases, not the regular FAS.
- The reactions resemble those catalyzed by the FAS complex, using the same 4-step pattern of thioester activation, reduction of the carbonyl group, dehydration, and reduction of the carbon-carbon double bond, but **CoA** rather than ACP **is the acyl carrier** in the reaction. **NADH or NADPH provide the reducing equivalents**, depending on the tissue and cellular site of the reactions.
- The reactions occur in the **mitochondria** or in the **endoplasmic reticulum**.

22.7. Desaturation of Fatty Acids

- Formation of monoenoic acids occurs in the endoplasmic reticulum, where **mixed-function oxidases** (fatty acyl-CoA desaturase) simultaneously oxidize the fatty acid (introducing the double bond) and oxidize NADPH. The path of electron flow includes a cytochrome (cytochrome b_5) and a flavoprotein (cytochrome b_5 reductase). The result is the formation of a *cis* double bond, in a position at least 6 carbons from the end of the chain.
- Polyunsaturated fatty acids (with *cis* double bonds) are formed by a combination of chain elongation reactions and desaturation reactions. Products include arachidonic acid, which is important as a precursor for prostaglandins and thromboxanes.
- In humans, some of these reactions do not occur, notably the desaturation of oleate to linoleate, and linoleate to α-linolenate (mammals can introduce double bonds at the Δ9 position of fatty acids but cannot introduce additional double bonds between C-10 and the methyl-terminal end); this is the basis for declaring **linoleate and α-linolenate** to be "**essential**" in the diet.

22.8. Other Modifications of Fatty Acids

- Branching: Branched-chain fatty acids are rare in higher animals, though common enough in plants. The "branch" is a methyl group, introduced when methylmalonyl-CoA is used instead of malonyl-CoA in a chain-elongation reaction.
  - "Branching" may occur pathologically in humans when there is a deficiency of vitamin B12 in the diet.
- Alcohols: The fatty acid may be reduced in a NADPH-dependent process to form the corresponding fatty alcohol. This occurs in the endoplasmic reticulum. The fatty alcohols

may then be incorporated into certain phospholipids. Fatty alcohols are found in skin oil, ear wax, etc., and are considered lubricants and as barriers to water and to bacteria.

23. METABOLISM OF COMPLEX LIPIDS

23.1. GLYCEROPHOSPHOLIPIDS (PHOSPHOGLYCERIDES)

Glycerophospholipids are membrane lipids, in which two fatty acids are attached in ester linkage to the first and second carbons of glycerol, and a highly polar and charged group is attached through a phosphodiester linkage to the third carbon (lecture fig.). Glycerophospholipids are named as derivatives of the parent compound, phosphatidic acid (diacylglycerol-phosphate), according to the polar alcohol in the head group. Phosphatidylserine and phosphatidylethanolamine have serine and ethanolamine as their polar head groups.

Phosphatidylcholine (with choline head group) is also known as lecithin. Inositides are phospholipids in which inositol is the attached alcohol. Inositides play a definitive role in biological signal transduction and in anchoring glycoproteins to the plasma membrane of the cell.

In all these compounds, the head group is joined to glycerol through a phosphodiester bond, in which the phosphate group bears a negative charge at neutral pH. The polar alcohol may be negatively charged (as in phosphatidylinositol 4,5 bisphosphate), neutral (phosphatidylserine), or positively charged (phosphatidylcholine, phosphatidylethanolamine). These charges contribute to the surface properties of membranes. The fatty acids in glycerophospholipids can be any of a wide variety, so a given phospholipid may consist of several molecular species, each with its unique component of fatty acids. In general, glycerophospholipids contain a C16 or C18 saturated fatty acid at C-1 and a C18 or C20 unsaturated faty acid at C-2.

If only one fatty acid is attached to the glycerol backbone, it is called lysophosphatidate (monoacylglycerol phosphate) (lecture fig.). The acyl group may be attached to either C1 or C2 of the glycerol backbone. Lysolecithin is 1-acylglycerolphosphorylcholine (the ′lyso″ part of the name comes from the detergent action of this compound, which readily solubilizes biomembranes and causes cell lysis).

Cardiolipin is a complex phosphoglyceride, in which the glycerol appears not only in the ′backbone″ but also as the biological alcohol esterified to the phosphate group (lecture fig.).It may help to stiffen certain biological membranes.

Some animal tissues and some unicellular organism are rich in ether lipids, in which one of the two acyl chains is attached to glycerol in ether linkage (lecture fig.). The ether–linked chain may be saturated as in platelet activating factor (PAF), or may contain a double bond between C1 and C2, as in plasmalogens. Vertebrate heart tissue is uniquely enriched in ether lipids and the membrane of bacteria and certain invertebrates also contain high proportions of ether lipids. The functional importance of ether lipids in these membranes is unknown; perhaps their resistance to phospholipases cleaving ester-linked fatty acids from membrane lipids is important in some cases.

At least one ether lipid, platelet activating factor, is a potent molecular signal. It is

released from leukocytes and stimulates platelet aggregation and the release of serotonin from platelets. It also plays an important role in inflammation and the allergic response.

23.2. MAJOR BIOLOGICAL FUNCTIONS OF PHOSPHOLIPIDS

1. Membrane components:
2. Surfactant action:

3. Communication:

- Phospholipids are a predominant constituent of most biomembranes. They also serve to anchor proteins to the lipid bilayer of the membrane (e.g., inositides and glycoproteins).
- Dipalmitoyllecithin in the lung serves to reduce surface tension in alveoli, and allow free expansion/contraction of lung tissue.
- Phosphatidylcholine acts as a detergent/solubilizing agent in bile, to dissolve and transport cholesterol.
- Phosphatidylinositol 4,5-bisphosphate plays a central role in one of the major signal transduction pathways.

23.3. BIOSYNTHESIS OF TRIACYLGLYCEROLS AND PHOSPHOLIPIDS

Most of the fatty acids synthesized or ingested by an organism characterized by one of two fates:

1. incorporation into triacylglycerols for the storage of metabolic energy-when an organism has a plentiful food supply, but is not actively growing

2. incorporation into phospholipid components of membranes, during rapid growth, synthesis of new membranes requires the production of membrane phospholipids.

Phosphatidic acid and 1,2-diacyl-glicerol are common intermediates in the pathways of phospholipid and triacylglycerol biosynthesis and both pathways share some of the same enzymes.

Synthesis of phosphatidic acid

Phosphatidic acid is synthesized from glycerol 3 phosphate and fatty acyl-CoA.

The vast majority of the the glycerol 3-phosphate is produced in a NADH dependent

reduction by the glycerol 3-phosphate dehydrogenase from the glycolytic intermediate dihydroxyacetone phosphate (DHAP).

Within the liver and kidney, a small amount of glycerol 3-phosphate is also formed from glycerol by the action of glycerol kinase.

The fatty acyl-CoAs formed from fatty acids by acyl-CoA synthetases (the same enzyme responsible for the activation of fatty acids for β-oxidation.

The two free hydroxyl groups of glycerol 3-phosphate, is in turn acylated by two molecules of fatty acyl-CoA to yield diacylglycerol 3-phosphate, more commonly known as phosphatidic acid or phosphatidate. These reactions are catalyzed by acyl transferases.

A second route to phosphatidic acid is through phosphorylation of a diacylglycerol.

In eukaryotes, the synthesis of phosphatidic acid is mainly associated with the endoplasmic reticulum, but also occurs in mitochondria.

Triglyceride Biosynthesis

- Triacylglycerol biosynthesis occurs only in the liver, adipose tissue and intestine.
- Triglycerides are synthesized from phosphatidic acid (which already carries two fatty acid chains).

- Phosphatidic acid is hydrolyzed by cytosolic phosphatidic acid phosphatase to form a 1, 2 diacylglycerol.
- Diacylglycerols are then converted to triacylglycerols by transesterification with a third fatty acyl-CoA.
- The three fatty acids in a triglyceride may all be distinct and different from one another.

Synthesis of Membrane Phospholipids

- Essentially all cells are capable of synthesizing phospholipids some degree (except mature erythrocytes)
- Membrane phospholipids are synthesized by two routes.
- One route adds an “alcohol” to an activated diacyl glycerol.
- The other route activates the alcohol, and adds it to a diacyl glycerol.

Biosynthesis of Phosphatidylserine

- In bacteria, first phosphatidic acid is activated (using CTP) to give the CDP-diacylglycerol.
  - This is similar to the use of UTP to activate sugars in biosynthesis, forming the UDP-sugar conjugate and releasing pyrophosphate.
- Next, cytidine monophosphate is displaced and a phosphoester link is made to the “alcohol” (e.g., serine), to yield the product, phosphatidylserine.
- In mammals phosphatidylserine is derived from phosphatidylethanolamine or phosphatidylcholine via one of two head-groups exchange reactions carried out in the endoplasmic reticulum.

Biosynthesis of Phosphatidylcholine

- First, the “alcohol” (choline) is activated, using ATP by the choline kinase, to create phosphorylcholine.
- Second, this is converted to CDP-choline.
- Third, the CDP-choline is added to a diacylglycerol in a choline phosphotransferase reaction, to produce phosphatidylcholine.
- In the liver, phosphatidylcholine is also produced by methylation of phosphatidylethanolamine (with S-adenosylmethionine as methyl group donor).

Biosynthesis of phosphatidylethanolamine

In one route similar to phosphatidylcholine, first the ethanolamine is activated (using ATP) by a kinase to phosphoethanolamine, which is converted to CDP-ethanolamine.

Finally this is added to a diacylglycerol in the ethanolamine phosphotransferase catalyzed reaction, to yield phosphatidylethanolamine.

In the other route, decarboxylation of the serine moiety in phosphatidylserine, catalyzed by phosphatidylserine decarboxylase, yielding phosphatidylethanolamine.

Biosynthesis of phosphatidylinositol

Phosphatidylinositol is synthesized by condensation of CDP-diacylglycerol with inositol in a phosphatidylinositol transferase catalyzed reaction.

Remodelling reactions

The asymmetric distribution of fatty acids in phospholipids is due to remodeling reactions.

Two phospholipases, phospholipase A1 and phospolipase A2, occur in many tissues and play a role in the formation of specific phospholipids structure containing appropriate fatty acids in sn-1 and sn-2 positions.

Following removal the undesirable fatty acid by phospholipase A1 or A2 resulting a

lysophophatide, insertion of a new fatty acid can be accomplished either by direct acylation from acyl-CoA involving fatty acid-specific acyl-CoA transferase or from some other phospholipids by an exchange-type reaction.

23.4. SPHINGOLIPIDS

Sphingolipids are derivatives of sphingosine. **Roles for sphingolipids** are diverse:

Certain sphingolipids act intracellularly as second messengers in signal transduction pathways, helping to regulate cell division, differentiation, migration, and apoptosis (programmed cell death).

As membrane components, they tend to rigidify or stiffen the lipid bilayer and to reduce diffusibility of integral membrane components.

With carbohydrate modifications, sphingolipids in cell membranes act as points for biological recognition, as in the human blood group types.

23.5. STRUCTURES OF SPHINGOLIPIDS

Sphingolipids, the fourth large class of membrane lipids, also have a polar head group and two nonpolar tails, but contain no glycerol. Sphingolipids are composed of one molecule of the long-chain amino alcohol spingosine, one molecule of a long-chain fatty acid, and a polar head group joined by a glycosidic linkage in some cases and a phosphodiester in others (lecture fig.).

Sphingosine is derived from palmitoyl-CoA and serine in a series of reactions.

It has a “tail”, a C-C double bond, and an amino group that can be used for forming amide linkages to fatty acids, to form ceramide.

In the body, there is virtually no free sphingosine; instead it is immediately derivatized, primarily through the intermediate ceramide (it is produced by the attachment a fatty acid in amide linkage to the –NH2 on C2 of sphingosine). Ceramid itself is only an intermediate and it is the structural parent of all sphingolipids.

There are three subclasses of sphingolipids, all derivatives of ceramide but differing in their head groups: sphingomyelins, neutral (uncharged) glycolipids and gangliosides.

Sphingomyelins contain phosphocholine or phosphoethanolamine as their polar head group and therefore are classified (along with the glycerophospholipids) as phospholipids.Sphingomyelins are neutral at physiological pH and may contain different fatty acids, depending on the tissue.They are present in the plasma membranes of animal cells and are especially prominent in myelin (a membranous sheath surrounding the axons of some neurons). In Niemann-Pick disease (caused by a genetic defect in the enzyme sphingomyelinase, which cleaves phosphocholine from sphingomyelin),

sphingomyelin accumulates in the brain, spleen and liver, and leads to mental retardation and early death.

Glycosphingolipids, which occurs largely in the outer face of plasma membranes, have head groups with one or more sugars connected directly to the –OH at C1 of the ceramide moiety; they do not contain phosphate.

Cerebrosides have a single sugar linked to ceramide: galactose (in the plasmamembrane of cells in neural tissue) or glucose (in the plasmamembrane of cells in noneural tissue).

Globosides are glycosphingolipids with two or more sugars, usually D-glucose, D-galactose or N-acetyl-D-galactosamine. Cerebrosides and globoside are usually neutral glycolipids, as they have no charge at pH7.

Gangliosides the most complex sphingolipids, have oligosaccharides as their polar head groups and one or more residues of N-acetylneuraminic acid, a sialic acid, at the termini. Sialic acid gives gangliosides the negative charge at pH7. Ganglioside with one sialic acid residue are in the GM (M for mono) series, those with two are in the GD (D for di) series, and so on. Gangliosides can be found in ganglion cells. They are concentrated in the outer surface of cells, where they present points of recognition for extracellular molecules or surfaces of neighboring cells. One of them found within intestinal mucosal cells, is effective at binding cholera toxin. Accumulation of gangliosides (caused by defects in lysomomal enzymes) leads to mental retardation, hepatospenomegaly and early death.

Sulfation reaction on galactocerebroside produce sulfatide, or sulfogalactocerebroside; the donor here is 3′-phosphoadenosine-5′-phosphosulfate (PAPS).

23.6. BIOSYNTHESIS OF SPHINGOLIPIDS

- Sphingolipid biosynthesis starts in the endoplasmic reticulum with the synthesis of **sphingosine**. This is **made from the activated fatty acid palmitoyl CoA and serine**. Serine is attached via decarboxylation, to the activated fatty acid, this reaction dependent on pyridoxal phosphate cofactor. Two successive redox reactions then follow, first the reduction using NADPH, and then an oxidation using FAD- to give sphingosine.
- Sphingosine is only an intermediate and does not accumulate appreciably. It is used to make **ceramide** by attachment of a fatty acid chain to the amino group of the sphingosine core.

Sphingomyelin results when a phosphorylcholine is transferred from phosphatidylcholine (lecithin) to the hydroxyl group on ceramide. Sphingomyelin is a major membrane component in some cells and organelles, and contributes to membrane rigidity.

If one or more sugars are linked to the hydroxyl group of the ceramide, the result is a **glycosphingolipid**. (Glycosphingolipids are made from ceramide and activated sugars (UDP-sugars)).

- If only a single sugar is attached the compound is called a **cerebroside**.
- More sugars may be attached, including modified sugars such as acetylgalactosamine and N-acetylneuraminic acid (sialic acid). **Globosides** are neutral glycosphingolipids as cerebrosides, with two or more sugars, usually D-glucose, D-galactose or N-acetyl-D-galactosamine. **Gangliosides** the most complex sphingolipids, have oligosaccharides as their polar head groups and one or more residues of N-acetylneuraminic acid, a sialic acid, at the termini. Sialic acid gives gangliosides the negative charge at pH7.
- These complex glycosphingolipids in cell membranes are oriented in which the saccharide moieties project outside the cell; they are important extracellular features for recognition (e.g., in the immune system for blood-group antigens).
- The Golgi apparatus is responsible for these further steps in biosynthesis.

23.7. DEGRADATION OF PHOSPHOLIPIDS AND SPHINGOLIPIDS

Most cells continually degrade and replace their membrane lipids. For each hydrolyzable bond in glycerophospholipid, there is a **specific hydrolytic enzyme in the lysosome** (lecture fig.).

Phosopholipases of the A type (A1 and A2) remove one of the two fatty acids, producing lysophospholipid. (These esterases do not attack the ether link of plasmalogens). **Lysophospholipases** remove the remaining fatty acid. **Phospholipases C and D** each split one of the phospphodiester bonds in the head group.

Gangliosides are degraded by a set of lysosomal enzymes catalyzing the stepwise removal of sugar units, finally yielding a ceramide.

Certain genetic diseases result in disturbances of lipid storage. These so-called **lipid storage diseases, or lipidoses** (lecture fig.), are due to a defect in the activity of one or another of several catabolic enzymes, notably those involved in sphingolipid and sterol metabolism.

23.8. BIOSYNTHESIS OF PROSTAGLANDINS, THROMBOXANES AND LEUKOTRIENES (EICOSANOIDS)

Eicosanoids (include prostaglandins, leukotrienes, and thromboxanes) are fatty acids with twenty carbons in the chain. These molecules act as second messengers inside the cells and between neighboring cells; they **are local hormones**.

An important **precursor to the eicosanoids is arachidonic acid**. Arachidonic acid a 20:4 FA, **derived from linoleoyl-CoA**. The pathway involves chain-lengthening and the introduction of two additional C-C double bonds. A dietary deficiency in linoleic acid often results in the blocking of biosynthesis of arachidonic acid.

- Arachidonic acid is incorporated into phospholipids of cell membranes. It is released by the action of **phospholipase A2** from the middle carbon of glycerol, and then undergoes reactions converting it to prostaglandins, prostacyclins, thromboxanes, and leukotrienes. Antiimflammatory steroids act by inhibiting the arachidonic acid release by the phospholipase A2.
- Distinct biosynthetic pathways leads from arachidonic acid to prostaglandins, thromboxanes and leukotrienes:

The cyclic pathway

Enzymes in the smooth ER convert arachidonate to prostaglandins, beginning with the formation of **prostaglandin H2**, the immediate precursor of many other prostaglandins and of thromboxanes.

The two reactions that lead to PGH2 are catalyzed by a bifunctional enzyme, **cyclooxygenase (COX)**, also called prostaglandin H2), the immediate precursor of many other (PGH2 synthase. In the first of two steps, the cyclooxygenase activity introduces molecular oxygen to convert arachidonate to PGG2

The second step, catalyzed by the peroxidase activity of COX, converts PGG2

- Mammals have two isozymes of prostaglandin H2 Synthase, COX-1 and COX-2 Both forms catalyze the same type of reaction: the formation of a peroxide and a cyclopentane ring on arachidonic acid. Aspirin and other non-steroidal antiimflammatory drugs (NSAIDs) inactivates the cyclooxygenase activity of both isozymes (Aspirin act irreversibly, by acetylating a Ser residue in active site of COX; ibuprophen, naproxen act probably by

mimicking the structure of substrate or intermediate). Because the undesirable side effects of COX1 inhibition, selective COX2 inhibitory NSAIDs are under development (See in clinical importance of lipids section))

- PGH2 is acted upon by a variety of enzymes (synthases), leading to prostacyclin, thromboxanes, and other prostaglandins.

The linear pathway

The linear pathway leads from arachidonate to **leukotrienes**, which are linear compounds. Leukotrienes are made primarily by inflammatory cells, such as macrophages, mast cells, and polymorphonuclear leukocytes.

Several lipooxygenases catalyze the incorporation of molecular oxygen into arachidonate.

- Lipoxygenases are not the same as cyclo-oxygenases; lipoxygenases are responsible for the synthesis of the leukotrienes.
  - There are various isozymes here, responsible for the diversity in leukotrienes.

These enzymes are found in leukocytes, the spleen, liver, heart, and brain. These are mixed-function oxidases of the cytochrome P450 family. Lipoxygenases are not inhibited by aspirin; it is the cyclo-oxygenases that are the targets for aspirin.

24. CHOLESTEROL METABOLISM

Cholesterol is a crucial component of cellular membranes and the precursor of steroid hormones and bile acids. Therefore it is an essential molecule in many animals, including humans, but it is not required in the mammalian diet- all cells can synthesize it from simpler precursors.

24.1. BIOSYNTHESIS OF CHOLESTEROL

- Although a significant amount of cholesterol derived from the diet (mainly from cholesterol rich foods such as meat, eggs and dairy products), the body also synthesizes it readily.
- The liver is the main organ for cholesterol biosynthesis, although other tissues synthesize it as well (e.g., glands producing steroid hormones).
- Biosynthesis of cholesterol takes place in the endoplasmic reticulum and the cytosol and starts from the common precursor, acetyl CoA..

Synthesis takes place in four stages:

1. Condensation of three acetate units to form a six-carbon intermediate, mevalonate
2. Conversion of mevalonate to activated isoprene units
3. Polymerization of six 5-carbon isoprene units to form the 30 carbon linear squalene
4. Cyclization of squalene to form the four rings of the steroid nucleus, with an additional series of changes (oxidations, removal or migration of methyl groups) to produce cholesterol

Stage 1. Synthesis of mevalonate from acetate

Two molecules of acetyl-CoA condenses to form acetoacetyl-CoA, which thereby condenses with a third molecule of acetyl-CoA to yield the six-carbon compound β-**hydroxy-β-methylglutaryl-CoA (HMG-CoA**). These first two reactions are catalyzed by thiolase and HMG-CoA synthase, respectively. The cytosolic HMG-CoA synthase in this pathway is distinct from the mitochondrial isozyme that catalyzes HMG-CoA synthesis in ketone body formation.

The third reaction is the committed and rate-limiting step: reduction of HMG-CoA to mevalonate, for which each of two molecules of NADPH donates two electrons. HMG-CoA reductase, an integral membrane protein of the smooth ER with a rapid turnover, is the major point of regulation on the pathway to cholesterol.

Stage 2. Conversion of mevalonate to two activated isoprenes

In this stage three phosphate groups are transferred from three ATP molecules to mevalonate. In the next step, the phosphate attached to the C-3 hydroxyl group of mevalonate and the nearby carboxyl group leave, producing a double bond in the five-carbon product, **Δ^3-isopentenyl pyrophosphate** (the first of the two activated isoprenes central to cholesterol formation). Isomerization of Δ^3-isopentenyl pyrophosphate yields the second activated isoprene, **dimethylallyl pyrophosphate**.

Stage 3. Condensation of six activated isoprene units to form squalene.

Isopentenyl pyrophosphate and dimethylallyl pyrophosphate now undergo a head-to-tail condensation, in which one pyrophosphate group is displaced, and a 10-carbon chain, **geranyl pyrophosphate**, is formed (the ″head″ is the end to which pyrophosphate is joined). Geranyl pyrophosphate undergoes another head to tail condensation with isopentenyl pyrophosphate, yielding the 15 carbon intermediate

farnesyl pyrophosphate. Finally, two molecules of farnesyl pyrophosphate join head-to-head, with the elimination of both pyrophosphate groups, to form squalene.

Stage 4. Conversion of squalene to the four-ring steroid nucleus

The action of squalene monooxygenase adds one oxygen atom from O_2 to the end of the squalene chain, forming an epoxide (this enzyme is a mixed-function oxidase; NADPH reduces the other oxigen atom of O_2 to H_2O).

The double bonds of the product, squalene 2,3-epoxid undergo a series of ring closures step, that gives the steroid nucleus; some hydride transfers and methyl group rearrangements gives lanosterol. Lanosterol undergoes three demethylations, a shift in the position of the double bond in the ring system, and saturation of the double bond in the alkyl tail, to give cholesterol (lecture fig.).

Esterification of cholesterol

- Cholesterol can be esterified at its hydroxyl group with a fatty acid (often with oleate or linoleate, in humans). Esterification may be catalyzed intracellularly by an acyl CoA:cholesterol transferase (ACAT), with the acyl CoA as a co-substrate.
- Alternatively, in serum the fatty acid moiety may be transferred from phosphatidylcholine, in a reaction catalyzed by lecithin-cholesterol acyltransferase (LCAT). The serum reaction takes place mainly within HDL particles and helps to trap newly-synthesized cholesterol inside the particle.

24.2. TRANSPORT OF CHOLESTEROL

Newly synthesized cholesterol is exported from the liver through the circulation to other tissues where it can be used in membrane synthesis or in hormone biosynthesis. Transport occurs via lipoproteins.

- VLDL (very low density lipoprotein) carries cholesterol, cholesteryl esters, and triacylglycerols from the liver to other tissues, where the triacylglycerols are degraded by lipoprotein lipase, converting VLDL to LDL (low density lipoprotein).
- The LDL, the major cholesterol transporter, rich in cholesterol and its esters, is taken up by receptor mediated endocytosis, in which the apolipoprotein B-100 of LDL is recognized by receptors (LDL receptor) in the plasma membrane.
- HDL (high density lipoprotein) originates in the liver and small intestine as small protein-rich particles that contain relatively little cholesterol and no cholesteryl esters and the enzyme LCAT, removes cholesterol from the tissue and plasma carrying it to the liver.
- Chylomicrons, synthesized in the small intestine, move dietary lipids and cholesterol from the intestine to other tissues. Chylomicrons that have had their triacylglycerol content reduced by plasma lipoprotein lipase become chylomicron remnant, which are rich in dietary cholesterol (free and esterified) and in fat soluble vitamins. They are taken up by receptor mediated endocytosis into liver cells.
- Transcription of the gene for the LDL receptor is responsive to the levels of cholesterol. Decreases in cholesterol levels result in greater gene transcription, more synthesis of LDL receptor protein, and hence greater uptake of cholesterol from serum.

24.3. REGULATION OF CHOLESTEROL BIOSYNTHESIS

- Cholesterol synthesis is a complex and energy expensive process, so it is clearly advantageous to an organism to regulate the biosynthesis to complement the dietary uptake

- Cholesterol biosynthesis is feedback-regulated at the step then catalyzed by the enzyme HMG-CoA reductase, which catalyzes the rate-limiting step in the pathway.
- Regulation by energy levels and hormones is mediated by covalent modification of HMG-CoA reductase. The enzyme exist in phosphorylated (inactive) and dephosphorylated (active) forms. Low energy levels (high AMP concentration and glucagon hormone stimulate phosphorylation (inactivation), and insulin promotes dephosphorylation (activation).
- Increases in intracellular cholesterol also tend to reduce transcription of the gene for HMG-CoA reductase (by a family of proteins called sterol regulatory element-binding proteins (SREBPs)), leading to less enzyme amount and hence less cholesterol biosynthesis. Intake of cholesterol through the diet can cause this.
- There is relatively rapid turnover of the enzyme (HMG-CoA reductase). The combination of direct enzyme inhibition and indirect loss of enzyme level can reduce overall biosynthetic activity in the liver within hours.
- The class of drugs called **statins** specifically inhibit HMG-CoA reductase (their structure resemble to mevalonate) and thereby blocks cholesterol synthesis
- Two other regulatory mechanisms influence cellular cholesterol level:First, high intracellular concentrations of cholesterol activate ACAT, which increases esterification of cholesterol for storage, and and secondly, high cellular cholesterol levels diminish (via SREBP) transcription of the gene that encodes the LDL receptor, reducing production of the receptor and thus the uptake of cholesterol from blood.

Unregulated cholesterol production can lead to serious human disease. When the sum of cholesterol synthesized and obtained in the diet exceeds the amount required for the synthesis of membranes, bile acids, and steroids, pathological accumulation of cholesterol in blood vessels (atherosclerotic plaques) can develop, resulting in obstruction of blood vessels (atherosclerosis).

- **Atherosclerosis** is linked to high level of cholesterol in the blood, and particularly to a **high level of LDL-bound cholesterol**; there is a negative correlation between HDL levels and arterial diseases. In familial hypercholesterolemia, a human genetic disorder, blood levels of cholesterol are extremely high and severe atherosclerosis develops in childhood. These individuals have a **defective LDL receptor** (or defects in apo B-100) and lack the receptor mediated uptake of cholesterol carried by LDL.
- In **familial HDL deficiency**, HDL levels are very low; they are almost undetectable in **Tangier disease** (both disease are very rare). Both genetic disorder are the result of **mutations in the ABC1 protein**. Cholesterol-depleted HDL cannot take up cholesterol from cells that lack the ABC1 protein, and cholesterol-poor HDL is rapidly removed from the blood and destroyed.

24.4. BILE ACIDS

- The bile acids are the end products of cholesterol metabolism
- They are synthesized from cholesterol in the liver, and then transported to the gall bladder for secretion.
- Three major bile acids are cholate (cholic acid), glycocholate, and taurocholate.
- Cholate is made, as cholyl-CoA, in several steps from cholesterol. Reaction with glycin gives glycocholate, which is the major digestive bile acid. Alternatively, the reaction of cholyl-CoA with taurine (a catabolite of the amino acid cysteine) gives taurocholate
- Transformation of cholesterol to bile acids requires, first, the epimerization of the 3β-OH group , and secondly, the reduction of the C-5 double bond and thirdly, the introduction of OH groups at C-7 and C-12 and finally, the conversion of the C-27 side chain into a C-24 carboxylic acid by elimination of a propyl equivalent..
- Bile acids are a major component of bile, the digestive fluid secreted by the gall bladder into the digestive tract.

- Bile acids are effective biological detergents, as they contain both nonpolar groups (the sterol ring) and a polar region (the ionic acid groups)
- They form micelles and help to solubilize dietary lipids, for further digestion.
- The capacity of liver to produce bile acids is insufficient to meet the physiological demands, so the body relies on an efficient enterohepatic circulation.
- Bile acids represent the only significant way in which cholesterol can be excreted; the carbon skeleton of cholesterol is not oxidized to CO_2 and H_2O in humans, but is excreted in bile as free cholesterol and bile acids. Humans do not have the capacity to degrade sterol rings, so cholesterol and other sterol are excreted with the ring system intact. Because of their poor aqueous solubility, sterols are excreted via the feces.

24.5. STEROID HORMONES

- Humans derive all their steroid hormones from cholesterol.
- There are three general classes of steroid hormones.
- Sex hormones are involved in sexual maturation and reproduction.
- Glucorticoids help regulate gluconeogenesis and the immune response.
- Mineralocorticoids help control reabsorption of small ions in the kidney (e.g., Na^+, Cl^-, and bicarbonate).
- Steroid hormones are effective at very low concentrations and are therefore synthesized in relatively small quantities. In comparison with the bile salts, their production consumes relatively little cholesterol
- All steroid hormones are derived from cholesterol through pregnenolone.
- Synthesis of steroid hormones requires removal of some or all the carbons in the side chain on C-17 of the D ring of cholesterol. Side chain removal takes place in the mitochondria of steroidogenic tissues. Removal involves hydroxylation of the adjacent carbons in the side chain (C-20 and C-22) followed by cleavage of the bond between them.
- Progesterone is then derived from pregnenolone by oxidizing the 3-hydroxyl group to a ketone and shifting the double bonds position. Formation of the various hormones also involves the introduction of oxygen atoms. All the hydroxylation and oxygenation reactions in steroid biosynthesis are catalyzed by mixed function oxidases, that use NADPH, O_2, and mitochondrial cytochrome P-450.
- The steroid hormones count for a little of catabolism of cholesterol. Following their inactivation (by reduction) and conjugation with glucoronides or sulfates, they are excreted in the urine.

24.6. ALTERNATIVE FATES OF THE INTERMEDIATES IN CHOLESTEROL BIOSYNTHESIS

- Cholesterol biosynthesis provides substrate for the photochemical production of vitamin D3 in skin. **7-Dehydrocholesterol** is an intermediate in the pathway of cholesterol biosynthesis and is converted in the skin to provitamin D3 by irradiation with UV rays of the sun. Vitamin D3 is not itself biologically active, but it is converted by enzymes in the liver and kidney to active hormone (calcitriol), which acting through nuclear receptors and involved in the calcium homeostasis.
- In addition to its role as intermediate in cholesterol biosynthesi, **isopentenyl pyrophosphate is the activated precursor** of a huge array of **biomolecules** with diverse biological roles. They include vitamins A, E, and K; dolichols, which serve as lipid soluble carriers in complex polysaccharide synthesis; ubiquinone, an electron carrier in mitochondria; plant pigments such as carotene and the phytol chain of chlorophyllfound in many essential oil. Collectively, these molecules are called **isoprenoids**.

- **Prenylation** (covalent attachment of an isoprenoid) is a common mechanism by which proteins are anchored to the inner surface of cellular membranes in mammals. In some of these proteins, the attached lipid is the 15-carbon farnesyl; others have the 20 carbon geranylgeranyl group. It is possible prenylation **target proteins to different membranes**, depending on the attached lipid.

25. AMINO ACIDS AND PROTEINS

25.1. AMINO ACIDS

Roles of the Amino Acids

Amino acids characteristically feature several biological roles:

- They may be polymerized to form peptides, most notably proteins (polypeptides);
- They serve as precursors for other small biomolecules, e.g., purines, pyrimidines, porphyrins, etc.;
- They may be oxidized to serve as an energy source (fuel) for the cell.

General Features of Amino Acids

Amino acids contain two characteristic functional groups: an amino group and a carboxylic acid .

- For amino acids used in proteins (α -amino acids), these are attached to a central carbon
- atom, C α , in which a hydrogen atom and an organic side chain group R is also attached.
- These (20) common amino acids are defined as those for which at least one specific codon
- exists withinin the DNA genetic code.
- The central carbon is a chiral center for 19 out of 20 of the common amino acids, and the L isomer is considered the common stereoisomeric form. Additionally, D isomers of amino acids are rarely found in the bacterial cell wall and peptide antibiotics) .

Other features of amino acids include the following:

- Amino acids are white crystalline ionic solids with high melting points and poor solubility in organic solvents (they are moderately soluble in water).
- Amino acids dissolve in water as dipolar ions, or zwitterions, due to ionization of the acid and amino groups (the side chains may also contain titrable groups and be ionized under
- physiological conditions).
- The α -carboxyl group will typically have a pKa around 2.0 - 2.4 and the α -amino group
- around 9.0 - 9.7.
- Titrable groups on the side chains vary considerably in their pKa values.

Classification of amino acids by properties

The 20 common amino acids can be grouped by properties of the side chain into nine classes:

- **Glycine** (Gly) is in a class by itself.
- **Alanine** (Ala), **Valine** (Val), **Leucine** (Leu), and **Isoleucine** (Ile) all have alkyl side chains.
- **Prolin** (Pro) is in a class by itself.
- **Phenylalanine** (Phe), **Tyrosine** (Tyr), and **Tryptophan** (Trp) have aromatic side chains.
- **Cysteine** (Cys) and **Methionine** (Met) have sulfur atoms.
- **Serine** (Ser) and **Threonine** (Thr) have –OH groups.
- **Glutamate** (Glu) and **Aspartate** (Asp) have acidic groups in the side chain.
- **Glutamine** (Gln) and **Asparagine** (Asn) have amides of those acid groups.
- **Lysine** (Lys), **Arginine** (Arg), and **Histidine** (His) have basic groups in side chains.

First Glycine, with its side chain of a **simple hydrogen atom**, is in a class by itself. The hydrogen atom presents the least steric hindrance to rotation or packing of neighboring group in protein, so Gly residues contribute strongly to protein **flexibility**. At acid pH, Gly is found mainly in the form of the cation; at alkaline pH, it is manly in the form of anion.

At physiological pH, around pH 7, (the isoelectric point-pI- of Gly is 5.97) the glycine exists mainly as a zwitterion (dipolar ion), and only small amounts of the electrically neutral, cationic or anionic species is present.

Secondly there are amino acids with **alkyl side chains**: Ala, Val, Leu, Ile. The **nonpolar character of the side chains** of these amino acids make their surface hydrophobic, and the packing of these side chains **in the interior of protein**, away from exposure of water, help to stabilize the structure. The bulkiness of the side chains also contribute to forming specific binding sites on the surface of proteins.

Thirdly , Pro is a group unto itself; it has an aliphatic side chain with a distinctive **cyclic structure**. The secondary amino (imino) group of proline residues is held in **rigid** conformation reducing the structural flexibility of polypeptide regions containing proline.

The *fourth* class comprises the aromatic amino acids: Phe, Tyr, and Trp. The **aromatic side chains** are bulky and hydrophobic; these side chains may also be used for molecular recognition in binding sites on protein surface. Tyr and Trp are significantly more polar than Phe,because of the Tyr hydroxyl group and the nitrogen of the Trp indole ring. Trp and Tyr, and to a much lesser extent, Phe **absorbs ultraviolet light**. This accounts for the characteristic strong absorbance of light by most of the proteins at a wavelenght of 280 nm.

The **sulfur-containing** amino acids Cys and Met form the *fifth* category. The thiol group on the side chain of Cys is a weak acid and may be ionized at a pH close to physiological ; this can be important for acid-base behavior in certain enzyme catalytic mechanism. Cysteine can form disulfide bonds with adjacent thiols, especially Cys-to-Cys **disulfide bridges**, which are often important in stabilizing the folded tertiary structure of proteins. The combination of two Cys residues joined by such a bridge is the amino acid cystine. Bridge formation requires oxidation of the thiols on both participants; disruption of the disulfide bridge requires reduction. The sulfur atom in Met is relatively polarizable, and can contribute to molecular recognition in binding sites.

The *sixth* category made up of two amino acid alcohols Ser and Thr. The **hydroxyl group on the side chain** is only very weakly acidic, but is an important site for protein modifications (e.g., phosphorylation, glycosylation) capable of affecting the functionality of the protein.

The *seventh* category includes the **acidic amino acids** Glu and Asp. The carboxyl groups of their side chains are ionized to anions at physiological pH, this phenomenon contributes to the overall charge on a protein, important for its solubility and for recognition of binding partners.

The *eight* category, containing Asn and Gln, is composed of the **amides of the acidic amino acids**. These amides are not titrable, but they are highly polar, and they participate in hydrogen bonding.

The *ninth* category collects the **basic amino acids** Lys, Arg and His.. The imidazole ring of His can accept or donate protons at pH values near neutral; this is an important factor in acid-base catalysis of many enzymes. The imidazol-ring nitrogens can also help bind metal ions. Lys has its amino group at the end of a relatively long chain, allowing flexibility in positioning the (usually positively charged) terminus in active sites, or for forming amide links to carboxyl groups of cofactors such as biotin. The positively charged guanidium group on the side chain of Arg is often involved in especially strong ionic or hydrogen bonding interactions with negative ions (such as carbxylates or phosphates.

Charge of amino acids and proteins

Amino acids are ionized (act as weak acids and bases) in aqueous solution (**amphoteric** or **ampholytes**). Ionizable groups common to proteins and amino acids are the NH2 in peptides, Lys; COOH-terminal residue in peptides, Glu, Asp;and side chain groups of Arg, Cys, His,and Tyr. The ionization state of the ionizable groups (and the charge of the amino acid or protein) depends on the pH of the solution. The titration curve of an amino acid without an ionizable side chain has two ionization stages with two ionization midpoint (lecture figure). Amino acids with an ionizable R group have more complex titration curves, with three stages, corresponding to the three possible ionization steps; they have three pK values. There is a characteristic pH at which the net electric charge is zero. It is called the **isoelectric point** or **isoelectric pH**, designated **pI**.

The isoelectric points reflect the nature of the ionizing R group present.

Peptides contain only one free α-aminogroup and one free α-carboxyl group at opposite ends of the chain, the others are covalently joined in the peptide bonds, which do not ionize and therefore do not contribute to the total acid-base behaviour of peptides. The total charge of a peptide (protein) depends on the nature and the number of the ionizable R groups at a given pH..

The techniques of electrophoresis, isoelectric focusing, and ion-exchange chromatography separate and characterize biological molecules on the basis of differences in their pI. (When the pH>pI, then the protein charge negative; when pH<pI, then the protein charge positive). In clinical medicine, separation of plasma proteins by electrophoresis has led to the classification of the proteins based on their relative electrophoretic mobility. The separation is commonly carried out at pH 8.6, which is higher than the pI values of the major plasma proteins. Accordingly, the proteins

are negatively charged and move toward the anode at a rate dependent on their net charge. The electrophoretic mobility patterns change characteristically in certain diseases (see lecture figure).

Some important but less common amino acids include the following

- **Hydroxyproline** and **hydroxylysine** are found in the connective tissue protein collagen.
- Ser, Thr, Tyr, and Arg can be **phosphorylated** on the side chain.
- • **Selenocysteine** resembles Cys in structure, but with a selenium atom replacing the sulfur
- atom in the side chain.
- **Ornithine** is similar to Lys, and is important in the urea cycle and elsewhere.
- **Homocysteine** is an intermediate in the catabolism of methionine.
- γ **-Aminobutyric acid (GABA**) is an important neurochemical.

Peptides

Two amino acid can be covalently joined through a *substituted amide linkage*, termed a **peptide bond**, to yield a **dipeptide**. Such a linkage is formed by the condensation of the α-carboxyl group of one amino acid with the α-amino group of another amino acid. Peptide bonds are *thermodynamically unstable* with respect to hydrolysis (equilibrium favours hydrolysis). However, the bonds are *kinetically stable* in neutral aqueous solution (the hydrolysis of peptide bond is

exergonic, but it needs a high activation energy). The peptide bond is planar due to the partial double bond character of the C-N bond, involving resonance hybridization and sharing of the lone pair of electrons on the amide nitrogen with the carbonyl carbon. Additionally, due to the sharing of nitrogen s electrons, they are not available to bond to a proton from solution; hence, they are characterized as amids resist titration and *elelctrically neutral functional groups*. *Rotation around the peptide bond is*

energeticallly restricted because of its partial double bond character. This is not the case, however for the carbon-carbon bond between Cα and the carboxyl group or for the nitrogen-carbon bond joining the amide group to the adjacent Cα. This relative free rotation around these single bonds leads to immense possible conformational freedom for the polypeptide chain.

Joining of amino acids in a peptide chain leaves one end the chain with a free amino group (N-terminus) and the other end with a free carboxylic acid group (the C-terminus). Conventionally, a **polypeptide's sequence** of amino acids is written **starting with the N terminus on the lef**t a C-terminus on the right.

Three amino acids can be joined by two peptide bonds to form a **tripeptide** and so forth.

When a few aminoacids are joined in this manner, the structure is identified as an **oligopeptide**. When many amino acids are joined, the product is characterized as a **polypeptide**. Typically, molecules referred as a polypeptide generally feature molecular weights below 10 000, and those called proteins have higher molecular weights.

The peptides differs from proteins in other respect as well:

Peptides can contain other amino acids beside the 20 common amino acids.

Some peptides contain amino acids (for example β-alanin), and do not contain α-amino group.

In peptides, not only the α-amino or α-carboxyl group of the amino acid can be involved in the formation of peptide bond.

Some peptides are cyclic compounds, and therefore, do not contain N-terminal or C-terminal amino acids, because every amino and carboxyl group involves peptide bonding. formation.

Additionally, peptides may contain D amino acids as well.

Biologically active peptides and polypeptide occur in a vast range of sizes and composition. Many small peptide exert their effects at very low concentration. For example, a number of vertebrate hormones are small peptides (insulin, oxytocin, TRH). Glutathione, which is present in a wide variety of cells, is an important tripeptide. Some extremely toxic mushroom poisons, such as amanitin, are also small peptides, as are many antibiotics.

25.2. PROTEINS

The structure of proteins

Proteins are long polymers of amino acids connected by peptide bond to each other.

Four levels of protein structure are commonly defined.

Primary structure is a description of all covalent bonds (mainly peptide bonds and disulfide bonds) linking amino acid residues in a polypeptide chain. The most important element of primary structure is the *sequence* of amino acid residues.

The **secondary structure** refers to a *regular spatial arrangement of the atoms along the chain backbone*. The secondary structure *is stabilized by weak, noncovalent interactions* such as hydrogen bonds, hydrophobic and electrostatic interactions. For polypeptide chains there are three principal secondary structures, the α-helix, the β-sheet and the collagen helix.

Tertiary structure refers the spatial relationship among all the amino acids in the polypeptide chain. Tertiary structure describes the *complete three dimensional structure of the polipeptide*.

When a protein has *two or more polypeptide chains or subunits*, their spatial relationship is referred to as **quaternary structure**.

Comparison of primary structures is commonly used to predict the similarity in structure and functionality between proteins. Sequence comparisons typically require aligning sequences to maximize the number of identical residues while minimazing the number of insertions or deletions required to achieve this alignment.

Two sequences are termed **homologous** when their sequences are highly alignable and they have a genetic (evolutionary) relationship. **Analogy** is used to desribe sequences from proteins structurally similar but for which no evolutionary relationship yet been demonstrated.

Substitution of an amino acid by another amino acid similar polarity is called a **conservative substitution** and is commonly observed in amino acid sequences of the same protein from different species. If a particular amino acid is always found in the same position, these are designated **invariant residues** and it can be assumed these residues have an essential role in the structure and function of the protein. Notably, by contrast, **non-conservative substitutions** requires the replacement of one amino acid with another featuring a distinct and contrasting polarity. In addition to the change in polarity, other physical properties of amino acids, as volume and surface area determines whether a substitution will significantly alter the protein´s function.

Secondary structure

The α-helix

The α-helix is formed from a **single strand of polypeptide**. It is **stabilized by** a characteristic pattern of **hydrogen bonding**, specifically, the carbonyl of an amide to the NH of another amide, **four residues farther along the chain**. These hydrogen bonds all point roughly in the same direction, parallel to the axis of the helix. All the backbone carbonyl and NH residues are hydrogen bonded, except those near the end of the hekix. The polypeptide backbone curls in space in a **right-handed** helix, the α-helix. There are 3.6 residues per turn of the helix, with one turn of the helix rising by 5.4 A (rise per residue of 1.5 A). The neighboring residues ends up on opposite sides of the helix.

The side chains of amino acids are exposed and project laterally away from the helix.

Amino acid sequence affects the stability of the helix. For example, Pro cannot parcipitate in the normal H-bonding scheme and cannot rotate around the N-Cα bond; this will cause the helix to kink or bend, so **Pro is unsuitable** for inclusion in an α-helix. **Gly occurs infrequently** in the α-helix for a different reason; it has more conformational flexibility when compared with other amino acids.

Polymers of Gly generally take up the coiled structures differently when compared to an α-helix.

Other primary factors affecting stability include the following:

- repulsion between successive residues
- bulkiness of adjacent side chain groups
- interaction of R groups 3-4 residues apart
- interaction of the ends

The β-sheet

The β-sheet structure is formed from two or more strands of amino acids. The strands are extended, and there is a roughly coplanar alignment of backbone atoms across the strands Like the α-helix, the β-sheet used H bonding between backbone carbonyl and amino groups on the strands. However here the hydrogen bonds are positioned at right angles across the strands to the general direction of the component strands. Hence, two potential directions are possible for the adjacent strands :aligned parallel to one another or in an non-parallel orientation.

The “sheet” description is taken from the the coplanarity of the backbone atoms for the participating strands. A ˝pleated sheet˝ describe the alternation of Cα atoms above and below the plane of the sheet. In real proteins, the sheets are not precisely planar, but tend to feature a right-hand twist. The side chains project above and below the plane of the sheet.

The β-turn

A high proportion of residues in tightly folded proteins are involved in turns of the backbone. These turns may **flexible loops** of larger segment of amino acids, serving to join different stretches of secondary structure, or may be **sharp turns** (shorter non-regular segments) rever sing the chain direction. One common structural element found in connection with antiparallel β-sheet structures is the β-turn. A few variation of this type of turn exist; in the two most common ones residue is H-bonded to residue i+3. This structure use four amino acids to effect a 180◦ turn in chain direction. Gly and Pro are often found in β-turns (Pro adopting a cis conformation to tighten the helix).

Tertiary structure

The tertiary structure is the **three dimensional structure** of a protein. It refers to a larger scale organization of the polipetide chain and describes how elements of the secondary structure are linked to and packed against one another. The tertiary structure is **stabilized by noncovalent interactions**, although the covalent linkages (disulfide bonds between Cys residues) can be also an important factor . **Two main types** of proteins, **fibrous** and **globular**, can be distinguished by their tertiary structures and by chemical properties.

Different proteins may share a common folding pattern for two or more secondary

structural elements: it is called a **motif**. A motif is a recognizable folding pattern involving two or more elements of secondary structure and the connection(s) between them (for example β-α-β loop). Motifs are tipically formed from adjacent chain segments, rather than from distant segments.

Notably, motifs are not considered independently stable. Once isolated from the remainder of the protein structure, they often unfold. There are several type of motifs.

The second structural/folding pattern is the **domain**. Domain is a compact structural unit within a polypeptide, and fold as independent units. Different domains often have different functions, such as binding of small molecules, or interaction with other proteins

Globular proteins

Globular proteins are water-soluable proteins with several function. Globular proteins and feature a variety of tertiary structures. As the name implies, these are roughly spherical in shape and generally compactly folded. Amino acids with nonpolar side chains are generally found in the interior of the protein. In contrast, amino acids with polar and ionic side chains predominate on the protein´s surface. In

this way the polar and ionic side chains help to solvate the protein, while the nonpolar, hydrophobic side chain are kept buried, thereby separated from potential contact with water.

Fibrous proteins (collagen, keratin, tropomyosin, silk fibroin)

- In fibrous proteins, the polypeptide chains are arranged in long strands or sheets.
- They usually consist largely of a single type of secondary structure, and their tertiary structure is relatively simple.
- There function differs from globular proteins: they provide support, shape and external
- protection to vertebrates; whereas most enzymes and regulatory proteins are globular protein.

Collagen

It is a typical fibrous protein with many subtypes (more than 20).

Collagen provide tensile strength in connective tissue such as tendon, cartilage, bone, cornea and skin.

It has high proportion of Gly, Ala, Pro, and hydroxyproline

The collagen helix is left-handed and has three amino acid residues per turn.

Collagen has a distinctive secondary structure, namely a triple helix. The chains are arranged in a right handed triple-stranded helix which is quite distinct from the α-helix

The amino acid sequence in collagen is generally a repeating tripletide unit, Gly-X-Y, where X is often Pro, and Y is often 4-Hyp.

Only Gly residues can be accommodated at the condensed intersections between the individual chains; the Pro and 4-Hyp residues permit the sharp twisting of the collagen helix.

Together, the repeating tripeptide units help wind the three strands into a rod-like bundle.

In connective tissue, collagen triple helices are packed together into fibrils, which are then cross-linked by covalent bonds between Lys, HyLys or His residues(which are present at several different positions). The collagen fibrils can aggregate to form cable-like bundles (collagen fiber)

The result is a very stable and strong pad of connective tissue.

Quaternary structure

Quaternary structure results from interactions between the subunits of multi-subunit (multi-meric) proteins or large protein assembles. The subunits may be identical or non-identical polypeptide chains, associated noncovalently. Some multimeric proteins have a repeated unit consisting of a single subunit or a group of subunits.

Unstructured proteins

Some proteins or protein segments are intrinsically disordered, lacking definitive structure.

These proteins feature distinctive amino acid compositions (rich in polar and charged amino acids, and Pro) allowing more flexibility to their stucture. They are dynamic structures and are often similar to the intermediates of folding pathways.

Some of these disordered proteins function as structural components or scavengers; others may interact with a variety of different protein partners, serving as versatile inhibitors or as central components of protein interaction networks. They often develop a distinct structure once they interact with other proteins, however, the structure may vary with different binding partners.

Protein complexes, networks

Some proteins form protein complexes consisting 5-10, 20-30 proteins. A complex with connection of more than 3 complexes is called hub; it can be a good target of drug therapy .

The functional network of interconnected complexes is referred as **interactome**.

Conjugated proteins

Some proteins contain permanently associated chemical components in addition to amino acids; these are defined as conjugated proteins. Conjugated proteins are classified on the basis of the chemical nature of their prosthetic groups; for example, lipoproteins contain lipids, glycoproteins contain sugar groups, and metalloproteins contain a specific metal.

25.3. PROTEIN FOLDING

Protein folding is a spontaneous, non-random process towards achieving a unique active conformation. The conformation of a protein is the conformation of the lowest free energy accessible to the amino acid sequence within a physiological time frame. Thus, folding is under thermodynamic and kinetic control. It is initiated by short range interactions (noncovalent interactions) forming secondary

structures in small regions of the polypeptide. The noncovalent forces, such as hydrophobic interaction forces (increased entropy of water molecules), hydrogen bonds formations, electrostatic interactions and Van der Walls forces, lead to protein folding and contribute to a protein´s stability. The folding proceeds through intermediates. Sections of polypeptides, known as initiation sites, fold into small regions of the secondary structure. Next, the partially folded structure condenses to one another forming a molten globule state (condensed inter-intermediate on the folding, contains much of the secondary structure elements of the native structure, but a large number of incorrect tertiary structure interactions). The correct medium and long-range interactions beween different initiation sites are found by rearrangements within the molten globule and the low free energy, native tertiary structure is formed. Upon formation of the native tertiary structure, the correct disulfide bonds are formed. In general, folding in the cell is rapid, occuring within seconds in vivo.

Molecules assist protein folding

Protein folding is often assisted by chaperone proteins, large multi-subunit complexes.

Chaperones perform several roles in connection with protein folding:

They assist newly synthesized chains to fold properly

They aid in the movement of proteins across membranes to target organelles or for

secretion to the cellś exterior

They help mis-folded proteins to unfold and re-fold correctly particularly after heat shock or stress.

They help to block the aggregation of misfolded poteins, and they assist in resolubilizing and refolding aggregated proteins

They assist in the assembly of multimeric protein structures

They are components of some signal transduction pathways in the cell.

Two major families of chaperones are the **Hsp70** family and the **chaperonine.**

Hsp70 proteins are abundant in cells stressed by heat. They bind to the hydrophobic

regions of unfolded polypeptides, and protect them. The Hsp70 proteins bind to and release polypeptides in a cycle using energy from ATP hydrolysis and involves several other proteins (including a class called Hsp40)

Chaperonins (GroEL/GroES in bacteria, hsp60 in eukaryotes) are long multisubunit

structures, required in the folding of some cellular proteins. They bind the unfolded proteins in their cavity and prevent inappropiate protein aggregation.

The folding pathway of some proteins requires two enzymes catalyzing isomerization reactions.

Protein disulfide isomerase (PDI) is a widely distributed enzyme which catalyzes the interchange, or shuffling, of disulfide bonds until the bonds of the native conformation are formed. Among its functions, PDI catalyzes the elimination of folding intermediates with inappropiate disulfide cross-links.

Prolin residues mostly have the trans geometry, but to facilitate the formation of β-turns, the cis geometry is preferred. The cell contains **peptide prolyl cis-trans isomerases (PPI**) to specifically isomerize proline residues from trans to cis geometry.

25.4. PROTEIN DENATURATION

Denaturation of a protein means the loss of higher structure (secondary, tertiary and quaternary structure) together in association with the intersection, or junction. (The denatured state doesn´t necessary equal with complete unfolding of the protein and randomization of conformation. Under most conditions, denatured proteins exist in a set of partially folded states.)

Even though conformational differences between denatured and native structure may be substantial, the free energy difference, may in same cases be as low as the free energy of three or four noncovalent bonds.

A change in stability of noncovalent bonds-denaturation- can be caused by a change in pH, ionic strength or temperature.

In vitro denaturation

1. **Thermal denaturation** of a protein:

Even at room temperature, the weak noncovalent interactions stabilizing the folded form of a proteins transiently break and reform. Such fluctuations can be integralin the proper functionality of a protein. Raising the temperature causes more thermal fluctuations in a protein´s structure as more of these weak interactions are disrupted, until the critical loss of stability occur. The

temperature of the midpoint of the transition from native to unfolded conformation is denoted as Tm (melting temperature).

2. Denaturation of the proteins by **extremes of pH**, with **strong acids or bases**, by certain **miscible organic solvents** (acetone, alcohol) by certain solutes (urea, guanidine hydrochloride), or by a **detergent**:

Organic solvents, urea and detergents act primarily by disrupting the hydrophobic

interactions consisting of the stable core of globular proteins,. Notably, urea also disrupts hydrogen bonds; pH extremes alter the net charge of the protein, causing electrostatic repulsion and the disruption of some hydrogen bonding.

The unfolding process can be monitored sprectroscopically by changes in protein absorbance or fluorescence, by heat absorption (caloriymetry), or by changes in solution viscosity.

The concentration of a protein in a cell is controlled by its rate of synthesis and degradation. Inmany circumstances, the denaturation of a protein is the rate controlling step in its degradation.

Cellular enzymes and organelles that digest proteins ˝recognize˝ denatured protein conformation and eliminate them rapidly.

25.5. PROTEIN TURNOVER

Turnover is the process of recycling proteins into their constituent amino acids, for the reuse by the cell.

Turnover is necessary for many reasons:

To eliminate misfolded or damaged proteins before they aggregate and interfere with cell processes;

To regulate the cell cycle by breaking down in a timely fashion proteins used transiently during the cell cycle; In the immune system, to provide the necessary cleavage of foreign antigenic proteins for presentation to and recognition by other immune system cells

The lifespan of proteins can vary from several moments up to minutes and or the lenght of life of the cell (e.g. eyelense proteins, hemoglobin). In contrary, the turnover of blood clotting proteins require several days, insulin several hours and transcription factors only several minutes.

Relationship between protein half-life and amino-terminal amino acid residues has been found

(lecture table). For many proteins, the identity of the first residue remaining following removal of the amino terminal Met residue, and any other posttranslational proteolytic processing of the amino-terminal end, has a profound influence on half-life. More complex signals, such as ˝destruction box˝ on the amino terminal also are being identified.

There are two main systems for protein degradation in mammalian cells: the lysosomal system and the proteosome/ubiquitin system.

The lysosomal system

Lysosomes are extremely acidic, with a pH in the range 3.8-5.0, which promotes denaturation of proteins and nucleic acids. Lysosomes also contain numerous hydrolytic enzymes bursting open thebacterial cells, digest lipids and the break down of the backbone bonds in the protein and nucleic acid polymers. The building block monomers of these macromolecules can then passed out of the lysosome for recycling by the cell

The ubiquitin/proteasome-system

It is primarily responsible for turnover of the cell´s own proteins, but also contributes to the breakdown of foreign proteins

Ubiquitin is a 76-residue highly conserved polypeptide that can be linked to other proteins to mark them for degradation. Ubiquitin is covalently linked to proteins slated for destruction via an ATP-dependent pathway involving three separate types of enzymes, called E1 activating enzymes, E2 conjugating enzymes, and E3 ligases (lecture figure)

Proteins with multiple ubiquitin groups are recognized by the **proteasome**. The proteasome is a very large, multisubunit protein assembly degrading protein chains into short oligopeptides.

The eukaryotic 26S proteasome contains two main types of subcomplexes, a barrel-like core particle and regulatory particles on either end of the barrel. The central 20S cylindrical core particle is composed of a stack of four rings. Each ring has even subunits, either α or β. The 19S cap serve as regulatory units and help to recognize, bind, unfold and translocate the unfolded polypeptide into the core particle for degradation.. The 19S particle also deubiquitinates the proteins as they are degraded in the proteasome. The central cylinder of the proteasome contains a

hollow central chamber, in which multiple proteolytic activities occur and are thereby associated with the β subunits (lecture figure).

Defects in protein breakdown

Defects in the degradation pathway have been implicated in a wide range of disease states:

cancer,

renal disease,

asthma,

neurodegenerative disorders such as Huntington disease (mutant huntingtin protein accumulation),

Alzheimer disease (amyloid β precursor protein (APP) product-beta amyloid-accumulation),

Parkinson disease (α-synuclein-a synaptic protein-deposits in neurons leads to formation of aggregates-Lewy bodies)

Juvenile Parkinsonism (Parkin protein mutation causing loss of E3-ubiquitin ligase-enzyme activity)

25.6. SEPARATION OF PROTEINS

Proteins can be purified according to size, charge, solubility and specific binding affinity

1. Separation methods for proteins based on charge:

Electrophoresis (migration of dissolved proteins in electric field)

Isoelectric focusing (separation of proteins according to their isoelectric point)

Ion-exchange chromatography (proteins bind to oppositely charged beads filled in a column)

2. Separation methods based on size:

Ultracentrifugation (proteins subjected to centrifugal force moves in the direction of the force at a velocity dependent on its mass. The rate of movement is measured with an appropriate optical detection system, and from the rate the sedimentation coefficient is calculated in Svedberg units (S,units of 10

Molecular exclusion chromatography (a porous gel in the form of small insoluble beads is filled in a column. Small proteins penetrates the pores once the gel features a larger solvent volume through which to travel in the column, than large proteins.)

3. HPLC-chromatographic techniques separate amino acids, peptides, and proteins (In high-performance liquid chromatography, a liquid solvent containing a mixture of components to be identified is passed through a column densely packed with a small-diameter insoluble bead-like resin. Resin beads are coated with charged chemical groups to separate compounds by ionic charge or with hydrophobic groups to retard hydrophobic nonpolar molecules. This latter type of chromatography over nonpolar resin beads is called reverse-phase HPLC)

4. Affinity chromatography (base on individual proteins´ high affinity for their substrates, prosthetic groups, membrane receptors, specific noncovalent inhibitors, and antibodies made against them)

5. Two-dimensional electrophoresis (the combination of isoeletric focusing with SDS-polyacrylamide gelelectrophoresis)

Other protein analysing methods

AA sequencing

Polypeptide chains are most commonly sequenced by the Edmann reaction, in which the polypeptide chain is reacted with phenylisothiocyanate, which forms a covalent bond to the NH2 terminal amino acid. In this derivative, acidic conditions catalyze an intramolecular cyclization resulting in a cleavage of the NH2

phenylthiohydantoin derivative. This derivative may be separated chromatographically and identified against standards. By repeating these steps the sequence of a shorter polypetide chain can be determined. Longer polypeptide chains have to be hydrolyzed into smaller fragments by chemical or enzymatic methods and sequenced in sections.

Mass spectrometry can also be used to sequence of short stretches of polypeptides (tandem MS, or MS/MS).

X-ray diffraction enables determination of the three dimensional structure of proteins at near atomic resolution. This technic requires the formation of a protein crystal, which contain solvents and is thus a concentrated solution, for use as target. The technical base on the electron scattering property of X-ray .

Spectroscopic methods

UV light spectroscopy

The side chains of Tyr, Phe, and Trp, as well as peptide bonds in proteins, absorb ultraviolet (UV) light. A peptide bond in α-helix conformation interacts with elelctron of other peptide bonds above and below it in the spiral conformation to create an exciton system with delocalized electrons. The result is a substantial shift in the potential increase in absorption .. Thus, UV spectroscopy can be used to study changes in a protein´s secondary and tertiary structure.

Fluorescence

The energy of an excited electron produced by light absorption is lost by various mechanisms, in some cases by fluorescence emission. The fluorescent emission is always at a longer wavelenght of light (lower energy) than the absorption wavelenght of a fluorophore. The aromatic groups in proteins have a distinct fluorescence. When a second chromophore is present within close proximity to the first one, the light energy emitted by the first molecule (donor) directly-terminal amino acid from the polypeptide chain as a transferred to the second (acceptor). If the acceptor molecule loses its excitation energy by a nonfluorescent process, it quenches the donor molecule fluorescence. Since the efficiency of this excitation transfer is dependent on the distance and orientation between donor and acceptor molecules, the fluorescence yield is dependent on the conformation of the proteins. In folded state the chromophore side chains are closer to each other, than in denatured state.

Circular dichroism (CD)

L-amino acids optically active molecules rotate the polarized light in certain direction. In proteins, the aromatic amino acids and the polypeptide chain generate an optical rotation and CD spectrum. Because of the differences between α-helical, β-structure, and random polypeptide spectra, circular dichroism has been s sensitive assay for the amount and type of secondary structure in a protein.

Nuclear Magnetic Resonance (NMR)

With two-dimensional (2-D) NMR and powerful NMR spectrometers it is possible to obtain the solution conformation of small proteins of approximately 150 amino acids or less. Multifunctional NMR and triple resonance can extend the NMR to solve protein structures with up to 250 amino acids.

26. DIGESTION OF PROTEINS AND AMINO ACID SYNTHESIS

Amino acids necessary for protein synthesis in animals could come either from the digestive system as hydrolytic products of dietary proteins, or from endogenous biosynthetic pathways. The biosynthetic pathways require carbon skeleton from carbohydrates and nitrogen in the form of ammonia (amino group). The carbon skeleton links the amino acid metabolism to carbohydrate and fatty acid metabolism; the amino group makes connection with the urea cycle.

Mammals including humans can synthesize only about half of the 20 amino acids necessary for protein synthesis. These are the **nonessential** amino acids, not needed in the diet. Others can be synthesized, however the speed of the synthesis is not enough to support the need. These are the **semi-essential** amino acids. Finally, the **essential** amino acids must be obtained from food.

In humans, the degradation of ingested proteins to amino acids occurs in gastrointestinal tract in several steps. In the stomach, **pepsin** hydrolyzes ingested proteins cleaving long polypeptide chains into a mixture of smaller peptides. In the small intestine, **trypsin, chymotrypsin, carboxypeptidases** and **elastase** – all are synthesized and secreted by the exocrine cells of the pancreas – further hydrolyze the peptides that were produced by pepsin in the stomach. The resulting mixture of free amino acids is transported into the epithelial cells lining the small intestine, through which the amino acids enter the blood capillaries in the villi and travel to the liver. The catabolism of most of the amino acids which have reached the liver starts with the separation of the amino group and the carbon skeleton.

Amino acid biosynthesis requires large amount of nitrogen. The nitrogen source could be $\mathbf{N_2}$ or $\mathbf{NO_3^-}$ and the processes to convert these sources to $\mathbf{NH_4^+}$ are called **nitrogen fixation** and **nitrate assimilation (reduction)**, respectively. Nitrogen fixation is carried out by a complex of proteins called the **nitrogenase** complex. It contains two Fe-S subunits and an FeMo cofactor. The reaction of nitrogen to ammonia is an exergonic reaction: $\mathbf{N_2 + 10H^+ + 8e^- + 16ATP \rightarrow 2\ NH_4^+ + 16ADP + 16\ P_i + H_2}$. Nitrate assimilation is a two-step process. The first step is catalyzed by nitrate reductase, the second by nitrite reductase and the electrons are donated by NADH and ferredoxin: $\mathbf{NO_3^- + 2e^- \rightarrow NO_2^- + 6e^- \rightarrow NH_4^+}$.

In living systems, reduced nitrogen is incorporated first into amino acids glutamate and glutamine and then into a variety of other biomolecules. The key entry point is the glutamate which is metabolized by **transaminases** and the enzyme **glutamate dehydrogenase**. This enzyme is present in the mitochondrial matrix and in humans the reversible reaction goes mainly into the direction of production of α-ketoglutarate. In this direction, ammonia and NADH are produced from glutamate; the reverse reaction adds ammonia to α-ketoglutarate forming glutamate and NADPH is oxidized to NADP. In humans, the most important entry point for ammonia is the **glutamine synthetase** reaction, where glutamate and ATP react to form an intermediate, which then reacts with ammonia to produce glutamine and inorganic phosphate. Glutamine synthatase is found in all organisms, and since it plays a central metabolic role it is regulated both allosterically (feedback) and covalently (in bacteria).

26.1. THE GLUTAMATE FAMILY (GLU, GLN, PRO, ARG)

The biosynthesis of **glutamate** from **α-ketoglutarate** (**transaminase**) and **glutamine** from glutamate (**glutamine synthetase**) was already discussed. Glutamate is the precursor for **proline** and **arginine** biosynthesis. These synthetic pathways share their first steps: with ATP and NADH a semialdehyde is produced. This semialdehyde is then reduced to proline using NADPH; or after acetylation and transamination ornithine is formed which in turn converted to arginine by the enzymes of the urea cycle. Arginine is the substrate of the **NO synthase** reaction in which **NO**, an important gas transmitter is produced. Its role as a neurotransmitter and vasodilator will be discussed later. Glutamate decarboxylation (removing CO_2 with **PLP** cofactor) gives rise to **γ-amino-butirate (GABA)**, an inhibitory neurotransmitter.

26.2. THE ASPARTATE FAMILY (ASP, ASN, MET, THR, ISO, LYS)

Aspartate is synthesized from oxaloacetate by **transamination** from glutamate. **Asparagine** is synthesized by **amidation** of aspartate, with glutamine donating the amino group. From aspartate a semialdehyde is produced using ATP and NADPH and this semialdehyde is the precursor for the essential **lysine, threonine, isoleucine** and **methionine** amino acids.

26.3. THE ALANINE FAMILY (ALA, VAL, LEU)

Alanine is synthesized from pyruvate by **transamination** from glutamate or from valine. In humans, the biosynthesis of **valine** and **leucine** from pyruvate does not exist that is why these are also essential amino acids.

26.4. SERINE FAMILY (SER, CYS, GLY)

The major pathway for the formation of serine is started from 3-phosphoglycerate. First, it is oxidized by a dehydrogenase using NAD and then after transamination and hydrolysis serine is formed. The phosphoglycerate dehydrogenase reaction is regulated allosterically by serine. Serine is the precursor of glycine through removal of a carbon atom by serine hydroxymethyltransferase. The reaction requires PLP and tetrahydrofolate (THF). In the liver of vertebrates, glycine can be made by another route using glycine synthase (also called glycine cleavage enzyme): **CO_2 + NH_4^+ + N^5,N^{10}-methylene-THF + NADH + H^+ <-> glicin + THF + NAD^+**. Glycine is the precursor for numerous biosynthetic pathways such as purine nucleotides, porphyrin ring, creatine, bile acids and glutathione. Plants and bacteria produce the reduced sulfur required for the synthesis of cysteine from environmental sulfates. Mammals synthesize cysteine using the sulfur of the amino acid methionine. Methionine is first converted to S-adenosylmethionine which loses its methyl group to form S-adenosylhomocysteine. This product is hydrolyzed to homocysteine, which undergoes a reaction with serine, catalyzed by cystathionine β-synthase, to yield cysthationine. Finally, a lyase enzyme cleaves the cysthationine to free cysteine and α-ketobutirate. Homocysteine could be converted to methionine as well with a methyl group of a methyl-THF. Serine is an important precursor in phospholipid biosynthesis as well.

26.5. RING-STRUCTURED AMINO ACIDS (PHE, TYR, TRP, HIS)

Aromatic amino acids (Phe, Tyr, Trp) are synthesized using **erythrose 4-phosphate** and **phosphoenolpyruvate** as precursors. Chorismate is the first branch point of the pathway, with one branch leading to **phenylalanine** through phenylpyruvate, the other to **tyrosine**. **Tryptophan** biosynthesis starts from chorismate as well. The indole ring is formed from the nitrogen of glutamine and the carbons of phosphoribosyl pyrophosphate. Animals cannot produce phenylalanine and tryptophan (essential amino acids), however tyrosine can be directly produced from phenylalanine through hydroxylation of the phenyl group by **phenylalanine hydroxylase**. This reaction needs oxygen and tetrahydrobiopterin (THB) which is oxidized to dihydrobiopterin (DHB), which in turn can be reduced back to THB by dihydrobiopterin reductase (NADPH). Tyrosine gives rise to a family of catecholamines that includes **dopa** (by tyrosine hydroxylase), **dopamine** (by aromatic amino acid decarboxylase), **norepinephrine** (by dopamine β-hydroxylase), and **epinephrine**. Another important neurotransmitter, **serotonin** is derived from tryptophan in a two-step pathway by a hydroxylase and a decarboxylase enzymes. **Histidine** is derived from three precursors: **PRPP**, **ATP** and **glutamine**. PRPP contributes five carbons, the purine ring of ATP contributes a nitrogen and a carbon, and glutamine supplies the second ring nitrogen. The amino group of histidine is coming from a glutamate by a transamination reaction. The pathway to histidine exists only in plants and bacteria so the amino acid is essential for animals and humans. Histidine undergoes decarboxylation to **histamine**, a powerful vasodilator.

Amino acid biosynthesis is under complex allosteric regulation. Regulation takes place in part through feedback inhibition of the first reaction by the end product of the pathway. This first reaction is often catalyzed by an allosteric enzyme that plays an important role in the overall control of flux through that pathway. When one molecule serves as a precursor for several different amino acids, independently controlled isozymes prevent one biosynthetic end product from shutting down key steps in a pathway when other products of the same pathway are required.

26.6. AMINO ACID DEGRADATION

The pathways for amino acid degradation include a key step in which the α-amino group is separated from the carbon skeleton. The amino group is then shunted into the pathways of amino acid metabolism; the carbon skeleton is into the citric acid cycle.

Amino acids are grouped according to their major degradative end product. **Ketogenic** amino acids are degraded to acetoacyl-CoA and/or acetyl-CoA, can yield ketone bodies in the liver (Leu, Lys). The amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl-CoA, fumarate, and/or oxaloacetate, can be converted to glucose and glycogen are the **glucogenic** amino acids. Five amino acids are both ketogenic and glucogenic: Trp, Phe, Tyr, Thr, Ile.

Removal of the α-amino groups from the carbon skeleton promoted by enzymes called **aminotransferases**. In these reactions, the α-amino group is transferred to an α-ketoglutarate, forming glutamate, leaving behind the corresponding α-keto acid. There is no net deamination in these reactions where the prosthetic group is **PLP**. PLP is a derivative of B_6 vitamin (or pyridoxine) and is a carrier of amino groups. It undergoes reversible transformations between pyridoxal phosphate, which can accept an amino group and pyridoxamine phosphate, which can donate its amino group to an α-keto acid. In hepatocytes, glutamate then undergoes **oxidative deamination** producing α-ketoglutarate and ammonia, which in turn excreted in the form of urea (uroetelic organisms). The combined action of an aminotransferase and oxidative deamination is referred to as **transdeamination**. Deamination can occur not only on glutamate but on serine, histidine and threonine producing the appropriate keto acids. Serine is converted to pyruvate, threonine is to α-ketobutyrate at that way. Threonine however can be further converted to 2-amino 3-ketobutyrate by the **threonine dehydrogenase**, which then forms glycine by decarboxylation or lactate by deamination.

Deamination of amino acids occurs in the kidney as well by **amino acid oxidases**, however these reactions have less importance in the metabolism of amino acids. Beside the oxidases specific for the L-isoform of amino acids animals and humans have D-amino acid specific oxidases, playing important role in the elimination of plant or bacterial D-amino acids. One of the pathways of glycine degradation uses **D-amino acid oxidase** producing glyoxylate, and in further oxidation oxalate, as an end product.

Glycine could be converted to serine by **serine hydroxymethyltransferase** which requires the coenzymes THF and PLP. The serine is converted to pyruvate by serine dehydratase as described previously. The last pathway, which predominates in animals, glycine undergoes oxidative cleavage to CO_2 and NH_4^+, and a methylene group. This reaction is catalyzed by **glycine cleavage enzyme**, also requires THF, which accepts the methylene group. The two carbon atoms of glycine do not enter the citric acid cycle. One carbon is lost as CO_2 and the other becomes the methylene group of methylene-THF, a one-carbon group donor in certain biosynthetic pathways. Free ammonium can be produced from the amide group of the glutamine by **glutaminase**. This mitochondrial enzyme is regulated by the energy status of the cell: high ATP, GTP and NADH concentration inhibits; high ADP, GDP concentration stimulates the enzyme. As we saw, glutamate plays a key role both in anabolic and catabolic processes of amino acids. This molecule is the entry site for ammonia and it is also the key site for ammonia production.

Although much of the metabolism of amino acids takes place in the liver, the three amino acids with **branched side chains (Val, Ile, Leu)** are oxidized in muscle, adipose, kidney and brain tissue. These extrahepatic tissues contain an **aminotransferase**, absent in liver, producing the corresponding α-keto acids. The **branched-chain α-keto acid dehydrogenase complex** then catalyzes oxidative decarboxylation of all three α-keto acids. This reaction is formally analogous to two other oxidative decarboxylations, mentioned earlier: pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase complex. Five cofactors participate in all three reactions: TPP, lipoate, CoA, NAD, FAD. Defective branched-chain α-keto acid dehydrogenase complex results the **maple syrup urine disease**.

27. UREA CYCLE

Ammonia, produced by catabolic processes is toxic. The molecular basis of this toxicity is not entirely understood, however the most likely mechanism is the imbalance of glutamate - α-ketoglutarate. High concentration of ammonia push this balance towards the glutamate decreasing the concentration of α-ketoglutarate and in turn the speed of the citric acid cycle. Elimination of ammonia takes place in the liver in the form of urea, made by the urea cycle. One amino group enters the urea cycle as **carbamoyl phosphate**, the other enters as **aspartate** and after a series of reactions **urea** is formed and excreted into the urine by the kidneys. Ammonia used for the first step of urea cycle is coming from **glutamate** or **glutamine** by the help of glutamate dehydrogenase and glutaminase reactions, respectively. The liver also receives some ammonia from peripheral tissues. Alanine is the major source of ammonia transported from muscle which is converted to pyruvate by the alanine aminotransferase, while the amino group is used to form glutamate.

1. The first reaction is an ATP-dependent reaction catalyzed by **carbamoyl phosphate synthetase I**. NH_4^+ together with CO_2 (as HCO_3^-) forms **carbamoyl phosphate** in the mitochondrial matrix while two ATP are hydrolyzed. The cytosolic isoform of the enzyme (carbamoyl phosphate synthetase II) has a separate function in pyrimidine biosynthesis with separate substrate (glutamine) and separate regulation.

2. Carbamoyl phosphate donates its carbamoyl group to ornithine to form **citrulline**, with the release of P_i. The reaction, still in the mitochondria, is catalyzed by **ornithine transcarbamoylase**.

3. The citrulline passes from the mitochondrion to the cytosol. A condensation reaction between the amino group of aspartate, the source of the second amino group, and the citrulline forms **argininosuccinate**. This cytosolic reaction, catalyzed by **argininosuccinate synthetase**, requires ATP and the immediate cleavage of PP_i makes this reaction **irreversible**.

4. The argininosuccinate is then cleaved by **argininosuccinase** to form free **arginine** and **fumarate**, the latter being converted to malate before entering mitochondria to join the citric acid cycle.

5. In the last reaction of the urea cycle, the **arginase** cleaves arginine to yield **urea** and **ornithine**. Ornithine is transported into the mitochondrion to initiate another round of the urea cycle and it plays similar role in the urea cycle than the oxaloacetate in the citric acid cycle. Urea is transported to blood and later it is excreted by the kidneys.

Citric acid cycle and urea cycle are linked. The pathways linking the two cycles are known as the aspartate-argininosuccinate shunt, these effectively link the fates of the amino groups and the carbon skeletons of amino acids. Since both pathways were described by Hans Krebs, the interconnected cycles are called the “Krebs bicycle”.

27.1. REGULATION

The activity of the urea cycle is regulated on the first enzyme of the pathway: **carbamoyl phosphate synthetase I**. This enzyme is allosterically activated by N-acetylglutamate, which is synthesized from acetyl-CoA and glutamate by N-acetylglutamate synthase. The steady-state levels of N-acetylglutamate are determined by the concentrations of glutamate and acetyl-CoA and arginine. Diet rich in proteins and/or long term fasting could accelerate the cycle as well.

The complete blockade of the urea cycle is lethal. The absence of a urea cycle enzyme can result in **hyperammonemia** or in the buildup of one or more urea cycle intermediates. Although the breakdown

of amino acids can have serious health consequences in individuals with urea cycle deficiencies, a protein-free diet is not a treatment option. Careful administration of proteins and keto acids in the diet could decrease the level of ammonia in the blood and is an option to replace essential amino acids.

28. CLINICAL ASPECTS OF AMINO ACID METABOLISM

In addition to their role as the building blocks of proteins, amino acids are precursors of many biomolecules, including complex lipids, hormones, coenzymes, nucleotides, neurotransmitters, porphyrins, alkaloids, antibiotics and pigments.

28.1. ROLE OF AMINO ACIDS IN THE SYNTHESIS OF LIPIDS:

Serine, ethanolamine and choline (both derivatives of serine) are components of phospholipids.

Serine (and palmitoyl CoA) are the precursors of ceramide synthesis, (ceramid is the structural parent of all the sphingolipids).

28.2. ROLE OF AMINO ACIDS IN NUCLEOTIDE BIOSYNTHESIS:

Gly is an important precursor in purine biosynthesis, while aspartate is the precursor in the synthesis of pyrimidines. Gln and Asp are important sources of amino groups in more steps of the de novo nucleotide biosynthesis.

28.3. IMPORTANT NEUROTRANSMITTERS, HORMONES AND PIGMENTS ARE THE DERIVATIVES OF TYROSINE

Tyrosin metabolism is closely related to phenylalanine metabolism, since tyrosine results from hydroxylation of phenylalanine. The reaction is catalyzed catalysed by **phenylalanine hydroxylase** (lecture fig.), which is tetrahydrobiopterin dependent enzyme. Biopterin, unlike folic acid, resembles, in containing a pteridin ring, and is not a vitamin. It is synthesized from GTP.)

Most tyrosine not incorporated into proteins is metabolized to acetoacetate and fumarate, but some is used as a precursor to **catecholamines** (lecture figure). Chatecholamine synthesis (lecture fig.) starts with tyrosine hydroxylase, which like phenylalanine hydroxylase, is dependent on tetrahydrobiopterin. This reaction produces **DOPA**. DOPA is converted to **dopamine**, the active neurotransmitter. In some parts of the brain, this is the last enzyme in this particular pathway. The adrenal cortex converts dopamine to **norepinephrine** and **epinephrine** (also called adrenaline). The methyl group of epinephrine is derived from S-adenosylmethionine. Levels of chatecholamines are correlated with, among other things, changes in blood pressure. The neurological disorder *Parkinson´s disease* is associated with a diminished production of dopamine, and it has traditionally been treated by administering L-DOPA. Increased production of dopamine in the brain may be linked to physiological disorders such as *schizophrenia*.

Chatecholamines are metabolized by monoamine oxidase and chatecholamine O-methyltransferase (lecture fig.). Absence of these meatbolites in urine is a symptom of a deficiency in the synthesis of catecholamines.

Tyrosine is also involved in the synthesis of melanin and thyroid hormone. Conversion of tyrosine to **melanin** requires **tyrosinase**, a copper containing protein (lecture fig.). The two-step reaction uses DOPA as a cofactor internal to the reaction and produces dopaquinone. During melanogenesis, following exposure to UV light, tyrosinase and a protein referred to as a tyrosinase-related protein are induced. The lack of tyrosinase activity produces *albinism*. There are various types of melanin (dark, yellow or colorless). All are aromatic quinones and the conjugated bond system gives rise to color.

Tyrosine is also a necessary precursor of **thyroid hormones**. Synthesis of thyroid hormones requires incorporation of iodine into a tyrosine of thyroglobulin (for additional details, please see the hormone chapter).

Phenylalanine and tyrosine also gives rise to many commercially significant natural products, including **tannins** that inhibit oxidation in wines; alkaloids such as **morphine**; and the **flavoring of cinnamon oil, nutmeg, cloves, vanilla, cayenne pepper**, and other products.

28.4. TRYPTOPHAN IS A PRECURSOR OF NAD, SEROTONIN AND MELATONIN

Some of the intermediates in tryptophan catabolism are precursors for synthesis of other biomolecules, including nicotinate, a precursor of **NAD** and **NADP** in animals; serotonin, a neurotransmitter in vertebrates; melatonin, a sleep inducing molecule; and indolacetate, a growth factor in plants.

Serotonin (5-hydroxytryptamine) results from the hydroxylation of tryptophan by a tetrahydrobiopterin-dependent enzyme and decarboxylation by a pyridoxal-phosphate containing enzyme (lecture fig.). It is a neurotransmitter within the brain and causes contraction of smooth muscles in support of arterioles and bronchioles. It is widely found in the body and has other physiological roles. Lack of synthesis of serotonine is indicated by lack of 5-hydroxyindole-3-acetic acid.

Melatonin, or N-acetyl-5-methoxytryptamine, is synthesized in the pineal gland and the retina.

It is involved in the regulation of circadian rhythm cirkadian rythm, and is mostly synthesized during the night. It appears to function by inhibiting synthesis and secretion of other neurotransmitters, such a dopamine and GABA.

28.5. OTHER BIOLOGICAL AMINES PRODUCED BY DECARBOXYLATION OF AMINO ACIDS

GABA (γ-amino-butirate), an inhibitory neurotransmitter, is synthesized from glutamate by decarboxylation. Its decreased production is associated with epileptic seizures. GABA analogs are used in the treatment of epilepsy and hypertension. Levels of GABA can also be increased by administering inhibitors of the GABA degrading enzyme GABA aminotransferase.

Histidine undergoes decarboxylation and becomes **histamine**, a powerful vasodilatator in animal tissues. Histamine is released in large amounts as part of the allergic response, and it also stimulates acid secretion in the stomach. A growing array of pharmaceutical agents are being designed to interfere with either the synthesis or the action of histamine (for example, **cimetidine** is a histamine receptor antagonist).

28.6. CARNITINE IS DERIVED FROM LYSINE

Medium and long chain fatty acids are transported into mitochondria for β-oxidation as carnitine conjugates. Carnitine is synthesized not from free lysine but rather from lysine residues in certain proteins. The first step is trimethylation of the ε-amino group of the lysine side chain, with AdoMet as the methyl donor. Free trimetillysine is obtained from hydrolysis of the protein and is metabolized in four steps to become carnitine.

28.7. AMINO ACIDS ARE PRECURSORS OF CREATINE AND GLUTHATHIONE

Creatine is synthesized from **glycine** and **arginine** (lecture fig.); **methionine**, in the form of S-adenosylmethionine, acts as methyl group donor. Phosphocreatine, derived from creatine, is an important energy buffer in skeletal muscles. The amount of creatine within the body is related to muscle mass, and a certain percentage of this undergoes significant turnover each day. About 1-2% of preexisting creatine phosphate is cyclized nonenzimatically to creatinine and excreted in urine, and new creatine is synthesized to replace it. The amount of creatinine in the daily urine sample truly represents the daily urinary output.

Glutathione, the tripeptide **γ-glutamyl-cysteinyl-glycin**, has several important biological functions. It is reductant, conjugated to drugs to make them more water soluble, involved in the transport of amino acids across cell membranes, a cofactor for some enzymatic reactions, and aids in the rearrangement of protein disulfide bonds.

Glutathione is synthesized from three amino acid, **glutamate**, **cysteine**, and **glycine** (lecture fig.). The synthesis starts by formation of the dipeptide γ-glutamylcysteine, and followed by the addition of glycine. Both reactions require activation of carboxyl groups by ATP. Synthesis of glutathione is largely regulated by cysteine availability.

The oxidized formation of glutathione (GSSG), produced in the course of its redox activities, contains two glutathione molecules linked by a disulfide bond. Glutathione is thought to aid in maintaining the sulfhydryl groups of proteins in the reduced state, and the iron of heme in the ferrous (Fe^{2+}) state. Its redox function is also used to remove toxic peroxides formed in the normal course of growth and metabolism under aerobic conditions (lecture fig.). The reaction is catalyzed by **glutathione peroxidase** (this enzyme contains a covalently bound selenium (Se) atom in the form of selenocysteine, which is essential for its activity).

The oxidized glutathione is rereduced in the **glutathione reductase** reaction, using NADPH as reducing equivalent.

Transport of amino acids by the γ-glutamyl cycle

γ-glutamyl cycle is an example of ″group transfer″ transport (lecture figure). It has more demand for energy when compared to other mechanisms, but it is rapid and has high capacity, and functions within the kidney and among other tissues. It is particularly important in renal epithelial cells.

The enzyme γ-glutamyl transpeptidase is located in the cell membrane. It shuttles GSH to the cell surface to interact with an amino acid. Γ-glutamyl amino acid is transported into the cell, and the complex hydrolysed to liberate the amino acid. Glutamate is released as 5-oxoplroline, and cysteinylglycine is cleaved to its component amino acids. To regenerate GSH, glutamate is reformed from oxoproline, and GSH is resynthesized from its three component parts. Three ATPs is used towards the regeneration of glutathione.

28.8. HEME BIOSYNTHESIS

Heme is produced in virtually all mammalian tissues (mostly in bone marrow and liver). It consists of one ferrous ion and a tetrapirrol ring, protoporphyrin IX. There are several important heme-containing components within the body (hemoglobin, mioglobin, catalase, peroxidase, cytochromes). The organic portion of heme is derived totally from eight residues each of **glycine** and **succinyl-CoA**. The enzymes in heme biosynthesis occur in both mitochondria and cytosol.

Steps of hem biosynthesis:

1. Delta aminolevulinate synthase (DALAS)
   In this first step, glycine reacts with succinyl CoA to yield α-amino-β-ketoadipate, which is then decarboxylated to δ-aminolevulinate. This is the rate limiting step of heme synthesis. DALAS is a mitochondrial, PLP requiring enzyme. Two isozymes exist for DALAS; only the erythrocytic form contains an IRE (iron response element). Both synthesis and activity of the enzyme are subject to regulation by a variety of substances. Heme allosterically inhibits the enzyme, glucose inhibits heme synthesis through inactivation of transcription factors. Certain steroid hormones activates the enzyme, and it is also induced by many drugs.

2. ALA dehydratase
   In this cytosolicstep, two molecules of ALA condense asymmetrically to form porphobilinogen. The ALA dehydratase is a sulfhydryl enzyme, and is very sensitive to inhibition by heavy metals.

3. Porphobilinogen deaminase
   This enzyme converts four porphobilinogens to a linear tetrapirrole, which closes in an enzyme independent step to form uroporphyrinogen I, if no additional factors are present. In the presence of uroporphyrinogen III, cosynthase the uroporphyrinogen III isomer is formed.

4. Uroporphirinogen decarboxylase
   This enzyme acts on the side chains of uroporphyrinogens to form the coproporphyrinogens (both I and III isomers). The enzyme is inhibited by iron salts. In a single gene, abnormality for this enzyme leads to the disease *porphyria cutanea tarda*, which shows cutaneous manifestations primarily featuring sensitivity to light. The condition is not expressed unless patients either take drugs (cause an increase in porphyrin synthesis) or drink large amounts of alcohol (leads to the accumulation of iron)

5. Coproporphyrinogen oxidase
   In this mitochondrial step, coproporphyrinogen III is converted to protoporphyrinogen III. A dominant disease associated with the deficiency of this enzyme leads to form a hereditary hepatic porphyria, known as *hereditary coproporphyria*.

6. Protoporphyrinogen oxidase
   This mitochondrial enzyme generates protoporphyrin IX, which, in contrast to other heme precursors, is extremely water insoluble. Excess amounts of protoporphyrine IX is excreted by bile. A dominant disease, *variegate porphyria*, is due to the deficiency of this enzyme.

7. Ferrochelatase
   Ferrochelatase inserts ferrous iron into protoporphyrin IX in the final step of the synthesis of heme. The enzyme is sensitive to heavy metals (especially leads), and iron deprivation.

28.9. DEGRADATION OF HEME

The heme group of (mostly) hemoglobin, released from dying erithrocytes in the spleen, is degraded to yield free Fe^{2+} and, ultimately, bilirubin in a two-step pathway.

1. The first step catalyzed by **heme oxygenase**, converts heme to **bilioverdin**, a linear tetrapirrol derivative (lecture fig). The other products of the reaction are free Fe^{3+} and CO, which is mostly released via respiratory tract.

   Humans have at least three isozymes of heme oxygenases (HO). HO-1 is inducible by a wide range of stress conditions. HO-2 is found mainly in brain and the testes, where it is continuously expressed.

2. Biliverdin is reduced to **bilirubin** in the second step, catalyzed by **biliverdin reductase**.

Bilirubin, produced in the reticuloendothelial system, is largely insoluble, and it travels in the bloodstream bound to albumin. Its high level is toxic, called kernicterus, which is manifested by the transfer of bilirubin to membrane lipids. In the liver, bilirubin is transformed to the bile pigment **bilirubin diglucoronide**. The reaction utilizes **uridine diphosphoglucuronate** derived from the oxidation of **uridine diphosphoglucose** (lecture fig.). Bilirubin diglucuronide is sufficiently water soluble to be secreted with bile into the small intestine, where microbial enzymes convert it to several products, predominantly urobilinogen. Some **urobilinogen** is oxidized to coloured products, **urobilins**, stercobilins, which are excreted in the feces; a small fraction of urobilinogen is reabsorbed into the blood to be removed by hepatic cells and resecreted by bile, or in the form of urobilin, and is excreted by the kidneys.

Impaired liver function or blocked bile secretion causes bilirubin to leak from the liver into the blood, resulting in a yellowing of the skin and eyeballs, a condition called **jaundice**.

In normal states, most of the plasma bilirubin is unconjugated (indirect bilrubin), which is lipid soluble, therefore it is not excreted by urine. Elevation of serum conjugated bilirubin level (*Rotor´s syndrome*) can be caused by liver and/or biliary tract disease (extra- or intrahepatic) or biliary tract occlusion. In *Doubin-Johnson syndrome,* the bilirubin transport (secretion) is defective from the liver to the bile.

Intravascular hemolysis

In certain diseases, destruction of red blood cell occurs in the intravascular compartments (not by RES). The appearance of free hemoglobin and heme in the plasma could lead to the excretion of these substances through the kidney with a loss of iron. Scavenging mechanisms help to prevent this: **transferrin** binds free iron, and thus permits its reutilization. Free hemoglobin, after oxygenation and dissociation into dimers, binds to **haptoglobins** (α_2-globulins), which delivers hemoglobin to the reticuloendothelial cells for degradation.

Free hem and hematin (heme with ferric iron) appearing in plasma are bound by a β-globulin, **hemopexin**, which transfers hem to the liver for further metabolism by hem oxygenase.

28.10. DISEASES OF AMINO ACID METABOLISM

Phenylketonuria (PKU)

Phenyketonuria is a classic example of inborn errors of metabolism-in this case the metabolism of phenylalanine. It is caused (97%) by the absence or deficiency of the enzyme **phenylalanine hydroxylase** (classic form), or in rare case by biopterin deficiency (the cofactor of the phenylalanine hydroxylase reaction). The absence of the enzyme activity blocks the normal conversion of phenylalanine to tyrosine, thereby resulting in the accumulation of Phe in all the body fluids. Many side reactions then occur, that normally would be at low level. Phenylalanine undergoes transamination with piruvate to yield **phenylpiruvate.** Much of the phenylpyruvate is decarboxylated to **phenylacetate** or reduced to **phenyllactate**. These products accumulate in the blood and tissues and are excreted in the urine (phenylacetate imparts a characteristic odor to the urine).

The accumulation of phenylalanine or its metabolites impairs the normal development of the brain, causing severe mental retardation. This may be caused by excess phenylalanine competing with other amino acids for transport through the blood-brain barrier.

Newborn screening is important for earlyrecognition. . Treatment involves a phenyalanine-free diet, Tyr and in some cases, biopterin supplementation.

Alkaptonuria

Another inheritable disease of phenylalanine and tyrosine catabolism is alkaptonuria, in which the defective enzyme is **homogentisate dioxygenase**. Homogentisate (Phe, and Tyr breakdown intermediate) is normally oxidized by this enzyme to maleylacetoacetate, which can be further broken down to fumarate and acetoacetate. In alkaptonuria, the homogentisate accumulates and is excreted in the urine. The homogentisate polymerizes upon standing to form a dark-colored substance, which produces an ink-colored urine. This condition is less serious than PKU, but arthritis can develop witholder age.

Maple syrup urine disease (MSUD)

The breakdown of Val, Ile, and Leu first involves transamination to the corresponding α-keto acids. The keto acids are normally oxidatively decarboxylated for further metabolism. In MSUD, however, there is typically a deficiency in the **branched-chain α-keto acid dehydrogenase (BCDH)**, that blocks further metabolism of the α-keto acids. This leads to accumulation of the α-keto acids, which causes the characteristic odor associated with the disease.

Some other inborn errors of amino acid metabolism is presented in the additional lecture figure.

29. NUCLEIC ACIDS

29.1. BUILDING BLOCKS AND STRUCTURES

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are large polymers composed of deoxyribonucleotides and ribonucleotides, respectively.

DNA is the central repository of genetic information. The storage and transmission of biological information are the only known functions of DNA.

RNAs have a broader range of functions, and several classes are found in cells. **Ribosomal RNAs (rRNA)** are components of ribosomes, the complexes that carry out the synthesis of proteins. **Messenger RNAs (mRNAs)** are intermediates, carrying genetic information from one or a few genes to a ribosome, where the corresponding proteins can be synthesized. **Transfer RNAs (tRNAs)** are adapter molecules that faithfully translate the information in mRNA into a specific sequence of amino acids. In addition to these major classes, there is a wide variety of RNAs with special functions.

Nucleotides play a variety of important roles in all cells:

- They are the precursors of DNA and RNA.
- They are essential carriers of chemical energy- a role primarily of ATP and to some extent GTP.
- They are component of cofactors, NADH, FADH, S-adenosylmethionine, and CoA.
- They serve in activating intermediates in metabolic reactions, for example, by activating glucose in sugar metabolism (UDP-glucose).
- They often function as metabolic regulators or signaling agents; for example, cyclic adenosine monophosphate (cAMP) serving as a ˝second messenger˝ in hormonal signaling.

Nucleotides have three characteristic components:

1. a nitrogenous (nitrogen containing) base
2. a pentose, and
3. one or more phosphates.

The molecule without a phosphate group is called **nucleoside**.

The nitrogenous bases are derivatives of two parent compounds, **pyrimidine** and **purine**. The bases and pentoses of the common nucleotides are heterocyclic compounds.

29.2. BASES, NUCLEOSIDES, AND NUCLEOTIDES

Both DNA and RNA contain two major purine bases, **adenine** (A) and **guanine** (G), and two major pyrimidines. In both DNA and RNA, one of the pyrimidine is **cytosine** (C), but the second common pyrimidine is not the same in both: it is **thymine** (T) in DNA and **uracil** (U) in RNA.

Some other notable members of the purine family include caffeine and theobromine (found in coffee and chocolate), and the anticancer agent 6-mercaptopurine. Some members of the pyrimidine family are also powerful anticancer agents, i.e., 5-fluorouracil or cytarabine.

Physico-chemical properties

Purines and pyrimidines are *weakly basic* because they have -NH_2 groups. In an acidic solution, these groups can be protonated (introduce positive charge on the base). In alkaline solution, a proton (on the N3 of pyrimidine, or on the N1 of a purine) can dissociate; the base then acts functionally as a weak acid. Additionally, bases can *tautomerize*. The keto form dominates this keto-enol tautomerization at the physiological pH.

In solution, *nucleotides can hydrogen-bonded with one another*. The most famous hydrogen-bonding scheme was proposed by James Watson and Frances Crick, in their discovery of the DNA double helix, in which adenine pairs with thymine, and guanine with cytosine (the keto form involved in the Watson-Crick base pairing). This type of base pairing is highly important for the association of polymerized strands of nucleic acid. In an aqueous solution of free single nucleotides, there is very little Watson-Crick pairing; instead, the bases associate by alternative hydrogen-bonding pattern and by stacking.

Bases can stack their heterocyclic rings *on top of one another*, enjoying hydrophobic interactions. The stacking also involves a combination of van der Walls and dipole-dipole interactions between bases. Base stacking help to minimize contact of the bases with water, and base-stacking interactions are very important in stabilizing the three-dimensional structure of nucleic acids.

The purine and pyrimidine *bases,* especially some purine derivatives (xantin and uric acid), are *not very water soluble*. Addition of a polar sugar moiety, and of ionic phosphoryl groups, significantly improves their aqueous solubility.

Nucleic acids have two kinds of pentoses. The recurring deoxyribonucleotide units of DNA contain **2´-deoxy-D-ribose** (lacks the hydroxy group of ribose at the C2 position), and the ribonucleotide units of RNA contain **D-ribose**. In nucleotides, both types of pentoses are in their β-furanose (close five-membered ring) form. The pentose ring is not planar but occurs in one of a variety of conformations generally described as ˝puckered˝ (four of the five atoms are in nearly in a single plane, the fifth is on side of the plane).

The base of a nucleotide is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an N-β-glycosyl bond to the 1´ carbon of the pentose, and the phosphate is esterified to the 5´carbon.

Nucleotides carry a negative electrical charge, due the ionization of the phosphate. When incorporated into nucleotides the monophosphate, diphosphate, and triphosphate groups will ionize. For example, adenosine triphosphate (ATP) has four dissociable protons, with pKa values for three of these below pH5, while the fourth has a pKa of approximately 6.9. In a solution at physiological pH, a solution of ATP will therefore, have molecular species in several different titrated forms carrying three or four negative charges.

Although nucleotides ,bearing the major purines and pyrimidines, are most common, both DNA and RNA also contain some minor bases. In DNA, the most common of these are methylated forms of the major bases; in some viral DNAs, certain bases may be hydroxymethylated or glucosylated. These bases in DNA often have roles in regulating or protecting genetic information. Minor bases of many types are also found in RNAs, especially in tRNAs (lecture Fig.).

29.3. PRIMARY STRUCTURE OF DNA AND RNA

The sequence of nucleotide residues, when covalently linked together in a linear chain in a polynucleotide, is called the primary structure of the polynucleotide.

The successive nucleotides of both RNA and DNA are covalently linked through phosphate-group ″bridges″, in which the 5´phosphate group of one nucleotide unit is joined to the 3´hydroxyl group of the next nucleotide, creating a **phosphodiester linkage**.

By convention, for single-stranded DNA or RNA, the **5'-terminus** of the chain is written on the left, the **3'-terminus** on the right; that is, the base sequence is written 5' → 3' (lecture fig.).

The covalent backbone of DNA and RNA is subject to slow, nonenzymatic hydrolysis of the phosphodiester bonds. In the test tube, RNA is hydrolyzed rapidly under alkaline conditions, but DNA is not; the 2´-hydroxyl groups in RNA (absent in DNA) are directly involved in the process. Cyclic 2´,3´-monophosphate nucleotides are the first products, then are rapidly hydrolyzed further to yield a mixture of 2´- and 3´-nucleoside monophosphates.

29.4. SECONDARY STRUCTURE

Secondary structure refers to the stable, repeating conformational pattern in a polymer. DNA and RNA both have highly distinctive secondary structures, deriving from the properties of their constituent nucleotides.

29.5. DNA SECONDARY STRUCTURE

The DNA double helix

- The stable solution conformation of DNA is the famous **B-form double-helix**.
- The bases lie to the interior of the helix, and they are stacked on top of one another.
- The sugar-phosphate backbone is largely exposed to solvent.
- The helix has a right-handed twist. It makes a complete turn every 3.4 nm, or about 10.5 residues. The overall diameter of the helix is about 2.0 nm.
- The strands are antiparallel; one strand runs 3'→5', while the other runs 5'→3'.
- There are two grooves (major and minor) in the helix, where the edges of the bases are exposed to solvent
- Adenine is paired with thymine, guanine is paired with cytosine.
- The base pairs are joined by hydrogen bonds, with two hydrogen bonds joining adenine with thymine, and three hydrogen bonds joining guanine and cytosine.
- The hydrogen bonding enforces the co-planarity of the two-paired bases.
- The base pairs stack on top of one another in overlapping fashion, with the stack resembling the treads in a spiral staircase.
- Because the two strands complement one another by base pairing, the base sequence of one strand necessarily specifies the sequence of the other strand.

DNA can also form a left handed (Z-form) helix

- This requires certain unusual solution conditions (high concentrations of certain salts or organic cosolvents).
- The bases still form Watson-Crick pairs, with the sugar-phosphate backbone exposed to solvent and the base pairs in the helix interior. The base pairs are still nearly perpendicular to the axis of the helix, and the strands are still antiparallel.
- The most striking feature of the Z-form is the helix features a left-handed twist, just the opposite from the B-form helix.
- There are 12 base pairs per turn of the helix, and one turn covers the length of about 4.4 nm.

- The phosphodiester backbone traces a zig-zag course (the origin of the ″Z″ designation), not a smooth curve.
- The helix is slimmer than the B-form helix, with a diameter of approximately 1.8 nm.
- The major groove is much shallower, and the minor groove is much deeper and narrower, than in the B-form helix.

It is thought that the vast majority of duplex DNA in solution is in the B-form, interrupted only rarely with runs of the Z-form helix. Tract of Z-form helix may, however, be important for regulating gene expression.

Under other rather artificial conditions (e.g., alcoholic solutions that dehydrate the polymer), DNA can adopt an **A-form helix**.

- This right handed, double stranded helix contains nonparallel strands, similar to the B-form helix.
- Compare to the B-form the minor groove is shallower, while the major groove is deeper.
- Also, the twist of the helix is more pronounced: the rise per base pair is only 0.26 nm.
- The base pairs are also tilted more sharply, than in the B-form, and the sugar ring has a different puckering.

Under in vivo (hydrated conditions) it is unlikely the A-form helix formation, so it is probably not important in the functioning of the cell.

29.6. UNUSUAL DNA STRUCTURES

Inverted repeated sequences, or **palindromes** (DNA sequences that read the same on both strands when read in the same direction), can be paired to form a double **hairpin** or **cruciform** structure (lecture fig.)

Nucleic acids can also form **triple helices or triplexes**, that is structures with three strands are held together by the stacking of the bases and extra hydrogen bonds to accommodate the third strand (lecture fig.).

- Thanks to the multiple possibilities for hydrogen bonding around the bases, the standard Watson-Crick pairs can accept a third base to form a triplex (acid-base behavior of pyrimidines is involved here). The third base attaches itself to a purine; thus triplex formation generally requires that one strand of a duplex has an extended series of only purine basis, to which the third strand can attach. Such structures are limited by pH, and are not stable outside of acidic conditions

Certain unusual DNA sequences have the ability to fold back on themselves repeatedly to form a **quadruplex** structure (lecture fig.).

- These sequences are enriched in guanine bases, and it is in fact the guanine residues that hydrogen-bond to each other to form the four-base structure, a G-quartet, which contains a monovalent ion coordinated to the four bases. To fold the strand back and forth the runs of guanine residues must be interrupted by other bases that will form the joining the single stranded loops.

G-quartets and quadruplex structures are important structural features of telomeres, the protein-DNA complexes that lie at the end of eucaryotic chromosomes.

29.7. RNA SECONDARY STRUCTURE

- RNA is generally found as single chains, whereas DNA naturally occurs as a duplex of two separate chains.

- The base uracil replaces thymine in RNA.
  - In duplex forms there is complementary pairing of uracil with adenine.
  - This base pair is stabilized by two hydrogen bonds, just as is the adenine-thymine pair.
- Duplex regions can be formed by folding an RNA chain back on itself and using complementary base-pairing. The strands are arranged in antiparallel fashion, as in DNA.

The RNA double helix differs from the B-helix of DNA in several ways.

- RNA duplexes mainly adopt an A-form helix, which differs significantly from the B-form helix of DNA.
- The diameter of the helix is thicker, at 2.6 nm.
- The base pairs are tilted at an appreciable angle to the axis of the helix (about 20º).
- A complete turn of the helix requires 11 base pairs, and occurs over a helical run of length 2.9 nm.
- The major groove is narrower and deeper, and the minor groove wider and shallower, than in the B-form helix.
- The extra –OH group on ribose, vs. deoxyribose, changes the puckering of the sugar and the overall hydration of the residues, leading to the above differences from the B-form helix.

The secondary structures of tRNA molecules resemble a cloverleaf. Notable features here include:

- Several (4-5) short helical runs of 4-6 base pairs each;
- Several single-stranded regions, some forming loops at the ends of runs of helix (called stem-loop structures);
- Several modified bases and sugars, often involving modification by methylation;
- A short stretch of single-stranded RNA, a bulge, that is not involved in a stem-loop structure, in the middle of the molecule (not all tRNA molecules have this feature, however);
- An exposed single-stranded region at the 3’ end of the RNA molecule.

Messenger RNA can also fold back on itself to form stem-loop structures.

In eukaryotes, the 5’ end of the molecule carries a “cap” of a 7-methylguanine residue, attached to the chain by an unusual 5’ phosphoryl group, rather than through the usual 3’ linkage. Additionally, at its 3’ end the mRNA often carries a long run of up to 200 adenylate residues (a poly A “tail”).

Like the smaller tRNA molecules, ribosomal RNA molecules fold back on themselves, forming multiple short runs of helix, stem-loop structures, and single-stranded “bulges”. Also like the tRNA molecules, these rRNA molecules are often modified to carry unusual bases.

29.8. DNA DENATURATION AND RENATURATION

The DNA double helix is stable under physiological conditions of temperature, salt concentration, and pH. However, DNA will change from a duplex helix to separated, single DNA strands (the bases unpair and unstack), if the temperature is too high, the pH is too acidic or too alkaline, or if the salt concentration in solution is too low. This referred to as **denaturation** or “**melting**” of the DNA, and the DNA is said to undergo a **helix-coil transition**. Reversing the solution conditions will promote re-association of the strands and re-formation of the double helix.

The ability of the DNA double helix to open and close is highly important for several fundamental processes, including replication of DNA, gene expression, and repair of damaged DNA.

This process occurs over a limited temperature range, and the midpoint of the transition is where half of the nucleotides are unpaired and unstacked is denoted as **Tm** called the **melting temperature** or, more formally known as the transition midpoint temperature.

When nucleic acid bases are stacked on one another, electronic interactions between the bases decrease their optical absorbance compared to an unstacked conformation. Conversely, when a polynucleotide helix is heated, the optical absorbance increases as the bases unpair and become unstacked (the largest change in absorbance occurs around 260 nm). This allows for monitoring of the helix-coil transition by UV absorbance measurements.

Major thermodynamic factors affecting helix stability:

- Coulomb repulsions along the backbone, from the charged phosphates, and the attraction of counterions to those phosphates;
- Hydrogen bonds between the base pairs;
- The entropically-favorable release of waters solvating individual nucleotides as the helix forms;
- The relative entropic favorability of a less-organized, “melted” state for the polynucleotide strands; and
- Stacking interactions between neighboring bases and base pairs.

Generally, longer helical molecules are more stable than shorter ones Short double helixes tend to ″fray″from the ends relatively easily, while end-fraying is relatively less important for the longer molecules. These tend to denature through the formation and enlargement of internal single-stranded regions, or ″denaturation bubbles″.

Base sequence, not just base percentage composition, will also affect stability and melting temperature.

30. BIOSYNTHESIS OF NUCLEOTIDES

Nucleotides have a variety of important functions in all cells. They are essential carriers of chemical energy as ATP and GTP and they play a role in the formation of activated biosynthetic intermediates such as UDP-glucose and CDP-diacylglycerol. They are components of the cofactors NAD, NADP, FAD and coenzyme A. They are the precursors of DNA and RNA and some of them such as cAMP and cGMP, are also cellular second messengers.

Nucleotides have three characteristic components: a nitrogen-containing **heterocyclic base** (purine or pyrimidine); a **pentose** (ribose or deoxyribose); and one or more **phosphates**. The molecule without a phosphate group is called **nucleoside**. Two types of pathways lead to nucleotides: the **de novo** pathways and the **salvage** pathway. De novo synthesis of nucleotides begins with their metabolic precursors: **amino acids, ribose 5-phosphate, CO_2, NH_3**. Salvage pathways recycle the **free bases** and **nucleosides** released from nucleic acid breakdown. Both pathways are important in cellular metabolism because the cellular nucleotide pool is limited. Rapidly dividing cells require an even faster nucleotide biosynthesis thus inhibiting these pathways are important strategies in tumor therapy.

30.1. DE NOVO SYNTHESIS OF PURINE NUCLEOTIDES

The carbon and nitrogen atoms of the purine ring system have different origin: two nitrogen from glutamine; one nitrogen from aspartate; one nitrogen from glycine which donates two carbons as well; two carbons from formate (THF); and one carbon from CO_2.

1. The first step in the biosynthesis is the formation of **5-phosphoribosyl 1-pyrophosphate (PRPP)** from ribose 5-phosphate (coming from the pentose phosphate pathway), by the **PRPP synthetase**.

2. In the second and **committed step**, an amino group donated by glutamine is attached to the PRPP, forming **5-phosphoribosyl amine** and glutamate. The reaction is catalyzed by **PRPP amidotransferase** and the amino group serves as a platform where the purine ring is built up. Both steps (synthetase and transferase) can be blocked by the intermediate and end products of the pathway: IMP, AMP and GMP.

3. The next ATP-dependent step is the addition of three atoms from glycine to form **glycinamide ribonucleotide (GAR)** by **GAR synthetase**.

4. The amino group is then formylated by formyl-THF forming **formylglycinamide ribonucleotide (FGAR)**.

5. Another amino group from glutamine is added to the substrate by **FGAR-amidotransferase**, forming **formylglycinamidine ribonucleotide (FGAM)**. This step is also requires ATP.

6. Dehydration and ring closure with another ATP hydrolysis yield the five-membered **imidazole ring (AIR)** of the purine nucleus. The reaction is catalyzed by **FGAM-cyclase** (AIR synthetase). At this point, three of the six atoms needed for the second ring in the purine structure are in place.

7. The carboxylation step (AIR carboxylase) is unusual in that it **does not require biotin and ATP**, but instead uses bicarbonate present in aqueous solutions. In higher eukaryotes, production of **CAIR** is a one-step reaction instead of two as in bacteria and fungi.

8-9. Aspartate now donates its amino group in two steps: formation of an amide bond between aspartate and CAIR (**SAICAR**), followed by elimination of the carbon skeleton of aspartate

as fumarate (**AICAR**). The first step (ATP-dependent) is catalyzed by **SAICAR synthetase** the second one by **SAICAR lyase**. Recall that aspartate plays an analogous role in two steps of the urea cycle.

10. The final carbon is contributed by formyl-THF catalyzed by **AICAR transformylase** forming **FAICAR**, and after dehydration a second ring closure takes place to yield the second ring of the purine nucleus and the first molecule with complete purine ring: **inosinate (IMP).**

IMP biosynthesis is a highly energetic process. **Two ATP** are used for PRPP biosynthesis and another **four** during the following steps. In bacteria, each step is catalyzed by separate proteins; in eukaryotic cells the steps are catalyzed by multienzyme complexes which are important for advanced regulation of the pathway and for the unstable intermediates.

Conversion of inosinate to **ATP** and **GTP** goes through the intermediates AMP and GMP, respectively. AMP biosynthesis requires the insertion of an amino group derived from aspartate and GTP is the source of energy. The two consecutive steps are catalyzed by a synthetase and a lyase enzyme. GMP biosynthesis requires the insertion of an amino group derived from glutamine and ATP is the source of energy. The two consecutive steps are catalyzed by a dehydrogenase (NAD) and an amidotransferase (ATP is cleaved to AMP and PP_i). As a feedback regulation, the synthetase step in the AMP biosynthetic pathway is inhibited by AMP; the dehydrogenase step in the GMP pathway is inhibited by GMP.

30.2. REGULATION

De novo purine nucleotide synthesis is regulated by the cellular AMP and GMP concentrations. The first regulatory point is the **PRPP amidotransferase** step, which is inhibited **allosterically** by the end products **IMP**, **AMP**, and **GMP**. AMP and GMP act synergistically in this concerted inhibition. Thus, whenever either AMP or GMP accumulates, the first step in its biosynthesis from PRPP is partially inhibited. In the second control mechanism, an excess of **GMP** inhibits **IMP dehydrogenase** without affecting the formation of AMP. Conversely, an accumulation of **AMP** inhibits **adenylosuccinate synthetase**, without affecting the biosynthesis of GMP. In the third mechanism, GTP is required in the conversion of IMP to AMP, whereas ATP is required for conversion of IMP to GMP, a reciprocal arrangement that tends to balance the synthesis of the two ribonucleotides. The final control mechanism is the allosteric inhibition of **PRPP synthesis by ADP and GDP**.

30.3. SALVAGE PATHWAYS

Salvage pathways recycle the free bases and nucleosides released from nucleic acid breakdown. One possible mechanism is the reaction of **PRPP** with a **free purine base**. The energy, necessary for the synthesis of the mononucleotide is produced by the hydrolysis of the pyrophosphate group of the PRPP. Depending on the bases involved in the reactions the enzymes are the **adenosine** or **hypoxanthine-guanine phosphoribosyltransferases**. In these salvage reactions AMP is produced from adenine, IMP from hypoxanthine and GMP from guanine. These products inhibit allosterically the corresponding transferase enzymes and also the de novo pathways, as we discussed earlier, creating a complex feedback regulatory loop. The other mechanism to produce nucleotides in the salvage pathway is the phosphorylation of **nucleosides** (adenosine, guanosine) to AMP and GMP using **ATP** as a phosphate donor. The corresponding enzymes are the **adenosine kinase** and the **guanosine kinase**.

30.4. BIOSYNTHESIS OF PYRIMIDINE NUCLEOTIDES

De novo pyrimidine nucleotide biosynthesis proceeds in a different manner from purine nucleotide synthesis. The six-membered pyrimidine ring is made first and then attached to ribose 5-phosphate.

Beside ribose 5-phosphate, originated from PRPP, **aspartate** and **carbamoyl phosphate** are necessary for the synthesis.

1. **Carbamoyl phosphate** required in pyrimidine biosynthesis is made by the **cytosolic carbamoyl phosphate synthetase II**. The mitochondrial isoform of this enzyme (carbamoyl phosphate synthetase I) plays an important role in the urea cycle as we discussed it earlier. The cytosolic isoform uses glutamine as an amino group donor instead of free ammonia. Two ATP are hydrolyzed in the reaction which is allosterically inhibited by UTP and stimulated by PRPP.

2. Carbamoyl phosphate reacts with aspartate to yield **N-carbamoylaspartate** in the first **committed** step of the biosynthesis of pyrimidine. This reaction is catalyzed by **aspartate transcarbamoylase** and it is allosterically inhibited by CTP. When ATP is present this inhibitory effect of CTP is abolished.

3-4. By removal of water by dihydroorotase, the pyrimidine ring is closed to form **dihydroorotate**, which is then oxidized to **orotate**, a reaction in which NAD is the electron acceptor and is catalyzed by **dihydroorotate dehydrogenase**. In eukaryotes, the first three enzymes of this pathway are part of a single multifunctional protein, called **CAD**.

5. Once orotate is formed, the ribose 5-phosphate chain, provided again by PRPP, is attached to yield **orotidylate** by **orotate phosphoribosyl transferase**.

6. Orotidylate is then decarboxylated to uridylate (**UMP**). In eukaryotes, the **orotidylate decayrboxylase** and the previous step (orotate phosphoribosyl transferase) are catalyzed by an enzyme complex.

7. UMP is phosphorylated to **UTP**. CTP is formed from UTP by the action of **cytidylate synthetase (CTP synthetase)** consuming one ATP. The nitrogen donor is normally glutamine, although the CTP synthetase enzyme can use other amino acids and NH_4^+ as a donor as well.

There are two major types of **salvage** pathways in pyrimidine nucleotide biosynthesis. The first one is the ATP-dependent nucleoside – nucleoside monophosphate conversion. These reactions are catalyzed by the **uridine-cytidine kinases**: **uridine +ATP -> UMP + ADP; cytidine + ATP -> CMP + ADP**. The other salvage pathway is the reaction of PRPP with pyrimidine base catalyzed by the **uracil phosphoribosyl transferase**: **uracil + PRPP -> UMP + PP_i**.

As we described earlier, the biosynthesis of pyrimidine nucleotides is regulated at the carbamoyl phosphate synthetase II reaction by **PRPP** (activator) and by **UTP** (inhibitor).

Nucleotides to be used in biosynthesis are generally converted to nucleoside triphosphates. The conversion pathways are common to all cells.

1. **Adenylate kinase: AMP + ATP <-> 2ADP**

   The ADP so formed is phosphorylated to ATP in one of the following mechanisms: (i) substrate-level phosphorylation (glycolysis); (ii) oxidative phosphorylation (respiratory chain); (iii) photophosphorylation (photosynthesis).

2. ATP also brings about the formation of other nucleoside diphosphates by the action of nucleoside monophosphate kinases: NMP + ATP <-> NDP + ADP. These enzymes are generally specific for a base but nonspecific for the sugar (ribose or deoxyribose).
3. Finally, nucleoside diphosphates are converted to triphosphates by the action of nucleoside diphosphate kinase: $NTP_D + NDP_A <-> NDP_D + NDP_A$. This enzyme is not specific for the

base (purine or pyrimidines) or the sugar (ribose or deoxyribose), the donor (D) is almost invariably ATP and so the acceptor (A) is ADP.

30.5. BIOSYNTHESIS OF DEOXYRIBONUCLEOTIDES

Deoxyribonucleotides are derived from the corresponding ribonucleotides by direct reduction at the 2'-carbon atom of the ribose. The reaction is catalyzed by **ribonucleotide reductase**, which enzyme works with all the ribonucleoside diphosphates. Water is produced using the oxygen of the sugar and a pair of hydrogen atoms, which are ultimately donated by NADPH. The enzyme consists of two subunits: a catalytic subunit and a regulatory subunit. The regulatory subunit contains two –SH groups (Cys) that supports hydrogen atoms for the reduction. The disulfide bridge, formed during the reaction is reduced back by the electrons of either **glutathione** or **thioredoxin**, however the ultimate electron donor in both cases is **NADPH**. These reactions are catalyzed by **glutathione reductase** and **thioredoxin reductase**, respectively. Regulation of ribonucleotide reductase is based on the concentrations of the nucleoside triphosphates in the cell and the final goal is to provide the proper amount of deoxyribonucleotides for DNA synthesis. In general, dATP inhibits the biosynthesis of deoxyribonucleotides, while dCTP has no significant effect.

30.6. SYNTHESIS OF THYMIDILATE

The immediate precursor of dTTP is dTMP which can be generated by two different pathways. The first one is the **methylation of dUMP**, the second one is the **direct phosphorylation of deoxythymidine**. Conversion of dUMP to dTMP is catalyzed by thymidylate synthase. The methyl group is transferred from **N^5, N^{10}-methylenetetrahydrofolate (THF)** to dUMP. **Dihydrofolate (DHF)**, produced in the reaction is reduced back to tetrahydrofolate by the expense of **NADPH** and by the **dihydrofolate reductase**. **Serine hydroxymethyltransferase** (with PLP cofactor) is required for regeneration of the N^5, N^{10}-methylenetetrahydrofolate.

Cancer cells have greater requirements for nucleotides as precursors of DNA and RNA. Useful targets for pharmaceutical agents in cancer therapy are thymidylate synthase and dihydrofolate reductase, enzymes that provide the only cellular pathway for thymine synthesis. One inhibitor that acts on thymidylate synthase, **fluorouracil**, is an important chemotherapeutic agent. In the cell, it is converted to deoxynucleotide monophosphate, which binds to and inactivates the enzyme. Another group contains **folate analogues**, which act as competitive inhibitors of the dihydrofolate reductase.

The second way to produce dTMP and dTTP is the **direct phosphorylation of deoxythymidine**. This ATP-dependent step is catalyzed by **thymidine kinase** yielding dTMP, which is further phosphorylated by **thymidylate kinase** to dTDP, and then by **nucleoside diphosphate kinase** to dTTP.

31. DEGRADATION OF NUCLEOTIDES. CLINICAL ASPECT OF NUCLEOTIDE METABOLISM

Turnover of nucleic acids results in the release of purine nucleotides and pyrimidine nucleotides. A continous turnover of nucleotides occurs within the cell. Some bases are recycled by salvage pathways, whereas others are degraded and the products are excreted.

31.1. DEGRADATION OF PURINS

The degradation of purine nucleotides, nucleosides and bases funnel through a common pathway leading to formation of uric acid. The enzymes involved in the degradation of these compounds vary in their specificity. **Nucleases** are specific to either RNA or DNA and also toward the bases, and position of cleavage site at the phosphodiester bonds. **Nucleotidases** range from high to broad specificity. **Purine nucleoside phosphorylases** catalyze reversible reactions. However, because of the low concentration of free purine basis and ribose 1-phosphate, they mostly function in the direction of degradation pathways.

The nucleotidase action on adenylate yields adenosine, which is deaminated to inosine by **adenosine deaminase**, and inosine is phosphorolyzed to hypoxanthine (its purine bases) and ribose-1 phosphate. Hypoxanthine is oxidized succesively to xanthine and then uric acid by xanthine oxidase (lecture fig.).

GMP is first hydrolyzed to guanosine by 5´nucleotidase, which is then cleaved to free guanine by nucleoside phosphorylase. Guanine undergoes hydrolytic removal of amino group to yield xantine, which is converted to uric acid by xanthine oxidase. The ammonia produced in the deamination steps converted to urea in the ornithin cycle.

Xanthine oxidase is a flavoenzyme with an atom of molybdenum and four iron-sulfur centers in its prosthetic group. In this reaction, molecular oxygen is the electron acceptor, which is converted to water forming hydrogen peroxide. The newly produced hydrogen peroxide is next degraded to water and oxygen through catalysis.

Uric acid is the excreted end product of purine catabolism in primates, birds, and other animals. Uric acid is an antioxidant and an efficient scavenger of reactive oxygen species. Unfortunately, urate is only moderately soluble, and urate levels in human serum are typically close to the solubility limit, which can lead to problems.

In most mammals and many other vertebrates, uric acid is further degraded to allantoin by the action of urate oxidase. Allantoin is also produced in humans by spontaneous (nonenzymatic) oxidation of uric acid, and can be a measure of oxidative stress. In other organisms, the pathway is further extended (lecture fig.).

31.2. DEGRADATION OF PYRIMIDINE NUCLEOTIDES

Pyrimidine nucleotides are degraded to β-amino acids

In these pathways the pyrimidine nucleotides are converted to nucleosides by nonspecific **phosphatases**. Cytidine and deoxycytidine are deaminated to uridine and deoxyuridine by **pyrimidine nucleoside deaminase**. **Uridine phosphorylase** catalyzes phosphorolysis of uridine,deoxyuridine, and thymidine resulting in the formation of uracil and thymine.

Uracil and thymine are then further degraded by analogous reactions (reduction, ring opening by hydrolysis, and another hydrolysis), although the final product is different (lecture fig.). Uracil degraded

to **β-alanine**, **NH_4^+**, and **CO_2**. Thymine degradation proceeds to **β-aminoisobutiric acid**, **NH_4^+,** and **CO_2**. β-aminoisobutiric acid is excreted in urine in humans and originates exclusively from degradation of thymine (its urinary level may increase in a high-DNA containing diet or among tumor diseases). β-aminoisobutiric acid can also undergo transamination to methyl-malonyl-CoA, which by conversion to succinyl-CoA enters the citric acid cycle.

31.3. CLINICAL ASPECTS OF PURINE AND PYRIMIDINE METABOLISM

Immunodeficiency diseases best defined as those associated with defects in purine nucleoside degradation. **Deficiency of adenosine deaminase** (ADA) and **purine nucleoside phosphorylase** (PNP) has been correlated with disease states in humans. A deficiency in ADA is associated with a **severe combined immunodeficiency** involved in both T-cell and B-cell function. PNP deficiency is associated with an immunodeficiency involving T-cell functions. In ADA deficient patients, intracellular concentration of dATP and S-adenosylhomocysteine are greatly increased: First, high levels of dATP inhibit ribonucleotidase activity and consequently inhibit DNA synthesis. Secondly, Deoxyadenosine inactivates S-adenosyl homocysteine hydrolase, leading decreased SAM (required for methylation of bases in RNA and DNA) and thirdly, increased levels of adenosine result in an increase in cAMP levels, which are possible explanations.

31.4. DEFECT IN THE SALVAGE PATHWAYS

Free guanine and hypoxanthine (the deamination product of adenine) are salvaged in the same way by **hypoxanthine-guanine phosphoribosyltransferase** (HGPRT) enzyme, which uses PRPP as a .partner substrate (Lecture fig.). (Adenin is salvaged in a similar reaction, catalyzed by adenine phosphorybosyltransferase). The genetic lack of HGPRT activity, seen almost exclusively in male children (X-linked characteristic), results in ***Lesch-Nyhan syndrome***. Symptoms of the syndrome include compulsive and self-destructive behavior, mental deficiency and spasticity.

Deficiency in activity of HGPRT may lead to a increased level of PRPP and decreased levels of IMP or GMP, which promote de novo synthesis of purine nucleotides, resulting in high levels of uric acid production and gout-like damage to tissue (see below). The products of purine degradation (hypoxanthine, xanthine, urate) may be toxic to the developing brain, or the lack of the enzyme leads to an imbalance in the concentration of purine nucleotides.

31.5. EXCESS OF URIC ACID CAUSES GOUT

Gout is characterized by **elevated uric acid levels** in blood and urine due to a variety of metabolic abnormalities: overproduction of purine nucleotides via the de novo pathway (increased PRPP synthetase activity, partial HGPRTase activity), decreased excretion of uric acid (kidney diseases) and glucose 6-phosphatase deficiency. Excessive cell death (cancer therapy), diet rich in protein or purine and alcohol consumption can also lead to increased uric acid levels. Consumption of purines would obviously raise urate levels; a high protein diet would likewise contribute to higher cc.-s because certain amino acids goes in the de novo synthesis of purines. Alcohol acts as diuretic and tends to close dehydration, leading to urate precipitation.

Due to its **poor solubility** and **high serum concentration**, **sodium urate** can **precipitate in kidneys and in joints**. The joints become inflammed, painful, and arthritic.

There are different approaches to the treatment of gout including allopurinol, colchicine and uricosuric agents:

- ***Allopurinol*** is an analog of hypoxanthine and is an **irreversible inhibitor of xanthine oxidase**. Allopurinol is a substrate of xanthine oxidase, which converts allopurinol to oxypurinol (alloxanthine). Oxypurinol inactivates the reduced form of the enzyme by remaining tightly bound to its active site. When xanthine oxidase is inhibited, the excreted end products of purine metabolism are xanthine and hypoxanthine, which are more water soluble and less likely to form crystalline deposits.

- ***Colchicine***, a plant alkaloid, relieves the symptoms of gout by **retarding the inflammation** associated with urate crystal deposition. It appears to inhibit the migration and phagocytic action of polymorphonuclear leukocytes, probably by binding to and depolymerizing tubulin (microtubules needed to cell migration and phagoctosis). Colchicine also inhibits the synthesis and the release of leukotrienes.

- ***Uricosuric agents*** (*probenecid, sulfinpyrazone*) **improves excretion of urate** by exerting their action in the kidneys. Urate is passed freely at the glomerulus in the kidney, but it is also reabsorbed, then reexcreted, in the proximal tubules by certain active transport proteins specific for weak acids, i.e., urate. Uricosuric drugs inhibit these active transporters, inasmuch the net result is to reduce the reabsorption of urate. In consideration of other weak acids, the net effect may be in the opposite direction, i.e., in the case of Probenecid which reduces the excretion of penicillin and several other drugs.

31.6. CHEMOTHERAPEUTIC AND ANTIBACTERIAL AGENTS TARGET ENZYMES IN THE NUCLEOTIDE METABOLISM

Glutamine antagonists inhibit enzymes utilizing glutamine as nitrogen donors.

Many reactions in mammalian cells utilize glutamine as the amino group donor such as amidation reactions in de novo synthesis of purine nucleotide, synthesis of GMP from IMP, synthesis of CTP from UTP, synthesis of NAD^+ and the formation cytosolic carbamoyl phosphate. This differs substantially from bacterial cells primarily utilizing ammonia as the amino donor .

Compounds inhibiting these reactions are referred to as glutamine antagonists. ***Azaserine (O-diazoacetyl-L-serine), Acivicin*** and ***6-diazo-5-oxo-L-norleucine (DON)*** are glutamine antagonists and are used as cancer chemotherapeutic agents, largely due to the cancer cells rapid growth rate when compared with normal cells and have greater requirements for nucleotide as precursors of DNA and RNA, and consequently are more sensitive to inhibitors of nucleotide biosynthesis.

31.7. INHIBITORS OF THYMIDILATE SYNTHESIS AND FOLATE METABOLISM

Notably, cancer cells are especially vulnerable to a block of dTMP synthesis. Two key enzymes in this process are **thymidylate synthase and dihydrofolate reductase**, and both are targets of several anticancer drugs (lecture fig.).

One inhibitor acting on **thymidilate synthase**, ***5-fluorouracil***, is a pyrimidine analog of uracil and is an important chemotherapeutic agent. Fluorouracil is itself not an active species. It must be converted to the active metabolites 5-fluorouridine 5´-triphosphate (FUTP) and 5-fluoro-2´-deoxyuridine 5´monophosphate (FdUMP). FdUMP binds to and inactivates the thymidilate synthase (mechanism-based enzyme inactivation).

31.8. ANTIFOLATES INHIBIT (RE)FORMATION OF TETRAHYDROFOLATE

Antifolates interfere with the formation of H_4folate from H_2folate or folate by inhibition of **H_2folate reductase (DHFR)**. ***Methotrexate*** (MTX), a folate analog, is used as an antitumor agent in the treatment of cancer among humans.This folate analog acts as a competitive inhibitor; the enzyme binds methotrexate with about 100 times higher affinity than (dihydro)folate. ***Aminopterin*** is a related compound which acts similarly. H_4folate is required to both the de novo purine biosynthesis and dTMP formation.

Antifolates are also useful as antibacterial agents. Bacterial DHFR differs from mammalian DHFR in the amino acid sequence and in the structure around the active site. The bacterial enzyme can be selectively inhibited by ***trimethrophin*** (TMP), a competitive inhibitor.

Bacteria synthesize their own folate from p-aminobenzoate (PABA), pteridine, and glutamate. Aryl ***sulfonamides*** (˝sulfa˝ drugs) are **analogs of PABA** and compete with the PABA in the bacterial enzyme dihydropteroate synthase (lecture figure); sulfonamides competitive inhibitors of this enzyme. The bacteria are then unable to synthesize adequate amounts of folate, and as a result, their growth is inhibited (bacterostatic action).

Co-trimethoxazole is a combination of sulfomethoxazole with trimethoprim (inhibit both folate formation and folate utilization).

31.9. OTHER ANTIMETABOLITES (STRUCTURAL ANALOGS OF BASES AND NUCLEOSIDES)

- **6-Mercaptopurine** (6-MP) is a useful antitumor drug in humans. It is converted to mercaptopurine ribonucleotide and 6-mercaptopurine ribonucleoside 5´monophosphate. The latter is a negative effector of PRPP amidotransferase; the nucleotide also inhibits the conversion of IMP to GMP and AMP.
- **Cytosine arabinoside** (araC) (see lecture fig) is used in the treatment of several forms of human cancer. AraC must be metabolized by cellular enzymes to araCTP to exert its cytotoxic effect. AraCTP competes with dCTP in the DNA polymerase reaction and araCMP is incorporated into DNA, resulting in the inhibition of synthesis of the growing DNA strand.

31.10. OTHER NUCLEOTIDE METABOLISM INTERFERING AGENTS

- **Hydroxyurea** specifically inhibits DNA synthesis. It is an inhibitor of ribonucleotide reductase, blocking reduction of CDP, UDP, GDP and ADP to the corresponding 2´-deoxyribonucleotides. Toxicity of this drug results from depletion of dNTP-s required for DNA replication.
- **Tiazofurin** is converted by cellular enzymes to the NAD^+ analog, tiazofurin adenin dinucleotide (TAD). TAD inhibits IMP dehydrogenase, consequently the concentration of GTP is markedly depressed.

31.11. PURINE AND PYRIMIDINE ANALOGS AS ANTIVIRAL AGENTS

One-one antimetabolites have been identified often used in the control/treatment (but not cure) of **herpesvirus** (HSV) and **human immundeficiency virus** (HIV) infections.

These drugs, acyclovir and acycloguanosine, a purine analog and ***3´-azido-3´deoxythymidine*** (AZT), a pyrimidine analog (lecture fig) require metabolism to phosphorylated compounds yielding the active drug.

- ***Acycloguanosine*** is activated to the monophosphate only by specific HSV-thymidine kinase, encoded by the HSV genome. Acycloguanosine monophosphate is then phosphorylated by cellular enzymes to the di- and triphosphate forms, which serves as substrate for the HSV specific DNA polymerase and is incorporated into the growing viral DNA chain causing early chain termination.
- ***AZT*** is phosphorylated by cellular kinases to AZT triphosphate, which blocks HIV replication by inhibiting HIV polymerase (an RNA-dependent polymerase, which is at least a hundred-fold more sensitive to AZT triphosphate than are host cells DNA-dependent DNA polymerase).

32. REGULATION OF NUCLEOTIDE AND AMINO ACID METABOLISM

33. METABOLISM OF IRON

34. BIOTRANSFORMATION

35. METABOLIC INTERRELATIONSHIP IN DIFFERENT NUTRITIONAL STATES (STARVE-FEED CYCLE)

35.1. WELL-FED STATE

In well-fed state, the diet supplies energy requirements.

After consumption and digestion of meals, glucose and amino acids are transported from the intestine to the blood. The dietary lipids are packaged into chylomicrons and transported to the blood by the lymphatic system. Thus, the fed condition leads to the secretion of insulin (induced by high blood glucose level and the parasympathetic nervous system). Insulin signals the fed state by stimulating the storage of fuels and the synthesis of proteins. For instance, insulin initiates protein kinase cascades- it stimulates glycogen synthesis in both muscle and liver, and suppresses glyconeogensis in the liver. Insulin also accelerates glycolysis in the liver, which in turn increases the synthesis of fatty acids. The high insulin level also promotes the entry of glucose into muscle and adipose tissues. The action of insulin also extends to amino acids and protein metabolism. Insulin has a general stimulating effect on protein synthesis, and it inhibits the intracellular degradation of proteins.

In the liver, dietary glucose can be converted to glycogen by glycogenesis, converted into pyruvate by glycolysis, or used in the pentose phosphate pathway for the generation of NADPH. Pyruvate converted to acetyl-CoA, which, in turn can be converted into triacylglycerol or oxidized to CO_2 and water.

Much of the glucose passes through the liver to reach other organs, including brain, which is almost completely dependent on glucose for ATP generation, red blood cells and renal medulla, which can only be carried out through glycolysis, and adipose tissues, which primarily converts it into the glycerol part of triacylglycerol.

Muscles also use glucose, converting it to glycogen or using it for energy production. Lactate and pyruvate produced by glycolysis in other tissues are taken up by the liver, and oxidized to CO_2 or converted to triacylglycerol. The Cori cycle is interrupted in this state because of the lack of gluconeogenesis in the liver.

The intestinal cells use some dietary amino acids as an energy source, but transport most of them into the portal blood. Liver removes some absorbed amino acids and uses it for protein synthesis, but most pass through. Excess amino acids in the liver can be oxidized completely to CO_2, urea, and water, or the intermediates generated can be used for lypogenesis. Amino acids escaping from the liver are used for protein synthesis or energy in other tissues.

Dietary triacylglycerols reaches the bloodstream as chylomicrons, which are acted upon by lipoprotein lipase in the capillaries of adipose tissue and muscle. This lipase hydrolyzes a large portion of the triacylglycerols of chylomicrons. The released fatty acids are taken up by the adipocytes, reesterified with glycerol 3-phosphate (derived from glucose), and stored as fat droplets within the adypocytes. Chylomicron remnants cleared from the blood by the liver, hydrolyzed by a lysomomal lipase, reesterified with glycerol 3-phosphate (derived from free glycerol or glucose) to triacylglycerol. This triacylglycerol, along with triacylglycerol produced by de novo synthesis from glucose and amino acids, are packaged into VLDL, and secreted into the blood. Triacylglycerol content of VLDL is hydrolyzed by lipoprotein lipase and free fatty acids are taken up and assembled into triacylglycerol in adipose tissue. In muscles, the fatty acids produced by lipase action are taken up and used as fuel.

35.2. EARLY FASTING STATE

Hepatic glycogenolysis maintains blood glucose during early fasting. Lactate, pyruvate and alanine are diverted from oxidation and fatty acid synthesis into glucose formation, completing the Cori and alanine cycle.

35.3. FASTING STATE

Since no dietary fuels enters from the gut and little glycogen is left in the liver after 10-12 hours fasting, the body is dependent upon **hepatic gluconeogenesis**, primarily from lactate, glycerol and alanine. The **Cori** and **alanine cycles** are important, but do not contribute to the net synthesis of glucose. The brain oxidizes glucose to CO_2 and water, and as a result, other sources of gluconeogenezis are needed. **Glycerol,** a byproduct of lipolysis in adipose tissue, is an important substrate for glucose synthesis, but the **proteins**, especially from the skeletal muscles, supplies most of carbons for net glucose synthesis. Proteins are hydrolyzed in muscles and amino acids are partially metabolized. Of the amino acids, mostly alanine and glutamine are released into the blood. The other amino acids mostly metabolized to intermediates, which can yield Ala and Gln. Branched-chain amino acids are the major source of nitrogen for the production of Ala and Gln in muscle. The branched-chain ketoacids produced are partially released into the blood for uptake by the liver, which uses them for glucose or ketone body synthesis. Part of the Gln released from muscles are used by intestinal epithelium, lymphocytes and macrophages (rapidly dividing cells require it for purin and pyrimidine synthesis). Gln is partially oxidized in these cells (called glutaminolysis see lecture fig.), leading to alanine (enterocytes) or aspartate (lymphocytes) (and ammonia) release. Glucose synthesis in the liver is also closely linked to synthesis of urea.

During fasting, **lipolysis** is greatly activated **in adipose tissue** due to the **low insulin/high glucagon** level. This elevates the blood fatty acid levels, which is used in preference to glucose by many tissues. In heart and muscles, oxidation of fatty acids inhibits glycolysis and pyruvate oxidation. In liver, fatty acid oxidation provides most of the ATP needed for gluconeogenesis. Little acetyl-CoA generated by fatty acid oxidation in liver oxidized completely, rather is converted into **ketone bodies,** which are released into the blood and are a source of energy for many tissues. Like fatty acids, they are preferred to glucose by many tissues. Brain cannot oxidize fatty acids because they cannot cross the blood-brain barrier. Once their blood concentration is high enough, ketone bodies enter the brain and serve as alternative fuel (they cannot replace completely the brain´s need for glucose). After weeks of starvation, ketone bodies become the major fuels for brain. Ketone bodies may also suppress proteolysis and branched-chain amino acid oxidation in muscle. This decreases muscle-wasting and reduces the amount of glucose synthesized in the liver during long-term fasting. These metabolic adaptions in a prolonged starvation minimize protein degradation.

The metabolic interrelationship between the liver, muscle and adipose tissue in providing fuel for the brain is essential in fasting. Liver synthesizes the glucose, muscle and gut supply the substrate (alanine, glutamine) and adipose tissue supplies the ATP (via fatty acid oxidation in the liver) needed for hepatic gluconeogenesis. This cooperation among major tissues is dependent on the appropriate blood hormone levels. Glucose levels are lower in fasting, reducing the secretion of insulin but favoring the release of glucagon from the pancreas and epinephrine from the adrenal medulla. Fasting also reduces formation of thyroid hormone, thus the daily basal energy requirement.

35.4. THE EARLY REFED STATE

In the early refed state, triacylglycerol is metabolized comparatively as to a well fed state, however the liver remains in the **gluconeogenic** mode for a few hours after feeding. Gluconeogenesis from lactate (periferal tissues) and amino acids (gut) results in the synthesis of glycogen instead of glucose,

reestablishing liver glycogen levels. After the rate of glucose synthesis declines, liver glycogen is sustained by direct synthesis from glucose.

35.5. CALORIC HOMEOSTASIS

The constant availability of fuels in the blood is termed caloric homeostasis. This means the blood levels of fuels which help to maintain the ATP levels between certain limits regardless of the actual nutritional state. The blood glucose concentrations are controlled within very tight limits, whereas fatty acid and ketone body concentration in the blood can vary by one-two orders of magnitude. The reason for this strict regulation of blood glucose level is the absolute needs of the brain for this substance. The effect of starvation on **glucose homeostasis** can be divided into five states (see lecture fig.).

Phase I is the well fed state, in which glucose is provided by dietary carbohydrates.

When this supply is exhausted, hepatic glcogenolysis maintains blood glucose levels during phase II.

As this supply of glucose is emptied, hepatic gluconeogenesis from lactate, glycerol and alanine becomes increasingly important until, in phase III, gluconeogenesis is the major source of blood glucose.

Several days of fasting lead to phase IV, when dependence on gluconeogensis decreases, and ketone bodies partly replace glucose as fuel for brain. Renal gluconeogenesis also becomes significant in this phase.

Phase V occurs after very prolonged starvation of extremely obese people and is characterized by even less dependence on gluconeogenesis. Here the energy needs of almost every tissue are met by fatty acid or ketone bodies oxidation.

After all the fat is used and ketone body levels fall, the body has to use the functional muscle proteins, which eventually leads to death.

36. METABOLISM OF ALCOHOL

Ethyl alcohol (ethanol) is ingested mostly as an ingredient found in alcoholic beverages, and is usually referred to simply as alcohol. Alcohol features both polar and nonpolar groups, and is capable of altering the polarity and fluidity of biomembranes which disrupts the processes of neurotransmission and signalling. Distinctively, this is primarily the reason for most of the effects of alcohol and its neural and psychological effects. Regular consumption of larger amounts of alcohol injures the entire organism. The central nervous system, the liver, the heart, the gastrointestinal tract and the pancreas are the main targets of ethanol toxicity. Chronic alcohol abuse can lead to alcoholism, alcohol dependence and addiction.

Ethanol is notably efficient in its ability to be absorbed from the digestive system into the blood, and only a small fraction leaves the body through the lung or kidney in unchanged form. The vast majority of ingested alcohol is converted to acetate and then either further oxidized and degraded or incorporated into lipids. About 10 g ethanol is metabolized within one hour in a healthy adult of average weight, dependent on nutritional status, gender, individual capacity and physical training. The enzymes participating in ethanol oxidation can be detected in a variety of cell types, but they are expressed at the highest level in hepatocytes. The liver metabolizes the majority of the ingested ethanol.

36.1. THE OXIDATIVE CATABOLISM OF ETHANOL

The first step in the ethanol catabolism is its oxidation to acetaldehyde. This process can be catalyzed by three alternative enzymes, which use different cofactors and are located in three different compartments of the cell:

1. Alcohol dehydrogenase (ADH) – cytosol
2. Microsomal ethanol oxidation system (MEOS) or Cytochrome P450 - endoplasmic reticulum
3. Catalase - peroxisome

Alcohol dehydrogenase

Alcohol dehydrogenase is a cytosolic enzyme oxidizing ethanol to acetaldehyde while NAD+ is reduced. The enzyme has a high affinity to ethanol (low Km), normally (low dose and nonregular comsumption) responsible for two-thirds of the total ethanol metabolism, but it becomes saturated. Only a small number of concentrated milimoles yields minor intoxication. There is remarkable genetic polymorphism involved in the activity of this enzyme.

Microsomal (cytochrome P450) oxidation of ethanol

Monooxygenation of ethanol is catalyzed by certain cytochrome P450 isozymes (mostly CYP2E1), utilizing molecular oxygen and NADPH and localized in the membrane of endoplasmic reticulum. The CYP2E1 affinity to ethanol is much lower (high Km), catalyzing only one-third of the total ethanol oxidation at low serum alcohol level. However, it is inducible by ethanol and other substrates. Consequently, it becomes the dominant metabolizer when larger amounts of ethanol is consumed and at repeated/consistent consumption.The acetaldehyde produced by the enzyme usually does not leave the active site because it is also substrate of CYP2E1 and can be further monooxygenated to acetate.

These enzymes (compared with other enzymes in biotransformation) exhibit a low substrate specificity. CYP enzymes metabolize a large number of drugs and hormones in addition to ethanol

(acetone, ethers, chloroform, carbon tetrachloride, acetaminophen, benzene, domestic pollutants, steroid hormones, etc.) The activity of CYP450 isozymes can increase up to severalfold in certain condition (by its substrates, in prolonged starvation, diabetes mellitus). In this case, the oxygen

requirement of the cell significantly increases, its NADPH producing capacity is challenged, and the generation of reactive free radicals is enhanced harmfully due to the carelessness of the cytochrome enzyme. The increased rate of monooxygenation can cause further problems in the liver (beside the hypoxia, oxidative stress and NADPH depletion):

Accumulation of toxic intermediates

The toxicity of endo/exogenous substances (toxins, procarcinogens) are often increased by conversion in the first phase of biotransformation (monooxygenation) and are usually decreased by conjugation in the second phase. The accelerated production of the toxic intermediate and the reduced glutathione conjugation (glutation depletion due to the oxidative stress) collectively make some substances (for example acetaminophen) hepatotoxic even at consistent dosage.

Alteration in the efficiency drugs/hormones

The accelerated metabolism often reduces the efficiency of the medicine (it must be administered at larger dose in alcoholic patients). However, some (pro)-drugs need to be activated by the phase I reactions of biotransformation. In situations such as these, their efficiency is increased in alcoholic patients.

Catalase dependent oxidation of ethanol

Catalase is located in the peroxisomes, where hydrogen peroxide is continuously generated. Its function is to eliminate hydrogen peroxide. Nevertheless, catalase can also function as an ethanol peroxidase, because it can oxidase the ethanol by hydrogen peroxide (lecture fig.). This activity is dependent on the H2O2 production of the hepatocyte, and in normal conditions, its contribution to ethanol oxidation is insignificant, however, it may be induced during conditions accompanied by an increase in fatty acid levels.

36.2. ACETALDEHYDE OXIDATION TO ACETATE

Aldehyde dehydrogenase converts acetaldehyde to acetate while NAD is reduced. The greatest activity is attributed to the mitochondrial isozyme, but there are other isozymes in the cytosol and peroxisomes, which are quantitively less significant.

The rate-limiting step in ethanol degradation is generally considered the first step: the oxidation of ethanol to acetaldehyde; therefore, acetaldehyde can only found at minute levels in the cell. If the oxidation of ethanol is accelerated (isozymes of ADH, or induction of CYP system) or the oxidation of acetaldehyde is hindered for some reason (mutations, drugs mitochondrial damage in regular drinker), harmful acetaldehydes accumulate both in the liver and the body.

36.3. ACETATE ACTIVATION TO ACETYL-COA

Acetate thiokinase (acetyl-CoA synthetase) catalyze the activation of acetate to acetyl-CoA (lecture fig.). This particular enzyme is mostly found within the cytosol, peroxisome and mitochondrion, and the reaction is limited by the availability of free CoA in the cell.

The produced acetyl-CoA enters the citrate cycle or is used in fatty acid, cholesterol or ketone body synthesis. Ethanol is a rather efficient fuel molecule (30kJ/g energy) when consumed at low doses.

However, alcoholic cellular injury lowers the efficiency of energy harvesting (less ATP, more heat) because of the membrane damage (caused by ethanol, acetaldehyde and induction of CYP2E1) at frequent and higher alcohol consumption.

36.4. METABOLIC CHANGES IN REGULAR, HIGH DOSE ALCOHOL CONSUMPTION

Elevating ethanol levels have deleterious metabolic consequences, primarily in the liver.

Alcohol breakdown is devoid of hormonal and allosteric regulation. The rate of oxidation is not adjusted to the requirement of the body, and only limited by the capacity of the enzymes, and availability of substrates and cofactors. The metabolic toxicity of ethanol is largely due to the overwhelming production of NADH and acetate. In this condition the NADH/NAD ratio is elevated in the hepatocytes (redox shift). The elevated level of NADH concentration inhibits gluconeogenesis by preventing the oxidation of lactate to pyruvate. Lactate will accumulate in the cell and the blood, and the consequences may be lactic acidosis and hypoglycemia.

The high NADH ratio also inhibits glucose and fatty acid breakdown, shifting the metabolism in the direction of fatty acid, triacylglycerol, cholesterol and ketone body synthesis. Triacylglicerols accumulate in the liver, leading to a condition known as fatty liver.

Both ethanol and acetaldahyde are membrane damaging agents, acetaldehyde is also a protein-denaturing and glutathione depleting the poison. Protein damage and enzyme inactivation caused by the acetaldehyde-protein adducts formation may interfere with a number of cellular function (DNA repair, vesicular transport, regulation of collagen formation, normal proteosomal degradation, and antioxidant defense). Free acetic acid/ acetate causes intracellular acidosis. In addition, the

enhanced CYP activity is accompanied by the generation of harmful by-products (free radicals and ROS), inducing oxidative stress.

37. DIABETES MELLITUS

Diabetes mellitus (DM) is a chronic disease accompanied with derangement in carbohydrate, fat and protein metabolism.

There are two major clinical classes of DM:

- **type 1diabetes** (insulin-dependent (IDDM) or childhood-onset diabetes) in which the metabolic defect stems from an autoimmune destruction of pancreatic β cells and a consequent inability to produce sufficient insulin,

- and **type 2 diabetes** (non-insulin-dependent diabetes mellitus (NIDDM) or insulin-resistant
- diabetes. Ultimately, this is a distinct group of diseases in which the regulatory activity of insulin is disrupted: insulin is produced, however, some characteristics for the insulin response system are consedirably defective during the process.

Characteristic symptoms of diabetes mellitus are:

- excessive thirst, and frequent urination (polyuria), leading to the intake of large volume of water (polydipsia),
- hyperglicemia (inability to absorb glucose efficiently from the blood)
- glucosuria
- ketosis (overproduction of ketone bodies), resulting in an immense concentration of ketone bodies in the blood (ketonemia), and urine (ketonuria) and a diminishedlevel in blood pH (ketoacidosis).

37.1. GESTATIONAL DIABETES MELLITUS

During pregnancy, an important metabolic adaptation is a decrease in insulin sensitivity, which is beneficial to providing adequate glucose in the developing fetus. However in a specific population of pregnant women, glucose intolerance develops (gestational diabetes mellitus, GDM). The resistance to insulin appears to be greater in the skeletal muscle and it seems it is due to defects in insulin action, rather than a decrease in insulin receptor binding affinity. The skeletal muscle cells of GDM subjects overexpress membrane glucoproteins, which inhibit the tyrosine kinase activity of the insulin receptor. These circumstances, coupled with a decreased expression and phosphorylation of the insulin receptor substrate-1, may lead to insulin resistance in GDM.

37.2. INSULIN PRODUCTION AND ITS EFFECT ON BLOOD GLUCOSE LEVEL

The insulin is produced by the pancreatic β cells and secreted in response to elevated blood glucose level.

Effects of insulin on blood glucose level:

Insulin increases glucose uptake of muscle and adipose cells (GLUT 4 glucose transporter activation), stimulate glucose uptake in liver (by increasing expression of glucokinase), stimulate glycolysis (by increasing the expression and activity of glycolytic enzymes), promotes the glucose storage as glycogen (activates glycogen synthase, inhibits phosphorylase) and inhibits hepatic glucose production. Additionally, insulin stimulates the synthesis of lipids (fatty acids, triacylglycerols and cholesterol) and the storage of excess fuel as fat in adipose tissue. In summary, the effect of insulin is to favor to conversion of excess blood glucose to two storage forms: glycogen (in the liver and muscle) and triacylglycerol (in adipose tissue).

37.3. INSULIN SIGNALING PATHWAYS

Insulin regulates both metabolic enzymes and gene expression. Insulin does not enter cells, but initiates a signal which travels upon a branching pathway from the plasma membrane receptor to insulin sensitive enzymes in the cytosol and to the nucleus, where it modulates the transcription of specific genes. Insulin binding to the insulin receptor inducing autophosphorylation of the receptor, which facilitates binding of cytosolic binding proteins, such as members of the IRS family, Shc and Cbl.

When phosphorylated , these substrates act as a docking protein for proteins mediating insulin action. Insulin stimulates the phosphorylation of some proteins and dephosphorylation of other proteins, which leads to activation or inhibition of specific enzymes. Separate signal transduction pathways originates from the insulin receptor, of which, several are not yet identified. Some of the activated pathways are the PI3 kinase (phosphatidylinozitol 3-kinase), Ras (small GTP binding protein), the

MAP kinase (mitogen activated protein kinase) and TC10 (a small GTP binding protein). These pathways act in a concerted fashion to coordinate the regulation of vesicle trafficking (incorporation of GLUT4 into plasma membrane (PI3 kinase and TC10), protein synthesis, enzyme activation and inactivation, and gene expression (see lecture figures). The net result of these diverse pathways is regulation of glucose, lipid and protein metabolism including cell growth, survival and differentiation.

37.4. OBESITY

Obesity, the most common nutritional problem in developed countries, is defined in terms of body mass index (BMI) (see lecture fig.). It is a life-threatening condition, significantly increases the chances of developing type 2 diabetes, including heart attack, stroke, and some cancers.

The **causes of obesity** are complex and involve both dietary and genetic components:

- Overeating plays an important role in many individuals, particularly inadequate exercise.
- Less common symptoms include tumors, vascular accidents, or abnormal development of the nervous system (inability to effectively control hunger within the hypothalamus are responsible).
- In rare cases, it can be characterized as secondary to endocrine disorders, such as hyperthyroidism or Cushings' disease.
- Also, however rare, the ineffective neural control of calorie intake required in the balance of energy expenditure is abnormal.
- mutations in genes coding hormones involved in the control of food intake, such as leptin or its receptor, may also be the reason.

Leptin (product of the OB gene) is an adipokine hormon. Its production by the adipose tissue is proportional to the mass and size of adipocytes. Following release in blood, leptin travels to the brain, where it acts upon receptors in the hypothalamus (the DB gene encodes the leptin receptor) to curtail appetite.

Two types of neurons in the arcuate nucleus (in hypothalamus) control fuel intake and metabolism.

The **orexigenic** (appetite stimulating) neurons stimulate eating by producing and releasing **neuropeptide Y (NPY)**, which triggers the next neuron to signal to the brain, "I am hungry and I want to eat."

The **anorexigenic** (appetite-suppressing) neurons in the arcuate nucleus produce **α-melanocyte-stimulating hormone (α-MSH)**, which transmits a signal to the next neuron within the circuit to stop eating .

Diferent hormones produced by different tissues control eating; insulin reduces the appetite in the activation of the anorexigenic neurons and inhibiting the orexigenic neurons. Additionally, leptin suppresses the appetite utilizing the indentical mechanism . Short term eating behaviors are also influenced by two other hormones, ghrelin and PYY . **Ghrelin** is a peptide hormone produced in stomach generally attributed to causing the sensation of hunger, particularly in the stimulation of orexigenic neurons, thereby inducing the release of NPY. **PYY** is a peptide hormone secreted by endocrine cells lining the small intestine and colon in response to food entering the stomach. It reduces the sensation of hunger by inhibiting the orexigenic neurons and NPY release.

37.5. OBESITY HAS SIGNIFICANT HEALTH COMPLICATIONS AND IS PREDISPOSED TO TYPE 2 DIABETES MELLITUS

Significantly, there is a corresponding increase in the levels of serum free fatty acids, cholesterol and triacylglycerol levels in people characterized as being obese . Why is this? Obesity is associated with an increased number and/or size of adypocytes.

Adipose tissue is an endocrine organ that produces peptid hormones, known as adipokines (leptin, adiponectin and resistin). In a person characterized as being obese, adipocytes overproduce hormones, such as leptin, resistin and cytokines, As a result, tumor necrosis factors alpha (TNFa), some of which appears to cause resistance to insulin by interfering with autophosphorylation of the insulin receptor (IR) and the subsequent phosphorylation of the insulin receptor substrate 1 (IRS-1). At the same time, the lipid-laden adipocytes decrease synthesis of hormones (adiponectin), which potentially enhances insulin responsiveness.

TNFα has paracrine effect on adipocytes:

1. It activates hormone sensitive lipase, which leads to an increase in free fatty acid levels in the blood.
2. It inhibits lipoprotein lipase which leads to a decrease in VLDL clearance from blood
3. 3. It decreases the LCAT expression and activity, ATP-binding casette and apo-A1 and apo-A4 expression which leads to a decrease in the HDL level.

TNFα also activates SREBP1-c transcription factor in liver which leads to an increase in the expression of genes of fatty acid and triacylglycerol synthesis, consequently an increase in VLDL production. These events lead to dyslipidemia (increased VLDL and decreased HDL levels).

Free fatty acids inhibits glucose uptake and utilization, inhibits mitochondrial biogenesis, increases ROS production, activates transcription factors and PKC isoforms, which contributes to insulin resistance.

Meanwhile, the pancreatic β cells secrete enough insulin to overcome the diminished insulin sensitivity found within certain muscles and the liver. Eventually, the β cells eventually fail, and the lack of enough insulin becomes apparent within the body's inability to effectively regulate blood glucose, and type 2 diabetes develops. The intermediate step, preceding type 2 diabetes mellitus is called metabolic syndrome, or syndrome X (lecture figure).

The **metabolic syndrome** is characterized by obesity (in the abdomen), hypertension (high blood pressure), abnormal blood lipids, elevated levels of blood glucose, and a reduced ability to clear glucose.

Abnormal clotting (high fibrinogen concentration) or inflammation, may also occur.

The progression of metabolic syndrome into type 2 diabetes does not occur in all patient characterized with obesity. Reportedly, estimates suggest insulin resistance evolves to diabetes within 5-10 years in at or about 40% of the patients .

37.6. METABOLIC INTERRELATIONSHIP OF TISSUES IN TYPE 2 DIABETES

These are middle-aged or older obese people with insulin resistance and higher but insufficient insulin production to compensate resistance (hyperinsulinemia: more hormones, less receptors and impaired β cell functionality). Insulin cannot control hepatic glucose production and muscular and adipose glucose uptake. Ketoacidosis rarely develops, because enough insulin is present to prevent uncontrolled release of fatty acids from adipocytes, and fatty acids reaching the liver are directed into triacylglycerol. Hypertriacylglycerolemia results from an increase in VLDL without

hyperchylomicronemia. The liver is continuously gluconeogenic and lipogenic, which results from a state of mixed insulin resistance of the signalling pathways (cannot control gluconeogenesis but there is some control on fatty acid synthesis and esterification which leads to overproduction of triacylglycerols).

37.7. METABOLIC INTERRELATIONSHIP IN TYPE 1 DIABETES MELLITUS

There is a c omplete absence of insulin production and the glucagon effect often is dominant. The liver is always gluconeogenic and ketogenic and cannot buffer blood glucose levels. There is no glucose uptake in muscle and adipose tissue (no GLUT-4 translocation). There is uncontrolled proteolysis in skeletal muscle, which fuels accelerated gluconeogenesis in liver. Uncontrolled lipolysis in adipose tissue increases plasma fatty acid levels and ketone body production by the liver (ketoacidosis). Fatty acid oxidation and ketogenesis cannot dispose of fatty acids taken up by liver, the excess is esterified and directed into VLDL synthesis. Hypertriacylglycerolemia develops (both VLDL and chylomicron level increase) in the absence of lipoprotein lipase (its expression depends on insulin). Every tissue plays a catabolic role which leads to severe wasting of body tissues.

The pathology of diabetes includes cardiovascular disease, renal failure, blindness, poor healing in extremities often resulting in amputations and neuropathy.

37.8. METABOLIC ABNORMALITIES RESPONSIBLE FOR THE COMPLICATION OF DIABETES

- **Increased protein glycation (advanced glycated end products (AGEP) formation):** glycation alters the activity, solubility, degradation of proteins contributing the damage of kidneys, retinas and cardiovascular system in diabetes. Glycated hemoglobin (A1c) levels provide a measure of the previous month's average glucose level

- **Activation of the polyol pathway**
  Lens, peripherial nerve, renal papillae, Schwann cells, glomerulus and retinal papillae contain two enzymes of polyol pathways; aldose reductase uses NADPH to reduce glucose to sorbitol, sorbitol dehydrogenase using NAD+ to oxidize sorbitol into fructose. Aldose reductase has a high Km for glucose; this pathway is only active during hyperglicemia. The sorbitol content of lens, nerve and glomerulus is elevated in diabetic individuals, which may damage these tissues by creating abnormal swelling.

- **Activation of protein kinase C**

- **Activation of the hexosamine pathway**

Each of these abnormalities has been linked to glucose induced formation of reactive oxygen species by the mitochondria, contributing to complications of diabetes.

37.9. TREATMENTS FOR TYPE 2 DIABETES

Three factors improve the health of individuals with type 2 DM: dietary restrictions, regular excercise, and pharmaceuticals used to increase insulin sensitivity or insulin production.

- **Dietary restriction** (weight loss) reduces the overall burden of handling fatty acids. The lipid
- composition of diet influences, through PPARs and other transcription factors, the expression of genes, capable of encoding proteins involved in the fatty acid oxidation and in energy expenditure via thermogenesis.
- **Exercise** activates AMPK, including adiponectin; AMPK shift metabolism toward fat oxidation and inhibits fat synthesis.
- **Several classes of pharmaceuticals** are used in the management of type 2 DM (lecture fig.).

Sulfonylureas act on the ATP-gated K activates AMPK. Thiazolidinediones act through PPARγ to increase adiponectin concentration in plasma and to stimulate adipocyte differentiation, thereby increasing the capacity for TAG store.

Inhibitors of dipeptide protease IV (DPPIV) prevent the proteolytic degradation of GLP-1, a peptide hormon produced in the lower abdomen stimulating pancreatic insulin secretion.

38. THE ROLE OF THE LIVER IN THE METABOLISM CONTROL

39. METABOLIC INTEGRATION

39.1. BRAIN

Glucose is virtually the sole fuel for the human brain, except during prolonged starvation. The brain lacks substantial fuel stores (glycogen, lipid protein) and hence requires a continuous supply of glucose (about 120 g daily). The brain features a distinctively active respiratory metabolism. Much of the energy is used to power the transport mechanism required in the maintenance of the Na essential for the effective transmission of the nerve impulses, including the synthesis of neurotransmitters and their receptors.

Fatty acids do not serve as fuel for the brain, because they are bound to albumin in plasma and therefore do not traverse the blood-brain barrier. During starvation, **ketone bodies** (β-hydroxybutirate) generated by the liver partially replace glucose as fuel intended in the brain.

39.2. SKELETAL MUSCLE

Metabolism of skeletal muscle is specialized to generate ATP as the immediate and effective source of energy required in support of contraction. There are two general classes of muscle tissue, which differ in physiological role and fuel utilization. **Slow–twitch muscle** (red muscle) -rich in mitochondria and is served by a dense network of blood vessels- provide relatively low tension but it is highly resistant to fatigue. It produces ATP by oxidative phosphorylation. **Fast-twitch muscle** (white muscle) has fewer mitochondria and is less well supplied with blood vessels, but it can develop greater tension, and does so much faster. It can obtain energy by anaerobic fermentation of glucose and by using its creatine phosphate stores.

Skeletal muscle can use **free fatty acids, ketone bodies, or glucose** as fuel depending on the degree of muscular activity. In dormant muscle, the primary fuels are free fatty acids from adipose tissues (85%) and ketone bodies from the liver. Moderately active muscle uses blood glucose in addition to fatty acids and ketone bodies. These fuels are metabolized by oxidative catabolism.

Maximally active fast-twitch muscles must perform under intensive anaerobic condition, when the stored **muscle glycogen** is broken down to lactate by fermentation. The lactate flows to the liver, where it converted into glucose (Cori cycle). Additionally, a large amount of alanine is formed in the active muscle by transamination of pyruvate. Alanine, like lactate, can be converted into glucose by the liver (alanine cycle).The relatively small amount of glycogen limits the amount of glycolytic energy available during full-trottle exertion, and the accumulation of lactate and consequent decrease in pH diminishes their efficiency. Skeletal muscle, however, contains another source of ATP, **phosphocreatine** (10-30 mM), which can rapidly regenerate ATP from ADP by the creatine kinase reaction (see lecture fig.). During periods of active and intensive contraction and glycolysis, this particular reaction proceeds distinctively in the direction of ATP synthesis; during recovery from exertion, the same enzyme resynthesizes phosphocreatine from creatine and ATP. Creatine and phosphocreatine spontaneously break down to form creatinine which is excreted through the kidney. To maintain high creatine levels, these losses have to replaced, either by dietary creatine, or by the de novo synthesis from glycine, arginine and methionine, which occurs primarily in liver and kidney.

Effect of exercise upon metabolic pathways in skeletal muscle: the energy demand of muscle contraction increase AMP levels and activates AMPK. AMPK stimulates the GLUT4 transport to plasma membrane for greater glucose uptake and catabolism for ATP production. AMPK-mediated phosphorylation also decrease malonyl-CoA levels by inactivating ACC and activating malonyl-CoA decarboxylase. Reduced levels of malonyl-CoA results in greater carnitine palmitoyltransferase 1 activity and fatty acid oxidation towards benefiting the muscles increased thirst for ATP.

39.3. HEART MUSCLE

Heart muscle differs from skeletal muscle in that it is continuously active, and it has a completely **aerobic metabolism** (it has a very high density of mitochondria). The heart uses mainly free fatty acids, but also some other fuels as sources of energy; these fuels are oxidized via citrate cycle and oxidative phosphorylation to generate ATP. Ultimately, a variety of metabolic fuels can be utilized by the heart in various physiological states. **Glucose , pyruvate and lactate** utilized preferentially following a meal, when the level of free fatty acids in the blood are low. **Free fatty acids and branched-chain amino acids** are utilized for energy in short term fasting. In long-term fasting, the **ketone bodies** become the preferred fuels. The utilization of glucose and endogenous glycogen by the heart increases about10-20-fold in anoxia. **Acetate**, present in blood in significant amounts immediately following ingestion of alcohol, is used as an energy source for heart.

Like skeletal muscle, heart muscle does not store lipids or glycogen in large amounts. It has some energy reserve in the form of phosphocreatine, enough for a few seconds of contraction.

39.4. KIDNEY

The major purpose of the kidney is to produce urine which serves as a vehicle for **excreting metabolic waste products** and for **maintaining the osmolarity of body fluids**. The blood plasma is filtered nearly 60 times each day in the renal tubules. Most of the materials filtered out from blood are reabsorbed. Water-soluble materials in the plasma, such as glucose, and water are reabsorbed to prevent wasteful loss. The kidney requires a large amount of energy to accomplish the reabsorption (consume 10% of oxygen used in cellular respiration).

During starvation, the kidney becomes an important site of **gluconeogenesis** and may contribute half of the blood glucose. The kidney is also involved (shared with the liver) in the **regulation of acid-base balance**. The complete catabolism of positively charged and sulfur containing amino acids produce protons and complete oxidation of negatively charged amino acids consume protons.

For acid –base balance, the kidney can take up glutamine effectively converting it to glucose coupled with the production of ammonium and bicarbonate ions (lecture fig.). The ammonium ions are excreted into the glomerule filtrate while the bicarbonate ions enter the blood to neutralize protons, and the resulted CO2 is blown off in the lungs, thereby eliminating excess protons.

Additional functions of the kidney involves the **synthesis of arginine, creatine and carnitine**. Citrulline released from the bowels and is converted to arginine by the kidney, which can be converted to creatine or released into the blood for use of other tissues.

Kidney (and the liver) can carry out the complete pathway of carnitine biosynthesis and supply other tissues with carnitine.

39.5. PREGNACY

The fetus is an energy demanding organism. The fetus primarily uses **glucose** for energy, but may also use **amino acids, lactate, fatty acids, and ketone bodies**. Lactate produced in the placenta by glycolysis is partly directed to the fetus, and the remainder enters the liver. Maternal LDL cholesterol is an important precursor of placental steroids (estradiol and progesterone).

During pregnancy, the starve-feed cycle is disturbed The placenta secretes placental lactogen and two steroid hormones. Placental lactogen stimulates lipolysis in adipose tissue which increases ketone body production in liver, and steroid hormones induce insulin resistance. After meals, pregnant women

enter the starved state more rapidly, plasma glucose, amino acids and insulin levels fall rapidly which may lead to maternal hypoglycemia.

39.6. LACTATION

In late pregnacy, placental progesterone and maternal prolactin hormones induce lipoprotein lipase in the mammary gland and promote the development of milk-secreting cells and ducts.

During lactation, breast-cells use glucose to form lactose and TAG, and energy; amino acids for protein synthesis; chylomicron and VLDL for TAG synthesis.

In low and diminished dietary supplies, proteolysis, gluconeogenesis and lipolysis cause maternal malnutrition and poor quality milk.

Lactating breast secretes Parathyroid Hormone-Related Protein (PTHrP) (mimics PTH effects) stimulating calcium and phosphorus absorption from the bowels and bones.

40. DIGESTION OF MACRONUTRIENTS

40.1. DIGESTION AND ABSORPTION OF PROTEINS

Digestion of the dietary proteins, digestive enzymes and slough-off epithelial cells to amino acids occurs in the gastrointestinal tract. In human species it is a highly effective process (about 1-2 g nitrogen is lost through feces per day). Entry of dietary proteins into the stomach stimulate the gastric mucosa to secrete the hormone **gastrin**, which in turn stimulates the secretion of **hydrochloric acid** by the parietal cells and **pepsinogen** by the chief cells of the gastric glands. The acidic gastric juice (pH 1.0-2.5) is both an antiseptic and a denaturing agent. Pepsinogen, an inactive precursor, or **zymogen,** is converted to active pepsin in an intramolecular reaction (autoactivation) below pH 5 or by active pepsin itself (autocatalysis). **Pepsins** only are active at acidic pH. They are **carboxyl proteases** (catalytic activity requires two carboxylic groups at the active site), and the major pepsin (pepsin A) hydrolyzes peptid bonds on the amino terminal of aromatic amino acids, cleaving the long polipeptide chains into a mixture of smaller peptides.

As the acidic stomach contents pass into the small intestine, the low pH triggers secretion of the hormone **secretin** into the blood. Secretin stimulates the pancreas to secrete **bicarbonate** into the small intestine to neutralize the gastric HCl, creating neutral pH. The digestion of proteins continues in the small intestine. Arrival of amino acids into the duodenum causes the release into the blood of the hormone **cholecystokinin**, which stimulates secretion of several pancreatic zymogens; endopeptidases **trypsinogen, chymotripsinogen proelastase, and carboxipeptidases A and B**. **Enteropeptidase**, a protease produced by duodenal epithelial cells, activates pancreatic trypsinogen to trypsin by proteolytic cleavage. **Trypsin**, in turn, autocatalytically activates more trypsinogen to trypsin and also acts on the other proenzymes (see lecture fig.). The pancreas protects itself against self digestion by making a specific inhibitor, the **pancreatic trypsin inhibitor**, preventing premature production of active proteolytic enzymes.

Trypsin, chymotrypsin and elastase have different substrate specificity (lecture fig.). They are active at a neutral pH only, and they are **serine proteases** (their catalytic mechanism involves an essential serine residue). Lecture figure demonstrates some natural proteins (**serpins**), which inhibit serine proteases´ activity. Additionally, pancreatic juice contains **carboxypeptidase A and B**, which are **Zn^{2+} metalloenzymes** (zinc petidases) and possess a different type of catalytic mechanism when compared with the carboxyl and serine peptidases. The combined action of pancreatic peptidases results in the formation of free amino acids and small peptides.

Digestion of small petides into free amino acids and di- and tripeptides are accomplished by small intestinal surface enzymes (**endopeptidases, aminopeptidases and dipeptidases**). These end products of the digestion are absorbed into the intestinal epithelial cells via specific amino acid and peptide transport systems. Transported di-and tripeptides are generally hydrolyzed in the cytoplasm prior to departing the cell and entering the blood capillaries.

Amino acids and peptides within the small intestine are **carrier mediated**.. Currently, there are seven known brush border specific transport systems in support of the uptake of amino acids/small peptides in the luminal membrane (lecture figure). Release of the amino acids into the blood involves other specific transporters. Many of transporters are **Na+ dependent cotransporter** (in the luminal membrane) and are energized by the electrochemical Na+ gradient, or **Na+ independent, facilitated diffusion type transporter**. Transporter type is dependent on the specific charge of the amino acid, and some amino acids are transported by more than one transporter. Neutral dipeptides are cotransported across the brush border membrane with a proton, and therefore are energized through the proton electrochemical gradient. The **dipeptide transporter** also accepts β-lactam antibiotics (aminopenicillins) and is important for absorption of orally administered antibiotics of this class.

40.2. DIGESTION AND ABSORPTION OF CARBOHYDRATES

Dietary carbohydrates provide the major portion of daily calorie intake (40-45%). They consist of polysaccharides (starch, glycogen), disaccharides (sucrose, lactose) and monosaccharides (glucose and fructose).

In most humans, starch is the major source of carbohydrates in our diet. Digestion of hydrated polysaccharides (starch and glycogen) begins within the mouth, where **salivary α-amylase** hydrolyzes the internal (α1→4) glycosidic linkage of polysaccharide (**endoglycosidase, specific for α1→4 linkage)**, producing short polysaccharide fragments, or oligosaccharides. (hydration of polysaccharides occurs during heating and is essential for efficient digestion). In the stomach, salivary α-amylase is inactivated by the low pH, but a second form of α-amylase, secreted by the pancreas into the small intestine, continues the breakdown process. **Pancreatic amylase** (which is more important than salivary enzyme from digestive point of view) yields mainly **maltose** and **maltotriose** (the di-and trisaccharides of glucose) and oligosaccharides called **α-limit dextrin**s, fragment of amylopectin containing (α1→6) branch points.

Final hydrolysis of di- and oligosaccharides to monosaccharides is accomplished by surface enzymes of the small intestinal epithelial cells (lecture fig.). Most of the **surface oligosaccharidases** are exoenzymes (cleave off one monosaccharides at a time from the nonreducing end). The capacity of the **α-glucosidases** and **sucrase** is normally much greater than needed for the digestion of dietary starch. However, **β-galactosidase (lactase)** can be rate limiting in humans for hydrolysis and utilization of lactose, the major milk carbohydrate (its deficiency, which is frequent in humans, leads to a condition, called **lactose intolerance**).

The non-hydrolyzed di-, oligo-, and polysaccharides cannot be absorbed; therefore they reach the lower tract of the intestine, which from the lower ileum contains bacteria, which can utilize many of the remaining carbohydrates (they posses many different types of saccharidases than humans). Next the resulted monosaccharides are metabolized anaerobically by the bacteria themselves, resulting in degradation products such as short-chain fatty acids, lactate, hydrogen gas (H_2), methane (CH_4), and carbon dioxide (CO_2). These compounds can cause fluid secretion, increased intestinal motility and cramps, because of increased intraluminal osmotic pressure, distension of gut, or irritable effects of the degradation products on intestinal mucosa. (Leguminous seeds cause flatulence, because their oligosaccharides (for example **raffinose**) contain galactose in α-linkage, which cannot be hydrolyzed by human enzymes).

The primary monosaccharides as a result from digestion of di- and polysaccharide are **D-glucose, D-galactose, and D-fructose**. Absorption of these and other minor monosaccharides are carrier mediated processes. At least two types of monosaccharide transporter catalyze monosaccharide uptake from the lumen into the cell:First, a **Na+ monosaccharide cotransporter (SGLT)**, which has high specificity for D-glucose and D-galactose and catalyze active transport (energized by Na+ electochemical gradient). Secondly, a **Na+-independent facilitated diffusion** type of monosaccharide transport system with specificity for D-fructose **(GLUT5)**. In addition, a **Na+-independent monosaccharide transporter (GLUT2)**, which accepts all three monosaccharides, is present in the contraluminal membrane. GLUT2 is also present in the liver and pancreas, (where it helps equilibrate the extra- and intracellular glucose concentration, and involved in the regulation of insulin release from the pancreas), while other glucose transporters (from GLUT1-12) with characteristic tissue distribution and function are located in different tissues.

40.3. DIGESTION AND ABSORPTION OF LIPIDS

An adult man ingests about 60-150g of lipids per day. **Triacylglycerols** constitute more than 90% of the dietary fats. The rest is made up of **phospholipids, cholesterol, cholesterol esters, and free**

fatty acids. In addition, 1-2 g of cholesterol and 7-22 g of phosphatydilcholine are secreted into the small intestine with bile.

In vertebrates, before ingested triacylglycerols can be absorbed through the intestinal wall, they must be converted from insoluble macroscopic fat particles to finely dispersed microscopic micelles. This **solubilization** is carried out **by bile salts**, which are synthesized from cholesterol in the liver, stored in the gallbladder, and released into the small intestine after ingestion of fatty meal. Bile salts are **amphipathic compounds**, that acts as **biological detergents**, converting dietary fats into mixed micelles of bile salts and triacylglycerols. Micelle formation greatly increases the fraction of lipid molecules accessible to the action of water-soluble lipases in the intestine. Micelles also provide a vehicle for moving lipids, cholesterol and lipid-soluble vitamins from the lumen to the cell surface for absorption. Thus, efficient absorption of lipids, cholesterol and lipid soluble vitamins depends on the presence of bile acids.

There are five different phases in the digestion and absorption of triacylglycerols:

1. hydrolysis of triacylglycerols to free fatty acids and monoacylglycerols
2. solubilization by detergents (bile acids) and transport from the intestinal lumen toward the cell surface
3. uptake of free fatty acids and monoacylglycerols into the cells and resynthesis to triacylglycerols
4. packaging of newly synthesized triacylglycerols (together with phospholipids and cholesterol) into chylomicrons, and
5. exocytosis of chylomicrons from cells and release into lymph.

Digestion of lipids is initiated in the stomach by an **acid-stable lipase** (most originate from glands at the back of the tongue). This converts some triacylglycerols into fatty acids and diacylglycerols, disperse the large droplets into smaller droplets , thus, an increase in the surface area to the action of more lipase.

The major enzyme for triacylglycerol hydrolysis is the **pancreatic lipase**, which is s**pecific for esters in the α-position** of glycerol and **prefers longer-chain fatty acids** (more than 10 carbon). The products of this enzyme are free fatty acids and β-monoacylglycerols, which are absorbed into the cells. Bile acids inhibit this enzyme, but the colipase solves the problem. It binds to the water-lipid interphase and to lipase, thereby anchoring and activating the lipase. It is secreted by the pancreas as procolipase and activated by triptic cleavage.

Other **less specific lipid esterases** act on cholesterol esters, monoglycerides or other lipid esters, such as esters of vitamin A with carboxylic acids. This lipid esterase requires bile acids for activity.

Phospholipids are hydrolyzed by specific **phospholipases**, for example phospholipase A2 (lecture fig.), which also secreted from the pancreas as proenzymes and activated by tripsin. Phospholipase A2 requires bile acids for activity.

Within intestinal cells, the fate of absorbed fatty acids depends on chain length. Medium-chain fatty acids (6-10 carbon) pass through the cell into the portal blood without modification. **Long chain fatty acids** (>12 carbon atom) become bound to a cytosolic **intestinal fatty acid binding protein (I-FABP)** and are transported to the ER, where they are resynthesized into triacylglycerol. Triacylglycerols are incorporated with cholesterol and apolipoproteins into **chylomicrons**, migrate through the Golgi to the basolateral membrane, and leave the intestine via lymph vessels. Chylomicrons move through the lymphatic system and bloodstream to tissues. **Lipoprotein lipase**, activated by apoC-II in the capillary converts triacylglycerols to fatty acids and glycerol. Fatty acids enter the cells, and oxidized as fuel

(myocytes) or reesterified for storage (adipocytes). Chylomicron remnants travel and are eventually absorbed by the liver.

Bile acids are mostly reabsorbed by the epithelium of the lower small intestine into the portal blood and are then extracted by the liver (**enterohepatic circulation**).

40.4. FIBERS

Dietary fibers are components of food that cannot be broken down by human digestive enzymes (some partially broken down by intestinal bacteria).

The lecture figure shows the major type of fibers and their properties.

Cellulose and **hemicelluloses** increase stool bulk and decrease transit time. They decrease intracolonic pressure and play a beneficial role, with respect to diverticular diseases. By diluting out potential carcinogens, and spreading their transit through the colon they reduce the risk of colon cancer.

Lignins have a bulk-enhancing effect, and they also absorb organic substances such as cholesterol (have a cholesterol-lowering effect).

Mucilaginous fibers, such as **pectin** and **gums**, tend to form viscous gels in the stomach and intestine and slow the rate of absorption of many nutrients (reduce the rate of carbohydrate digestion and absorption, therefore diminish the rise in blood sugar and insulin level!).

Water soluble fibers (pectin, gums, some hemicelluloses, and storage polysaccharides) also help to lower serum cholesterol levels.

41. BIOCHEMISTRY OF HEMOGLOBIN

42. BIOCHEMISTRY OF SENSES

42.1. OLFACTION

The olfactory membrane lies in the superior part of each **nostril**. The receptor cells for the smell sensation are the **olfactory cells**. The mucosal end of the olfactory cells forms a knob from which **olfactory cilia** project into the mucus that coats the inner surface of the nasal cavity. These cilia react to odors in the air and stimulate the olfactory cells. The receptors for olfactory stimuli have G protein-coupled receptor (GPCR) structure with seven transmembrane α helices (7TM). The olfactory signal can be any one of the many volatile compounds. Human beings can detect and distinguish thousands of different compounds by smell. The smell produced by an odorant depends on the compound's shape and so on its interaction with a specific receptor. Our ability to discriminate odors based on the different olfactory receptors and on the brain's ability to integrate input from different types of olfactory receptors. Almost every odorant activates a number of receptors and almost every receptor is activated by more than one odorant. However, each odorant activates a unique combination of receptors. Odorants are decoded by a combinatorial mechanism and the resulting pattern is the basis of the much higher number of discriminating odors then the number of receptors.

The molecular events of olfaction are described in the lecture slide and those steps occur in the cilia of olfactory receptor cells.

42.2. TASTE

The sense of taste is a number of independent senses all utilizing the same organ, the tongue, for their expression. Tastants are detected by specialized structures called **taste buds**, which contain sensory neurons. These neurons extend **microvilli**, rich in taste receptors, to the surface of the tongue, where they interact with tastants. In some sensory neurons (sweet, bitter, umami) GPCRs are coupled to the heterotrimeric G protein **gustducin**. Five primary tastes are recognized: **bitter, sweet, sour, salty, and umami** (the taste of glutamate). Tastants are quite distinct for the different groups.

Bitter and sweet receptors are 7TM receptors with the specific G protein, gustducin. Each taste receptor cell expresses many different members of receptor genes. This pattern of expression stands in sharp contrast to the pattern of one receptor type per cell that characterizes the olfactory system. The difference in expression patterns accounts for the greater specificity of our perceptions of smells compared with tastes. Each odorant stimulates a unique pattern of neurons, in contrast, many tastants stimulate the same neurons. Sweet-tasting molecules are those that bind to receptors in sweet taste buds. The transduction mechanism for sweet tastants is explained in the lecture slide.

Salty tastants are not detected by 7TM receptors. Salty tastes are detected primarily by the passage of **sodium ions** through channels, expressed on the surface of cells in the tongue. **Amiloride** is a compound, which could mute the taste of salt and reduce sensory neuron activation in response to sodium. Sodium ions passing through the **amiloride-sensitive channels** produce a significant transmembrane current. Amiloride blocks this current, accounting for its effect on taste. Beside these channels, other ion channels could also contribute to salt detection.

Sour tastes are also detected by direct interactions with **ion channels**, but at that case **hydrogen ions** are detected instead of sodium ions. Hydrogen ions are also sensed by other mechanisms since hydrogen ions can block some potassium channels and activate other types of channels. Together, these mechanisms lead to changes in membrane polarization in sensory neurons that produce the sensation of sour taste.

42.3. VISION

Visual perception is the ability to interpret the surrounding environment by processing information that is contained in visible light. Visible light has wavelengths between 300 and 850 nanometers. In vertebrate eye, light entering through the pupil is focused on a collection of **light-sensitive neurons**. These neurons have to types: **rods** (100 million per retina in humans) function in dim light but cannot discriminate colors, and **cones** (3 million per retina in humans) function in bright light and can discriminate colors. Both cell types are long, specialized sensory neurons with two cellular compartments: the outer segment contains membranous disks loaded with receptor proteins and their photosensitive chromophore **retinal**; the inner segment contains the nucleus, mitochondria, and other cellular compartments. The **photoreceptor** molecule in rods is **rhodopsin**, which contains the protein **opsin** linked to **11-*cis*-retinal**, a prosthetic group. Rhodopsin is an integral membrane protein with 7TM structure characteristic to GPCRs. The light-absorbing pigment 11-*cis*-retinal is covalently attached to opsin. When a photon is absorbed by the rhodopsin, the energy causes a photochemical change; 11-*cis*-retinal is converted to **all-*trans*-retinal**. This molecular change leads to conformational changes in the rhodopsin and this is the first step in visual transduction.

Rods and cones have a transmembrane electrical potential, produced by **Na^+K^+ATPase** by pumping 3 Na^+ out for every 2 K^+ pumped in. The membrane potential is reduced by the inflow of Na^+ and Ca^{2+} through **cGMP-gated cation channels** in the outer segment of the neurons. When rhodopsin absorbs light, it triggers degradation of cGMP in the outer segment, causing closure of the ion channel. Without cation influx through this channel, the cell becomes **hyperpolarized**. This electrical signal is passed to the brain through interconnecting and ganglion neurons.

The molecular consequences of photon absorption by rhodopsin (excitation and recovery) in the rod outer segment, is described in the lecture slide.

The molecular events in **color vision** (in cone cells) are essentially identical to that described in the rod cells with slightly different light receptors. Three types of cone cells are specialized to detect light from different regions of the spectrum: cone cells with **red, green, and blue photoreceptors**. We discriminate colors by integrating the output from these three types of cone cells, each containing one of the three photoreceptors.

Color blindness is the inability to distinguish red from green and is a common, genetically inherited trait in humans. The various types of color blindness result from different opsin mutations. The genes for the green and red pigments lie adjacent to each other. These genes are more than 98% identical in nucleotide sequence and they are very susceptible to unequal homologous recombination. Recombination can take place either between or within transcribed regions of the gene. Rearrangements in the course of DNA replication may lead to the loss of visual pigment genes or the formation of hybrid pigment genes that encode photoreceptors with anomalous absorption spectra.

42.4. HEARING

Hearing is based on the detection of **mechanical stimuli**. We hear frequencies ranging from 200 to 20,000 Hz, corresponding the times of 5 to 0.05 ms. Sound waves are detected inside the **cochlea** of the inner ear. The cochlea is a fluid-filled, membranous, coiled sac. The primary detection is accomplished by specialized neurons inside the cochlea called **hair cells**. Each cochlea contains thousands of hair cells and each hair cell contains hundreds of projections called **stereocilia**. Mechanical deflection of the hair bundle by the sound wave arrives at the ear, creates change in the membrane potential of the hair cell. The movement of the hair bundle acts on **ion channels** directly. Adjacent stereocilia are linked by individual filaments called **tip links**, which are coupled to ion channels in the membranes of the stereocilia that are gated by mechanical stress. In the absence of stimulus, only a small fraction of these channels are open. When the hair bundle is displaced toward its tallest part, the stereocilia

slide across one another and the tension on the tip links increases, leading to additional channel opening, which depolarizes the membrane. Conversely, if the displacement is in the opposite direction, the tension on the tip links decreases, the open channels close, and the membrane hyperpolarizes. The mechanical motion of the hair bundle is directly converted into current flow across the hair-cell membrane.

42.5. TOUCH

Touch, detected by the skin, senses pressure, temperature, and pain. Specialized nerve cells called **nociceptors** transmit signals that are interpreted in the brain as pain. A receptor responsible for the perception of pain has been isolated on the basis of its ability to bind **capsaicin**, the molecule responsible for the hot taste of spicy food. The capsaicin receptor, also called **VR1** (for vanilloid receptor 1), functions as a **cation channel** that initiates a nerve impulse. VR1 receptors are activated not only by capsaicin but by temperature and acidity as well, suggesting that these responses are not independent. VR1 acts to integrate these noxious stimuli as pain, to avoid the potentially destructive conditions that caused the unpleasant sensation.

All the signal-transducing systems that act through heterotrimeric G proteins share some common features. The receptors have 7TM structure. Ligand binding induces interaction between receptor and G protein and heterotrimeric G proteins activate or inhibit effector enzymes: adenylyl cyclase (AC), phospholipase C (PLC), and phosphodiesterases (PDEs), which change the concentration of a second messenger (cAMP, cGMP, IP_3, or Ca^{2+}). The final output is either an activated protein kinase or a change in membrane potential and a consequent electrical signal that passes to the brain.

43. BIOCHEMISTRY OF BLOOD COAGULATION

44. BIOCHEMISTRY OF HORMONES

45. NEUROTRANSMITTERS

46. DRUG DEVELOPMENT