

Flexibook



Color Atlas of Biochemistry

Jan Koolman
Klaus-Heinrich Roehm

3rd edition

basic sciences

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A. Important elements				
	Sym- bol	V. d. Waals radius (pm)	Relative atomic mass	Coval. radius (pm)
○ Hydrogen	H	100	1.008	37
● Carbon	C	170	12.011	77
● Nitrogen	N	150	14.007	70
● Oxygen	O	140	15.999	66
● Phosphorus	P	190	30.974	110
● Sulfur	S	180	32.060	104
● Metal	Me	—	—	—

B. Biomolecules	
Carbohydrate	Signal molecule
Protein	Outside
Membrane	Inside
RNA	DNA

C. Processes, reactions, and metabolites				
Metabolic process	Reaction	Catalysis	Activation	
General process	Several steps	Anabolic	Inhibition	
Metabolite	Transport	Catabolic reaction pathways	Complex effect	
Carbohydrate	① Transport process	① Metabolic process	① Other process	
Nucleotide	① Metabolic process	① Other process	① Process takes place	
Lipid	① Other process	① Process takes place	① Molecule active	
Protein/amino acid	① Process does not take place	① Molecule inactive	① Concentration increases	
Storage substance	① Concentration decreases	① High-energy bond	① Suggests pathology	
Pathological process	① High-energy bond	① Suggests pathology		

D. Coenzymes and nucleotides (examples)				
Redox coenzymes	NAD	NADP	FMN	FAD
Nucleotides	AMP	ADP	ATP	dTMP
Other coenzymes	CoA	TPP	PLP	THF

A. Representation of enzyme-catalyzed reactions				
Methanol	Effector	Effect	Enzyme	
Ethanol	Substrate	NAD	Coenzyme	
Ethanal	Product	NADH	Cofactor	
1	Alcohol dehydrogenase [Zn ²⁺]			
1	Enzyme			
1	Protein kinase			
1	Protein phosphatase			
Enzyme classes:				
1	Oxidoreductase			
2	Transferase			
3	Hydrolase			
4	Lyase			
5	Isomerase			
6	Ligase			

B. Tissue, organs, organelles	
Liver	Muscle
Kidney	Adipose tissue
Intestine	Lungs
Erythrocyte	Mitochondrion
Golgi apparatus	Cell with nucleus
C. Structures and complex molecules	
Membrane	Transporter
Ion channel	Transport ATPase
1-helix membrane receptor	7-helix membrane receptor
Ribosome	Transcription factor
Antibody	

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Color Atlas of Biochemistry

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Third edition, revised and updated

With 220 color plates prepared by Juergen Wirth

Thieme

Stuttgart · New York

Koolman and Roehm, Color Atlas of Biochemistry (ISBN 978-3-13-100373-7),
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Library of Congress Cataloging-in-Publication Data is available from the publisher.

1st French edition 1994
 1st German edition 1994
 1st English edition 1996
 2nd German edition 1997
 1st Italian edition 1997
 1st Japanese edition 1997
 2nd French edition 1999
 1st Greek edition 1999
 1st Russian edition 2000
 1st Indonesian edition 2002
 1st Turkish edition 2002
 1st Dutch edition 2004
 3rd French edition 2004
 1st Spanish edition 2004
 2nd English edition 2005
 1st Polish edition 2005
 1st Portuguese edition 2005
 2nd Greek edition 2007
 2nd Japanese edition 2007
 1st Korean edition 2008
 1st Chinese edition (Taiwan) 2008
 4th French edition 2011
 1st Czech edition 2012
 2nd Portuguese edition 2012
 2nd Spanish edition 2012

© 2013 Georg Thieme Verlag KG,
 Rüdigerstrasse 14, 70469 Stuttgart, Germany
<http://www.thieme.de>
 Thieme Medical Publishers, Inc.,
 333 Seventh Avenue,
 New York, NY 10001, USA
<http://www.thieme.com>

Color plates prepared by Juergen Wirth,
 Professor of Visual Communication, Dreieich,
 Germany

Cover design: Thieme Publishing Group
 Typesetting by primustype Hurler GmbH,
 Notzingen, Germany
 Printed in China by Everbest Co., Ltd

ISBN 978-3-13-100373-7
 eISBN 978-3-13-169693-9

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About the Authors



Jan Koolman (left) was born in the Hanseatic city of Lübeck, Germany, and grew up with the sea wind blowing off the Baltic. The high school he attended was providing a classical education, which left its mark on him. From 1963 to 1969, he studied biochemistry at the University of Tübingen. His doctoral work at the University of Marburg under supervision of the biochemist Peter Karlson focused on the biochemistry of insects and other invertebrates. He took his postdoctoral degree in 1977 in the field of human medicine, and was appointed Professor in 1984. In 2010 he retired from active duty. His fields of interest are biochemical endocrinology and educational methods in biochemistry. He is married to an art teacher.

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Jürgen Wirth (center) studied in Berlin and in Offenbach, Germany. His studies focused on free graphic arts and illustration. From 1963 to 1977 he was involved in designing the exhibition space in the Senckenberg Museum of Natural History in Frankfurt am Main, while at the same time working as a freelance associate with several publishing companies, providing illustrations for schoolbooks, non-fiction titles, and scientific publications. He has received several awards for book illustration and design. In 1978, he was appointed to a professorship at the University of Applied Sciences in Schwäbisch Gmünd, Germany, and in 1986 he became Professor of communication design at the University of Applied Sciences in Darmstadt, Germany. His fields of interest include scientific graphics/information graphics and illustration methods.

Preface

Biochemistry is a dynamic, rapidly growing field, and the goal of this color atlas is to illustrate this visually. It is difficult to define precisely the cut-off points between biochemistry and its neighboring fields such as cell biology, anatomy, physiology, genetics, or pharmacology. For this reason this color atlas can be placed in the middle of the range of Thieme Flexi books.

Owing to space limitations this book focuses on human biochemistry, although the biochemistry of animals, plants, and microorganisms is no less interesting. The topics chosen concentrate on information that is relevant to students of human medicine. Again, because of the space restrictions it was impossible to avoid some gaps in the information included. It must be emphasized, therefore, that this color atlas is not intended as a substitute for a good, comprehensive textbook of biochemistry.

As in all Thieme Flexi books, color plates outlining the fundamental principles of the subject matter are the essential elements. Great emphasis has been placed on the graphic presentation—the texts can primarily be considered as expanded and complementary legends to the illustrations. As in former editions, color codes and symbols are used to aid the reader.

The key to their use will be found inside the front and back covers. We have made strenuous efforts to use these codes consistently.

The fourth edition of the color atlas has been extensively revised and expanded with students of human medicine in mind. Some material from the natural sciences has been shed, while more pathobiochemical information has been added. We have, however, endeavored to ensure that the changes fit the original concept of the book.

Our special thanks go to Thieme, especially Angelika Findgott, Annie Hollins and Sophia Hengst, for their guidance and support. We also wish to thank many colleagues, coworkers and—last but not least—interested readers for their constructive criticism, and for their positive comments and detailed recommendations for improvement. We continue to welcome further comments and suggestions in order to continue improving our color atlas for you, the reader. Please feel free to email us at koolman@staff.uni-marburg.de or roehm@staff.uni-marburg.de.

*Jan Koolman,
Klaus-Heinrich Röhm,
Jürgen Wirth*

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Basics

Periodic Table

A. Biologically important elements

There are 81 stable elements in nature. Fifteen of these are present in all living things, and a further 8–10 are only found in particular organisms. The illustration shows the first half of the **periodic table**, containing all of the biologically important elements. In addition to physical and chemical data (atomic number, relative atomic mass, group and electron configuration), it also provides information about the distribution of the elements in the living world and how abundantly they occur in the human body.

More than 99% of the atoms in animals' bodies are accounted for by just four elements—hydrogen (H), oxygen (O), carbon (C) and nitrogen (N). Hydrogen and oxygen are the constituents of **water**, which alone makes up 60–70% of cell mass (see p. 196). Together with carbon and nitrogen, these atoms are also the major constituents of the **organic compounds** on which most living processes depend. Many biomolecules also contain sulfur (S) or phosphorus (P). The above **macroelements** are essential for all organisms.

A second biologically important group of elements, which together represent only about 0.5% of the body, are present almost exclusively in the form of inorganic ions. These are known as **electrolytes** and include the *alkali metals* sodium (Na) and potassium (K), and the *alkaline earth metals* magnesium (Mg) and calcium (Ca). The halogen *chlorine* (Cl) is also always ionized in the cell. All other elements important for life are present in such small quantities that they are referred to as **trace elements** (p. 384). These include transition metals such as iron (Fe), zinc (Zn), copper (Cu), cobalt (Co) and manganese (Mn). A few *nonmetals*, such as iodine (I) and selenium (Se), can also be classed as essential trace elements.

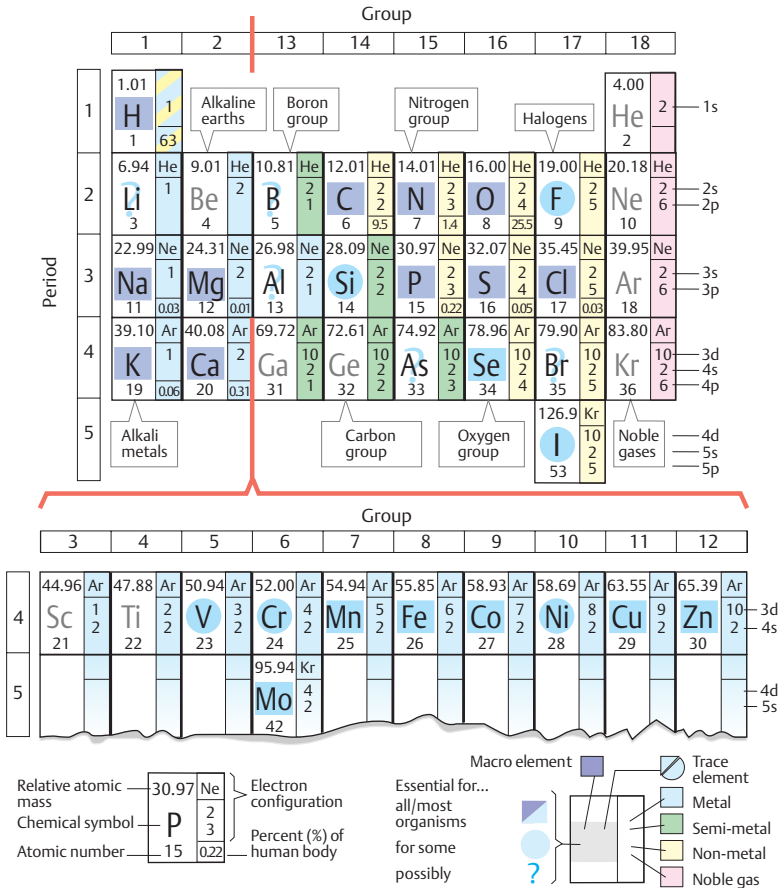
B. Electron configurations: examples

The chemical properties of atoms and the types of bond they form with each other are determined by the structure of their electron shells. The **electron configurations** of the elements are therefore also shown in **A**, while **B** explains the symbols and abbreviations used. More detailed discussions of the subject are available in chemistry textbooks.

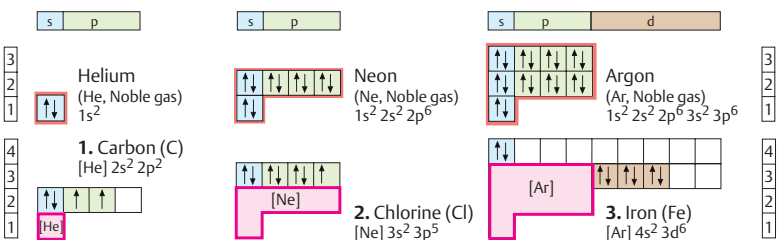
The possible states of electrons are called **orbitals**. These are indicated by what is known as the principal quantum number (1, 2, 3 and so

on) and by a letter—s, p, or d. The orbitals are filled one by one as the number of electrons increases. Each orbital can hold a maximum of two electrons, which must have oppositely directed “spins.” Figure **A** shows the distribution of the electrons among the orbitals for each of the elements. For example, the six electrons of carbon (**B1**) occupy the 1s orbital, the 2s orbital, and two 2p orbitals. A filled 1s orbital has the same electron configuration as the noble gas helium (He). This region of the electron shell of carbon is therefore abbreviated as “He” in figure **A**. Below this, the numbers of electrons in each of the other filled orbitals (2s and 2p in the case of carbon) are shown on the right margin. For example, the electron shell of chlorine (**B2**) consists of that of neon (Ne) and seven additional electrons in 3s and 3p orbitals. In iron (**B3**), a transition metal of the first series, electrons occupy the 4s orbital even though the 3d orbitals are still partly empty. Many reactions of the transition metals involve empty d orbitals—e.g., redox reactions or the formation of complexes with bases. Particularly stable electron arrangements arise when the outermost shell of elements of the second and third periods is fully occupied with eight electrons (the “**octet rule**”). This applies, for example, to the noble gases, as well as to ions such as Cl^- ($3s^2 3p^6$) and Na^+ ($2s^2 2p^6$). It is only in the case of hydrogen and helium that two electrons suffice to fill the outermost 1s orbital, thus achieving a stable electron configuration.

A. Biologically important elements



B. Electron configurations: examples



Isomerism

A. Isomerism: definition

Isomer is the name given to molecules that contain the same type and number of atoms (i.e., they have the same *molecular formula*), but that differ in structure. If isomers differ in the way in which their atoms are bonded in the molecule, they are described as **structural isomers**. Leucine and isoleucine (p. 50) or citrate and isocitrate (p. 114) are examples of structural isomers. In **stereoisomers**, the spatial arrangement of substituents differs relative to a bond (**B, C**), or the isomerism is due to the presence of a chiral center in the molecule (**D**). When stereoisomers behave like an image and its mirror-image, they are called **optical isomers**; all other stereoisomers are called **diastereomers**.

B. *cis-trans* isomers

Double bonds are not freely rotatable. If double-bonded atoms have different substituents, there are therefore two possible orientations for these groups. In **fumaric acid**, an intermediate of the tricarboxylic acid cycle (p. 114), the carboxyl groups lie on *different* sides of the double bond (*trans* or *E* position). In its isomer **maleic acid**, which is not produced in animal metabolic processes, the carboxyl groups lie on the *same* side of the bond (*cis* or *Z* position). *Cis-trans* isomers (**geometric isomers**) have markedly different chemical and physical properties—e.g., their melting points (*Fp*) and *pK_a* values. They can only be interconverted by a chemical reaction.

In lipid metabolism, *cis-trans* isomerism is particularly important. For example, the double bonds in naturally occurring fatty acids (see p. 38) usually have a *cis* configuration, while they have a *trans* configuration in unsaturated intermediates of β -oxidation.

C. Conformers

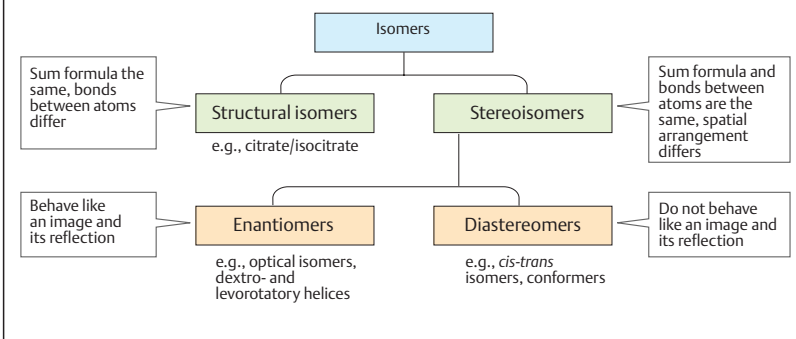
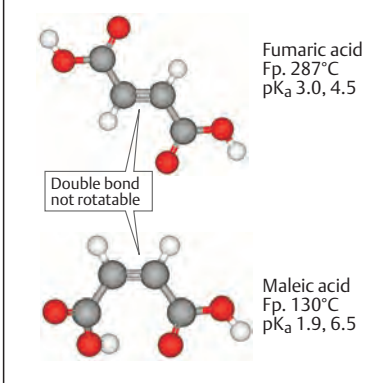
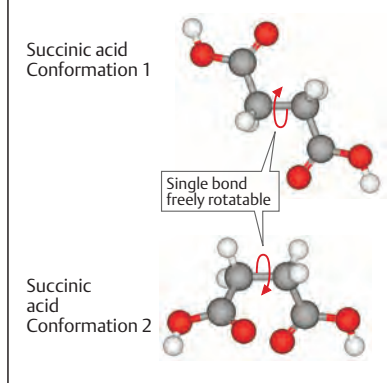
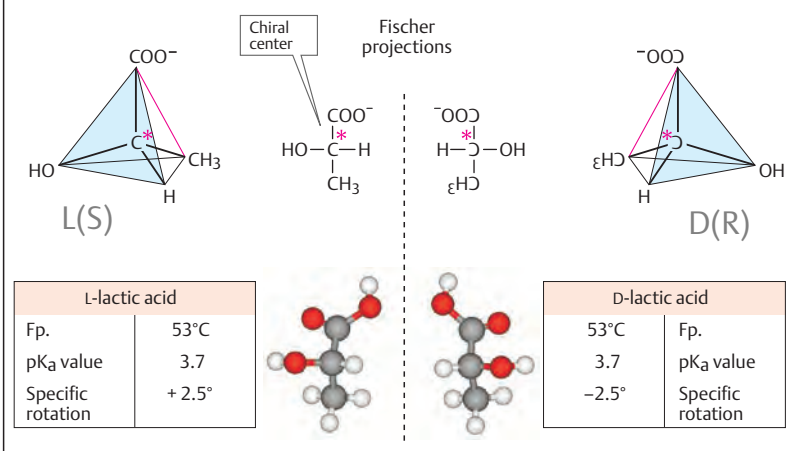
Molecular forms that arise as a result of rotation around freely rotatable bonds (e.g., simple C–C bonds) are known as **conformers**. Even small molecules can have many different conformations in solution. In the two conformations of **succinic acid** illustrated opposite, the atoms are arranged in a similar way to fumaric acid and maleic acid. These two forms occur in solution, along with many others, although conformation 1 (top) is more favored due to the greater distance between the COOH groups

and therefore occurs more frequently. Biologically active macromolecules such as proteins or nucleic acids contain thousands of freely rotatable bonds and therefore can theoretically have an extremely large number of conformations. Nevertheless, they are usually present only in a well-defined (“native”) conformation, which is stabilized by interactions in the molecule (see pp. 60, 68). If the native conformation of a macromolecule is lost because of **denaturation**, its biological activity also disappears.

D. Enantiomers

Another type of isomerism arises when a molecule contains a **chiral center** or is chiral as a whole. Chirality (from the Greek *cheir*, hand) leads to the appearance of structures that behave like image and mirror-image and that cannot be superimposed (“*mirror*” isomers). The most frequent cause of chiral behavior is the presence of an asymmetric C atom—i.e., a saturated carbon atom with four *different* substituents. The compound then occurs in two forms (**enantiomers**) with different **configurations**. Often, the enantiomers of a molecule are designated as *l* and *d* forms.

Unequivocal classification of the configuration is made possible by the *R/S* system (see chemistry textbooks). The *Fischer projection* (p. 48) is used to represent the formulas of chiral centers. Enantiomers have very similar chemical properties and are therefore very difficult to separate using chemical means. The fact that enantiomers rotate polarized light in opposite directions (**optical activity**) can be used to distinguish them. The enantiomers of **lactic acid** also possess this property. The dextrorotatory *l*-lactic acid occurs in animal muscle and blood, while the levorotatory *d*-form produced by microorganisms is found in milk products, for example.

A. Isomerism: definition**B. *cis-trans* isomers****C. Conformers****D. Enantiomers**

Substance Classes I

A. Important classes of compounds

Most biomolecules are derivatives of simple compounds of the nonmetals carbon (C), oxygen (O), hydrogen (H), nitrogen (N), sulfur (S), and phosphorus (P). The oxygen, nitrogen, and sulfur compounds important in biochemistry can be formally derived from their compounds with hydrogen (i.e., H_2O , NH_3 , and H_2S). In biological systems, phosphorus is found almost exclusively in derivatives of phosphoric acid, H_3PO_4 .

If one or more of the hydrogen atoms in the hydrogen compounds of the nonmetals are replaced formally with another group—e.g., alkyl residues—then derived compounds of the type $R-XH_{n-1}$, $R-XH_{n-2}R$, etc., are obtained. In this way, **alcohols** ($R-OH$) and **ethers** ($R-O-R$) are derived from water (H_2O); primary **amines** ($R-NH_2$), secondary amines ($R-NH-R$) and tertiary amines ($R-N-R'R''$) are obtained in this way from ammonia (NH_3); **thiols** ($R-SH$) and **thioethers** ($R-S-R'$) arise from hydrogen sulfide (H_2S). Polar groups such as $-OH$ and $-NH_2$ are found as substituents in many organic compounds. As such groups are much more reactive than the hydrocarbon structures to which they are attached, they are referred to as **functional groups**.

New functional groups can arise as a result of **oxidation** of the compounds mentioned above. For example, the oxidation of a thiol yields a **disulfide** ($R-S-S-R$). Double oxidation of a primary alcohol ($R-CH_2-OH$) gives rise initially to an **aldehyde** ($R-C(O)-H$), and then to a **carboxylic acid** ($R-C(O)-OH$). In contrast, the oxidation of a secondary alcohol yields a **ketone** ($R-C(O)-R$). The carbonyl group ($C=O$) is characteristic of aldehydes and ketones.

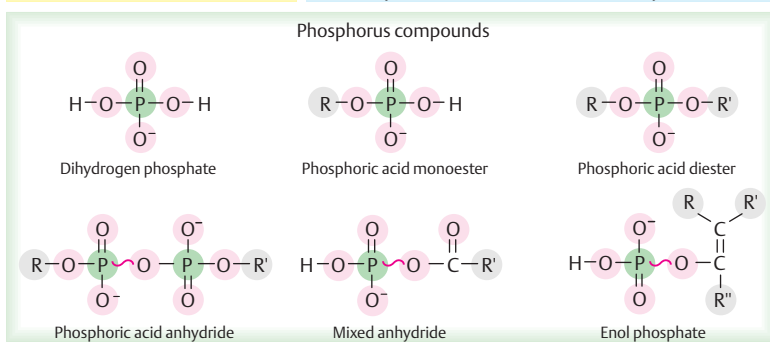
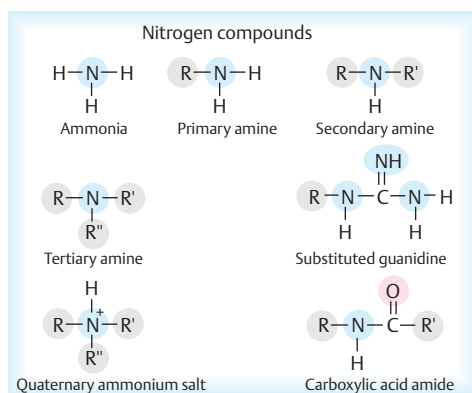
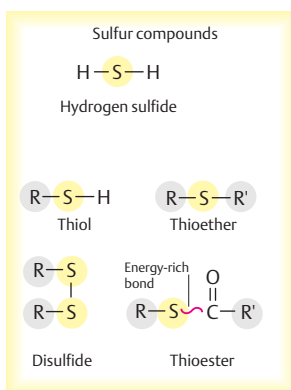
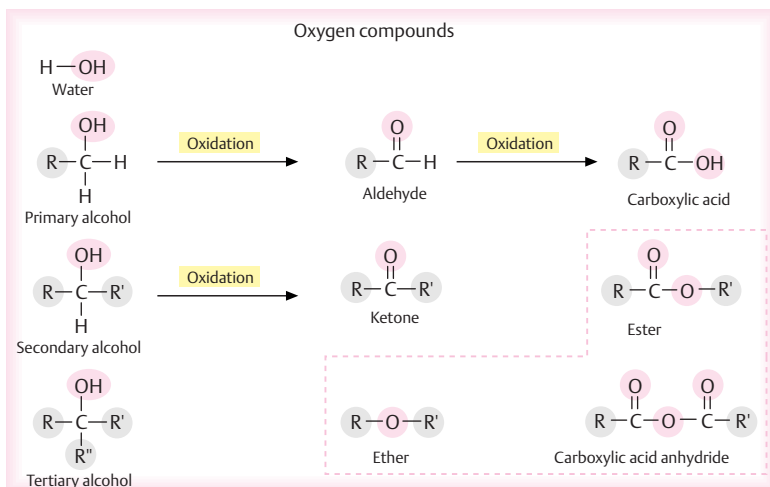
The addition of an amine to the carbonyl group of an aldehyde yields—after removal of water—an **aldimine** (not shown). Aldimines are intermediates in amino acid metabolism and serve, among other things, to bond aldehydes to amino groups in proteins (p. 62). The addition of an alcohol to the carbonyl group of an aldehyde yields a **hemiacetal** ($R-O-C(H)(OH)-R$). The cyclic forms of sugars are well-known examples of hemiacetals (see p. 28). The oxidation of hemiacetals produces carboxylic acid esters.

Very important compounds are the **carboxylic acids** and their derivatives, which can be formally obtained by exchanging the $-OH$ group for another group. In fact, derivatives of this type are formed by nucleophilic substitutions of activated intermediate compounds with the

release of water (p. 10). **Carboxylic acid esters** ($R-O-CO-R'$) arise in this way from carboxylic acids and alcohols. This group includes the fats, for example (p. 38). Similarly, a carboxylic acid and a thiol yield a **thioester** ($R-S-CO-R'$). Thioesters play an extremely important role in carboxylic acid metabolism. The best-known compound of this type is acetyl-coenzyme A (p. 8). Carboxylic acids and primary amines react to form **carboxylic acid amides** ($R-NH-CO-R'$). The amino acid constituents of peptides and proteins are linked by carboxylic acid amide bonds, which are therefore also known as **peptide bonds** (p. 56).

Phosphoric acid, H_3PO_4 , is a tribasic (three-protic) acid—i.e., it contains three hydroxyl groups able to donate H^+ ions. At least one of these three groups is fully dissociated under normal physiological conditions, while the other two can react with alcohols. The resulting products are phosphoric acid monoesters ($R-O-P(O)(OH)-O-R'$) and diesters ($R-O-P(O)(OR'')_2$). **Phosphoric acid monoesters** are found in carbohydrate metabolism, for example (p. 28), whereas **phosphate diester** bonds occur in phospholipids (p. 40) and nucleic acids (p. 64). Compounds of one acid with another are referred to as **acid anhydrides**. A particularly large amount of energy is required for the formation of an acid-anhydride bond. Phosphoric anhydride bonds therefore play a central role in the storage and release of chemical energy in the cell (pp. 104, 122). Mixed anhydrides between carboxylic acids and phosphoric acid along with enol phosphates are “*energy-rich metabolites*” in cellular metabolism.

A. Important classes of compounds



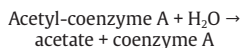
Substance Classes II

Many biomolecules are made up of smaller units in a modular fashion, and they can be broken down into these units again. The assembly of these molecules usually takes place through condensation reactions involving the removal of water. Conversely, their breakdown functions in a hydrolytic fashion—i.e., as a result of water uptake. The page opposite illustrates this modular principle using the example of an important coenzyme.

A. Acetyl CoA

Coenzyme A (p. 88) is a nucleotide with a complex structure. It serves to activate acyl residues (carboxylic acid residues). Bonding of the carboxyl group of the carboxylic acid with the thiol group of the coenzyme creates a **thioester bond** ($-S-CO-R$; see p. 8) in which the acyl residue has a **high chemical potential**. It can therefore be transferred to other molecules in exergonic reactions. This fact plays an important role in lipid metabolism in particular (see pp. 318ff.), as well as in two reactions of the tricarboxylic acid cycle (p. 114).

As discussed on page 18, the **group transfer potential** can be expressed quantitatively as the change in free enthalpy (ΔG) during hydrolytic cleavage of the compound concerned. This is an arbitrary definition, but it provides important indications of the **chemical energy** stored in such a bond. In the case of the acetyl group in acetyl-CoA, the reaction to be considered is:



In standard conditions and at pH7, the change in the chemical potential, G (ΔG° , p. 18), in this reaction amounts to $-32 \text{ kJ} \cdot \text{mol}^{-1}$ and it is therefore as high as the ΔG° of ATP hydrolysis (p. 104). In addition to the “energy-rich” **thioester bond**, acetyl-CoA also has seven other hydrolyzable bonds with different degrees of stability. These bonds, and the fragments that arise when they are hydrolyzed, will be discussed here in sequence.

- (1) The reactive thiol group in the thioester bond of coenzyme A is derived from **cysteamine**. Cysteamine is a *biogenic amine* (p. 52) formed by decarboxylation of the amino acid cysteine.
- (2) The amino group of cysteamine is bound to the carboxyl group of another biogenic

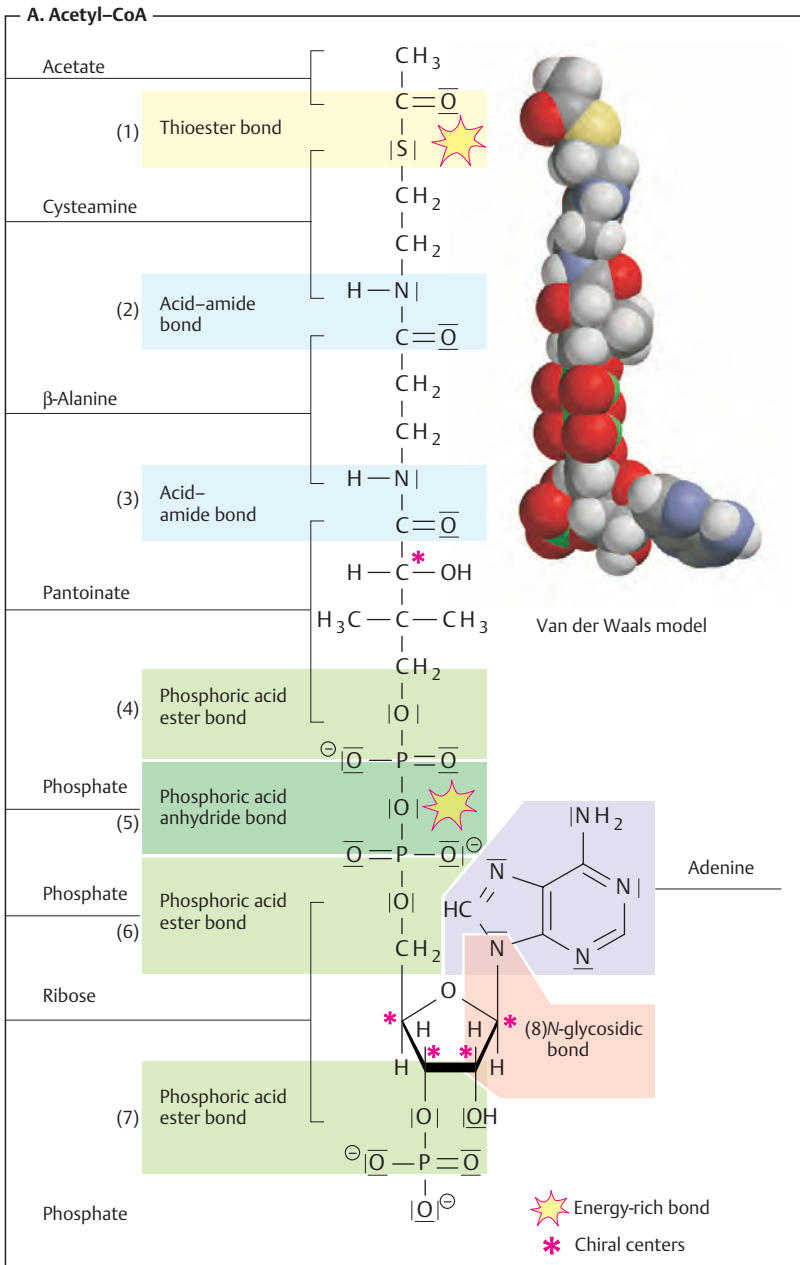
amine via an **acid amide bond** ($-CO-NH-$). **β -Alanine** arises through decarboxylation of the amino acid aspartate, but it can also be formed by breakdown of pyrimidine bases (p. 180).

- (3) Another **acid amide bond** ($-CO-NH-$) creates the connection to the next constituent, **pantoinate**. This compound contains a *chiral center* and can therefore appear in two enantiomeric forms (p. 4). In natural coenzyme A, only one of the two forms is found, the (*R*)-pantoinate. Human metabolism is not capable of producing pantoinate itself, and it therefore has to take up a compound of β -alanine and pantoinate—**pantothenate** (“pantothenic acid”)—in the form of a vitamin in food (p. 394).

- (4) The hydroxyl group at C-4 of pantoinate is bound to a **phosphate** residue by an **ester bond**.

The section of the molecule discussed so far represents a functional unit. In the cell, it is produced from pantothenate. The molecule also occurs in a protein-bound form as **4'-phosphopantetheine** in the enzyme *fatty acid synthase* (p. 150). In coenzyme A, however, it is bound to 3',5'-adenosine diphosphate.

- (5) When two phosphate residues are connected, they do not form an ester, but an “energy-rich” **phosphoric acid anhydride bond**, as also occurs in other nucleoside phosphates. By contrast, (6) and (7) are ester bonds again.
- (8) The base **adenine** is bound to C-1 of **ribose** by an **N-glycosidic bond** (pp. 34, 64). In addition to the carbon atoms C-2 to C-4 of ribose, C-1 also represents a *chiral center* (p. 4).



Chemical Reactions

Chemical reactions are processes in which electrons or groups of atoms are taken up into molecules, exchanged between molecules, or shifted within molecules. Illustrated here are the most important types of reaction in organic chemistry, using simple examples. Electron shifts are indicated by red arrows.

A. Redox reactions

In redox reactions (p. 12), **electrons** are **transferred** from one molecule (the reducing agent) to another (the oxidizing agent). One or two protons are often also transferred in the process, but the decisive criterion for the presence of a redox reaction is the electron transfer. The reducing agent is oxidized during the reaction, and the oxidizing agent is reduced.

Figure A shows the oxidation of an alcohol into an aldehyde and the reduction of the aldehyde to an alcohol. In the process, one *hydride ion* is transferred (two electrons and one proton; see p. 12), which moves from the alcohol to the oxidizing agent A. The surplus proton is bound by a base B that acts as a catalyst. In the reduction of the aldehyde, A-H serves as the reducing agent and the acid H-B is involved as the catalyst.

B. Acid–base reactions

In contrast to redox reactions, protons only (**H⁺ ions**) and not electrons are transferred in acid–base reactions (p. 14). When an acid dissociates (in this case, hydrochloric acid, HCl), water serves as a proton acceptor and is converted to the oxonium (hydronium) ion H₃O⁺. In the reverse reaction, water has the function of an acid in the protonation of the conjugated base Cl[−]. When the base NH₃ (ammonia) reacts with water, a hydroxide ion (OH[−]) is produced together with the ammonium ion (NH₄⁺) as conjugated acid.

C. Additions/eliminations

A reaction in which atoms or molecules are taken up by a multiple bond is described as **addition**. The converse of addition—i.e., the removal of groups with the formation of a double bond, is termed **elimination**.

When water is added to an alkene, a proton is first transferred to the reactant. The unstable *carbenium ion* that occurs as an intermediate initially takes up water, before the release of a

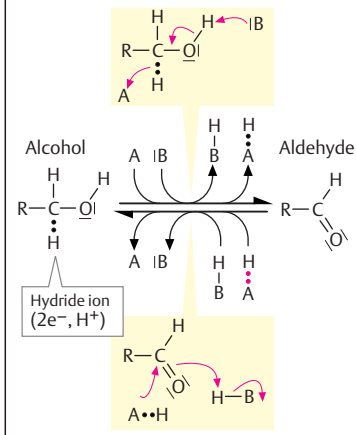
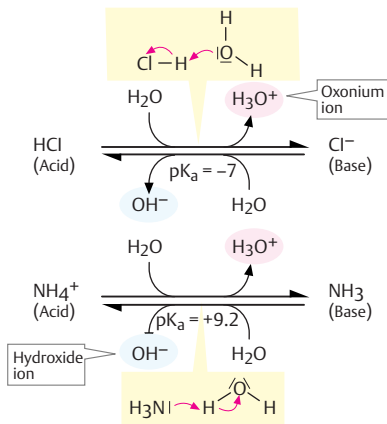
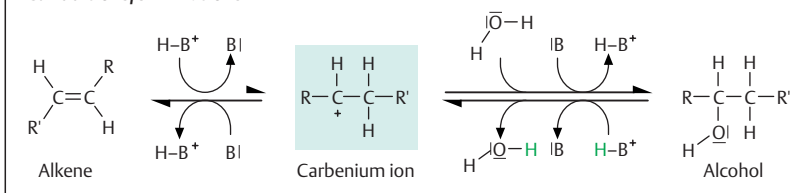
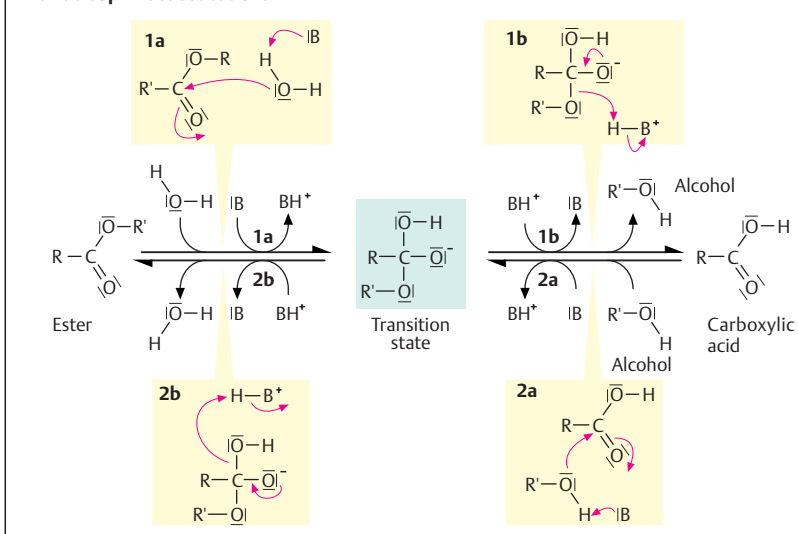
proton produces the alcohol. The reverse reaction, i.e., the elimination of water from the alcohol (*dehydration*) is also catalyzed by an acid and passes via the same intermediate as the addition reaction.

D. Nucleophilic substitutions

A reaction in which one functional group (p. 6) is replaced by another is termed **substitution**. Depending on how the reaction proceeds, a distinction is made between *nucleophilic* and *electrophilic substitution* (see chemistry textbooks). Nucleophilic substitutions start with the addition of one molecule to another, followed by elimination of the *leaving group*.

The hydrolysis of an ester to an alcohol and acid and the esterification of an acid with an alcohol are examples of the S_N2 mechanism. Both reactions are made easier by the marked polarity of the C=O double bond. In the illustrated mechanism of ester hydrolysis, a proton is removed from a water molecule by the catalytic effect of the base B. The resulting strongly nucleophilic OH[−] ion attacks the positively charged carbonyl C of the ester (1a), and an unstable sp³-hybridized *transition state* is produced. From this, either water is eliminated again (2b) and the ester re-forms, or the alcohol ROH is eliminated (1b) and the free acid results. In esterification (2), the same steps take place in reverse.

In **rearrangements** (isomerizations, not shown), groups are shifted within one and the same molecule. Examples of this in biochemistry include the isomerization of sugar phosphates (p. 128) and the rearrangement of methylmalonyl-CoA to succinyl CoA (p. 170).

A. Redox reactions**B. Acid-base reactions****C. Additions/eliminations****D. Nucleophilic substitutions**

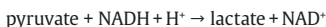
Redox Processes

A. Redox reactions

Redox reactions are chemical changes in which electrons are transferred from one reaction partner to another (p. 10). Like acid–base reactions, redox reactions always involve *pairs* of compounds. A pair of this type is referred to as a **redox system**. The essential difference between the two components of a redox system (A_{ox} and A_{red}) is the number of electrons they contain. The more electron-rich component (A_{red}) is called the **reduced form** of the compound concerned, while the other one is referred to as the **oxidized form** (A_{ox}).

The **redox potential**, E , of a system is a measure of its electron transfer potential, that is, of its capacity to donate or accept electrons. The Nernst equation links the potential, E , of the system with the normal potential, E^0 , which is independent of concentration, and with the concentrations of the two components. The redox potential, measured in volts, V , has a sign; it can be more negative or more positive than a reference potential arbitrarily set at zero.

In **redox reactions**, the reduced form of one system (the **reducing agent**, B_{red}) donates electrons to the oxidized form of another one (the **oxidizing agent**, A_{ox}). In the process, the reducing agent becomes oxidized and the oxidizing agent is reduced. Any given reducing agent can reduce only certain other redox systems. Potential differences between two redox systems can be determined by **galvanic cells**. This is shown below using the reaction



as an example. Under normal conditions, the $\text{NAD}^+/\text{NADH} + \text{H}^+$ system has a more negative potential. Therefore, the reverse reaction ($\text{lactate} + \text{NAD}^+ \rightarrow \text{pyruvate} + \text{NADH} + \text{H}^+$) is not possible in this case. On the basis of their redox potentials, redox systems can be arranged to form what are known as **redox series**. Spontaneous electron transfers are only possible if the redox potential of the donor is *more negative* than that of the acceptor (see, e.g., the respiratory chain, p. 120).

B. Normal potentials

The table shows the normal potentials of biologically important redox systems. Under standard conditions (all concentrations, including that of H_2O , are $1 \text{ mol} \cdot \text{L}^{-1}$), the system $[2 \text{H}^+$

$\text{H}_2]$ is arbitrarily assigned the normal potential $E^0 = 0 \text{ V}$. In biochemistry, the corresponding potentials at $\text{pH} = 7$ (E^0') are generally used.

C. Biological redox systems

In the cell, most redox reactions are catalyzed by enzymes, which work together with soluble or bound redox cofactors.

Some of these factors contain **metal ions** as redox-active components. In these cases, it is usually single electrons (e^-) that are transferred, so that the metal ion changes its valency. Unpaired electrons often occur in this process, but these are located in d orbitals (p. 2) and are therefore less dangerous than single electrons in non-metal atoms ("free radicals"; see below).

Other redox systems consist of **disulfides** (R-S-S-R) and the corresponding **thiols** (R-SH). $2e^-$ and 2H^+ are needed to reduce a disulfide. This occurs in two separate steps, with a highly reactive thiol radical occurring as an intermediate. In the cell, there are special protective systems that render free radicals harmless (p. 288).

In the complete reduction of the **flavins** FMN and FAD (p. 86), $2e^-$ and 2H^+ are required, with a *semiquinone radical* appearing as an intermediate.

In the reduction or oxidation of **quinone/quinol systems**, free radicals also appear as intermediate steps, but these are less reactive than flavin radicals.

The **pyridine nucleotides** NAD^+ and NADP^+ (p. 86) always function in soluble form. The oxidized coenzymes contain an aromatic nicotinamide ring in which the positive charge is delocalized. The right-hand *resonance structure* shown contains an electron-poor, positively charged C atom at the *para* position to nitrogen. If a **hydride ion** (H^-) is added at this point, the reduced forms NADH or NADPH arise. No radical intermediate steps occur. Since a H^+ ion is released at the same time, the correct expression for reduced pyridine nucleotides is $\text{NAD(P)H} + \text{H}^+$ and not NAD(P)H_2 .

A. Redox reactions

For a redox system

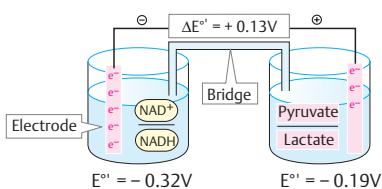


$$E = E^\circ + \frac{R \cdot T}{n \cdot F} \cdot \ln \frac{[A_{ox}]}{[A_{red}]}$$

For a redox reaction



$$\Delta E = E_{\text{Acceptor}} - E_{\text{Donor}} \quad \Delta G = -n \cdot F \cdot \Delta E$$

**B. Normal potentials**

Redox system	n	E° [V] pH 0	E°' [V] pH 7
Ferredoxin Fe ³⁺ /Fe ²⁺	1	-0.43	-0.43
H ⁺ /1/2 H ₂	1	0	-0.41
NAD(P) ⁺ /NAD(P)H+H ⁺	2	+0.09	-0.32
Liponamide _{ox} +2 H ⁺ /Liponamide _{red}	2	+0.21	-0.23
Pyruvate+2 H ⁺ /lactate	2	+0.24	-0.19
FAD(FMN)/FADH ₂ (FMNH ₂)	2	+0.22	-0.13*
GSSG/ 2 GSH+2 H ⁺	2	+0.31	-0.10
Fumarate/succinate+2 H ⁺	2	+0.38	-0.03
Ubiquinone+2H ⁺ /ubiquinol	2	+0.51	+0.13
Cyt c (Fe ³⁺)/Cyt c (Fe ²⁺)	1	+0.24	+0.24
1/2 O ₂ +2 H ⁺ /H ₂ O	2	+1.23	+0.82

GSH – glutathione; GSSH – glutathione disulfide

* depending on protein environment

C. Biological redox systems

1e ⁻	Metal complexes	Oxidized $\xrightarrow{e^-}$ Reduced
1e ⁻ 1H ⁺	Disulfide/ 2 thiols	Disulfide $\xrightarrow{e^-, H^+}$ Thiol $\xrightarrow{e^-, H^+}$ Thiol radical $\xrightarrow{e^-, H^+}$ 2 thiols
	Flavin	Oxidized flavin $\xrightarrow{e^-, H^+}$ Semiquinone radical $\xrightarrow{e^-, H^+}$ Reduced flavin
	Quinone/ hydro-quinone	p-benzoquinone $\xrightarrow{e^-, H^+}$ Semiquinone radical $\xrightarrow{e^-, H^+}$ Hydroquinone
1H ⁻	NAD(P) ⁺	NAD(P) ⁺ (resonance structures) $\xrightarrow{\text{Hydride ion}}$ NAD(P)H + H ⁺

Acids and Bases

A. Acids and bases: definition

According to Brønsted's definition, **acids** are substances that can donate hydrogen ions (protons, H^+ ions), while **bases** are compounds that accept protons.

Water enhances the acidic or basic properties of dissolved substances, as water itself can act as either an acid or a base. In aqueous solution, the acid HA donates a proton to the solvent. This results in the formation of the acid anion A^- and protonated water molecules (**oxonium ions**, H_3O^+ , usually simply referred to as H^+). Bases, on the other hand, accept H^+ ions from water molecules. As a result of this, **hydroxide ions** (OH^-) and protonated bases form (not shown, see p. 10).

If the law of mass action is applied to an acid–base reaction, the **acidity constant** K_a arises as equilibrium constant. The **Henderson–Hasselbalch equation** is obtained when the negative logarithms to base ten, pH and pK_a , are used to express the concentration of H^+ and the K_a respectively. This equation describes the dissociation state of the acid depending on its pH. In the form of a graph, this gives the **dissociation curve** of the acid (bottom). The pK_a of the system corresponds to the pH at the point of inflection of the curve.

B. Acid–base pairs

Acid–base reactions always involve *pairs* consisting of an **acid** and the associated **conjugated base**. The pK_a of an acid is a measure of its strength. The lower the pK_a , the *stronger* the acid. Strong acids are conjugated with weak bases and vice versa. For example, the very strongly acidic hydrogen chloride belongs to the very weakly basic chloride ion, while the very weakly acidic H_2O is conjugated with the very strong base OH^- .

C. The pH scale

The pK_a of water is 15.7 (**B**), so that the product $[H^+] \cdot [OH^-]$ —the **ion product** of water—has a constant value of $1 \cdot 10^{-14} \text{ mol} \cdot \text{L}^{-1}$ even when additional acids or bases are dissolved in the water. At 25°C , pure water contains H^+ and OH^- at concentrations of $1 \cdot 10^{-7} \text{ mol} \cdot \text{L}^{-1}$ each; it is **neutral** and has a pH of 7. Aqueous solutions with a higher H^+ concentration ($0 < \text{pH} < 7$) are acidic, and those with a lower H^+ concentration ($7 < \text{pH} < 14$) are **alkaline**.

D. pH values in the organism

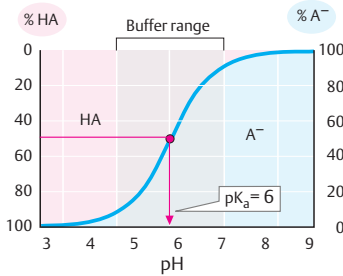
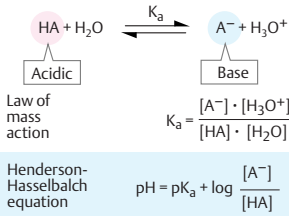
pH values in the cell and in the extracellular fluid are kept constant within narrow limits. In the blood, the pH value normally ranges only between 7.35 and 7.45 (p. 292). This corresponds to a maximum change in the H^+ concentration of ca. 30%. The pH value of cytoplasm is slightly lower than that of blood, at 7.0–7.3. In lysosomes (pH 4.5–5.5; p. 224), the H^+ concentration is several hundred times higher than in the cytoplasm. Extreme values are found in the stomach (ca. pH 2) and in the small intestine (pH > 8). Since the kidney can excrete either acids or bases (p. 336), the pH of urine has a particularly wide range of variation (pH 4.8–7.5).

E. Buffers

Short-term pH changes in the organism are cushioned by **buffer systems** (p. 292). These are mixtures of a weak acid, HA, with its conjugate base, A^- , or of a weak base with its conjugate acid. This type of system can neutralize both oxonium ions and hydroxide ions.

In the first case (left), the base (A^-) binds a large proportion of the added protons and HA and water are formed. If hydroxide ions (OH^-) are added, they react with HA to give A^- and water (right). In both cases, it is primarily the $[HA]/[A^-]$ ratio that shifts, while the pH value only changes slightly. The **dissociation curve** (top) shows that buffer systems are most effective at the pH values that correspond to the pK_a value of the acid. This is where the curve is at its steepest, so that the pH change, ΔpH , is at its smallest with a given increase Δc in $[H^+]$ or $[OH^-]$. In other words, the **buffer capacity** $\Delta c / \Delta\text{pH}$ of the system is highest in the region of the pK_a value.

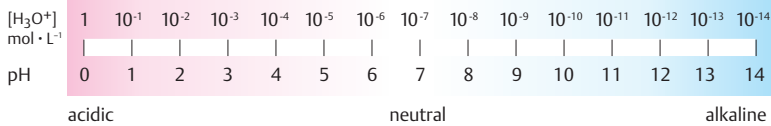
A. Acids and bases: definition



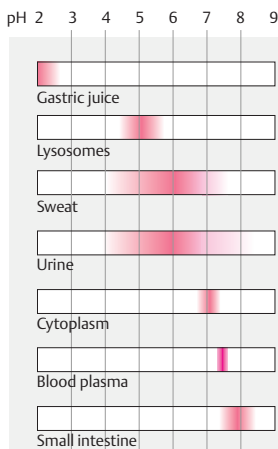
B. Acid-base pairs

Acid	Conjugated base	K_a (mol · L ⁻¹)	$\text{p}K_a$
HCl	Cl ⁻	$9 \cdot 10^6$	-7
H ₃ PO ₄	H ₂ PO ₄ ⁻	$7 \cdot 10^{-3}$	2.2
CH ₃ COOH	CH ₃ COO ⁻	$1.7 \cdot 10^{-5}$	4.8
H ₂ CO ₃	HCO ₃ ⁻	$4.3 \cdot 10^{-7}$	6.4
H ₂ PO ₄ ⁻	HPO ₄ ²⁻	$6.3 \cdot 10^{-8}$	7.2
NH ₄ ⁺	NH ₃	$5.6 \cdot 10^{-10}$	9.2
HCO ₃ ⁻	CO ₃ ²⁻	$5.6 \cdot 10^{-11}$	10.2
HPO ₄ ²⁻	PO ₄ ³⁻	$2.2 \cdot 10^{-13}$	12.7
H ₂ O	OH ⁻	$2.0 \cdot 10^{-16}$	15.7

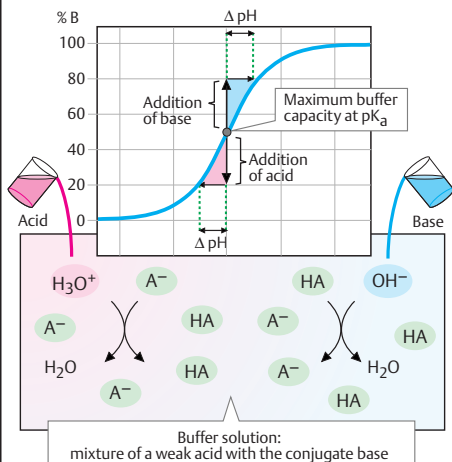
C. The pH scale



D. pH values in the organism



E. Buffers



Energetics

To obtain a better understanding of the processes involved in energy storage and conversion in living cells, it may be useful first to recall the physical basis underlying these processes.

A. Work and energy

There is essentially no difference between work and energy. Both are measured in **joule** units ($J = N \cdot m$). An outdated unit is the **calorie** ($1 \text{ cal} = 4.187 \text{ J}$). **Energy** is defined as the ability of a system to perform work.

A system performs work when matter moves along a potential gradient. This definition can be illustrated by an example involving mechanical work (1). Due to the earth's gravitational pull, the mechanical potential energy of an object is the greater the further the object is away from the center of the earth. A **potential difference** (ΔP) therefore exists between a higher location and a lower one. In a waterfall, the water spontaneously follows this potential gradient and, in doing so, is able to perform work—e.g., turning a mill.

Work and energy consist of two quantities: an **intensity** factor, which is a measure of the potential difference—i.e., the “driving force” of the process—(here it is the height difference) and a **capacity** factor, which is a measure of the quantity of the substance being transported (here it is the weight of the water). In the case of electrical work (2), the intensity factor is the voltage—i.e., the electrical potential difference between the source of the electrical current and the “ground,” while the capacity factor is the amount of charge that is flowing.

Chemical work (3) and chemical energy are defined in an analogous way. The intensity factor here is the **chemical potential** of a molecule or combination of molecules. This is termed **free enthalpy**, **G**. When molecules spontaneously react with one another, the result is products at lower potential. The difference in the chemical potentials of the reactants and products (the **change in free enthalpy**, ΔG) is a measure of the “driving force” of the reaction. The capacity factor in chemical work is the amount of matter reacting (in mol).

B. Conversion and conservation of energy

Energy can occur in different forms—e.g., as mechanical, electric, chemical, or radiation energy. Energy cannot be created or destroyed, but it is possible to convert different forms of energy into one another. In **photosynthesis**, for instance, light energy is converted into chemical energy. Conversely, a chemical reaction is utilized in the light organs of various animals to produce light. In **muscle** (p. 340), chemical energy is converted to mechanical work and heat, while chemical energy is converted to electrical energy when **electrochemical gradients** (p. 108) are produced.

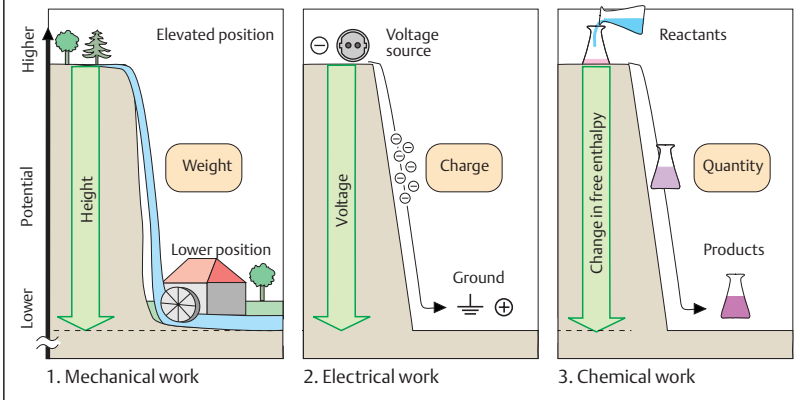
C. Energetics and the course of processes

Everyday experience shows that water never flows uphill *spontaneously*. Whether a particular process can occur spontaneously or not depends on whether the potential difference between the final and the initial state, $\Delta P = P_2 - P_1$, is positive or negative. If P_2 is smaller than P_1 , then ΔP will be negative, and the process will take place and perform work. Processes of this type are called **exergonic** (1). If there is no potential difference, then the system is in **equilibrium** (2). In the case of **endergonic** processes, ΔP is positive (3). Processes of this type do *not* proceed spontaneously.

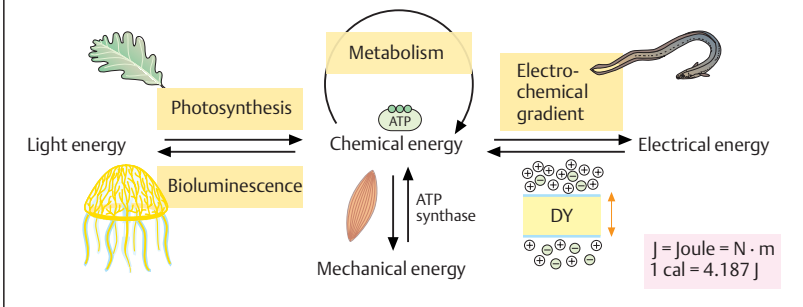
Forcing endergonic processes to take place requires the use of the principle of **energetic coupling**. When two masses M_1 and M_2 are connected by a rope, M_1 moves upward even though this part of the process is endergonic. The *sum* of the two potential differences ($\Delta P_{\text{eff}} = \Delta P_1 + \Delta P_2$) is the determining factor in coupled processes. When ΔP_{eff} is negative, the entire process can proceed.

A form of storage for chemical energy that is used in all forms of life is **adenosine triphosphate** (ATP; p. 104). Endergonic processes are usually driven by coupling to the strongly exergonic breakdown of ATP (p. 106).

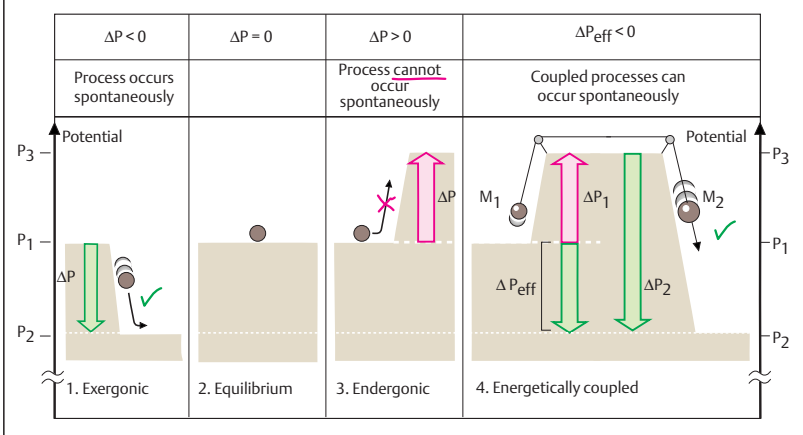
A. Work and energy



B. Conversion and conservation of energy



C. Energetics and the course of processes



Thermodynamics

A. ΔG and chemical equilibrium

Every chemical reaction reaches a **state of equilibrium** after a time, in which the forward and back reactions proceed at the same speed. The **law of mass action** describes the concentrations of the reactants (A, B) and products (C, D) *at equilibrium*. The **equilibrium constant K** is directly related to ΔG^0 , the change in free enthalpy, G , involved in the reaction (p. 16) under standard conditions ($\Delta G^0 = -R \cdot T \cdot \ln K$). For any given concentrations, the lowermost equation applies. At $\Delta G < 0$, the reaction proceeds spontaneously for as long as it takes for equilibrium to be reached (i.e., until $\Delta G = 0$). At $\Delta G > 0$, a *spontaneous* reaction is no longer possible (endergonic case; p. 16). In biochemistry, ΔG is usually related to pH 7, and this is indicated by the “prime” symbol ($\Delta G''$ or $\Delta G'$).

As examples, we can look at two different group transfer reactions (on the right). In ATP (p. 104), the terminal phosphate residue is at a high chemical potential. Its transfer to water (reaction **a**, below) is therefore strongly **exergonic**. Equilibrium ($\Delta G = 0$) is only reached when more than 99.5% of the originally available ATP has been hydrolyzed. ATP and similar compounds have a high **group transfer potential** for phosphate residues. Quantitatively, this is expressed as the **ΔG of hydrolysis** ($\Delta G'' = -32 \text{ kJ} \cdot \text{mol}^{-1}$).

In contrast, the **endergonic** transfer of ammonium (NH_4^+) to glutamate (Glu, reaction **b**, above, $\Delta G'' = +14 \text{ kJ} \cdot \text{mol}^{-1}$) reaches equilibrium after only 4% turnover so that only minimal amounts of the product glutamine (Gln) can be formed in this way. The efficient synthesis of glutamine from Glu and NH_4^+ is only possible through **energetic coupling** (pp. 16, 106).

B. Gibbs–Helmholtz equation

The change in free enthalpy, ΔG , depends on three factors, and the **Gibbs–Helmholtz equation** describes the connection between them. First of all, the heat of reaction is important, expressed as the **change in enthalpy, ΔH** . If heat is released during the reaction (exothermic), ΔH is < 0 , and if heat is consumed (endothermic), $\Delta H > 0$.

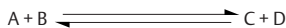
Endothermic reactions can also occur spontaneously because the **change in entropy, ΔS** , as well as the **temperature, T** , play a part, too. Entropy is a physical parameter that describes the **degree of order of a system**. The greater

the entropy, the lower the degree of order. Thus, when a process leads to an increase in disorder—and everyday experience shows that this is the normal state of affairs— ΔS is positive for this process. An increase in the order in a system ($\Delta S < 0$) always requires an input of energy. Both of these statements are consequences of an important natural law, the Second Law of Thermodynamics.

C Enthalpy and entropy-driven processes

In the **knall-gas (oxyhydrogen) reaction (1)**, gaseous oxygen and gaseous hydrogen react to form liquid water. Like many redox reactions, this reaction is strongly exothermic (i.e., $\Delta H < 0$). However, during the reaction, the degree of order increases. The total number of molecules is reduced by one-third, and a more highly ordered liquid is formed from freely moving gas molecules. As a result of the increase in the degree of order ($\Delta S < 0$), the term $-T \cdot \Delta S$ becomes positive. However, this is more than compensated for by the high heat of reaction, and the reaction is strongly exergonic ($\Delta G < 0$).

The **dissolution of salt in water (2)** is endothermic ($\Delta H > 0$)—i.e., the container and liquid cool. Nevertheless, the process still occurs spontaneously, since the degree of order in the system *decreases*. The Na^+ and Cl^- ions are initially fixed in a crystal lattice. In solution, they move about independently and in random directions through the fluid. The decrease in order ($\Delta S > 0$) leads to a negative term $-T \cdot \Delta S$, which compensates for the positive ΔH and results in a negative ΔG overall. The formation of ordered lipid structures in water (p. 24) and the folding of proteins (p. 60) are also mainly entropy-driven.

A. ΔG and chemical equilibrium

In chemical equilibrium:

Law of mass action

$$K = \frac{[C] \cdot [D]}{[A] \cdot [B]}$$

Relationship between K and ΔG° under standard conditions:

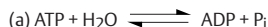
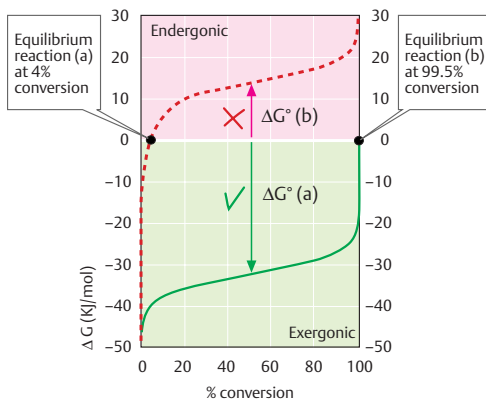
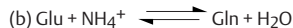
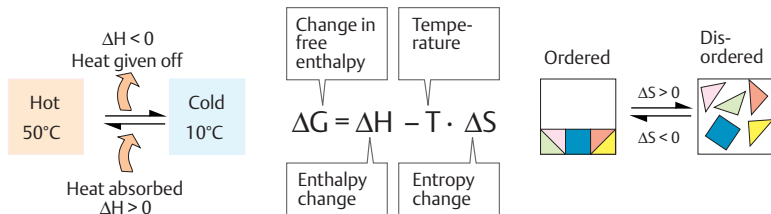
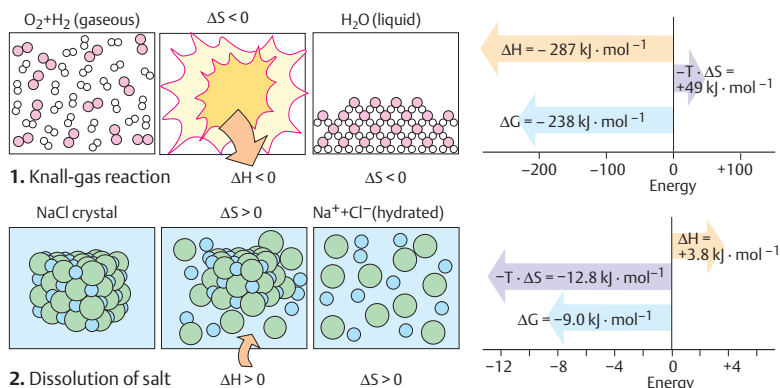
$$\Delta G^\circ = -R \cdot T \cdot \ln K$$

For any reactant concentration:

$$\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \frac{[C] \cdot [D]}{[A] \cdot [B]}$$

R = general gas constant ($8314 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$);

T = absolute temperature (in K)

**B. Gibbs–Helmholtz equation****C. Enthalpy and entropy-driven processes**

Catalysis

Catalysts are substances that accelerate chemical reactions without themselves being consumed in the process. In the cell, **enzymes** generally serve as catalysts. A few chemical changes are catalyzed by special RNA molecules, known as *ribozymes* (p. 72).

A. Activation energy

Most organic chemical reactions take place very slowly in solution, regardless of their ΔG value. This is because the reactants have to have a certain minimum energy before they can react at all. This can be shown by an energy diagram (1); the simplest reaction $A \rightarrow B$ was chosen. Reactant A and product B are each located at a certain **chemical potential** (G_a and G_b respectively). The reaction enthalpy ΔG is the difference between these potentials. To be converted to B, A must first overcome a potential barrier, whose peak G_a is far higher than G_e . The potential difference $G_a - G_e$ is the **activation energy**, E_a , of the reaction (in $\text{kJ} \cdot \text{mol}^{-1}$). The fact that A can be converted to B at all is because individual molecules attain a much higher potential occasionally, for instance, by colliding with other molecules. If the increase in energy is greater than E_a , these molecules can be converted to B.

The energy distribution calculated for such an assembly of molecules is shown in (2) and (3). $\Delta n/n$ is the fraction of the molecules that reach or exceed energy E; for example, at 27°C ca. 10% of the molecules have energies over $6 \text{ kJ} \cdot \text{mol}^{-1}$. The course of the energy function at energies around $50 \text{ kJ} \cdot \text{mol}^{-1}$ is shown in (3). Statistically, only 2 out of 10^9 molecules attain this energy at 27°C , while four molecules do so at 37°C (3).

B. Catalysis: principle

A catalyst creates a new pathway for the reaction. When the transition states arising have lower activation energies than that of the uncatalyzed reaction, the reaction will proceed more rapidly along the alternative pathway (2), even when the number of intermediates is greater. Since the reactants and products are the same in both routes, the ΔG of the reaction is not influenced by the catalyst. Catalysts—including enzymes—are in principle *not* capable of altering the equilibrium state of the catalyzed reaction.

The often-encountered statement that “a catalyst reduces the activation energy of a reaction” is incorrect, strictly speaking, since a *completely different* reaction takes place in the presence of a catalyst than in its absence.

C. Catalysis of H_2O_2 —breakdown by iodide

As a simple example of a catalyzed reaction, we can look at the disproportionation of hydrogen peroxide (H_2O_2) into oxygen and water. In the uncatalyzed reaction (1a, 2a), an H_2O_2 molecule initially decays into H_2O and atomic oxygen [O], which reacts with a second H_2O_2 molecule to form water and molecular oxygen (O_2). The activation energy E_a required for this reaction is relatively high, at $75 \text{ kJ} \cdot \text{mol}^{-1}$.

In the presence of **iodide** (I^-) as a catalyst, the intermediate that arises instead of [O] is hypoiodide (OI^-), which also forms H_2O and O_2 with another H_2O_2 molecule (1b, 2b). In this step, the I^- ion is released and can once again take part in the reaction. The lower activation energy of the reaction catalyzed by iodide ($E_a = 56 \text{ kJ} \cdot \text{mol}^{-1}$) causes acceleration of the reaction by a factor of 2100, as the reaction rate depends exponentially on E_a ($v \sim e^{-E_a/R \cdot T}$).

Catalase, an enzyme that protects cells against the toxic effects of hydrogen peroxide (p. 288), is much more catalytically effective. The activation energy of the enzyme-catalyzed reaction is only $23 \text{ kJ} \cdot \text{mol}^{-1}$, which in comparison with the uncatalyzed reaction leads to acceleration by a factor of 1.3×10^9 .

Catalase is one of the most efficient enzymes that exist. A single molecule can convert up to 10^8 (a hundred million) H_2O_2 molecules per second.

Water as a Solvent

Life as we know it evolved in water and is still absolutely dependent on it. The properties of water are therefore of fundamental importance to all living things.

A. Water and methane

The special properties of **water** (H_2O) become apparent when it is compared to **methane** (CH_4). The two molecules have a similar mass and size. Nevertheless, the boiling point of water ($+100^\circ\text{C}$) is far higher than that of methane (-162°C). At temperatures on the earth's surface, water therefore is liquid, whereas methane is gaseous. The high boiling point of water results from its high vaporization enthalpy, which in turn is due to the fact that the density of the electrons within the H_2O molecule is unevenly distributed. Two corners of the tetrahedrally shaped water molecule are occupied by unshared electrons (green), and the other two by hydrogen atoms. As a result, the $\text{H}-\text{O}-\text{H}$ molecule is v-shaped. In addition, the $\text{O}-\text{H}$ bonds are polarized due to the high electronegativity of oxygen. One side of the molecule carries a partial charge (δ) of about -0.4 units, whereas the other is correspondingly positively charged. The spatial separation of the positive and negative charges gives the molecule the properties of an **electrical dipole**. Water molecules are therefore attracted to one another like tiny magnets. When liquid water vaporizes, a large amount of energy has to be expended to disrupt these interactions. By contrast, methane molecules are not dipolar, and therefore interact with one another only weakly. This is why liquid methane vaporizes at very low temperatures.

B. Hydrogen bonds

In addition to the dipolar interactions, hydrogen bonds also make a major contribution to the cohesion between water molecules. These are noncovalent bonds that occur in proteins and DNA as well as in water (p. 56ff., p. 68). Hydrogen bonds can be regarded as the result of an incomplete acid–base reaction: protons of OH , NH or SH groups (hydrogen bond donors) interact with free electron pairs (especially O , N or S) without leaving the donor atom. The binding energies of H bonds are $10\text{--}40\text{ kJ}\cdot\text{mol}^{-1}$ and thus much lower than those of covalent bonds ($\sim 400\text{ kJ}\cdot\text{mol}^{-1}$); however, as there are large numbers of H bonds in water and macro-

molecules, they make an important contribution to the stability of such molecules.

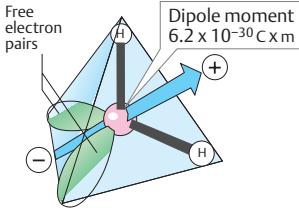
C. Structure of water and ice

In liquid water, the molecules are in a state of constant fluctuation. Tetrahedral networks of molecules, known as water “clusters,” often arise. As the temperature decreases, the proportion of water clusters increases until the water begins to crystallize at 0°C . In **ice**, most of the water molecules are fixed in a **hexagonal lattice** (on the right). Since the distance between the individual molecules in the frozen state is on average greater than in the liquid state, the density of ice is lower than that of liquid water.

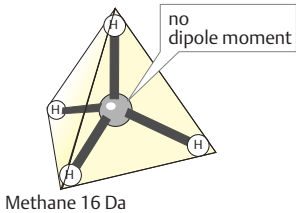
D. Hydration

In contrast to other liquids, water is an excellent **solvent for ions**. In the electrical field of cations and anions, the dipolar water molecules arrange themselves in a regular fashion corresponding to the charge of the ion. They form **hydration shells** and shield the central ion from oppositely charged ions. Metal ions are therefore often present as hexahydrates ($[\text{Me}(\text{H}_2\text{O})_6]^{2+}$, on the right). In the inner hydration sphere of this type of ion, the water molecules are practically immobilized and follow the central ion. Water has a high dielectric constant of 78—i.e., the electrostatic attraction force between ions is reduced to $1/78$ by the solvent. Electrically charged groups in organic molecules (e.g., carboxylate, phosphate, and ammonium groups) are also well hydrated and contribute to water solubility. Neutral molecules with several hydroxyl groups, such as glycerol (on the left) or sugars, are also easily soluble, because they can form H bonds with water molecules. Such molecules are called **hydrophilic** (“water-loving”).

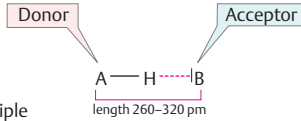
A. Water and methane



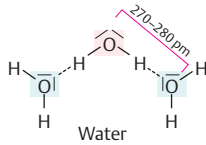
Water 18 Da



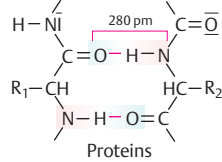
B. Hydrogen bonds



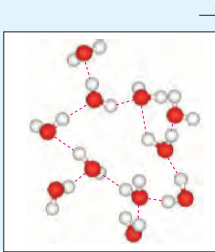
1. Principle



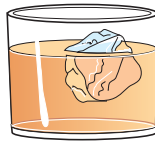
2. Examples



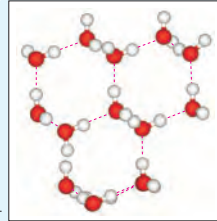
C. Structure of water and ice



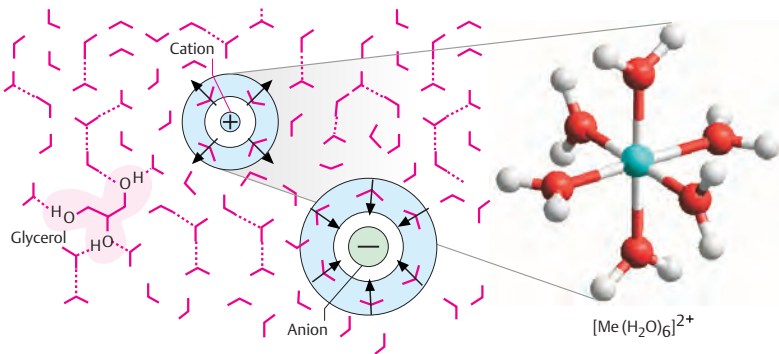
Liquid water
Density $1.00 \text{ g} \cdot \text{cm}^{-3}$
Short-lived "clusters"



Ice
Density $0.92 \text{ g} \cdot \text{cm}^{-3}$
Hexagonal lattice



D. Hydration



Hydrophobic Interactions

Water is an excellent solvent for ions and for substances that contain polarized bonds (p. 22). Substances of this type are referred to as **polar** or **hydrophilic** ("water-loving"). In contrast, substances that consist mainly of hydrocarbon structures dissolve only poorly in water. Substances of this type are said to be **nonpolar** or **hydrophobic** ("water-fearing").

A. Solubility of methane

To understand the reasons for the hydrophobic properties of hydrocarbons, it is useful first to examine the energetics (see p. 16) of the dissolution process involved. In the upper part of the figure, the terms of the Gibbs-Helmholtz equation (p. 18) for the simplest hydrocarbon, **methane**, are shown. As can be seen, the transition from gaseous methane to water is exothermic ($\Delta H^\circ < 0$). Nevertheless, the change in the free enthalpy, ΔG° , is positive (the process is endergonic), because the entropy term $T \cdot \Delta S^\circ$ has a strongly positive value. The entropy change in the process (ΔS°) is evidently negative—i. e., a solution of methane in water has a *higher* degree of order than either water or gaseous methane. One reason for this is that the methane molecules are less mobile when surrounded by water. More importantly, however, the water around the nonpolar molecules forms cagelike "**clathrate**" structures, which—as in ice—are stabilized by H bonds. This strongly increases the degree of order in the water—and the more so the larger the area of surface contact between the water and the nonpolar phase.

B. The hydrophobic effect

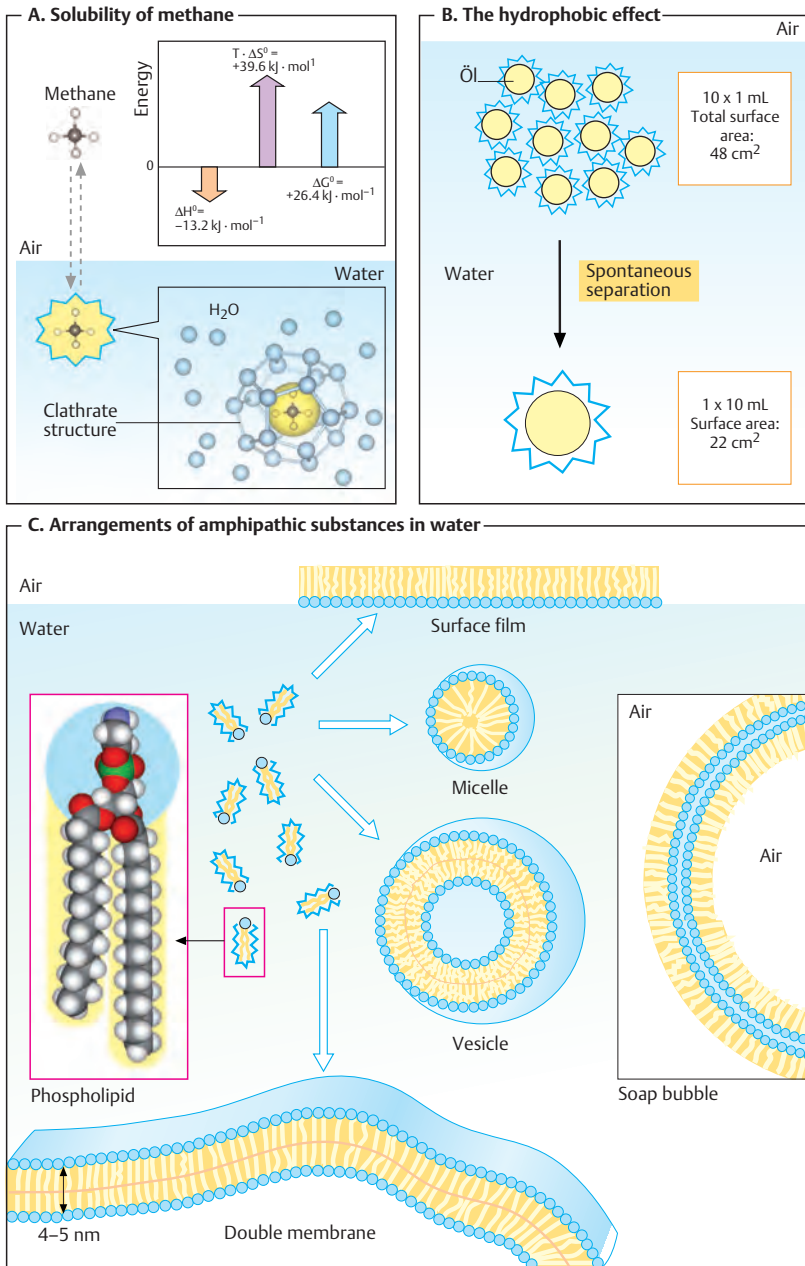
The spontaneous separation of oil and water, a familiar observation in everyday life, is due to the energetically unfavorable formation of clathrate structures. When a mixture of water and oil is firmly shaken, lots of tiny oil drops form to begin with, but these quickly coalesce spontaneously to form larger drops—the two phases separate. A larger drop has a smaller surface area than several small drops with the same volume. Separation therefore reduces the area of surface contact between the water and the oil, and consequently also the extent of clathrate formation. The ΔS for this process is therefore positive (the *disorder* in the water increases), and the negative term $-T \cdot \Delta S$ makes

the separation process exergonic ($\Delta G < 0$), so that it proceeds spontaneously.

C. Arrangements of amphipathic substances in water

Molecules that contain both polar *and* nonpolar groups are called **amphipathic** or amphiphilic. This group includes soaps (p. 38), phospholipids (p. 40), and bile acids (p. 320).

As a result of the "hydrophobic effect" amphipathic substances in water tend to arrange themselves in such a way as to minimize the area of surface contact between the nonpolar regions of the molecule and water. On water surfaces, they usually form single-layer **films** (top) in which the polar "head groups" face toward the water. **Soap bubbles** (right) consist of double films, with a thin layer of water enclosed between them. In water, depending on their concentration, amphipathic compounds form **micelles**—i.e., spherical aggregates with their head groups facing toward the outside, or extended bilayered **double membranes**. Most biological membranes are assembled according to this principle (p. 208). Closed hollow membrane sacs are known as **vesicles**. This type of structure serves to transport substances within cells and in the blood (p. 214). The separation of oil and water can be prevented by adding a strongly amphipathic substance. During shaking, a more or less stable **emulsion** then forms, in which the surface of the oil drops is occupied by amphipathic molecules that provide it with polar properties externally (not shown). The emulsification of fats in food by bile acids and phospholipids is a vital precondition for the digestion of fats (p. 274).



Biomolecules

Chemistry of Sugars

The **carbohydrates** are a group of naturally occurring carbonyl compounds (aldehydes or ketones) that also contain several hydroxyl groups. This group includes **single sugars** (**monosaccharides**) and their polymers, the **oligosaccharides** and **polysaccharides**.

A. Monosaccharides: structure

The most important natural monosaccharide, **D-glucose**, is an aliphatic aldehyde with six C atoms, five of which carry a hydroxyl group (1). Since C atoms 2 to 5 represent chiral centers (p. 4), there are 15 further isomeric **aldohexoses** in addition to D-glucose, although only a few of these are important in nature (p. 30). Most natural monosaccharides have the same configuration at C-5 as D-glyceraldehyde—they belong to the **D-series**.

The open-chained form of glucose shown in 1 is found in neutral solution in less than 0.1% of the molecules. The reason for this is an intramolecular reaction in which one of the OH groups of the sugar is added to the aldehyde group of the *same* molecule (2). This gives rise to a cyclic **hemiacetal** (p. 6). In aldohexoses, the hydroxyl group at C-5 reacts preferentially, and a six-membered pyran ring is formed. Sugars that contain this ring are called **pyranoses**. By contrast, if the OH group at C-4 reacts, a five-membered **furan ring** is formed.

The **Haworth projection** (2) is usually used to depict monosaccharides in the cyclic form, with the ring being shown in perspective as viewed from above. Depending on the configuration, the substituents of the chiral C atoms are then found above or below this level. OH groups that lie on the *right* in the Fischer projection (1) appear *under* the ring level in the Haworth projection, while those on the *left* appear *above* it.

The pyran ring is not planar, but usually has a *chair conformation*. In 3, two frequent conformations of D-glucopyranose are shown. In the $^1C_4$ conformation (bottom), most of the OH groups appear vertical to the ring level, as in the Haworth projection (**axial** or **a** position). In the slightly more stable $^4C_1$ conformation (top), the OH groups take the **equatorial** or **e** position.

B. Reactions of the monosaccharides

The sugars (monosaccharides) occur in many forms (derivatives) in metabolism. Only a few important conversion reactions are discussed here, using **D-glucose** as an example.

1. Mutarotation. In the cyclic form, as opposed to the open-chain form, aldoses have an additional chiral center at C-1 (see A, center). The corresponding isomeric forms are called **anomers**. In the β -anomer (center left), the OH group at C-1 (the anomeric OH group) and the CH₂OH group (C-6) lie on the *same* side of the ring. In the α -anomer (center right), they are on different sides. The reaction that interconverts anomers into each other is known as **mutarotation**.

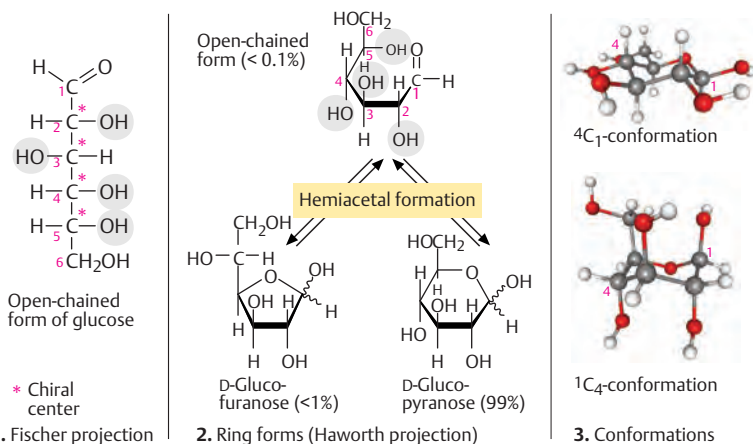
2. Oxidation and reduction. Reduction of the anomeric center at C-1 produces the sugar alcohol **sorbitol**. Oxidation of the aldehyde group at C-1 gives gluconolactone, the intramolecular ester (lactone) of **gluconic acid**. When glucose is oxidized at C-6, the highly polar **glucuronic acid** is formed. It plays an important role in biotransformations in the liver (p. 322).

3. Glycoside formation. When the anomeric OH group of a sugar reacts with an alcohol, with elimination of water, it yields an *O-glycoside* (in the case shown, α -methylglucoside). The glycosidic bond is not a normal ether bond, because the OH group at C-1 has a hemiacetal quality. Oligosaccharides and polysaccharides also contain *O-glycosidic* bonds. Reaction of the anomeric OH group with an NH₂ or NH group yields an *N-glycoside* (not shown).

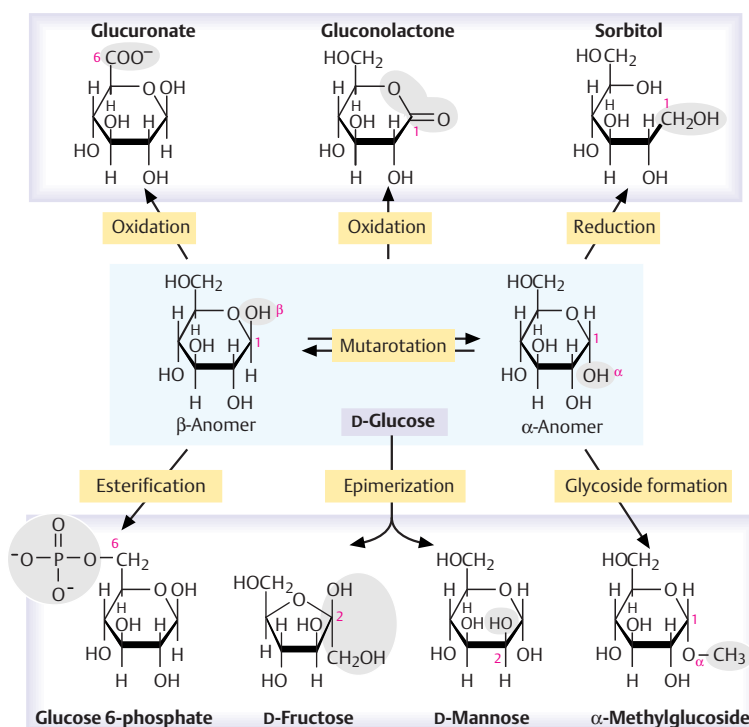
4. Epimerization. In weakly alkaline solutions, glucose is in equilibrium with **D-fructose** and **D-mannose**, via an enediol intermediate (not shown). The only difference between glucose and mannose is the configuration at C-2. Pairs of sugars of this type are referred to as **epimers**, and their interconversion is called **epimerization**.

5. Esterification. The hydroxyl groups of monosaccharides can form **esters** with acids. In metabolism, phosphoric acid esters such as **glucose 6-phosphate** and **glucose 1-phosphate** are particularly important (p. 130).

A. Monosaccharides: structure



B. Reactions of the monosaccharides



Monosaccharides and Disaccharides

Only the most important of the large number of naturally occurring **monosaccharides** are mentioned here. They are classified according to the number of C atoms (into pentoses, hexoses, etc.) and according to the chemical nature of the carbonyl function into aldoses and ketoses. Likewise, only a few of the many possible **disaccharides** are important for humans.

A. Important monosaccharides

The best-known **aldopentose** (1), *D-ribose*, is a component of RNA and of nucleotide coenzymes and is widely distributed. In these compounds, ribose always exists in the furanose form (p. 64). *D-xylose* and *L-arabinose* are found in large amounts as constituents of polysaccharides in the walls of plant cells.

The most important of the **aldohexoses** (1) is *D-glucose*. A substantial proportion of the biomass is accounted for by glucose polymers—above all, cellulose and starch. Free *D-glucose* is found in plant juices (“grape sugar”) and as “blood sugar” in the blood of animals. As a constituent of lactose (milk sugar), *D-galactose* is part of the human diet. Together with *D-mannose*, it is also found in many glycolipids and glycoproteins (p. 34).

Phosphoric acid esters of the **ketopentose** *D-ribulose* (2) are intermediates in the pentose phosphate pathway (see p. 132) and in photosynthesis. The most widely distributed of the **ketohehexoses** is *D-fructose*. In free form, it is present in fruit juices and in honey. Bound fructose is found in sucrose (see B) and plant polysaccharides (e.g., inulin).

In the **deoxyaldoses** (3), an OH group is replaced by a hydrogen atom. In addition to *2-deoxy-D-ribose*, a component of DNA (p. 68) that is reduced at C-2, *L-fucose* is shown as another example of these. Fucose, a sugar in the *L*-series is reduced at C-6.

The **acetylated amino sugars** *N-acetyl-D-glucosamine* and *N-acetyl-D-galactosamine* (4) are often encountered as components of glycoproteins.

N-acetylneuraminic acid (sialic acid, 5) is also a characteristic component of glycoproteins. Other **acidic monosaccharides** such as *D-glucuronic acid*, *D-galacturonic acid*, and *L-iduronic acid*, are typical constituents of the glycosaminoglycans found in connective tissue.

Sugar alcohols (6) such as *sorbitol* and *mannitol* do not play an important role in animal metabolism.

B. Disaccharides

When the anomeric hydroxyl group of one monosaccharide is bound glycosidically with an OH group of another sugar, a **disaccharide** is formed. Since this type of bond is formed in nature by enzymes, only *one* of the possible configurations (α or β) is found in each case. As in all glycosides, *no mutarotation* is possible in disaccharides.

Maltose (1) occurs as a breakdown product of starch in malt and in the intestine. In this molecule, the anomeric OH group of one glucose molecule forms an α -glycosidic bond with C-4 in a second glucose residue. **Trehalose** (2), which occurs in plants, fungi and insects, results from the $\alpha 1 \rightarrow 1$ bonding of two glucose residues. By contrast, a $\beta 1 \rightarrow 4$ bond between glucose residues produces **cellobiose** (3), a component of cellulose (p. 32).

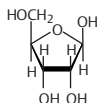
Lactose (*milk sugar*, 4) is the most important carbohydrate in the milk of mammals. Cow's milk contains 4.5% lactose, while human milk contains up to 7.5%. In lactose, the anomeric OH group of galactose forms a β -glycosidic bond with C-4 of glucose.

Sucrose (5) serves in plants as the form in which carbohydrates are transported, and as a soluble carbohydrate reserve. Humans value it because of its intensely sweet taste. Sources used for sucrose are plants that contain high amounts of it, such as sugarcane and sugar beet (*cane sugar*, *beet sugar*). Enzymatic hydrolysis of sucrose-containing flower nectar in the digestive tract of bees—catalyzed by the enzyme *invertase*—produces **honey**, a mixture of glucose and fructose. In sucrose, the two anomeric OH groups of glucose and fructose form a glycosidic bond; it is therefore one of the nonreducing sugars.

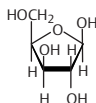
A. Important monosaccharides

1. Aldoses

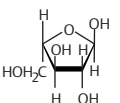
D-Ribose (Rib)



D-Xylose (Xyl)

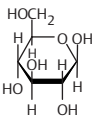


L-Arabinose (Ara)

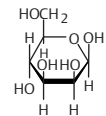


Pentoses

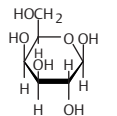
D-Glucose (Glc)



D-Mannose (Man)



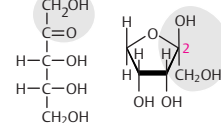
D-Galactose (Gal)



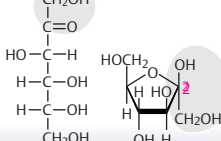
Hexoses

2. Ketoses

D-Ribulose (Ru)

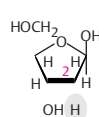


D-Fructose (Fru)

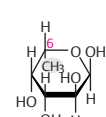


3. Deoxyaldoses

2-Deoxyribose (dRib)

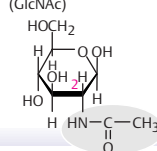


L-Fucose (Fuc)

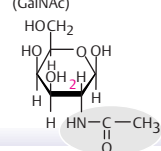


4. Acetylated amino sugars

N-Acetylglucosamine (GlcNAc)

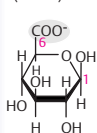


N-Acetylgalactosamine (GalNAc)

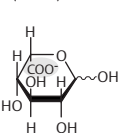


5. Acidic monosaccharides

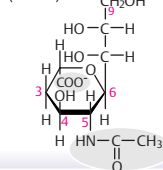
D-Glucuronic acid (GlcUA)



L-Iduronic acid (IdUA)

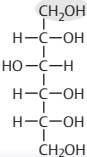


N-Acetylneuraminic acid (NeuAc)

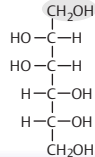


6. Sugar alcohols (alditols)

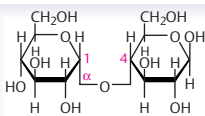
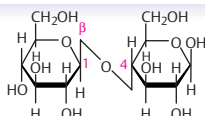
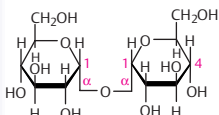
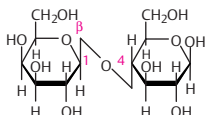
D-Sorbitol



D-Mannitol



B. Disaccharides


1. Maltose $\alpha(1 \rightarrow 4)$

3. Cellobiose $\beta(1 \rightarrow 4)$

2. Trehalose $\alpha(1 \rightarrow 1)$

4. Lactose $\beta(1 \rightarrow 4)$

5. Sucrose $\alpha(1 \rightarrow 2)\beta$

Polysaccharides

Polysaccharides are ubiquitous in nature. They can be classified into three separate groups, based on their different *functions*. **Structural polysaccharides** provide mechanical stability to cells, organs, and organisms. **Water-binding polysaccharides** are strongly hydrated and prevent cells and tissues from drying out. Finally, **reserve polysaccharides** serve as carbohydrate stores that release monosaccharides as required. Owing to their polymeric nature, reserve carbohydrates are osmotically less active, and they can therefore be stored in large quantities within the cell.

A. Polysaccharides: structure

Polysaccharides that are formed from only one type of monosaccharide are called **homoglycans**, while those formed from different sugar constituents are called **heteroglycans**. Both forms can exist as either linear or branched chains.

A section of a **glycogen** molecule is shown here as an example of a branched homoglycan. Amylopectin, the branched component of vegetable starch, has a very similar structure. Both molecules mainly consist of $\alpha 1 \rightarrow 4$ -linked glucose residues. In glycogen, on average every 8th to 10th residue carries—via an $\alpha 1 \rightarrow 6$ bond—another 1,4-linked chain of glucose residues. This gives rise to branched, tree-like structures, which in animal glycogen are covalently bound to a protein, *glycogenin* (p. 136).

Cellulose, a linear heteroglycan of $\beta 1 \rightarrow 4$ -linked glucose residues, is by far the *most abundant organic substance in nature*. Almost half of the entire biomass consists of cellulose and 40%–50% of *plant cell walls* are formed by it. In fact, the proportion of cellulose in *cotton fibers*, an important raw material, is 98%. Cellulose molecules can contain more than 10^4 glucose residues (mass $1\text{--}2 \times 10^6$ Da) and can reach lengths of 6–8 μm . In higher animals, including humans, cellulose is indigestible but is important as dietary fiber (p. 266).

B. Important polysaccharides

The table gives an overview of the composition and structure both of the glycans mentioned above and of several more.

In addition to the structural polysaccharide **murein**, bacterial polysaccharides include **dextrans**—glucose polymers that are mostly $\alpha 1 \rightarrow 6$ -linked and $\alpha 1 \rightarrow 3$ -branched. In water,

dextrans form viscous slimes or gels that after chemical treatment are used for chromatographic separation of macromolecules in the laboratory. Dextrans are also used as components of blood plasma substitutes (plasma expanders) and foodstuffs.

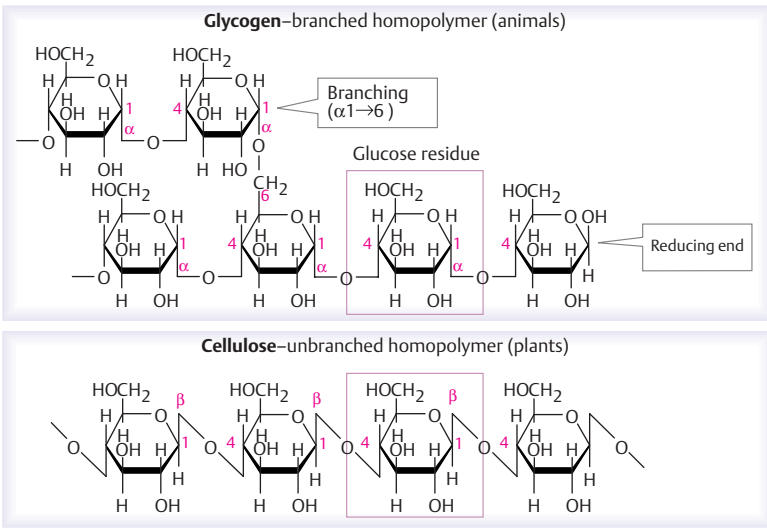
Carbohydrates from algae (e.g., **agarose** and **carrageenan**) are also used to produce gels. Agarose has been used in microbiology for more than 100 years to reinforce culture media (“agar-agar”). Algal polysaccharides are also added to cosmetics and ready-made foods to modify the consistency of these products.

Starch, a widely distributed vegetable reserve carbohydrate, is *the most important carbohydrate in the human diet*. Vegetable starch can consist of water-soluble amylose and water-insoluble amylopectin. It occurs in leaves, fruits, seeds or tubers. The starch content is especially high in cereal grains (up to 75% of the dry weight), potato tubers (~65%), and in other plant storage organs.

Inulin, a fructose polymer, is used as a starch substitute in diabetics’ dietary products. In addition, it serves as a test substance for measuring renal clearance (p. 334).

Chitin, a homopolymer of $\beta 1 \rightarrow 4$ -linked *N*-acetylglucosamine, is the most important structural substance in insect, crustacean, and spider shells, and is thus the most common animal polysaccharide. It also occurs in the cell wall of fungi.

Glycogen, the reserve carbohydrate of higher animals (A), is stored in the liver and musculature in particular (p. 136). The formation and breakdown of glycogen are subject to complex regulation by hormones and other factors (p. 140).

A. Polysaccharides: structure**B. Important polysaccharides**

Poly-saccharide	Mono-saccharide 1	Mono-saccharide 2	Linkage	Branching	Occurrence	Function
<i>Bacteria</i>						
Murein	D-GlcNAc	D-MurNAc ¹⁾	β 1 $\rightarrow$ 4	—	Cell wall	SC
Dextran	D-Glc	—	α 1 $\rightarrow$ 6	α 1 $\rightarrow$ 3	Slime	WB
<i>Plants</i>						
Agarose	D-Gal	L-aGal ²⁾	β 1 $\rightarrow$ 4	α 1 $\rightarrow$ 3	Red algae (agar)	WB
Carrageenan	D-Gal	—	β 1 $\rightarrow$ 3	α 1 $\rightarrow$ 4	Red algae	WB
Cellulose	D-Glc	—	β 1 $\rightarrow$ 4	—	Cell wall	SC
Xyloglucan	D-Glc	D-Xyl (D-Gal, L-Fuc)	β 1 $\rightarrow$ 4	β 1 $\rightarrow$ 6 (β 1 $\rightarrow$ 2)	Cell wall (Hemicellulose)	SC
Arabinan	L-Ara	—	α 1 $\rightarrow$ 5	α 1 $\rightarrow$ 3	Cell wall (pectin)	SC
Amylose	D-Glc	—	α 1 $\rightarrow$ 4	—	Amyloplasts	RC
Amylopectin	D-Glc	—	α 1 $\rightarrow$ 4	α 1 $\rightarrow$ 6	Amyloplasts	RC
Inulin	D-Fru	—	β 2 $\rightarrow$ 1	—	Storage cells	RC
<i>Animals</i>						
Chitin	D-GlcNAc	—	β 1 $\rightarrow$ 4	—	Insects, crabs	SC
Glycogen	D-Glc	—	α 1 $\rightarrow$ 4	α 1 $\rightarrow$ 6	Liver, muscle	RC
Hyaluronic-acid	D-GlcUA	D-GlcNAc	β 1 $\rightarrow$ 4 β 1 $\rightarrow$ 3	—	Connective tissue	SC, WB

SC = structural carbohydrate, RC = reserve carbohydrate,

WB = water-binding carbohydrate; ¹⁾N-acetylmuramic acid, ²⁾3,6-anhydrogalactose

Glycoproteins and Glycosaminoglycans

A. Glycoproteins: forms

Many proteins on the outer surface of plasma membranes and the majority of secreted proteins contain covalently bound oligosaccharide residues, which are added post-translationally in the endoplasmic reticulum (ER) and Golgi apparatus (p. 222). By contrast, cytoplasmic proteins are rarely glycosylated. **Glycoproteins** (glycans, glycoconjugates) can contain more than 70% carbohydrate; however, the proportion of protein is generally much greater.

The glycoproteins are divided into two groups, *N*-glycosylated and *O*-glycosylated, depending on how they are bound to the protein. There are two types of oligosaccharide structures with ***N*-glycosidic** links, which arise through different biosynthetic pathways. During glycosylation in the ER, the protein is initially linked to an oligosaccharide that contains six further mannose residues and three terminal glucose residues, in addition to its core structure (p. 222). The simpler form of oligosaccharide (the **mannose-rich type**) is produced when only the glucose residues are cleaved from the primary product and no additional residues are added. In other cases, the mannose residues located outside the core structure are also removed and replaced by other sugars. This produces oligosaccharides such as those shown in the figure (the **complex type**). Glycoproteins of the complex type often contain *N*-acetylneuraminic acid residues at the external end of the structure; these confer a negative charge on the oligosaccharide components. Certain glycoproteins on the outer cell surface and on large secreted proteins contain ***O*-glycosidic** bonds between the carbohydrate part and a serine or threonine residue, instead of *N*-glycosidic links to asparagine residues. This type of link is less common than the *N*-glycosidic one. It is found, for example, in mucins (p. 268ff.), 50%–80% of which consists of carbohydrate, and in the juxtamembrane region of the low-density lipoprotein (LDL) receptor (p. 284). The disaccharide residues of collagen (p. 350) are linked to hydroxylysine residues by *O*-glycosidic bonds.

B. Oligosaccharide in immunoglobulin G

As an example of the carbohydrate component of a glycoprotein, the structure of one of the oligosaccharide chains of immunoglobulin G (IgG; p. 310) is shown here. The oligosaccharide has an ***N*-glycosidic** link to the amide group of an asparagine residue in the F_c part of the protein.

Like all *N*-linked carbohydrates, the oligosaccharide in IgG contains a T-shaped **core structure** consisting of two *N*-acetylglucosamines and three mannose residues (shown in violet and green). In addition, in this case the structure contains two further *N*-acetylglucosamine residues, as well as a *fucose* residue and a *galactose* residue. Glycoproteins show many different types of branching. In this case, we not only have $\beta 1 \rightarrow 4$ linkage, but also $\beta 1 \rightarrow 2$, $\alpha 1 \rightarrow 3$, and $\alpha 1 \rightarrow 6$ bonds.

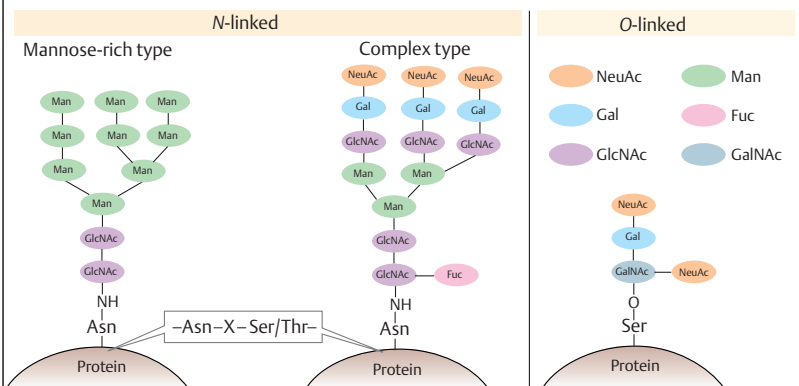
C. Hyaluronic acid

As constituents of proteoglycans, the glycosaminoglycans—a group of acidic heteropolysaccharides—are important structural elements of the extracellular matrix (p. 352).

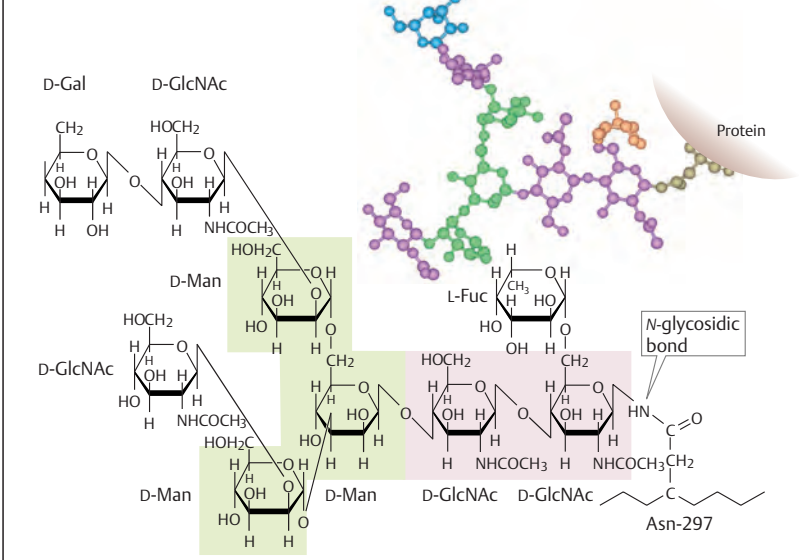
Glycosaminoglycans contain **amino sugars** as well as *glucuronic acid* and *iduronic acid* as characteristic components (p. 30). In addition, most polysaccharides in this group are esterified to varying extents by sulfuric acid, increasing their acidic quality. Glycosaminoglycans are found in free form, or as components of proteoglycans throughout the organism.

Hyaluronic acid, an unesterified glycosaminoglycan with a relatively simple structure, consists of disaccharide units in which *N*-acetylglucosamine and *glucuronic acid* are alternately $\beta 1 \rightarrow 4$ -linked and $\beta 1 \rightarrow 3$ -linked. Due to the unusual $\beta 1 \rightarrow 3$ linkage, hyaluronic acid molecules—which may contain several thousand monosaccharide residues—are coiled like a helix. Three disaccharide units form each turn of the helix. The outward-facing hydrophilic carboxylate groups of the glucuronic acid residues are able to bind Ca^{2+} ions. The **strong hydration** of these groups enables hyaluronic acid and other glycosaminoglycans to bind water up to 10 000 times their own volume in gel form. This is the function that hyaluronic acid has in the *vitreous body* of the eye, which contains about 1% hyaluronic acid and 98% water.

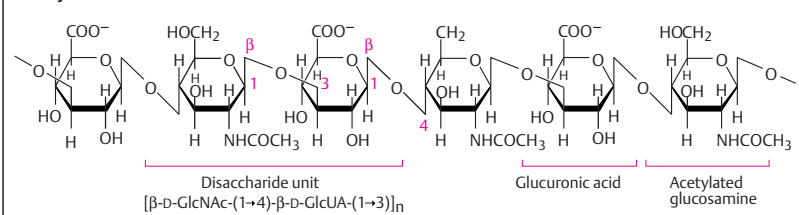
A. Glycoproteins: forms



B. Oligosaccharide in immunoglobulin G



C. Hyaluronic acid



Overview

Lipids are a large and heterogeneous group of substances of biological origin that are easily dissolved in organic solvents such as methanol, acetone, chloroform, and benzene. By contrast, they are either insoluble or only poorly soluble in water. Their low water solubility is due to a lack of polarizing atoms such as O, N, S, and P (p. 6).

A. Classification

Lipids can be classified into substances that are either *hydrolyzable*—i.e., able to undergo hydrolytic cleavage—or *nonhydrolyzable*. Only a few examples of the many lipids known can be mentioned here. The individual classes of lipids are discussed in more detail in the following pages.

1. Hydrolyzable lipids (components shown in parentheses below). The **simple esters** include the *fats* (triacylglycerol; 1 glycerol + 3 acyl residues), the *waxes* (1 fatty alcohol + 1 acyl residue), and the *sterol esters* (1 sterol + 1 acyl residue). The **phospholipids** are esters with more complex structures. Their characteristic component is a phosphate residue. The phospholipids include the *phosphatidic acids* (1 glycerol + 2 acyl residues + 1 phosphate) and the *phosphatides* (1 glycerol + 2 acyl residues + 1 phosphate + 1 amino alcohol). In the **sphingolipids**, glycerol and one acyl residue are replaced by sphingosine. In the **glycolipids**, the phosphate in the sphingolipids is replaced by one or more sugars (1 sphingosine + 1 fatty acid + sugar). The *cerebrosides* (1 sphingosine + 1 fatty acid + 1 sugar) and *gangliosides* (1 sphingosine + 1 fatty acid + several different sugars, including neuraminic acid) are representatives of this group.

Alternatively, the hydrolyzable lipids can be classified according to their basic components into **glycerolipids** (p. 40) and **sphingolipids** (p. 42). Their components are linked to one another by ester bonds, which are easily broken down either enzymatically or chemically.

2. Nonhydrolyzable lipids. The **hydrocarbons** include the *alkanes* and *carotenoids*. The **lipid alcohols** are also not hydrolyzable. They include long-chained *alkanols* and cyclic *sterols* such as cholesterol, and *steroids* such as estradiol and testosterone. The most important **acids** among the lipids are *fatty acids*. The *eicosanoids* also belong to this group; these are derivatives of the polyunsaturated fatty acid arachidonic acid (p. 38).

B. Biological roles

1. Fuel. Lipids are an important source of energy in the diet. In quantitative terms, they represent the principal energy reserve in animals (p. 332). Neutral fats in particular are stored in specialized cells, known as *adipocytes*. Fatty acids are released from these again as needed, and, following transport to the organs using them, they are oxidized in the mitochondria to form water and carbon dioxide, with oxygen being consumed. This process also gives rise to reduced coenzymes, which are used for ATP production in the respiratory chain.

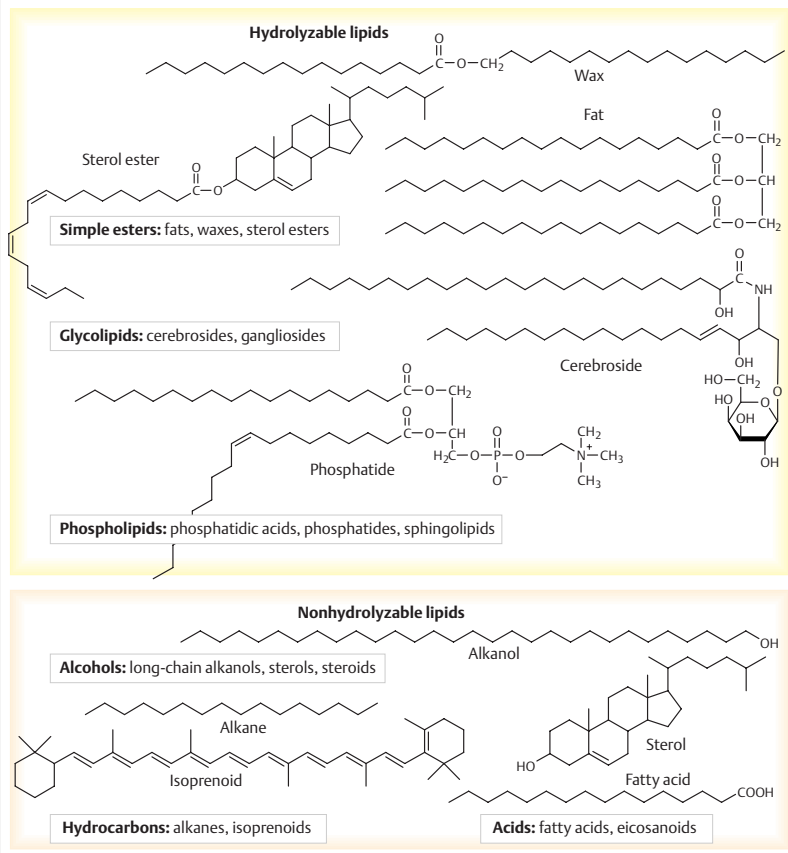
2. Cell components. Amphipathic lipids are used by cells to build membranes (p. 208 ff.). Typical membrane lipids include phospholipids, glycolipids, and cholesterol. Fats are only weakly amphipathic and are therefore not suitable as membrane components.

3. Insulation. Lipids are excellent insulators. In the higher animals, neutral fats are found in the subcutaneous tissue and around various organs, where they serve as mechanical and thermal insulators. As the principal constituent of cell membranes, lipids also insulate cells from their environment mechanically and electrically. The impermeability of lipid membranes to ions allows the formation of the membrane potential (p. 360).

4. Special tasks. Some lipids have adopted special roles in the body. Steroids, eicosanoids, and some metabolites of phospholipids have *signaling functions*. They serve as hormones, mediators, and second messengers (p. 414 ff.). Other lipids form *anchors* to attach proteins to membranes (p. 210). The lipids also produce *cofactors for enzymatic reactions*—e.g., vitamin K (p. 392) and ubiquinone (p. 86). The carotenoid retinal, a light-sensitive lipid, is of central importance in the *process of vision* (p. 368).

Several lipids are not formed independently in the human body. These substances, as **essential fatty acids** and **fat-soluble vitamins**, are indispensable components of nutrition (pp. 382, 392).

A. Classification



B. Biological roles

	Substance	Function
Fuel	Fats, fatty acids	Store and provide energy
Cell components	Phospholipids, sphingolipids, and cholesterol	Membrane components
Insulation	Fats Phospholipids, sphingolipids, and cholesterol	Mechanical cushion and thermal insulator Electrical insulator
Special tasks	Steroid hormones, glycerolipids, fatty acids, eicosanoids Fatty acids, isoprenoids Isoprenoids Retinal	Signal function: hormone, mediator, second messenger Membrane anchor Cofactor for enzymes Visual pigment

Fatty Acids and Fats

Fatty acids are important components of many lipids. They can be synthesized by all organisms.

A. Carboxylic acids

The naturally occurring **fatty acids** are carboxylic acids with unbranched hydrocarbon chains of 3–24 carbon atoms. In fats and membrane lipids, they are esterified with alcohols (glycerol, sphingosine, or cholesterol). Small amounts of fatty acids are also found in unesterified form. In this case, they are known as *free fatty acids* (FFAs). As FFAs have strongly amphipathic properties (p. 24), they are usually present in protein-bound forms (p. 280).

The table shows the full series of aliphatic carboxylic acids that are found in plants and animals. Carboxylic acids with at least three carbon atoms are known as fatty acids. In higher plants and animals, unbranched, long-chain fatty acids with either 16 or 18 C atoms are the most common—e.g., **palmitic** and **stearic** acid. The number of carbon atoms in the natural fatty acids is usually even. This is because they are biosynthesized from C_2 building blocks (p. 150).

Some fatty acids contain one or more isolated *double bonds*, and are therefore “unsaturated.” Common *unsaturated fatty acids* include **oleic acid** and **linoleic acid**. Of the two possible *cis-trans* isomers (p. 4), usually only the *cis* forms are found in natural lipids. Branched fatty acids only occur in bacteria. A shorthand notation with several numbers is used for precise characterization of the structure of fatty acids—e.g., 18:2; 9,12 for linoleic acid. The first figure stands for the number of C atoms, while the second gives the number of double bonds. The positions of the double bonds follow after the semicolon. As usual, numbering starts at the carbon with the highest oxidation state (i.e., the carboxyl group corresponds to C-1). Greek letters are also commonly used ($\alpha = C-2$; $\beta = C-3$; $\omega =$ the last carbon, $\omega-3 =$ the third last carbon).

Because of their different behavior in metabolism, fatty acids are often classified as **short-chain** (C_3 – C_6), **medium-chain** (C_8 – C_{10}), **long-chain** (C_{12} – C_{18}), and **very long-chain** ($> C_{18}$) fatty acids.

Essential fatty acids are fatty acids that have to be supplied in the diet because humans cannot produce them. These are polyunsaturated fatty acids: the C_{20} fatty acid **arachidonic acid** (20:4; 5,8,11,14) and the two C_{18} acids **linoleic acid** (18:2; 9,12) and **linolenic acid** (18:3; 9,12,15). The animal organism requires arachidonic acid to synthesize eicosanoids (p. 438). As the organism is capable of elongating fatty acids by adding C_2 units, but is not able to introduce double bonds into the end sections of fatty acids (after C-9), arachidonic acid has to be supplied in the diet. Since linoleic and linolenic acid can be converted into arachidonic acid, they can replace arachidonic acid.

B. Structure of fats

Fats are esters of the trivalent alcohol *glycerol* with three fatty acids. When a single fatty acid is esterified with glycerol, the product is referred to as a **monoacylglycerol** (fatty acid residue = acyl residue). Formally, esterification with additional fatty acids leads to **diacylglycerols** and ultimately to **triacylglycerols**, the actual fat (formerly termed “*triglycerides*”). As triacylglycerols are uncharged, they are also referred to as *neutral fats*.

The three acyl residues of a fat molecule may differ in terms of their chain length and the number of double bonds they contain. This results in a large number of possible combinations of individual fat molecules. When extracted from biological materials, fats therefore always represent mixtures of very similar compounds, which differ in their fatty acid residues. Dietary fats often contain palmitic, stearic, oleic, and linoleic acids in particular. Unsaturated fatty acids are usually found at the central C-atom of glycerol.

The length of the fatty acid residues and the number of their double bonds affect the melting point of the fats. The shorter the fatty acid residues and the more double bonds they contain, the lower their melting points. Fats that are liquid at room temperature are called **oils**, e.g., olive oil.

Glycerolipids

Most lipids in the human body are composite compounds in the form of esters of fatty acids with alcohols. They are named according to these structural components. Thus, all lipids that contain the trivalent alcohol glycerol are **glycerolipids**. If the basic component is sphingosine instead of glycerol, this is called a **sphingolipid** (p. 42). Not only fatty acid residues can be attached to glycerol and sphingosine; phosphate groups or carbohydrates can also be present in addition. These lipids are then called **phospholipids** and **glycolipids**.

A. Structure of glycerolipids

Fats (triacylglycerols, 1) are esters of glycerol with three fatty acids. As important energy sources, they are mainly stored in the form of fat droplets within the cell. For transport, the nonpolar fats are bound to proteins; in the blood, for example, they are transported in the hydrophobic interior of lipoproteins (p. 282ff.). **Phospholipids** are the main constituents of biological membranes (p. 208). Their common feature is a phosphate residue that is esterified with the hydroxyl group at C-3 of a diacylglycerol or acyl sphingosine. Due to the phosphate group, phospholipids have at least one negative charge at neutral pH. The phospholipids include the glycerophospholipids listed below, and also the sphingophospholipids described in the next section.

The simplest phospholipids are phosphate esters of diacylglycerol, the **phosphatidates** (anions of the phosphatidic acids; 2). They are important intermediates in the biosynthesis of fats and phospholipids (p. 154). Phosphatidates can also be released from phospholipids by the action of *phospholipases*.

The **phosphatides** (3) and other phospholipids can be derived from phosphatidates (residue = phosphatidyl). In the phosphatides, an alcohol is attached to the phosphate residue through an ester bond. The phosphate residue can be esterified with an amino alcohol (choline, ethanolamine, or serine) or with the cyclohexane derivative **myo-inositol**. A **phosphatidylcholine** (**lecithin**, **Ptd-choline**; 4) is shown here as the most important example of this type of compound. When two phosphatidyl residues are linked with one glycerol, the result is **cardiolipin** (diphosphatidylglycerol; 6), a phospholipid that is found only in the inner mitochondrial membrane. The highly amphipathic **lyso-phospholipids** (5) arise from phospholipids by

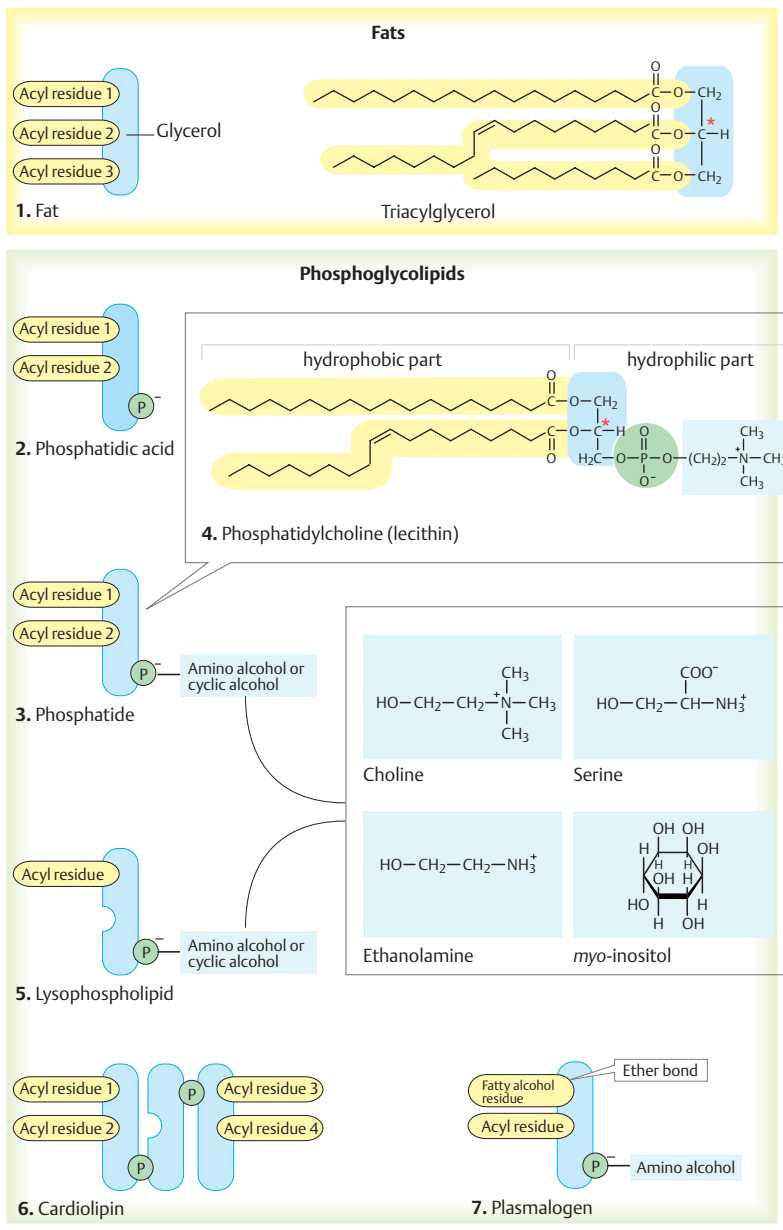
enzymatic cleavage of an acyl residue. The hemolytic effect of bee and snake venoms is due in part to this reaction.

Phosphatidylcholine is the most abundant phospholipid in membranes. Its amphipathic properties make it an important constituent of lung surfactant, which is required for lung development and function. **Phosphatidylethanolamine** (**cephalin**) has an ethanolamine residue instead of choline, and **phosphatidylserine** has a serine residue. In **phosphatidylinositol**, phosphatide is esterified with the cyclic polyalcohol myo-inositol. A doubly phosphorylated derivative of this phospholipid, **phosphatidylinositol 4,5-bisphosphate**, is a special component of membranes, which, by enzymatic cleavage, can give rise to two *second messengers*, **diacylglycerol** (DAG) and **inositol 1,4,5-trisphosphate** (InsP₃; p. 406).

The **plasmalogens** (7) are a special group of phospholipids. They resemble lecithin and cephalin but instead of the fatty acid at C-1 of glycerol, they have an *unsaturated fatty alcohol* linked by an *ether bond*. Plasmalogens and other **ether phospholipids** esterified with a saturated fatty alcohol account for some 18% of all phospholipids in humans. They are found in specific tissues and are located, for instance, in the membranes of myelin sheaths. Certain plasmalogens play a part as a *platelet-activating factor* (PAF).

Some phospholipids carry additional charges, in addition to the negative charge at the phosphate residue. In phosphatidylcholine and phosphatidylethanolamine, the N-atom of the amino alcohol is positively charged. As a whole, these two phosphatides therefore appear to be neutral. In contrast, phosphatidylserine—with one additional positive charge and one additional negative charge in the serine residue—and phosphatidylinositol (with no additional charge) have a negative net charge, due to the phosphate residue. This plays a part in their positioning within the membrane (p. 208).

A. Structure of glycerolipids



Sphingolipids

Besides the phosphoglycerolipids (previous page), **sphingolipids** are other important membrane lipids. They are found in very high quantities in the membranes of nerve cells in the brain and in neural tissues. Sphingolipids differ in structure from the membrane lipids discussed so far; **sphingosine**, an amino dialcohol with a long unsaturated alkyl side chain, replaces glycerol and one of the fatty acid residues. Thus, sphingosine roughly resembles a monoacylglycerol.

A. Structure of sphingolipids

Sphingosine (1) is the basic component of the sphingolipids. It is an unsaturated, doubly hydroxylated C₁₈ compound with a long aliphatic residue. It is synthesized from serine and palmityl-CoA.

When sphingosine forms an amide bond with a single fatty acid, the resulting compound is called **ceramide** (2). It is the precursor of all sphingolipids.

Among the **sphingophospholipids** (3), **sphingomyelin** (4) is the most abundant sphingophospholipid in membranes. It arises from ceramide by addition of a phosphate group and a choline residue. Sphingomyelin is therefore a phospholipid, similar to lecithin (previous page). Its name is derived from its high concentrations in the myelin sheaths of the nervous system.

Glycolipids, as the name suggests, contain carbohydrate residues. They occur in all tissues, predominantly on the outside of the plasma membrane. Mono- or oligosaccharide residues are attached to the ceramide residue (sphingosine + fatty acid) of the glycolipids. In contrast, the glycolipids lack the phosphate residue of the phospholipids. **Galactosylceramide** and **glucosylceramide**, known as **cerebrosides** (5), are simple representatives of this group. Cerebrosides in which the sugar is esterified with sulfuric acid are called **sulfatides** (6). They are synthesized particularly in the CNS.

Gangliosides (7) with a branched-chain oligosaccharide are the most complex glycolipids. Their characteristic constituent is **N-acetylneuraminic acid** (NeuAc, sialic acid, p. 30).

Glycolipids on the cell membrane form a hydrophilic layer known as the **glycocalyx**. This protects cells from being digested and from the unwanted uptake of lipophilic substances. The variety of glycolipids on the plasma membrane also serves for cell recognition and is respon-

sible for the formation of the different A, B and O **blood groups** (p. 298). Glycolipids on the cell surface are also used by **viruses** and bacterial **toxins**, for example, **cholera toxin**, as specific binding sites to penetrate into the cells.

There are no known diseases caused by disturbance of phospholipid metabolism, probably owing to the central role of phospholipids in membranes. On the other hand, there is a range of **sphingolipidoses**, in which the metabolism of sphingolipids is disturbed so that they accumulate. Neural cells are affected in particular.

Degradation of the sphingolipids by specific hydrolases takes place in lysosomes (p. 224). First, the carbohydrate chain of the glycolipids is shortened gradually. Besides enzymes, this also requires **sphingolipid activator proteins** (SAPs), as the glycosidases only become active in their presence. The phosphocholine residue is cleaved from sphingomyelin by a **sphingomyelinase**. The resulting ceramide is then broken down to sphingosine and fatty acid by **ceramidase**.

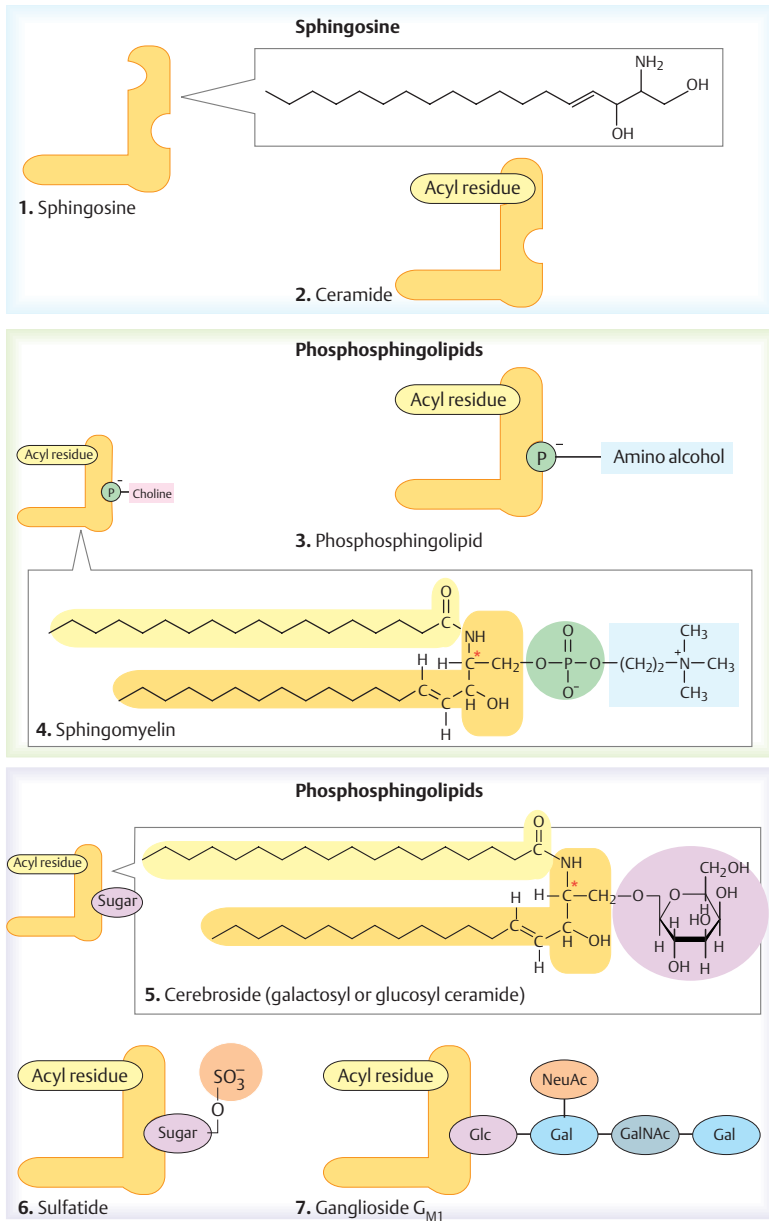
Just a few of the many diseases involving sphingolipids are mentioned here.

In **Tay-Sachs disease** (*Sandhoff disease*, GM₂ gangliosidosis), the activity of **hexosaminidase** is diminished or absent; this enzyme cleaves the glycosidic binding of **N-acetylglucosamine** and **N-acetylgalactosamine** to gangliosides. Because of the deposition of unmetabolized gangliosides, degeneration of the nervous system occurs.

In **Gaucher disease** (*cerebrosidosis*), β -glucosidase, which cleaves glucosylceramide to ceramide and glucose, is defective. The enzyme requires the activator protein SAP-C. As a result of the disorder, glucosylceramides accumulate in cells of the monocyte and macrophage system.

In **Niemann-Pick disease**, **sphingomyelinase** or its activator protein SAP-C is affected so that sphingomyelin accumulates in monocytes, macrophages, and histiocytes.

A. Structure of sphingolipids



Isoprenoids

A. Acetyl CoA as a building block of lipids

Although the lipids found in plant and animal organisms occur in many different forms, they are all closely related biogenetically; they are all derived from **acetyl-CoA**, “activated acetic acid” (p. 8).

1. One major pathway leads from acetyl-CoA to the activated fatty acids (**acyl-CoA**; for details, see p. 144ff.). *Fats, phospholipids, and glycolipids* are synthesized from these, and fatty acid derivatives in particular are formed. Quantitatively, this is the most important pathway in animals and most plants.

2. The second pathway leads from acetyl-CoA to isopentenyl diphosphate (“activated isoprene”), the basic component for the **isoprenoids**. Its biosynthesis is discussed in connection with the isoprenoid, cholesterol (p. 156).

B. Isoprenoids

Formally, isoprenoids are derived from a single common building block, isoprene (2-methyl-1,3-butadiene), a methyl-branched compound with five carbon atoms. Activated isoprene, *isopentenyl diphosphate*, is used by plants and animals to biosynthesize linear and cyclic oligomers and polymers. For the isoprenoids listed here—which only represent a small selection—the number of isoprene units (I) is shown.

From activated isoprene, the metabolic pathway leads via dimerization to activated **geraniol** (I = 2) and then to activated **farnesol** (I = 3). At this point, the pathway divides into two. Further extension of farnesol leads to chains with increasing numbers of isoprene units—e.g., **phytol** (I = 4), **dolichol** (I = 14–24), and **rubber** (I = 700–5000). The other pathway involves a “head-to-head” linkage between two farnesol residues, giving rise to **squalene** (I = 6), which, in turn, is converted to **cholesterol** (I = 6) and the other **steroids**.

The ability to synthesize particular isoprenoids is limited to a few species of plants and animals. For example, rubber is only formed by a few plant species, including the rubber tree (*Hevea brasiliensis*). Several isoprenoids that are required by animals for metabolism, but cannot be produced by them independently, are vitamins; this group includes **vitamins A, D, E**, and **K**. Due to its structure and function, vitamin D, or calcitriol, is now usually classified as a steroid hormone (p. 424), as biosyn-

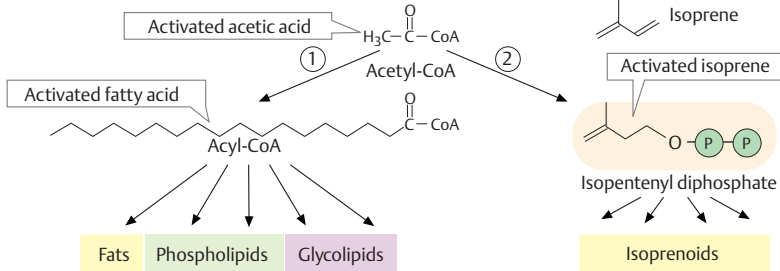
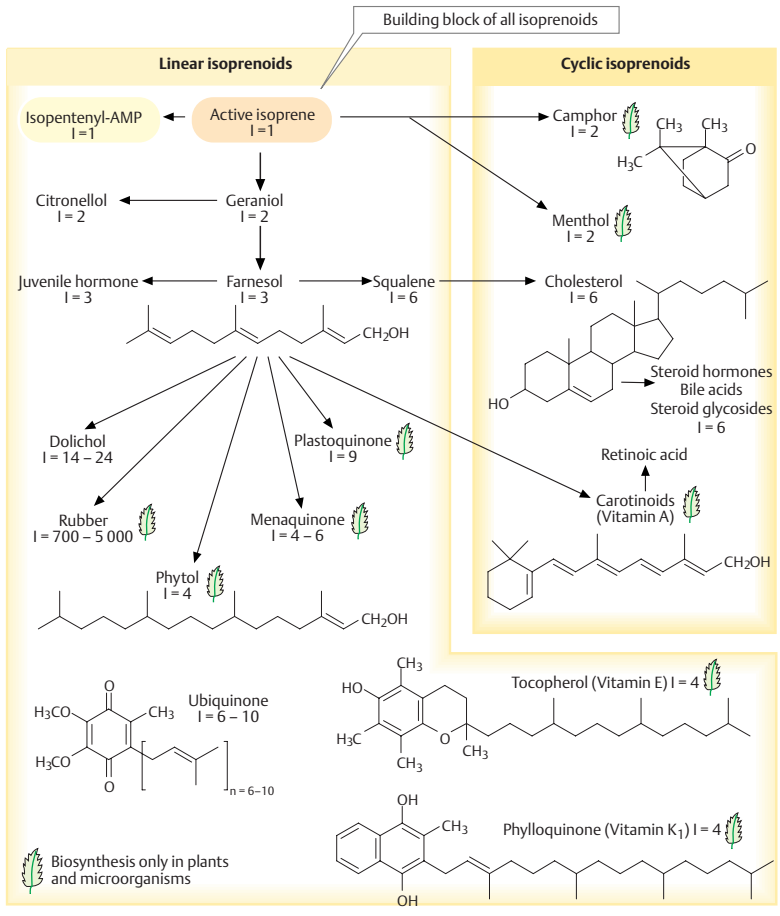
thesis of its precursor calcidiol (vitamin D₃) is possible in humans with adequate exposure to UV radiation.

Isoprene metabolism in plants is very complex. Many types of aromatic substances and volatile oils can be synthesized from isoprenoids. Examples shown include **menthol** (I = 2), **camphor** (I = 2), and **citronellol** (I = 2). These C₁₀ compounds are also called *monoterpenes*. Similarly, compounds consisting of three isoprene units (I = 3) are termed *sesquiterpenes*, and the steroids (I = 6) are called *triterpenes*.

Isoprenoids that have hormonal and signaling functions form an important group. These include **steroid hormones** (I = 6) and **retinoate** (the anion of retinoic acid; I = 3) in vertebrates, and *juvenile hormone* (I = 3) in arthropods. Some plant hormones also belong to the isoprenoids—e.g., *cytokinins*, *abscisic acid*, and *brassinosteroids*.

Isoprene chains are sometimes used as “*lipid anchors*” to fix molecules to membranes. Chlorophyll has a *phytyl* residue (I = 4) as a lipid anchor. Coenzymes with isoprenoid anchors of various lengths include **ubiquinone** (coenzyme Q; I = 6–10), **plastoquinone** (I = 9), and **phylloquinone** (vitamin K₁; I = 4). Proteins can also be anchored to membranes by *isoprenylation* (p. 210).

In some cases, an isoprene residue is used as an element to modify molecules chemically. One example of this is *N*-**isopentenyl AMP**, which occurs as a modified component in tRNA (p. 66).

A. Acetyl-CoA as a building block of lipids**B. Isoprenoids**

Steroids

The three most important groups of steroids are the *sterols*, the *bile acids*, and the *steroid hormones*. Compounds with a steroid structure are also found, especially in plants, which may have a marked pharmacological action; these include *steroid alcohols*, *digitaloids* and *saponins*.

A. Steroid building blocks

Common to all of the steroids is a molecular core structure consisting of four saturated rings, known as a *sterane*. (Steranes in which rings B and C and rings C and D are linked in *trans* position are called *gonanes*). At the end of the steroid core, many steroids also carry a side chain, as seen in **cholestane**, the basic component of the *sterols*.

B. Steroids

Sterols are *steroid alcohols*. They have a β -positioned hydroxyl group at C-3 and one or more double bonds in ring B and in the side chain. There are no further oxygen functions, such as carboxyl groups.

The most important sterol in animals is **cholesterol**. Plants and microorganisms have a wide variety of closely related sterols instead of cholesterol—e.g., *ergosterol*, *β -sitosterol*, and *stigmasterol*.

Cholesterol is present in all animal tissues, and particularly in neural tissue. It is a major constituent of cellular membranes, in which it regulates fluidity (p. 208). The storage and transport forms of cholesterol are its esters with fatty acids. In lipoproteins, cholesterol and its fatty acid esters are associated with other lipids (p. 282ff.). Cholesterol is a constituent of the bile and is therefore found in many gallstones. Its biosynthesis, metabolism, and transport are discussed elsewhere (pp. 156, 282ff., 320).

Cholesterol-rich *lipoproteins* of the LDL-type are particularly important in the development of *arteriosclerosis*, in which the arterial walls are altered in connection with an excess plasma cholesterol level. In terms of dietary physiology, it is important that plant foodstuffs are low in cholesterol. By contrast, animal foods can contain large amounts of cholesterol—particularly butter, egg yolk, meat, liver, and brain.

Bile acids are synthesized from cholesterol in the liver (p. 320). Their structures can there-

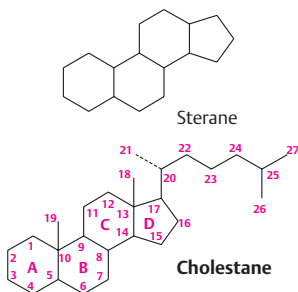
fore be derived from that of cholesterol. Characteristic for the bile acids is a side chain shortened by three C atoms in which the last carbon atom is oxidized to a carboxyl group. The double bond in ring B is reduced and rings A and B are in *cis* position relative to each other (p. 320). One to three hydroxyl groups (in α position) are found in the steroid core at positions 3, 7, and 12. Bile acids keep bile cholesterol in a soluble state as micelles and promote the digestion of lipids in the intestine (p. 274). **Cholic acid** and **chenodeoxycholic acid** are *primary bile acids* that are formed by the liver. Their dehydroxylation at C-7 by microorganisms from the intestinal flora gives rise to the *secondary bile acids* **lithocholic acid** and **deoxycholic acid**.

The conversion of cholesterol to **steroid hormones** (p. 424) is of minor importance quantitatively, but of major importance in terms of physiology. The steroid hormones are a group of lipophilic signal substances that, among other things, regulate metabolism, growth, and reproduction.

Humans have six families of steroid hormones, the most important of which are **progesterone**, **cortisol**, **aldosterone**, **testosterone**, **estradiol**, and **calcitriol** (old name: calciferol, vitamin D hormone). With the exception of calcitriol, these steroids have either no side chain or only a short one consisting of two carbons. Characteristic for most of them is an oxo group at C-3, conjugated with a double bond between C-4 and C-5 of ring A. Differences occur in rings C and D. Estradiol is aromatic in ring A, and its hydroxyl group at C-3 is therefore phenolic. Calcitriol differs from other vertebrate steroid hormones; it still contains the complete carbon framework of cholesterol, but light-dependent opening of ring B turns it into what is termed a “secosteroid” (a steroid with an open ring).

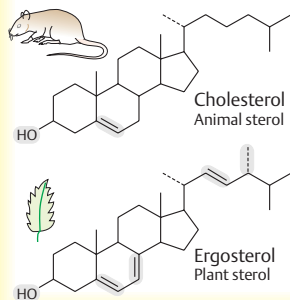
Ecdysone is the steroid hormone of arthropods. In evolutionary terms it can be regarded as an early form of the steroid hormones. Steroid hormones with signaling functions also occur in plants.

A. Steroid building blocks

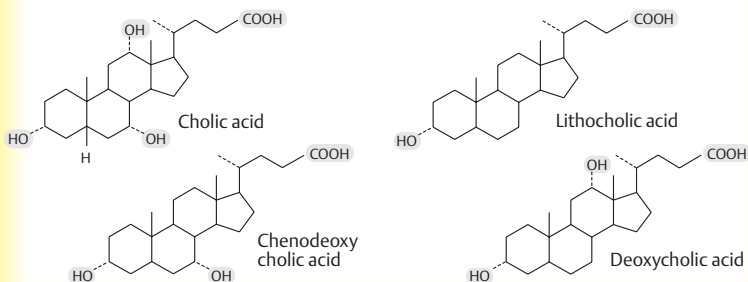


B. Steroids

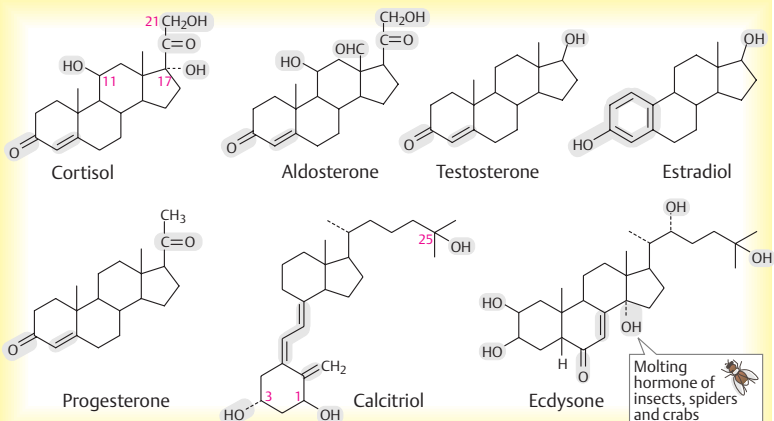
Sterols



Bile acids



Steroid hormones



Properties

A. Definition

The amino acids (aminocarboxylic acids) are an important group of biomolecules with various functions (see **B**). They are divided into α , β and γ amino acids, depending on the position of the amino group (**A**). Most of the natural amino acids are α amino acids (2-aminocarboxylic acids). These are the building blocks of peptides and proteins. β -Amino acids (e.g., β -alanine) and γ amino acids such as GABA (γ -aminobutyric acid; p. 362ff.) are less common. They have special functions, for example as components of biomolecules or as neurotransmitters.

B. Functions

The α -amino acids serve primarily as the **components of peptides and proteins**. The genetic code (p. 246) includes 20 different amino acids. Only these *proteinogenic amino acids* (p. 50) are regularly found in proteins. Some amino acids undergo further (post-translational) change following their incorporation into proteins (p. 52). Amino acids or their derivatives are also found as building blocks of **lipids**—e.g., serine in phospholipids and glycine in bile salts. Several amino acids function as **neurotransmitters** themselves (p. 362), while others act as precursors of neurotransmitters, mediators, or hormones (p. 366, 434ff.). Amino acids are also important or even essential components of food (p. 382). Specific amino acids are **precursors** for other metabolites—e.g., for glucose in gluconeogenesis, for purine and pyrimidine bases, for heme, and for other molecules. Several nonproteinogenic amino acids serve as intermediates in the synthesis and breakdown of other proteinogenic amino acids (p. 174) or as intermediates in other metabolic pathways (p. 116).

C. Enantiomers

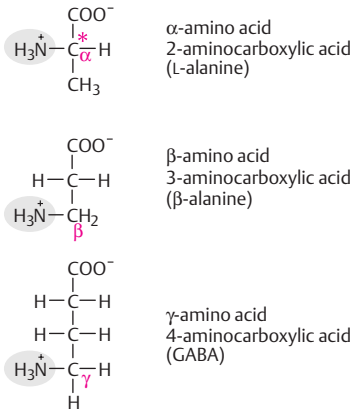
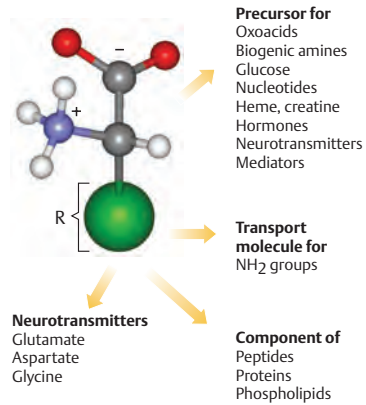
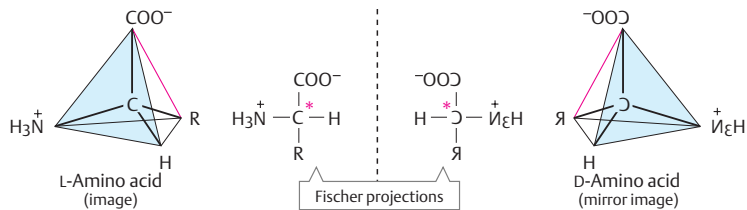
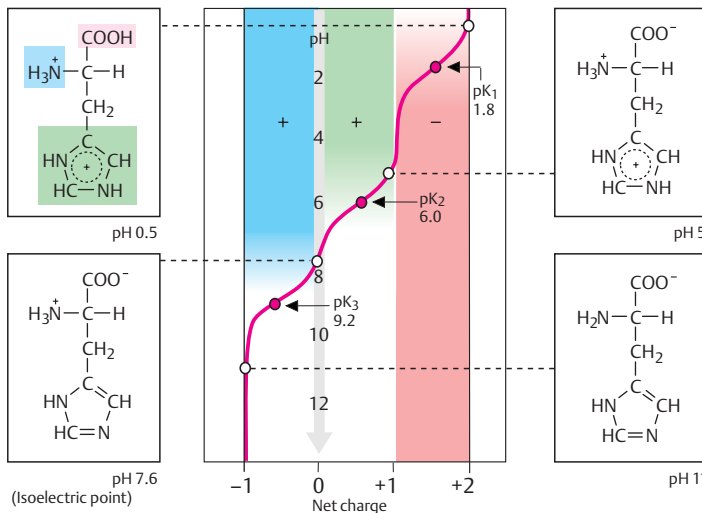
α -Amino acids have four different substituents at C-2 (C_α). The α atom therefore represents a *chiral center*—i.e., there are two different **enantiomers** (L- and D-amino acids; p. 4). Among the proteinogenic amino acids, only glycine is *not* chiral ($\text{R} = \text{H}$). In nature, it is almost exclusively **L-amino acids** that are found, whereas D-amino acids occur only in bacteria—e.g., in murein (as a component of the cell wall) or in *peptide antibiotics* (p. 252).

The **Fischer projection** (center) is used to represent the configurations of chiral centers in biomolecules. It is derived from their three-dimensional structure as follows: first, the tetrahedron is rotated in such a way that the most highly oxidized group (the carboxylate group in this case) is at the top. Rotation is then continued until the line (red) connecting COO^- and R is level with the page. In L-amino acids, the NH_3^+ group is then on the left, while in D-amino acids it is on the right.

D. Dissociation curve of histidine

All amino acids have at least two ionizable groups, and their net charge therefore depends on the pH value (p. 14). The COOH groups at the C_α atom have pK_a values of between 1.8 and 2.8 and are therefore more acidic than simple monocarboxylic acids. The basicity of the α -amino function also varies, with pK_a values of between 8.8 and 10.6, depending on the amino acid. Acidic and basic amino acids have additional ionizable groups in their side chain. The pK_a values of these side chains are listed on page 51. The electrical charges of peptides and proteins are mainly determined by groups in the side chains, as most α -carboxyl and α -amino functions are linked in peptide bonds. The proteinogenic amino acid **histidine** is used here as an example of the pH-dependence of the net charge of an amino acid. In addition to the carboxyl group and the amino group at the α -C atom with pK_a values of 1.8 and 9.2, respectively, histidine also has an *imidazole residue* in its side chain with a pK_a value of 6.0. As the pH increases, the net charge (the sum of the positive and negative charges) therefore changes from +2 to -1. At pH 7.6, the net charge is zero, even though the molecule contains two almost completely ionized groups in these conditions. This pH value is called the **isoelectric point**.

At its isoelectric point, histidine is said to be **zwitterionic**, as it has both anionic and cationic properties. Most other amino acids are also zwitterionic at neutral pH. Peptides and proteins have isoelectric points, too, which can vary widely depending on their amino acid composition.

A. Definition

B. Functions

C. Enantiomers

C. Dissociation curve of histidine


Proteinogenic Amino Acids

A. Proteinogenic amino acids: classification and nomenclature

The amino acids that are included in the genetic code (p. 246) are described as “proteinogenic.” With a few exceptions (p. 52), only these amino acids can be incorporated into proteins through *translation* (p. 248). The classification of the 20 proteinogenic amino acids is based on the chemical **structure** of their side chains, on the one hand, and on their **polarity** on the other. The literature includes several slightly different systems for classifying amino acids, and details may differ from those in the system used here.

For each amino acid, the illustration names:

- *Membership of structural classes* (aliphatic, sulfur-containing, aromatic, cyclic, neutral, acid, or basic)
- *Name and abbreviation*, formed from the first three letters of the name (e.g., histidine, His)
- The *one-letter symbol* introduced to save space in the electronic processing of sequence data (H for histidine)
- *The polarity of the side chain*, indicated by color. The polarity increases from orange, through yellow and light green, to bluish green.
- The pK_a of the functional group in the side group (red numbers)

Nearly half of the proteinogenic amino acids cannot be synthesized by the human body and must therefore be supplied in the diet. The amino acids **essential** for healthy adults (p. 174) are indicated by red triangles. For babies and young children, *cysteine* and possibly *arginine* are essential, in addition.

The **aliphatic** amino acids include *glycine*, *alanine*, *valine*, *leucine*, and *isoleucine*. These amino acids do not contain heteroatoms (N, O, or S) in their side chains and do not contain a ring system. Their side chains are markedly nonpolar. Valine, leucine, and isoleucine form the group of *branched-chain amino acids*. They are all essential.

Glycine is the only proteinogenic amino acid without a chiral center. In contrast, threonine and isoleucine contain two chiral centers. Of the four possible stereoisomers, however, only (2S,3R)-threonine and (2S,3S)-isoleucine are found in nature.

The **sulfur-containing amino acids**, *cysteine* and *methionine*, are also nonpolar. However, in the case of cysteine, this only applies to the undissociated state. Owing to its ability to form

disulfide bonds, cysteine plays an important role in the stabilization of proteins (p. 60). Two cysteine residues linked by a disulfide bridge are referred to as *cystine* (not shown, p. 174).

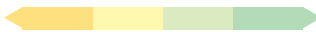
The **aromatic amino acids** contain resonance-stabilized ring systems. In this group, only *phenylalanine* has strongly nonpolar properties. *Tyrosine* and *tryptophan* are moderately polar, and *histidine* is even strongly polar. The imidazole ring of histidine is protonated even at weakly acidic pH values. Histidine, which is only aromatic in protonated form, can therefore also be classified as a basic amino acid.

The **neutral** amino acids have hydroxyl groups (*serine*, *threonine*) or amide groups (*asparagine*, *glutamine*). Despite their nonionic nature, the amide groups of asparagine and glutamine are markedly polar.

The carboxyl groups in the side chains of the **acidic** amino acids *aspartic acid* and *glutamic acid* are almost completely ionized at physiological pH values. These amino acids are therefore usually called *aspartate* and *glutamate*. The side chains of the **basic** amino acids *lysine* and *arginine* are also fully protonated—i.e., positively charged—at neutral pH. Arginine, with its positively charged guanidinium group, is particularly strongly basic, and therefore extremely polar.

Proline, which is **cyclic**, is a special case. Together with the α -C atom and the α -NH₂ group, its side chain forms a five-membered ring. Due to its ring structure, proline residues cause *bending of the peptide chain* in proteins (this is important in collagen, for example, see p. 58).

A. Proteinogenic amino acids: classification and nomenclature

Aliphatic					Sulfur-containing								
$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{H}_3\text{C}-\text{CH} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{H}_3\text{C}-\text{CH} \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{H}_3\text{C}-\text{CH} \\ \\ \text{CH}_2 \\ \\ \text{CH}_3 \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{SH} \end{array}$ 8.3 Thiol group	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{S}-\text{CH}_3 \end{array}$ Thioether	Glycine (Gly, G)	Alanine (Ala, A)	►Valine (Val, V)	►Leucine (Leu, L)	►Isoleucine (Ile, I)	Cysteine (Cys, C)	►Methionine (Met, M)
Aromatic					Cyclic		Neutral						
$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$ Benzene ring	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4\text{OH} \end{array}$ Phenol ring	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_8\text{H}_6\text{N} \end{array}$ Indole ring	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_2\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \end{array}$ Pyrrolidine ring	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{OH} \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{H}_3\text{C}-\text{C}-\text{H} \\ \\ \text{OH} \end{array}$	►Phenylalanine (Phe, F)	Tyrosine (Tyr, Y)	►Tryptophan (Trp, W)	Proline (Pro, P)	Serine (Ser, S)	►Threonine (Thr, T)		
Neutral		Acidic		Basic									
$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CONH}_2 \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CONH}_2 \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{COO}^- \end{array}$ 4.0	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COO}^- \end{array}$ 4.3	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_3\text{H}_3\text{N} \end{array}$ 6.0 Imidazole ring	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{NH}_3^+ \end{array}$ 10.8	$\begin{array}{c} \text{COO}^- \\ \\ ^+\text{H}_3\text{N}-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{NH} \\ \\ \text{C}=\text{NH}_2^+ \end{array}$ 12.5	Asparagine (Asn, N)	Glutamine (Gln, Q)	Aspartic acid (Asp, D)	Glutamic acid (Glu, E)	►Histidine (His, H)	►Lysine (Lys, K)	Arginine (Arg, R)
 nonpolar polar					4.0 pKa value * Chiral center ► Essential amino acids								

Selenocysteine and Nonproteinogenic Amino Acids

In addition to the proteinogenic amino acids (p. 50), there are also many more compounds of the same type in nature. These arise, for example, during metabolic reactions (**B**) or as a result of subsequent enzymatic modifications of amino acid residues in peptides or proteins (p. 62). The “biogenic amines” (**C**) are synthesized from α -amino acids by decarboxylation.

A. Selenocysteine

A small number of proteins (~10 in humans) contain **selenocysteine** (Sec) as the 21st proteinogenic amino acid, besides the “classical” amino acid components. Sec is a cysteine analogue in which the sulfur is replaced by the more reactive trace element selenium. Because of its markedly lower pK_a value compared with the SH group in cysteine, the SeH group is present in the cell mainly in dissociated form. During the translation of proteins containing Sec, the corresponding tRNA ($tRNA^{Sec}$, p. 246) is first formed from a special serine tRNA. The $tRNA^{Sec}$ then binds to the mRNA codon UGA, which is actually a stop codon but is interpreted in this case as a signal for incorporation of Sec. The Sec-containing proteins generally have redox functions. They include *glutathione peroxidase*, an enzyme for “detoxification” of lipid peroxidases (p. 290), *deiodinase* (p. 426) and *thioredoxin reductase*, which is required for the synthesis of DNA precursors (p. 184).

B. Nonproteinogenic amino acids

Only a few representatives of the nonproteinogenic α -amino acids are mentioned here. **Homocysteine** is an intermediate in the breakdown of methionine (p. 176). In contrast to cysteine, its side chain is longer by one CH_2 group. **Dopa** (an acronym of 3,4-dihydroxyphenylalanine) is synthesized by hydroxylation of tyrosine. It is an intermediate in the biosynthesis of *catecholamines* (p. 434) and of the brown pigment *melanin* (p. 170). The basic amino acid **ornithine** as well as **citrulline**, which is produced from it, are intermediates in the *urea cycle* (p. 172).

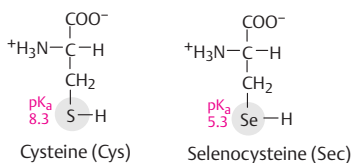
C. Biogenic amines

Many amino acids are broken down by *decarboxylation*. This reaction, which is catalyzed by

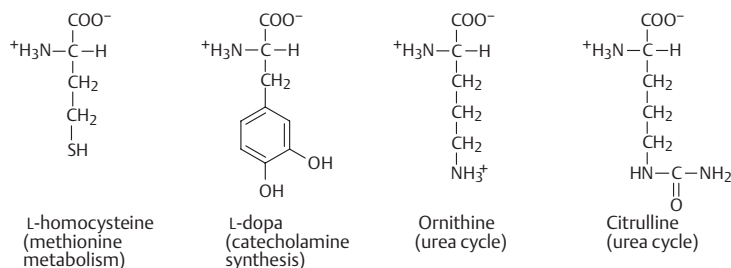
amino acid decarboxylases (**1**) gives rise to primary amines, known as “biogenic amines.” Substances in this group have various functions in the body. Some of them are **components of biomolecules**, such as the *ethanolamine* contained in some membrane lipids (p. 40). *Cysteamine* and β -alanine are components of coenzyme A (p. 8) and of pantotheine (p. 150). β -Alanine is produced not only during decarboxylation of aspartate but also when the pyrimidine base uracil is broken down (p. 180). Other biogenic amines function as **neurotransmitters**, for example, *4-aminobutyrate*, a γ -amino acid derived from glutamate (GABA, p. 362f.). GABA is the most important inhibitory neurotransmitter in the brain. Its sedating and anxiolytic action is reinforced by small amounts of alcohol and by the benzodiazepines (p. 322). Dopamine, produced by decarboxylation of dopa (**B**), is itself a neurotransmitter and is also a precursor for the catecholamines epinephrine and norepinephrine (p. 434).

Disorders of dopamine synthesis in the *substantia nigra* of the midbrain lead to Parkinson disease (p. 370). Some psychiatric disorders are also associated with changes in the brain dopamine level. The biogenic amine *serotonin*, a signaling substance that has many effects, is synthesized from tryptophan via 5-hydroxytryptophan. For example, it regulates vascular tone and thus blood pressure, promotes intestinal peristalsis and platelet aggregation (p. 294) and acts as a neurohormone (p. 358) in the brain. *Histamine* is a central factor in the development of allergic reactions (pp. 312, 436).

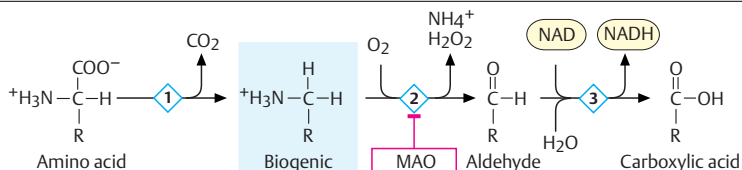
Several important biogenic amines are broken down into aldehydes and inactivated by *amine oxidase* (monoamine oxidase, “MAO”, [2]) with deamination and simultaneous oxidation. Further oxidation of these aldehydes by dehydrogenases (**3**) results in the corresponding carboxylic acids (p. 434). MAO inhibitors play an important role in pharmacological interventions in neurotransmitter metabolism.

A. Selenocysteine

Enzyme	Function
Glutathione peroxidase	Degradation of lipid hydroperoxides (R-O-O-H)
Deiodinases	Conversion of thyroxine (T ₄) to triiodothyronine (T ₃)
Thioredoxin reductases	Oxidation protection, synthesis of deoxy-ribonucleotides

1. Properties**2. Occurrence****B. Nonproteinogenic amino acids****C. Biogenic amines**

Amino acid	Amine	Function	Amino acid	Amine	Function
Serine	Ethanol-amine	Component of phospholipids	Glutamate	γ -Amino-butyrate	Neurotransmitter (GABA)
Cysteine	Cysteamine	Component of coenzyme A	Histidine	Histamine	Mediator, neurotransmitter
Threonine	Amino-propanol	Component of vitamin B ₁₂	Dopa	Dopamine	Neurotransmitter
Aspartate	β -Alanine	Component of coenzyme A	5-Hydroxy-tryptophan	Serotonin	Mediator, neurotransmitter
Ornithine	Putrescine	Polyamine precursor	Arginine	Agmatine	Neuromodulator

**1** Amino acid decarboxylase**2** Monoamine oxidase**3** Aldehyde dehydrogenase

Peptides and Proteins: Overview

When amino acids are linked together by acid–amide bonds, linear chain molecules (peptides) are produced. Those with a length of up to about 100 residues are called **peptides** (oligo- and polypeptides), while polypeptides containing more than 100 amino acid residues are described as **proteins**.

A. Proteins

Every organism contains thousands of different proteins, which have a variety of functions. At a magnification of ca. 1.5 million, the semi-schematic illustration shows the structures of a few intra- and extracellular proteins, giving an impression of their variety. The proteins can be classified as follows, according to their functions:

Structural proteins. Structural proteins are responsible for the *shape and stability* of cells and tissues. A small part of a **tropocollagen** molecule (collagen triple helix) is shown on the right as an example (p. 58). The complete molecule is 1.5×300 nm in size, and at the magnification used here it would be as long as three pages of the book. The **histones** (top right) organize the packing of DNA in the cell nucleus and regulate transcription. The basic components of chromatin (p. 234), the **nucleosomes**, consist of a complex of histones, around which DNA is coiled.

Transport proteins. A well-known transport protein is **hemoglobin** in the erythrocytes (bottom left). It facilitates the transport of oxygen and carbon dioxide between the lungs and tissues (p. 286). The blood plasma also contains many other proteins with transport functions. **Prealbumin** (transferrin; middle), for example, transports the thyroid hormones thyroxine and triiodothyronine. **Ion channels** (middle) and other integral membrane proteins facilitate the transport of ions and metabolites across biological membranes (p. 212).

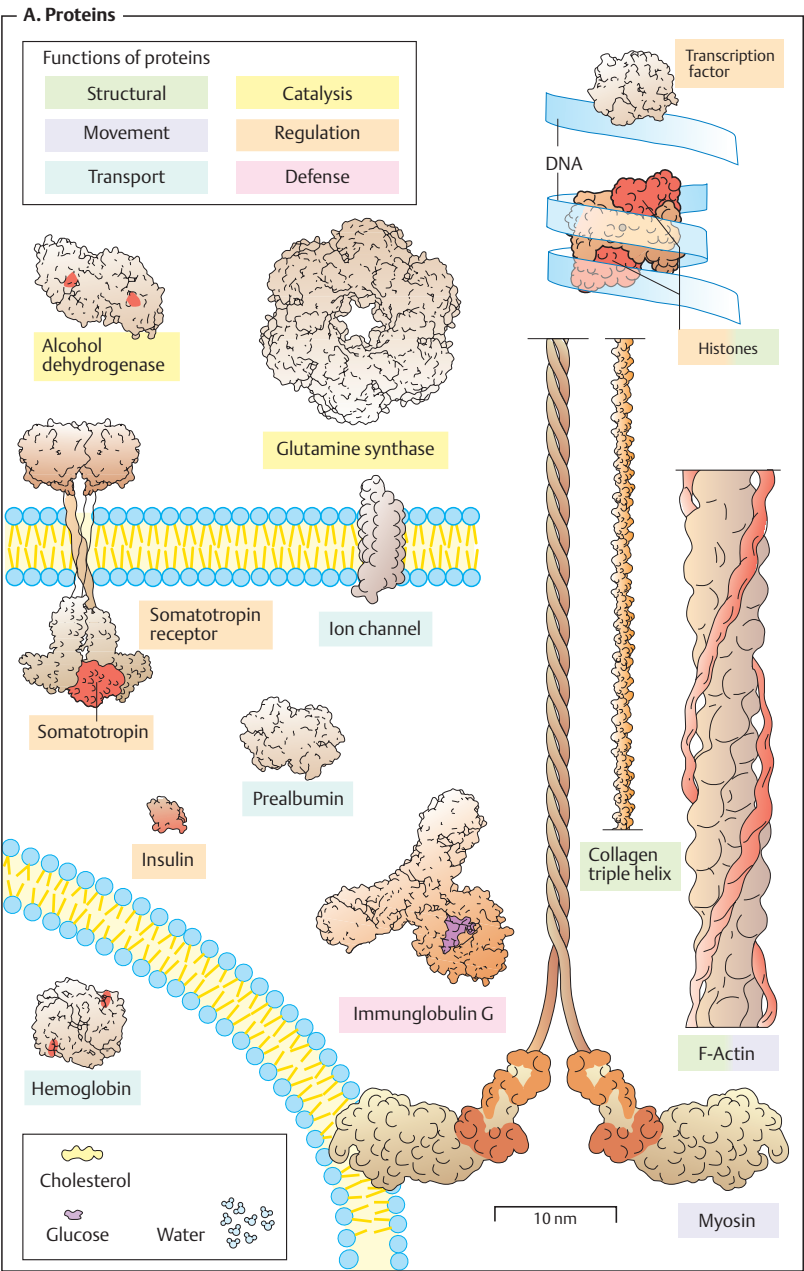
Defense proteins. The immune system protects the body from pathogens and foreign substances. An important component of the immune system is **immunoglobulin G**. It functions as an antibody (p. 310) to support the specific immune defense.

Regulatory proteins. In biochemical signal chains, proteins function as signaling substances (hormones) and also as hormone receptors. The complex between the growth hormone **somatotropin** (p. 432) and its **receptor** is shown here as an example (middle left). Here,

the extracellular domains of two receptor molecules bind one molecule of the hormone. This binding activates the cytoplasmic domains of the complex, leading to further conduction of the signal to the interior of the cell (signal transduction, p. 398). The hormone **insulin** (lower middle left) is discussed in detail elsewhere (p. 428). DNA-binding proteins (*transcription factors*; p. 240f.) are decisively involved in regulating the metabolism and in differentiation processes. The structure and function of the **catabolite activator protein** and similar bacterial transcription factors (top right) have been particularly well investigated. **Catalytic proteins.** *Enzymes*, with more than 2000 known representatives, are the largest group of proteins in terms of numbers (p. 72ff.). The smallest enzymes have molecular masses of 10–15 kDa. Intermediate-sized enzymes, such as **alcohol dehydrogenase** (top left) are around 100–200 kDa, and the largest—including **glutamine synthetase** with its 12 monomers (top middle)—can reach more than 500 kDa in size.

Motor proteins. The interaction between actin and myosin is responsible for muscle contraction and cell movement (pp. 202, 340). **Myosin** (right), with a length of over 150 nm, is among the largest proteins there are. Actin filaments (**F-actin**) arise due to the polymerization of relatively small protein subunits (G-actin). Along with other proteins, **tropomyosin**, which is associated with F-actin, controls contraction.

Storage proteins (not shown). Plants contain special storage proteins, which are also important for human nutrition, for example gluten in cereals. In animals, *muscle proteins* constitute a nutrient reserve that can be mobilized in emergencies. Thus, during prolonged periods of starvation, humans can ensure their glucose supply by metabolizing up to 6 kg of muscle protein (p. 378).



Protein Structures

A. Peptides

The amino acid components of peptides and proteins are linked together by *amide* bonds (p. 6) between α -carboxyl and α -amino groups. This type of bond is therefore also known as a **peptide bond**. In the **dipeptide** shown here (1), the serine residue has a free ammonium group, while the carboxylate group in alanine is free. Since the amino acid with the free NH_3^+ group is named first (see below), the peptide is known as **seryl alanine**, or in abbreviated form Ser-Ala or SA.

Like all acid–amide bonds, the peptide bond is **stabilized by resonance** and is therefore **planar**. Rotation around the C–N bond would only be possible at the expense of large amounts of energy, and the bond is therefore *not freely rotatable*.

Peptide chains have a **direction** and therefore two different ends (2). The amino terminus (**N-terminus**) has a free ammonium group, while the carboxyl terminus (**C-terminus**) is formed by the carboxylate group of the last amino acid. In peptides and proteins, the amino acid components are usually linked in linear fashion. To express the **amino acid sequence** of a peptide, it is therefore sufficient to combine the three-letter or single-letter abbreviations for the amino acid residues (p. 51). This sequence always starts on the left with the N-terminus. For example, the peptide hormone *angiotensin II* (p. 338) has the sequence Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, or DRVYIHFP.

B. Structural levels of proteins

There are various different structural levels in proteins; these will be explained using hemoglobin as an example.

The **primary structure** of a protein is its **amino acid sequence** (B). A partial sequence from the α subunit of hemoglobin is shown, involving amino acid residues 53–74.

Secondary structures are *regions of the peptide chain with a defined conformation*, stabilized by H bonds. The partial sequence 53–74 referred to above is folded into an α helix (see C). The **three-dimensional conformation** of a protein, made up of secondary structural elements and unordered sections, is referred to as the **tertiary structure**. In the case of the hemoglobin α subunit, this results in a compact and almost rectangular structure.

Quaternary structure. Due to non-covalent interactions, many proteins come together to

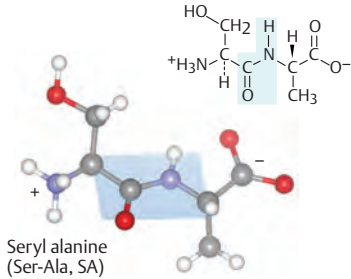
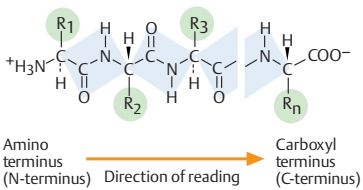
form symmetrical complexes (oligomers). The individual components of oligomeric proteins (usually 2–12) are termed *subunits* or *monomers*. In hemoglobin tetramer, two α (brown) and two β (green) subunits form a tetramer.

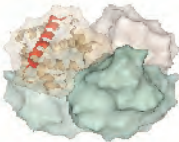
C. Secondary structures

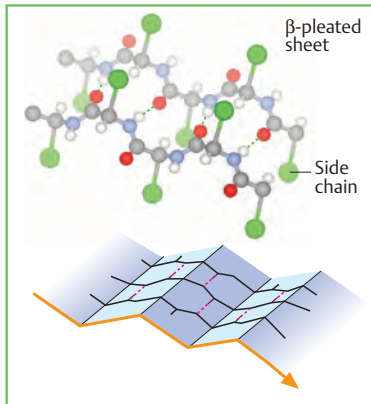
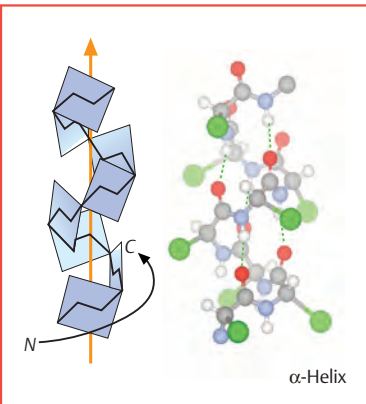
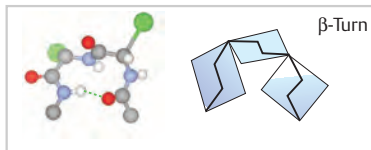
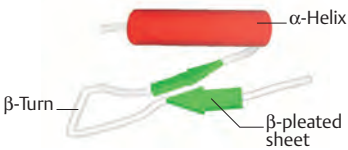
The **right-handed α -helix** (α_R) is one of the most common secondary structures. In this conformation, the peptide chain is wound like a screw. Each turn of the screw (the screw axis is shown in orange) covers about 3.6 amino acid residues. The **pitch** of the screw (i.e., the smallest distance between two equivalent points) is 0.54 nm. α -Helices are stabilized by almost linear **hydrogen bonds** between the NH and CO groups of residues, which are four positions apart from one another in the sequence (indicated by green dotted lines).

Two additional, almost fully extended, conformations of the peptide chain are known as **β -pleated sheets**, as the peptide planes are arranged like a regularly folded sheet of paper. In pleated sheets, H bonds can only form between **neighboring chains** (“strands”). When the two strands run in opposite directions, the structure is referred to as an **antiparallel pleated sheet** (β_A). When they run in the same direction, it is a **parallel pleated sheet** (β_P). In both cases, the α -C atoms occupy the highest and lowest points in the structure, and the side chains point alternately straight up or straight down. The β_A structure, with its almost linear H bonds, is energetically more favorable.

β -Turns are often found at sites where the peptide chain changes direction. These are sections in which four amino acid residues are arranged in such a way that the course of the chain reverses into the opposite direction. β -Turns are stabilized by a H bond between residues 1 and 4. They are often located between the individual strands of β -pleated sheets, or between strands of pleated sheets and α -helices.

A. Peptides**1. Peptide bonds****2. Peptide nomenclature****B. Structural levels of proteins**

Primary structure	-Ala-Gln-Val-lys-Gly-His-Gly-lys-Lys-Val-Ala-Asp-Ala-Leu-Thr-Asn-Ala-Val-Ala-His-Val-
Secondary structure	 Helix 53-74
Tertiary structure	 Hemoglobin- α_1 -subunit
Quaternary structure	 Hemoglobin tetramer

C. Secondary structures

Structural Proteins

Fibrillary proteins and protein complexes act as **structural proteins**. They give extracellular structures mechanical stability, and are also involved in the structure of the cytoskeleton (p. 198ff.). Most structural proteins contain a high percentage of specific secondary structures (p. 56). For this reason, their amino acid composition is also usually characteristic (see below).

A. α -Keratin

α -Keratin is a structural protein that predominantly consists of α -helices. Hair (wool), feathers, nails, claws and the hooves of animals consist largely of keratin. It is also an important component of the cytoskeleton (cytokeratin), where it appears in intermediate filaments (p. 198).

In the keratins, large parts of the peptide chain show right-handed α -helical coiling. Two chains each form a left-handed *superhelix*, as is also seen in myosin (p. 340). The superhelical keratin dimers in turn join to form tetramers, and these aggregate further to form **protofilaments**, with a diameter of 3 nm. Finally, eight protofilaments then form an **intermediate filament**, with a diameter of 10 nm.

Similar keratin filaments are found in **hair**. In a single wool fiber with a diameter of 20 μ m, millions of filaments are bundled together within dead cells. The individual keratin helices are cross-linked and stabilized by numerous disulfide bonds (p. 60). The chemical properties of disulfide bonds are exploited in the *perming* of hair. Initially, the disulfide bonds of hair keratin are disrupted by reduction with thiol compounds (see p. 12). The hair is then styled in the desired shape and heat-dried. In the process, new disulfide bonds are formed by oxidation, which maintain the hairstyle for some time.

B. Collagen

Collagen is quantitatively the most important protein in mammals, making up about 25% of the total protein. There are many different forms of collagen, particularly in connective tissue (p. 350). Collagen has an unusual amino acid composition. Approximately one-third of the amino acids are *glycine* (Gly), about 10% *proline* (Pro), 10% *hydroxyproline* (Hyp) and 10% *hydroxylysine* (Hyl). The two latter amino acids are only formed during collagen biosyn-

thesis as a result of *post-translational modification* (p. 62).

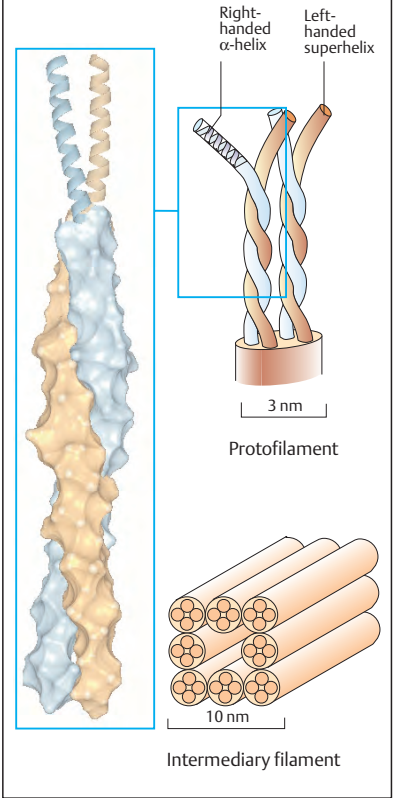
The triplet Gly-X-Y is constantly repeated in the sequence of collagen, with the X position often being occupied by Pro and the Y position by Hyp (1). The reason for this is that collagen is largely present as a **triple helix** made up of three individual collagen helices held together by hydrogen bonds (2). In triple helices, every third residue lies on the inside of the molecule, where for steric reasons there is only room for glycine residues (3; the glycine residues are shown in yellow). Only a small section of a triple helix is illustrated here. The complete collagen molecule is approximately 300 nm long.

C. Silk fibroin

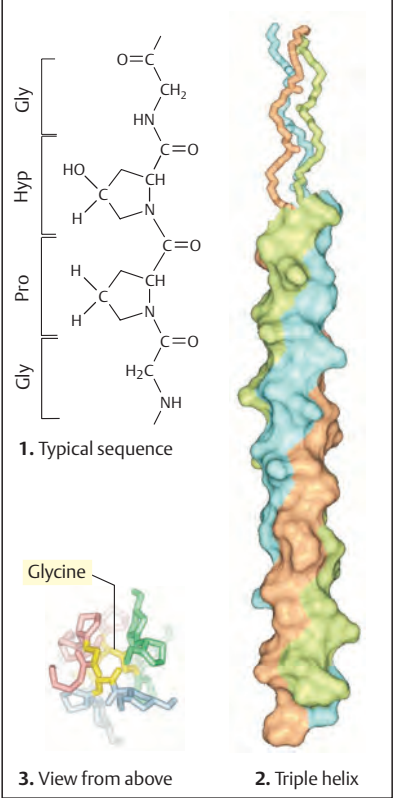
Silk is produced from the spun threads of silkworms (the larvae of the moth *Bombyx mori* and related species). Silk fibers contain elastic as well as highly ordered ("crystalline") segments and 80% of the fibers consist of the protein **fibroin**.

In fibroin, antiparallel β -pleated sheet structures are arranged one on top of the other in numerous layers (on the right). Since the amino acid side chains in pleated sheets point either straight up or straight down (p. 56), only compact side chains fit between the layers. In fact, more than 80% of fibroin consists of glycine, alanine, and serine—the three amino acids with the shortest side chains. A typical repetitive amino acid sequence is (Gly-Ala-Gly-Ala-Gly-Ser) $_n$. The individual pleated sheet layers in fibroin have been found to lie alternately 0.35 nm and 0.57 nm apart. In the first case, only glycine residues (R = H) are opposed to one another. The slightly greater distance of 0.57 nm results from repulsion forces between the side chains of alanine and serine residues.

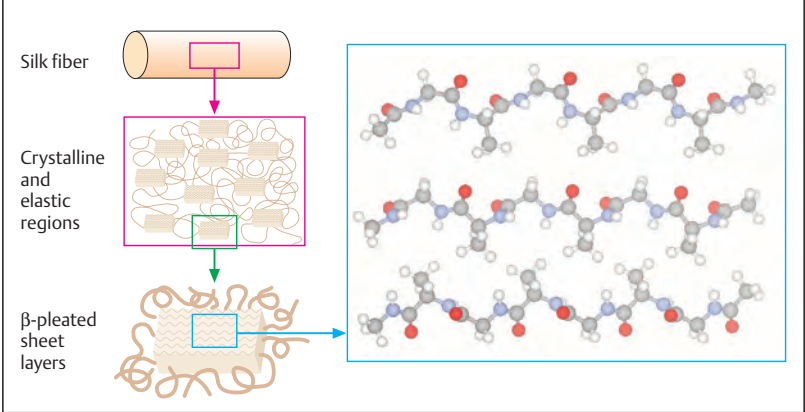
A. α -Keratin



B. Collagen



C. Silk fibroin



Soluble Proteins

Soluble proteins have a more complex structure than the fibrous, insoluble structural proteins (p. 58). The shape of soluble proteins is more or less spherical (globular). In their biologically active form, **globular proteins** have a defined spatial structure (the **native conformation**). If this structure is destroyed by **denaturation** (see below), not only does the biological function disappear, but also the protein usually precipitates in insoluble form. This happens, for example, when eggs are boiled; the proteins dissolved in the egg white are denatured by the heat and produce the solid egg-white.

To illustrate protein conformations in a clear (but extremely simplified) way, *Richardson diagrams* are often used (see, e.g., bottom right). In these diagrams, α -helices are symbolized by red cylinders or spirals and strands of pleated sheets by green arrows. Less structured areas of the chain, including the β -turns, are shown as sections of gray tubing.

A. Soluble proteins

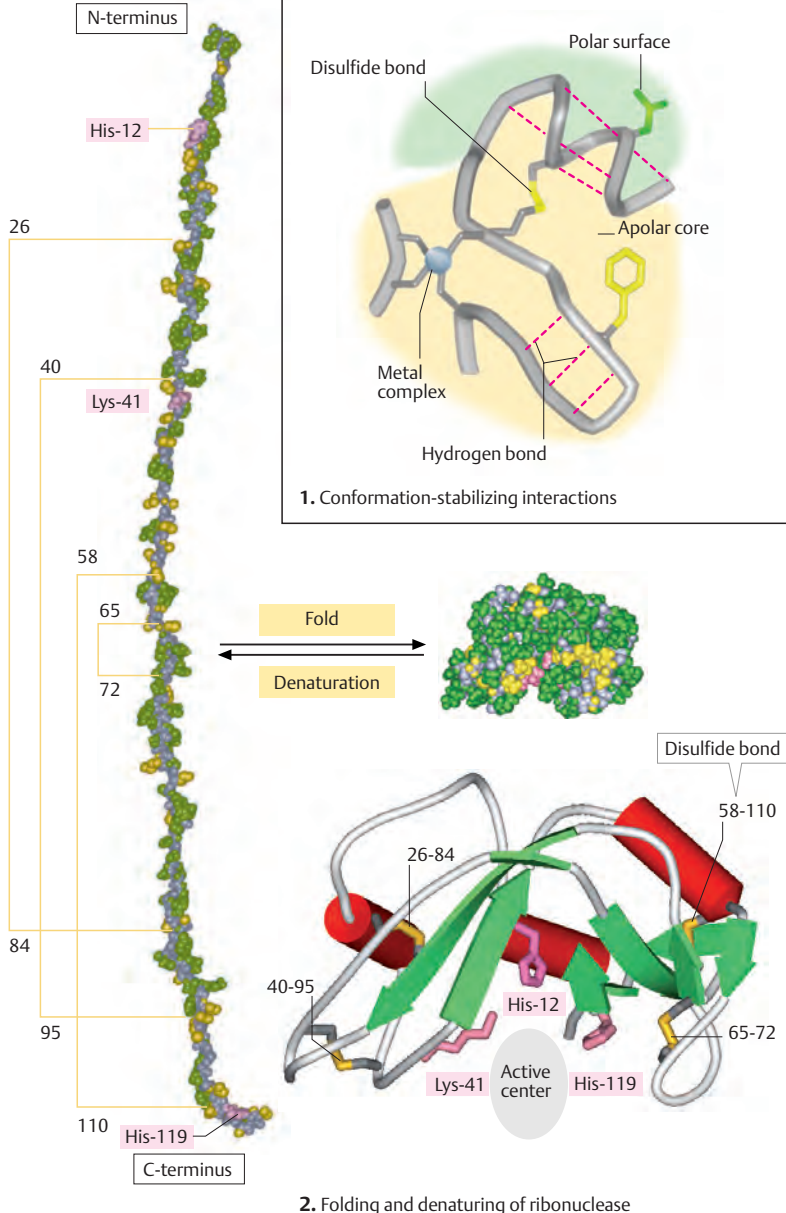
The native conformation of proteins is stabilized by a broad range of different interactions (1). In all proteins, large numbers of **hydrogen bonds** (p. 22) are involved in maintaining the native conformation. They form not only inside secondary structures (p. 56), but also between side chains of more distant residues. Many proteins are also stabilized by **complex formation** with metal ions (p. 78). The **hydrophobic effect** (p. 24) is particularly important for protein stability. In globular proteins, most hydrophobic amino acid residues are arranged in the interior of the structure in the native conformation, while the majority of the polar amino acids are on the surface in contact with the surrounding water (see below). The only covalent bonds that contribute to protein stability are **disulfide bonds** between cysteine residues. They are found particularly in extracellular proteins, since reducing conditions prevail in the interior of the cell, and disulfide bridges are cleaved under these conditions (p. 12).

Physiological conditions promote proteins to **fold** into their native form. **Denaturation**, that is, loss of the native conformation, occurs at extreme pH values, high temperatures, or under the influence of organic solvents, detergents, and other denaturing substances such as urea.

It was shown for the first time with **ribonuclease (2)**, a digestive enzyme (p. 268) consisting of 124 amino acids, that a denatured protein can revert spontaneously to its native conformation. In the native form (bottom right), there are extended pleated sheet structures and three α -helices. The protein's eight cysteine residues are connected to four disulfide bonds. Residues His-12, Lys-41 and His-119 (pink) are especially important for catalysis. Together with other amino acids, they form the **active center** of the enzyme.

The disulfide bonds of ribonuclease can be cleaved by reduction with **thiols**. If **urea** is also added at high concentration, the protein unfolds completely. In this form (on the left), it is up to 35 nm in length. In the denatured enzyme, polar (green) and nonpolar (yellow) side chains are distributed evenly. Because the amino acid residues important for catalysis (pink) are too far apart in the denatured protein to be able to interact with one another and with the substrate, the denatured protein is completely inactive.

When urea and the thiol are removed, the secondary and tertiary structures form again spontaneously. As a result, the cysteine residues come into close spatial proximity so that disulfide bonds can form again under the oxidative action of atmospheric oxygen. The active center is also reconstituted. Compared with the denatured protein, the native form is astonishingly compact, measuring 4.5×2.5 nm. In this state, the nonpolar side chains (yellow) are mainly in the interior of the protein, while the polar residues (green) are found predominantly on the surface. As mentioned above, this distribution is the result of the "hydrophobic effect" (p. 24). It plays an important part in the stability of the native configuration.

A. Soluble proteins

Protein Modification

A. Post-translational protein modification

Subsequent alteration of amino acid residues in finished peptides and proteins is referred to as *post-translational modification*. These reactions are catalyzed by enzymes in a specific manner and usually only involve the more reactive polar amino acid residues. Modifications serve very varied purposes (see below). Many of these reactions take place in the endoplasmic reticulum shortly after translation (p. 216f.), but others take place only at the site of action of the protein in question. The upper part of the illustration provides an overview of the most important post-translational modifications in eukaryotic cells. "D" indication a derivative and "B" a bond with the amino acid. Several products of modification reactions are shown below in formula form.

The free α -amino group at the *N-terminus* of many proteins is often blocked by an acetyl residue or a longer acyl residue—e.g., the myristyl residue (p. 38, **acylation**). It is estimated that 80% of all proteins in animals are acylated at the N-terminus, with soluble proteins usually being acetylated. N-terminal *glutamate* can cyclize into a pyroglutamate residue, while the C-terminal *carboxylate group* of some peptides and proteins can be converted to an acid amide.

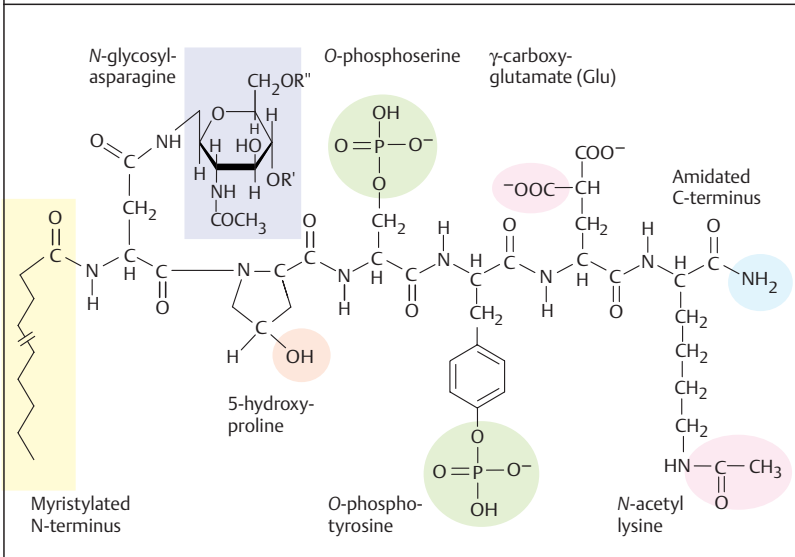
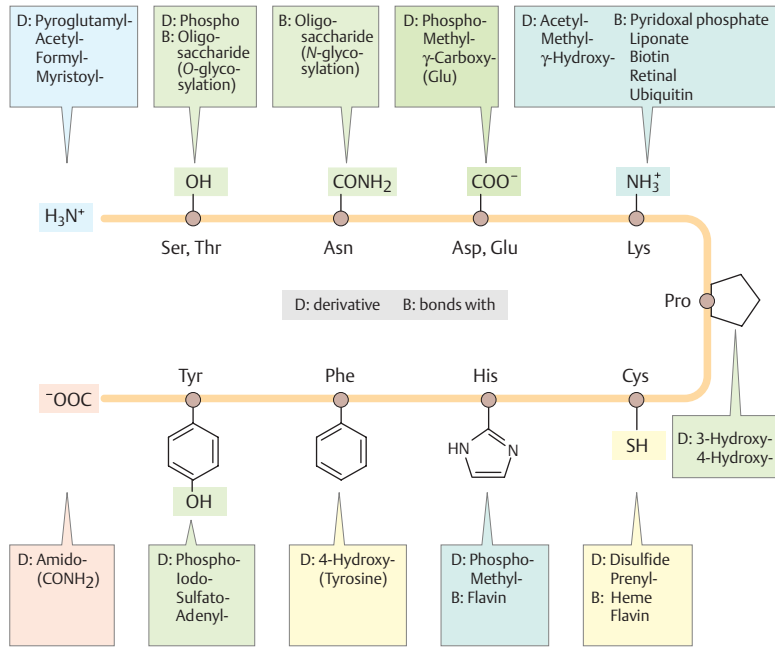
The side chains of *asparagine* and *serine* residues are often linked to oligosaccharides (**glycosylation**, pp. 34, 220). Extracellular proteins in particular are glycosylated and the proportion of carbohydrate can even exceed that of protein. The functional significance of protein glycosylation is not yet fully understood. In some cases, it is necessary for correct folding of the protein in the endoplasmic reticulum (p. 220f.). The plasma proteins, with the exception of albumin, are all glycosylated, and the carbohydrate moiety is used for recognition by liver receptors (p. 280), while in mucins (p. 268f.) and proteoglycans (p. 352) it increases the water-binding capacity. The carbohydrate moiety usually has hardly any effect on the actual functions of glycosylated proteins, e.g., the catalytic properties of enzymes. **Phosphorylation** of proteins mainly affects *serine* and *tyrosine* residues, which are thereby converted to phosphoric acid monoesters. *Aspartate* and *histidine* residues of enzymes are sometimes phosphorylated, too. Phosphorylation and dephosphorylation (interconversion) of proteins is a central mechanism in the regu-

lation of metabolism, cell proliferation and cell differentiation (pp. 100, 410).

A special modification of *glutamate* residues, vitamin K-dependent **γ -carboxylation**, is found in coagulation factors. It increases the ability of these factors to bind Ca^{2+} and is therefore essential for normal blood coagulation (p. 294). The ϵ -amino group of lysine residues is subject to a particularly large number of modifications. Its **acetylation** (or deacetylation) is an important mechanism for controlling gene activity (p. 234). Many *coenzymes* and *cofactors* are covalently linked to lysine residues. These include biotin (p. 88), lipoic acid (p. 112) and pyridoxal phosphate (p. 166), as well as the photoreceptor retinal (p. 368).

In collagen and some other proteins, *lysine* and *proline* residues are modified by **hydroxylation** to prepare for the formation of stable fibrils (p. 350). Hydroxylation of *asparagine* residues is involved in the reaction of cells to hypoxia (p. 126). Covalent bonding of a *cysteine* residue with heme is found in cytochrome c, for example (p. 122). Flavin coenzymes are also sometimes bound covalently to *cysteine* or *histidine* residues of enzymes.

Disulfide bonds between cysteine residues stabilize the tertiary and quaternary structure of many proteins. **Prenylation** is the term used for the bonding of the SH group of cysteine residues with the isoprenoids farnesol and geranylgeranol (p. 44). Like N-terminal acetylation, this serves to anchor proteins in membranes (p. 210). Among the modifications of *tyrosine* residues, besides phosphorylation, iodination is particularly interesting. This results in the hormones **thyroxine** and **triiodothyronine** (p. 426)

A. Post-translational protein modification

Bases and Nucleotides

The nucleic acids play a central role in the storage and expression of genetic information (p. 230f.). They are divided into two major classes: **deoxyribonucleic acid (DNA)** functions solely in information storage, while **ribonucleic acids (RNAs)** are involved in most steps of gene expression and protein biosynthesis. All nucleic acids are made up from **nucleotide components**, which in turn consist of a *base*, a *sugar*, and a *phosphate residue*. DNA and RNA differ from one another in the type of the sugar and in one of the bases that they contain.

A. Nucleic acid bases

The bases that occur in nucleic acids are *aromatic heterocyclic compounds* derived from either a **pyrimidine** or a **purine**. Five of these bases are the main components of nucleic acids in all living creatures. The purine bases **adenine** (abbreviation Ade, not "A"!) and **guanine** (Gua) and the pyrimidine base **cytosine** (Cyt) are present in both RNA and DNA. In contrast, **uracil** (Ura) is only found in RNA. In DNA, **uracil** is replaced by **thymine** (Thy), the 5-methyl derivative of uracil. 5-methylcytosine also occurs in small amounts in the DNA of the higher animals (p. 235). A large number of other modified bases occur in tRNA (p. 66) and in other types of RNA.

B. Nucleosides, nucleotides

When a nucleic acid base is *N*-glycosidically linked to ribose or 2-deoxyribose (p. 30), it yields a **nucleoside**. The nucleoside **adenosine** (abbreviation: A) is formed in this way from adenine and ribose, for example. The corresponding derivatives of the other bases are called **guanosine** (G), **uridine** (U), **thymidine** (T) and **cytidine** (C). When the sugar component is 2-deoxyribose, the product is a **deoxyribonucleoside**—e.g., 2'-deoxyadenosine (dA, not shown). In the cell, the 5'-OH group of the sugar component of the nucleoside is usually esterified with phosphoric acid. 2'-Deoxythymidine (dT, 2) for instance gives rise to **2'-deoxythymidine-5'-monophosphate (dTMP)**, one of the components of DNA. If the 5'-phosphate residue of a mononucleotide is linked via an acid-anhydride bond to additional phosphate residues, it yields nucleoside diphosphates and triphosphates—e.g., ADP and ATP, which are important coenzymes in energy metabolism (p. 88). All of these nucleoside

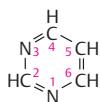
phosphates are classified as **nucleotides**. Cyclic nucleotides such as 3',5'-cyclic AMP (**cAMP**, 3) serve as second messengers in signaling systems (pp. 398, 406).

C. Oligonucleotides, polynucleotides

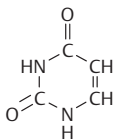
Phosphoric acid molecules can form acid-anhydride bonds with each other. It is therefore possible for two nucleotides to be linked via the phosphate residues. This gives rise to **dinucleotides with a phosphoric acid-anhydride structure** (1). This group includes the coenzymes NAD(P)⁺ and CoA, as well as the flavin FAD (p. 86).

If the phosphate residue of a nucleotide reacts with the 3'-OH group of a second nucleotide, the result is a **dinucleotide with a phosphate diester structure** (2). Dinucleotides of this type have a free phosphate residue at the 5' end and a free OH group at the 3' end. They can therefore be extended with additional mononucleotides by adding further phosphate diester bonds. This is the way in which **oligonucleotides**, and ultimately **polynucleotides**, are synthesized. In nucleosides and nucleotides, the pentose residues are present in furanose form (p. 28). The sugar and base are linked by a *N*-glycosidic bond between C-1 of the sugar and N-9 of the purine ring or N-1 of the pyrimidine ring. This bond always has the β configuration (p. 28).

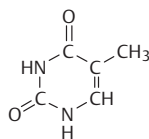
Polynucleotides consisting of ribonucleotide components are called **ribonucleic acid (RNA)** (p. 66), while those consisting of deoxyribonucleotide monomers are called **deoxyribonucleic acids (DNA)** (p. 68). To describe the base sequence of polynucleotides, the abbreviations for the *nucleoside* components are written from left to right in the 5'→3' direction. The position of the phosphate residue is also sometimes indicated by a "p". According to this system, the structure of the RNA segment shown in (3) is abbreviated as „ApUpG.. or simply as.. AUG...

A. Nucleic acid bases

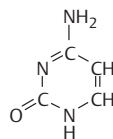
Pyrimidine



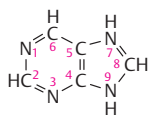
Uracil (Ura)



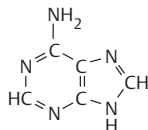
Thymine (Thy)



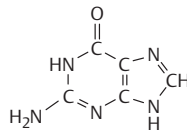
Cytosine (Cyt)



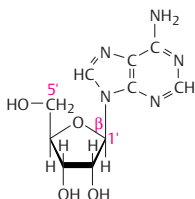
Purine



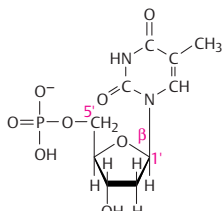
Adenine (Ade)



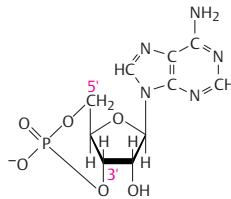
Guanine (Gua)

B. Nucleosides, nucleotides

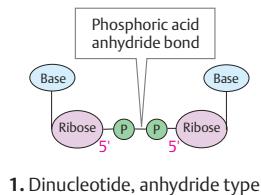
1. Adenosine, A



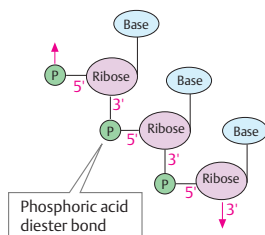
2. 2'-deoxythymidine-5'-monophosphate (dTMP)



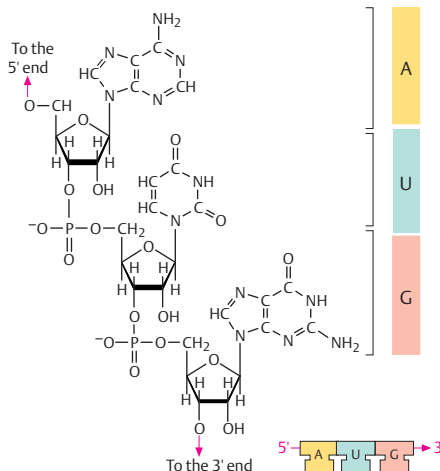
3. 3',5'-cyclo-adenosine-5'-monophosphate (cAMP)

C. Oligonucleotides, polynucleotides

1. Dinucleotide, anhydride type



2. Oligonucleotide, diester type



3. RNA (section)

RNA

Ribonucleic acids (RNAs) are polymers consisting of nucleoside phosphate components that are linked by phosphate diester bonds (p. 64). The bases they contain are mainly *uracil*, *cytosine*, *adenine*, and *guanine*, but also unusual and modified bases, which occur more often in tRNAs (see C). All RNAs are produced in the cell nucleus by transcription (p. 240) of DNA sequences. Thus, genes code not only for proteins but also for RNAs.

A. Ribonucleic acids (RNAs)

RNAs are involved in all the individual steps of gene expression and protein biosynthesis (p. 230). The properties of the most important forms of RNA are summarized in the table. The schematic diagram also gives an idea of the secondary structure of these molecules. Cellular RNAs vary widely in their size, structure, and lifespan. The great majority of them are ribosomal RNAs (**rRNAs**), which are also the most long-lived; they are the main structural and functional component of *ribosomes* (see B). Ribosomal RNAs are produced from DNA by transcription in the nucleolus, and they are processed there and finally assembled with proteins to form ribosome subunits (p. 204).

Messenger RNAs (**mRNAs**) transfer genetic information from the cell nucleus to the cytoplasm. The primary transcripts are substantially modified while still in the nucleus (mRNA maturation; p. 244). Due to the varying amounts of information that they carry, the lengths of mRNAs also vary widely. Their lifespan is usually short, as they are quickly broken down after translation.

Small nuclear RNAs (**snRNAs**) are involved in the splicing of mRNA precursors (p. 244). They associate with numerous proteins to form “spliceosomes.”

Even smaller RNA molecules (**miRNAs** and **siRNAs**) have been discovered recently, which are also produced by transcription and, together with other factors, control gene expression (not shown, see p. 262).

B. Ribosomal RNA

Ribosomes are large complexes consisting of several kinds of rRNA and numerous ribosomal proteins. They organize and catalyze the translation of mRNA (p. 248f.). As the illustration of a bacterial ribosome shows, the two

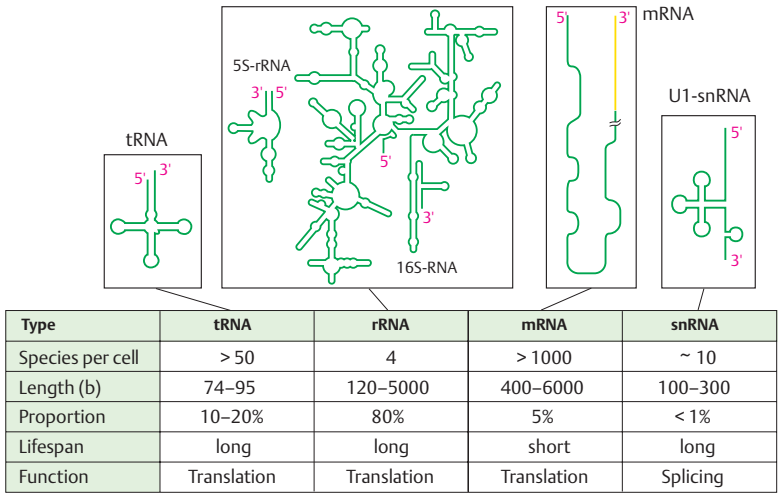
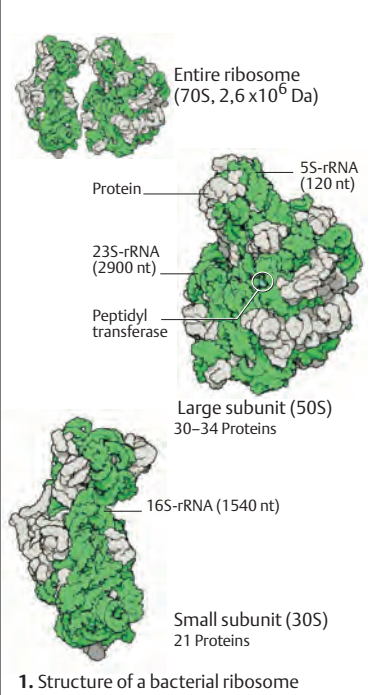
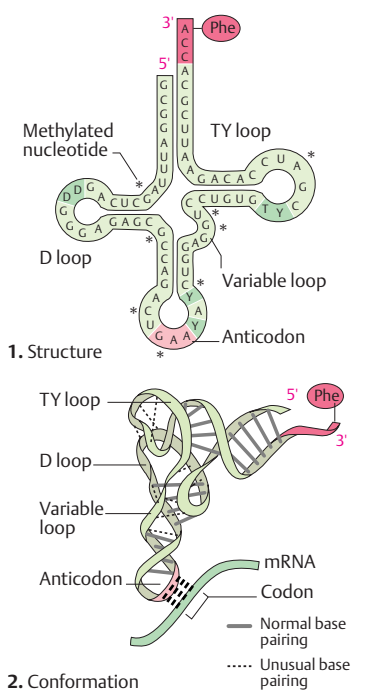
large rRNAs (**23S-rRNA** and **16S-rRNA**, green, p. 248) form the core of the two subunits, to which large numbers of small ribosomal proteins are attached (brown). The small **5S-rRNA** is found in addition on the outside of the large subunit. “S” indicates the sedimentation coefficient (p. 248). It is interesting that the *peptidyltransferase* activity of the ribosomes (p. 250) is not based in one of the ribosomal proteins but is located in a segment of the 23S-rRNA. Peptidyltransferase is therefore a *ribozyme* (p. 72).

Eukaryotic and prokaryotic ribosomes have a similar structure but they differ in the number and size of the rRNAs and ribosomal proteins (p. 248)

C. Transfer RNAs (tRNA^{Phe})

The transfer RNAs (**tRNAs**) function during translation (p. 250) as links between the nucleic acids and proteins. They are small RNA molecules consisting of 70–90 nucleotides (nt), which contain several “stem-loop” structures. tRNAs bound to the ribosome “recognize” specific mRNA codons with their **anticodons** through base pairing. At the same time, at their 3′ end (constant sequence, CCA) they carry the amino acid that is assigned to the relevant mRNA codon according to the genetic code (p. 246).

The base sequence and the structure of the yeast tRNA (**1**) specific for phenylalanine (tRNA^{Phe}) is typical of all tRNAs. The molecule contains a high proportion of unusual and modified components (shaded in dark green). These include *pseudouridine* (Y), *dihydrouridine* (D), *thymidine* (T), which otherwise only occurs in DNA, and many methylated nucleotides such as *7-methylguanine* (m⁷G) or—in the anticodon—*2′-O-methylguanine* (m²G). Numerous base pairs, sometimes deviating from the usual pattern, stabilize the molecule’s conformation (**2**).

A. Ribonucleic acids (RNAs)**B. Ribosomal RNA****C. Transfer RNAs (tRNA^{Phe})**

DNA

A. Base pairing in DNA

Like RNAs (p. 66), **deoxyribonucleic acids (DNAs)** are polymeric molecules consisting of nucleotide building blocks. Instead of ribose, however, DNA contains *2'-deoxyribose* as sugar, and the *uracil* base in RNA is replaced by *thymine* (5-methyluracil). The spatial structure of the two molecule types also differs.

Intact DNA consists of *two* polydeoxyribonucleotide molecules ("*strands*"). Each base in one strand is linked to a *complementary* base in the other strand by hydrogen bonds (p. 22). Adenine is complementary to thymine, and guanine is complementary to cytosine. One purine base and one pyrimidine base are thus involved in each **base pair**.

The complementarity of A with T and of G with C can be understood by considering the H bonds that are possible between the different bases. Potential *donors* are amino groups (in A, C and G) and ring NH groups (in G and T). Possible *acceptors* are carbonyl oxygen atoms (in T, C, G) and ring nitrogen atoms. *Two* linear and therefore highly stable bonds can thus be formed in A–T pairs, and *three* in G–C pairs.

Base pairings of this type are only possible, however, when the two strands run in opposite directions. In addition, they have to be intertwined to form a **double helix (B)**. Due to steric hindrance by the 2'-OH groups of the ribose residues, RNA is unable to form long double helices. The structure of RNA is therefore less regular than that of DNA (p. 66). The base pairing in DNA and RNA does not serve only to stabilize the molecules. Even more important is the fact that it allows specific "recognition" of complementary nucleotides during replication, transcription, and translation (p. 238ff.).

B. Structure of B-DNA

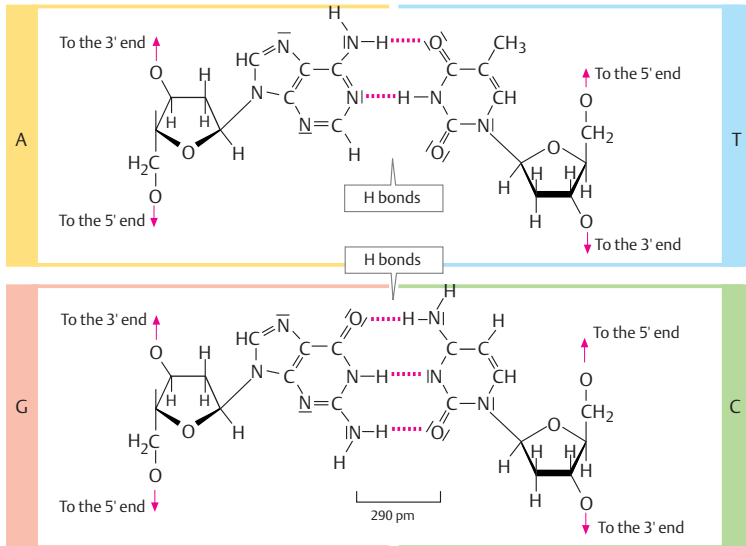
The conformation of DNA that predominates within the cell (known as **B-DNA**) is shown on the left as a space-filling model. The aromatic bases (light blue) are arranged on the inside of the **double helix** almost perpendicular to the axis of the helix. The central area of the DNA double helix is therefore nonpolar. By contrast, the molecule's surface is markedly polar and negatively charged, due to the hydrophilic sugar and phosphate residues in the backbone (dark blue). Along the whole length of the DNA molecule, there are two depressions—referred to as the "*minor groove*" and the "*major*

groove"—that lie between the strands. The schematic structure (center) shows their position, also demonstrating that the two strands run antiparallel.

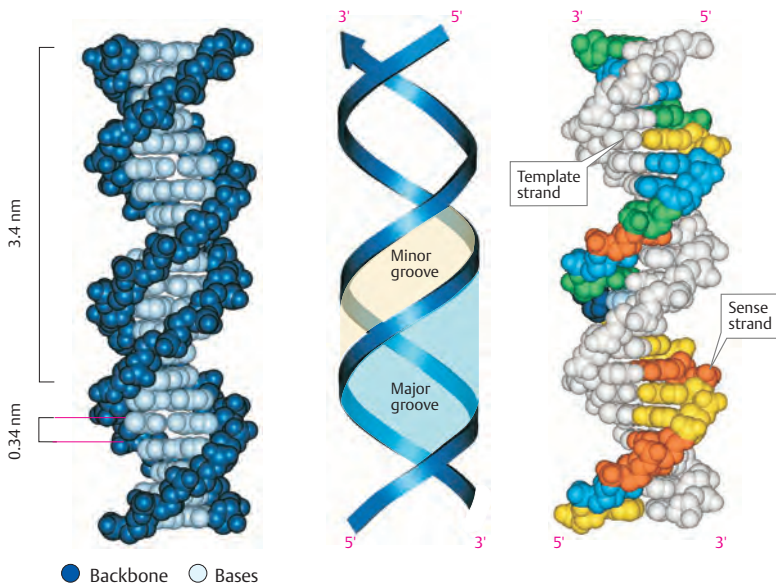
In all living cells, DNA serves to **store genetic information**. Specific segments of DNA ("*genes*," p. 232) are transcribed as needed into RNAs, which either carry out structural or catalytic tasks themselves or provide the basis for synthesizing proteins. In the latter case, the DNA codes for the primary structure of proteins. The "language" used in this process has four letters (A, G, C, and T). A total of 64 words ("*codons*"), each containing three letters, can be formed which stand for one of the 20 proteinogenic amino acids or signal the end of the "text" (p. 246).

The diagram on the right shows that the two strands of DNA are not functionally equivalent. The **template strand** (the (–) strand or "coding strand," shown in light gray) is the one that is copied during transcription (p. 240). Its sequence is therefore complementary to the mRNA formed. By contrast, the **sense strand** (the (+) strand or "coding strand," shown in color) has the *same sequence as the mRNA*, except that T is exchanged for U. By convention, it is agreed that gene sequences are expressed by reading the sequence of the sense strand in the 5'→3' direction. Using the genetic code (p. 246), in this case the protein sequence is obtained directly in the reading direction usual for proteins—i.e., from the N-terminus to the C-terminus.

A. Base pairing in DNA



B. Structure of B-DNA



Metabolism

Basics

A. Biological catalysts

Biological catalysts are substances of biological origin that accelerate chemical reactions (p. 20). Nearly all biological catalysts are **enzymes**—i.e., proteins with a catalytic action. However, there are also catalytically active ribonucleic acids, the **ribozymes**. The orderly course of metabolic processes is only possible because each cell is equipped with its own genetically determined set of enzymes. It is only because of this that coordinated sequences of reactions take place in cells (**metabolic pathways**; p. 96ff.). Enzymes are also involved in many regulatory mechanisms that allow the metabolism to adapt to changing conditions (p. 100). The schema symbolizes an enzyme with its active center (p. 75 B).

B. Specificity of enzyme catalysis

The action of enzymes is usually highly *specific*. This applies not only to the type of reaction being catalyzed (**reaction specificity**), but also to the nature of the reactants (“substrates”) that are involved (**substrate specificity**). Moreover, most enzymes are able to distinguish between stereoisomers (**stereospecificity**; p. 4). In the diagram, this is illustrated schematically using an important redox reaction as an example.

When glucose is metabolized within the cell, pyruvate is produced, the anion of a 2-oxoacid (p. 130). Under anaerobic conditions, pyruvate is reduced further to lactate, the anion of the corresponding 2-hydroxy acid, with the aid of the coenzyme NADH (p. 86). Under aerobic conditions, the reaction goes in the reverse direction. Both conversions are catalyzed by the enzyme *lactate dehydrogenase*.

Lactate dehydrogenase is not entirely specific with regard to its substrates. Apart from pyruvate, it also accepts other short-chain 2-oxoacids (or 2-hydroxy acids). As the table shows, the kinetic parameters k_{cat} and K_{m} for the reduction of different 2-oxoacids are quite different (see p. 76 for an explanation of these parameters). The enzyme has greatest activity (expressed as the $k_{\text{cat}}/K_{\text{m}}$ ratio) on pyruvate ($R = \text{CH}_3$). By comparison, the substrate with the larger branched chain ($R = -\text{CH}_2-\text{CH}(\text{CH}_3)_2$) is converted 200 000 times more slowly. Since lactate has a chiral center, there are two *enantiomers* (L-lactate and D-lactate). In animals, the reduction of pyruvate by the stereospecific

L-lactate dehydrogenase produces L-lactate almost exclusively, whereas the D-enantiomer is formed during bacterial lactic acid fermentation.

C. Enzyme classification

More than 2000 different enzymes are currently known. A system of *classification* has been developed that takes into account both their *reaction specificity* and their *substrate specificity*. Each enzyme is entered in the *Enzyme Catalogue* with a four-digit Enzyme Commission number (**EC number**). The first digit indicates membership of one of the six **major classes**. The next two indicate subclass and sub-subclass. The last digit indicates where the enzyme belongs in the sub-subclass. For example, lactate dehydrogenase (see **B**) has the EC number 1.1.1.27 (class 1, oxidoreductases; subclass 1.1, CH–OH group as electron *donor*; sub-subclass 1.1.1, NAD(P)⁺ as electron *acceptor*).

Enzymes with similar reaction specificities are grouped into each of the six major classes. The table lists their names, the types of reaction catalyzed, and the names of important sub-classes.

The **oxidoreductases** (class 1) catalyze redox reactions (i.e., the transfer of electrons between redox systems).

The **transferases** (class 2) are responsible for the transfer of other groups, e.g., amino groups or phosphate residues. Oxidoreductases and transferases always require coenzymes (p. 86ff.).

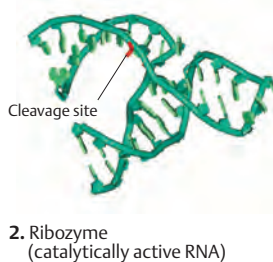
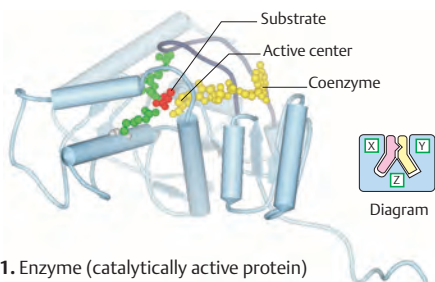
The **hydrolases** (class 3) are also involved in group transfer, but the acceptor is not a coenzyme but a *water molecule*.

Lyases (class 4, also referred to as “synthases” depending on the preferred reaction direction) catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing.

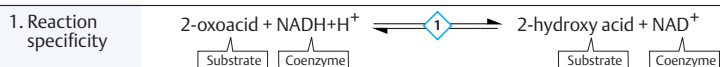
The **isomerases** (class 5) move groups within a molecule, without changing the molecular formula of the substrate.

The ligation reactions catalyzed by **ligases** (“synthetases,” class 6) are endergonic and they are therefore energetically coupled to the cleavage of nucleoside triphosphates (usually ATP).

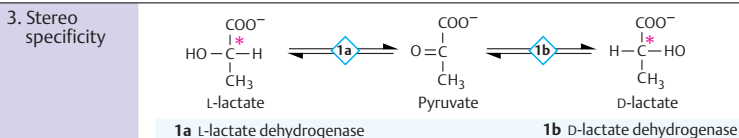
A. Biological catalysts



B. Specificity of enzyme catalysis



2. Substrate specificity	R	rel. k_{cat}	K_m (mM)
COO^-	-CH ₃	100	0.09
$\text{O}=\text{C}$	-H	60	3.2
R	-CH ₂ -CH ₃	50	0.6
	-CH ₂ -CH ₂ -CH ₃	6	1.9
	-CH ₂ -CH(CH ₃) ₂	0.02	3.6



C. Enzyme classification

Class	Reaction type	Important subclasses
1 Oxidoreductases	$\text{A}_{\text{red}} + \text{B}_{\text{ox}} \rightleftharpoons \text{A}_{\text{ox}} + \text{B}_{\text{red}}$	Dehydrogenases Oxidases, Peroxidases Oxygenases
2 Transferases	$\text{A} - \text{B} + \text{C} \rightleftharpoons \text{A} + \text{B} - \text{C}$	C ₁ -Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	$\text{A} + \text{H}_2\text{O} \rightleftharpoons \text{A} - \text{H} + \text{B} - \text{OH}$	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	$\text{A} + \text{B} \rightleftharpoons \text{A} - \text{B}$	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	$\text{A} \rightleftharpoons \text{A}'$	Epimerases <i>cis-trans</i> Isomerases Intramolecular transferases
6 Ligases ("synthetases")	$\text{A} + \text{B} + \text{ATP} \rightleftharpoons \text{A} + \text{B} + \text{ADP} + \text{P}_i$	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Enzyme Catalysis

Enzymes are extremely effective **catalysts**. They can increase the rate of a catalyzed reaction by a factor of 10^{15} or more (p. 20). To grasp the mechanisms involved in enzyme catalysis, we can start by looking at the course of an uncatalyzed reaction.

A. Uncatalyzed reaction

The reaction $A + B \rightarrow C + D$ is used as an example. In solution, reactants A and B are surrounded by a shell of water molecules (the **hydration shell**), and they move in random directions due to thermal agitation. They can only react with each other if they collide in a favorable orientation. This is unlikely, and therefore occurs very rarely. Before conversion into the products C + D, the **collision complex A–B** has to pass through a **transition state**, the formation of which requires a large amount of **activation energy**, E_a (p. 20). Since only a few A–B complexes can produce this amount of energy, a productive transition state arises much less often than a collision complex. In solution, a large proportion of the activation energy is required for the *removal of the hydration shells* between A and B. However, charge displacements and other *chemical processes* within the reactants also play a role. As a result of these limitations, conversion only happens occasionally in the absence of a catalyst, and the reaction rate, v , is low, even when the reaction is thermodynamically possible—i.e., when $\Delta G < 0$ (p. 16f.).

B. Enzyme-catalyzed reaction

Shown here is a *sequential mechanism* in which substrates A and B are bound and products C and D are released, in that order. Another possible reaction sequence is the so-called “*ping-pong mechanism*” (p. 76).

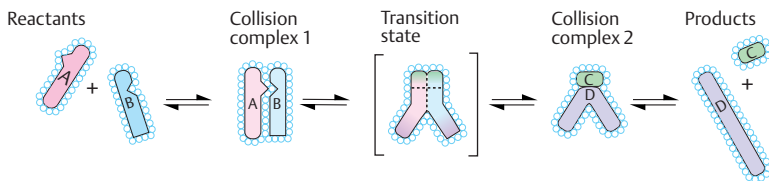
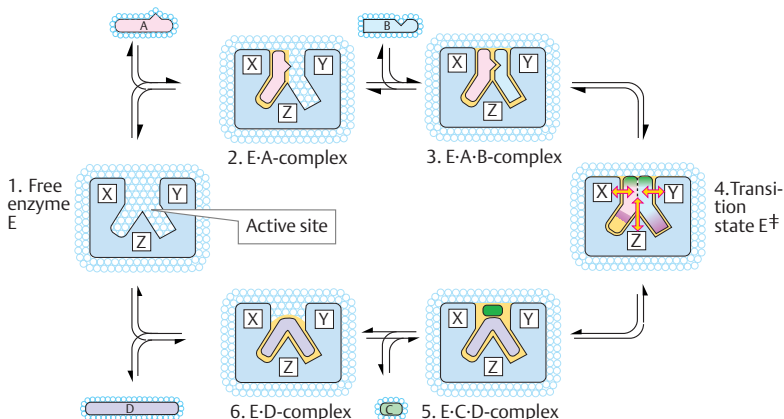
Enzymes are able to bind the reactants (their *substrates*) specifically at the **active center**. In the process, the substrates are oriented in relation to each other in such a way that they take on the *optimal orientation* for the formation of the transition state. The **proximity and orientation of the substrates** therefore strongly increase the likelihood that *productive* A–B complexes will arise. In addition, binding of the substrates results in removal of their hydration shells. As a result of the **exclusion of water**, very different conditions apply in the active center of the enzyme during catalysis than in

solution. A third important factor is the **stabilization of the transition state** as a result of interactions between the amino acid residues of the protein and the substrate. Coenzymes and other cofactors are often involved in the course of the reaction. This further reduces the activation energy needed to create the transition state. Moreover, many enzymes take up groups from the substrates or transfer them to the substrates during catalysis.

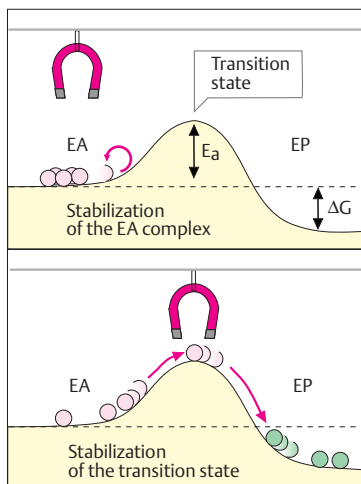
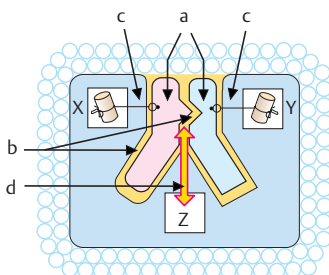
Proton transfers are particularly common. This **acid–base catalysis** by enzymes is much more effective than the exchange of protons between acids and bases in solution. In many cases, chemical groups are temporarily bound covalently to the amino acid residues of the enzyme during the catalytic cycle. This effect is referred to as **covalent catalysis** (see the transaminases, e.g., p. 166).

C. Principles of enzyme catalysis

Although it is difficult to provide quantitative estimates of the contributions made by individual catalytic effects, it is now thought that the enzyme's **stabilization of the transition state** is the most important factor. Therefore it is not tight binding of the *substrate* that is important for the effectiveness of catalysis—this would increase the activation energy required by the reaction, rather than reducing it—but rather the binding of the transition state. This conclusion is supported by the very high affinity of many enzymes for analogues of the transition state. A simple mechanical analogy may help clarify this (right). To transfer the metal balls (the reactants) from location EA (the substrate state) via the higher-energy transition state to EP (the product state), the magnet (the catalyst) has to be orientated in such a way that its attractive force acts on the transition state (below) rather than on EA (above).

A. Uncatalyzed reaction**B. Enzyme-catalyzed reaction****C. Principles of enzyme catalysis**

- Approximation and orientation of the substrates
- Exclusion of water
- Stabilization of the transition state
- Group transfer



Enzyme Kinetics I

The **kinetics** of enzyme-catalyzed reactions (the dependence of the reaction rate on the reaction conditions) is mainly determined by the *properties of the catalyst*. It is therefore more complex than the kinetics of an uncatalyzed reaction.

A. Michaelis–Menten kinetics

In the absence of an enzyme (on the left), the reaction rate, v , is proportional to the concentration of substance A. The constant k is the *rate constant* of the uncatalyzed reaction. Since the rate constant k_{cat} of the catalyzed reaction is much higher than k , its rate, v_e , is also much greater than v . The difference $v_e - v$ is called the **enzyme activity**. The recommended unit is the katal (kat, the activity that catalyzes the turnover of 1 mol of substrate per second). The **International Unit** (U, 1 μmol turnover per minute) is used more often as it is more practical.

Like all catalysts, the enzyme E creates a new reaction pathway (on right). Initially, A is bound to the free enzyme. Assuming that this reaction is in chemical equilibrium, the law of mass action can be applied and the concentrations $[E]$, $[A]$ and $[EA]$ can be linked. The **Michaelis constant** K_m thus describes the state of equilibrium of the substrate binding. If the total concentration of the enzyme $[E]_t$ is introduced and $[E]$ is eliminated, the expression $[EA] = [E]_t \cdot [A]/(K_m + [A])$ is obtained for the concentration $[EA]$. Like the conversion $A \rightarrow B$, the formation of B from EA is a first-order reaction—i.e., $v = k \cdot [EA]$ applies. When this equation is combined with the expression already derived for EA, the result is the **Michaelis–Menten equation** $v = k_{\text{cat}} \cdot [E]_t \cdot [A]/(K_m + [A])$.

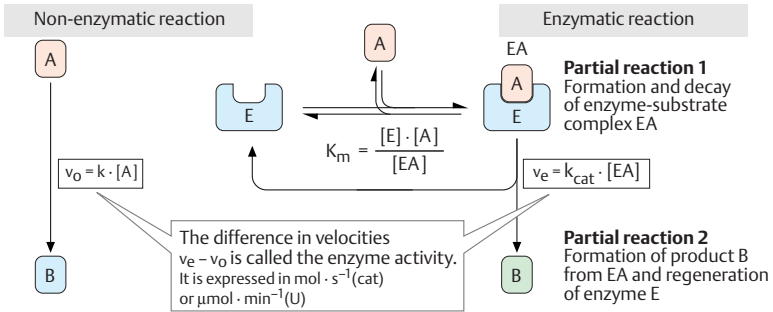
The equation contains two variables (v and $[A]$) and two *parameters* that do not depend on the substrate concentration $[A]$; the product $k_{\text{cat}} \cdot [E]_t$ is the limiting value for the reaction rate at a very high $[A]$, the **maximum velocity** V_{max} . The **Michaelis constant** K_m characterizes the *affinity* of the enzyme for a substrate. It corresponds to the substrate concentration at which v reaches half of V_{max} . A *high* affinity of the enzyme for a substrate therefore leads to a *low* K_m value, and vice versa. Of the two enzymes whose *substrate saturation curves* are shown in the middle diagrams 1 and 2, enzyme (b) has the higher affinity for A and its V_{max} , by contrast, is lower than that of enzyme (a).

The Michaelis–Menten model contains simplifying assumptions (substrate binding is in equilibrium, formation of B is irreversible, E and EA are the only enzyme forms). K_m corresponds to the dissociation constant of EA and k_{cat} to the product formation rate constants only when these assumptions are valid.

Since v approaches V_{max} *asymptotically*, it is difficult to read off reliable values for V_{max} or for K_m from diagrams plotting v against $[A]$. To get around this, the Michaelis–Menten equation can be arranged in such a way that the measured points lie on a *straight line*. In the **Lineweaver–Burk plot** (2), $1/v$ is plotted against $1/[A]$. The intersections of the line of best fit with the axes then produce $1/V_{\text{max}}$ and $-1/K_m$. This type of diagram is very clear, but for practical purposes it is less suitable for determining V_{max} and K_m . Computer programs are used today for this purpose.

B. Bisubstrate reactions

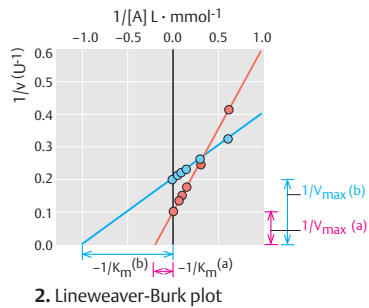
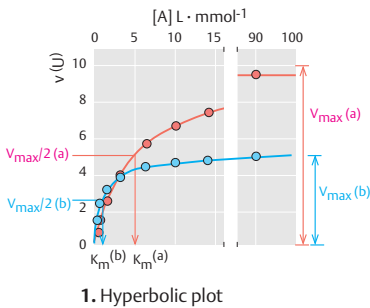
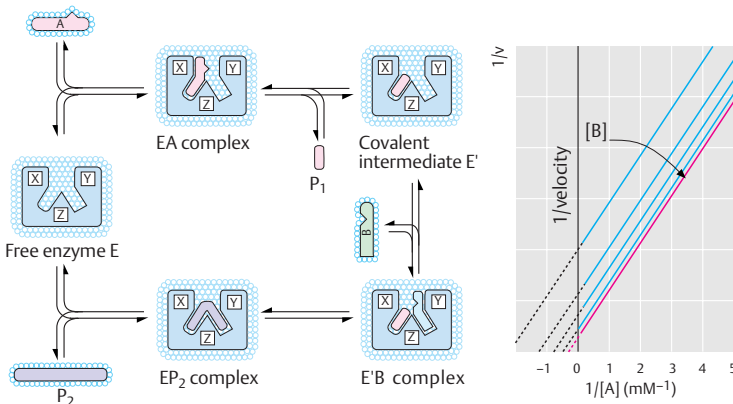
Almost all enzymes have more than one substrate or product. On the other hand, it is rare for more than two substrates to be bound *simultaneously*. In bisubstrate reactions of the type $A + B \rightarrow C + D$, several reaction sequences are possible. In addition to the *sequential mechanisms* (p. 74), in which all substrates are bound before the product is released, there are also mechanisms in which the first substrate A is bound and immediately cleaved. A part of this substrate remains bound to the enzyme, and is then transferred to the second substrate B after the first product P_1 has been released. This is known as the **ping-pong mechanism**, and it is used by *transaminases*, e.g., (p. 166). In the Lineweaver–Burk plot (right), it can be recognized in the parallel shifting of the lines when $[B]$ is varied.

A. Michaelis-Menten kinetics**Michaelis-Menten equation**

Maximum velocity
 $V_{\max} (\text{mol} \cdot \text{s}^{-1})$

$$v = \frac{V_{\max} \cdot [A]}{K_m + [A]}$$

Michaelis constant
 $K_m (\text{mol} \cdot \text{L}^{-1})$

**B. Bisubstrate reactions**

Enzyme Kinetics II

The catalytic properties of enzymes, and consequently their activity (p. 76), are influenced by numerous factors, which all have to be optimized and controlled if activity measurements are to be performed in a useful and reproducible fashion. These factors include physical quantities (temperature, possibly pressure), the chemical properties of the solution (pH value, ionic strength), and the concentrations of all relevant substrates, cofactors, and inhibitors.

A. pH and temperature dependency of enzyme activity

The effect of enzymes is strongly dependent on the pH value (p. 14). When the activity is plotted against pH, a *bell-shaped curve* is usually obtained (on the left). With animal enzymes, the **pH optimum**—i.e., the pH value at which enzyme activity is at its maximum—is often close to the pH value of the cells (i.e., pH 7). However, there are also exceptions to this. For example, the proteinase *pepsin* (p. 268), which is active in the acidic gastric lumen, has a pH optimum of 2, while other enzymes are at their most active at pH values higher than 9. The bell shape of the activity–pH profile results from the fact that amino acid residues with ionizable groups in the side chain are essential for catalysis. In the example, these are a basic group B ($pK_s = 8$), which has to be protonated to become active, and a second amino acid AH ($pK_s = 6$), which is only active in a dissociated state. At the optimum pH of 7, around 90% of both groups are present in the active form; at higher and lower values, one or the other of the groups increasingly passes into the inactive state.

The **temperature dependency** of enzymatic activity (on the right) is usually asymmetric. With increasing temperature, the increased thermal movement of the molecules initially leads to a rate acceleration (p. 20). At a certain temperature, the enzyme then becomes unstable, and its activity is lost within a narrow temperature range as a result of denaturation (p. 60). The optimal temperatures of the enzymes in higher organisms rarely exceed 50°C, while enzymes from thermophilic bacteria may still be active at 100°C.

B. Coenzymes

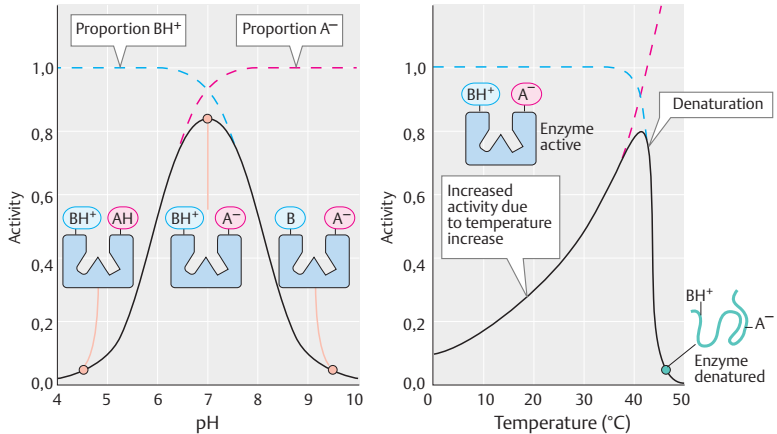
Enzymes that catalyze reactions in which groups are transferred usually require helper molecules called **coenzymes** (p. 86ff.). Their function is to provide the group to be transferred or accept it temporarily in the course of the reaction. As coenzymes are not catalytically active themselves, the less frequently used term “*cosubstrate*” would be more appropriate. In contrast to substrates for which a given enzyme is specific (p. 72), coenzymes cooperate with many enzymes of varying substrate specificity. Depending on the type of interaction with the enzyme, a distinction is made between soluble coenzymes and prosthetic groups. **Soluble coenzymes (1)** are *bound like substrates* during a reaction, undergo a chemical change, and are then *released again*. The original form of the coenzyme is regenerated by a second, independent reaction. **Prosthetic groups (2)**, on the other hand, are coenzymes that are *tightly bound to the enzyme* (sometimes covalently) and remain associated with it during the reaction. The part of the substrate bound by the coenzyme after release of the first product is then transferred in a second reaction to another substrate or coenzyme of the *same* enzyme.

Many coenzymes are aromatic compounds that cannot be synthesized from scratch by animal cells. Their precursors must therefore be provided in the diet as **vitamins** (p. 392f.).

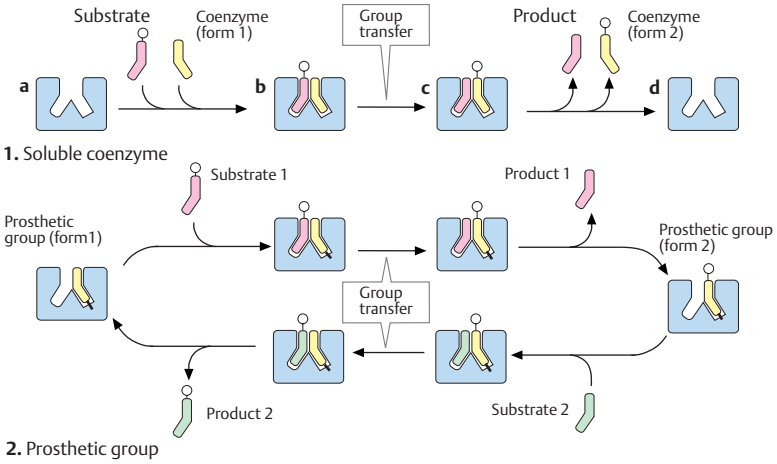
C. Metals as cofactors

Metal ions can also serve as enzyme cofactors. Their functions are very varied; some stabilize the native conformation of the active center, while others are involved in redox reactions (p. 12) or facilitate catalysis by polarizing chemical bonds in the substrate. The table lists important examples of metal-dependent enzymes. Metals that function as cofactors are required only in small amounts. They therefore belong to the group of **trace elements** (pp. 2, 384).

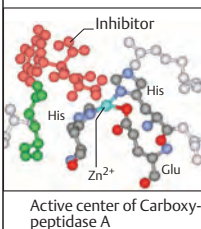
A. pH and temperature dependency of enzyme activity



B. Coenzymes



C. Metals as cofactors



Cofactor	Enzymes (examples)
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, peptidases
Mg^{2+}	ATP-dependent enzymes, phosphohydrolases
Mn^{2+}	Arginase, superoxide dismutase, photosystem II
Fe^{2+}/Fe^{3+}	Cytochrome, catalase, peroxidases, aconitase
Cu^{2+}	Cytochrome oxidase, amino oxidases, tyrosinase
Mo^{2+}	Xanthine dehydrogenase
Na^+, K^+	Na^+/K^+ ATPase, H^+/K^+ ATPase

Allosteric Regulation

A. Isosteric and allosteric enzymes

The Michaelis–Menten model of enzyme catalysis (p. 76) assumes that the enzyme's spatial structure (its conformation; p. 60) does not alter. However, many enzymes can occur in various *conformations*, which have different catalytic properties.

Allosteric enzymes can be recognized by their S-shaped (*sigmoidal*) saturation curves, which cannot be described using the Michaelis–Menten equation. In the case of **isosteric enzymes** (with only *one* enzyme conformation, **1**), the efficiency of substrate binding (the slope of the saturation curve, dashed curve) declines constantly with increasing $[A]$, because the number of free binding sites is constantly decreasing. In **allosteric enzymes** (**2**), on the other hand, the binding efficiency initially rises with increasing $[A]$, because the free enzyme is present in a low-affinity conformation (square symbols), which is gradually converted into a higher-affinity form (round symbols). It is only at high $[A]$ values that a lack of free binding sites becomes noticeable and the binding efficiency decreases again. The affinity of allosteric enzymes is therefore not constant, but depends on the type and concentration of the ligand. Inhibitors and activators (**effectors**) influence the activity of allosteric enzymes by stabilizing certain conformations (**B**). These effects play an important part in regulating metabolism (p. 100f.).

B. Allosteric effects

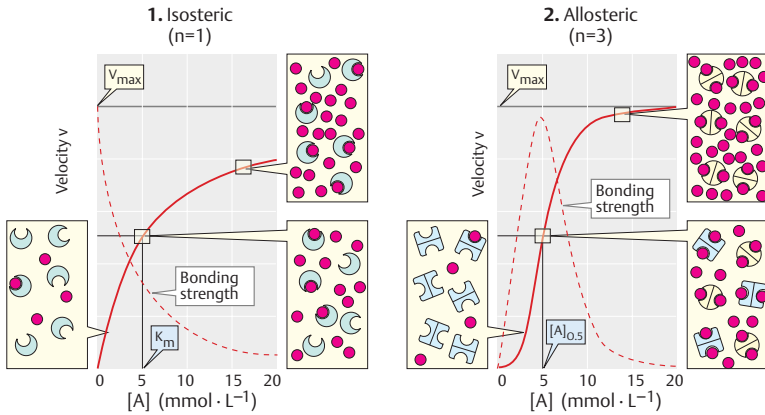
The allosteric regulation of enzyme activity is discussed here using the example of **aspartate carbamyltransferase** (ACTase) of the bacterium *Escherichia coli*.

ACTase is a key enzyme (p. 100) in pyrimidine biosynthesis (p. 182) and catalyzes the transfer of a carbamyl residue from carbamyl phosphate to the amino group of L-aspartate. Allosteric enzymes such as ACTase can be recognized by their **sigmoidal substrate saturation curves** (see **A**). Since the affinity of the enzyme for the substrate depends on the latter's concentration $[A]$ in these cases, the *substrate concentration at half-maximal rate* ($[A]_{0.5}$) is given instead of the Michaelis constant K_m (p. 76). The sigmoidal character of the curve is described by the **Hill coefficient**, h . In isosteric systems, $h = 1$, and h increases with increasing sigmoidicity.

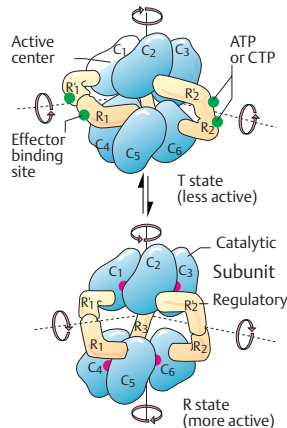
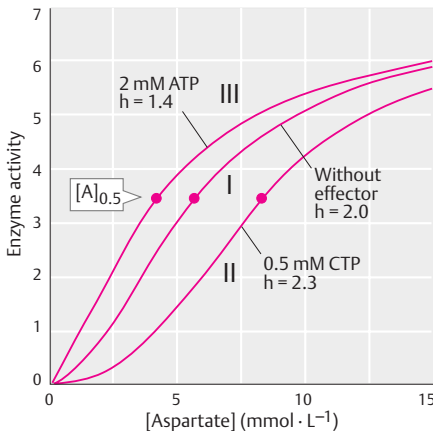
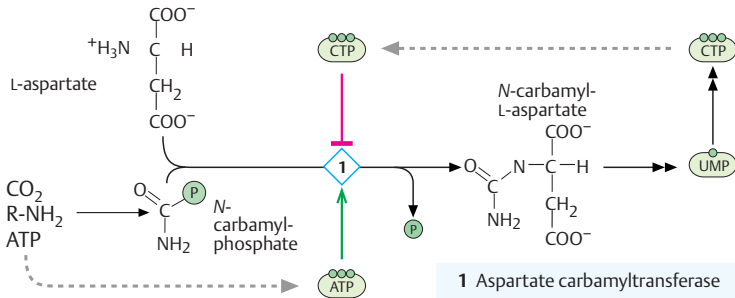
Depending on the enzyme, *allosteric effectors* influence the maximum rate V , the half-saturation concentration $[A]_{0.5}$ and the Hill coefficient h . If V is altered in particular, this is called a "**V system**." Much more common are "**K systems**," in which allosteric effects act only on $[A]_{0.5}$ and h . ACTase belongs to this group. It is inhibited by cytidine triphosphate (CTP), an endproduct of anabolic pyrimidine metabolism, and activated by the precursor ATP. The inhibitor CTP leads to a *right shift* of the curve, with an increase in $[A]_{0.5}$ and h (curve II). By contrast, the activator ATP produces a *left shift*; it reduces both $[A]_{0.5}$ and h (curve III).

Allosteric enzymes are almost always *oligomers* with 2–12 subunits (p. 56). ACTase consists of six catalytic subunits (blue) and six regulatory subunits (yellow). The latter bind the allosteric effectors CTP and ATP (green). Like hemoglobin (p. 286), ACTase can be present in two conformations—the less active **T state** (for "tense") and the more active **R state** (for "relaxed"). Substrates and effectors influence the equilibrium between the two states, and thereby give rise to sigmoidal saturation behavior. With increasing aspartate concentration, the equilibrium is shifted more and more toward the more active R form. ATP also stabilizes the R conformation by binding to the regulatory subunits. By contrast, binding of CTP to the same sites promotes a transition to the T state. In the case of ACTase, the structural differences between the R and T conformations are particularly dramatic. In $T \rightarrow R$ conversion, the catalytic subunits separate from one another by 1.2 nm, and the subunits also rotate around the axis of symmetry. The conformations of the subunits themselves change only slightly, however.

A. Isosteric and allosteric enzymes



B. Allosteric effects



Inhibitors

Many substances affect metabolic processes by influencing enzyme activity. **Enzyme inhibitors** are particularly important here. A large proportion of *medicines* act as enzyme inhibitors. Enzyme-kinetic experiments are therefore an important aspect of drug development and testing procedures. Natural *metabolites* are also involved in regulatory processes as inhibitors (p. 100f.).

A. Enzyme inhibition

As discussed on p. 78, physical and chemical influences interfere with the activity of most enzymes in a nonspecific manner. These include extremes of temperature and pH, and also organic solvents and heavy metals. Other inhibitors affect only some enzymes specifically. Most enzyme inhibitors act **reversibly**—i.e., they do not cause any permanent changes in the enzyme. However, there are also **irreversible** inhibitors that modify the target enzyme covalently and therefore permanently, e.g., penicillin (p. 252) or the prazoles (p. 270).

B. Types of inhibition

The mechanism of action of an inhibitor—its **inhibition type**—can be determined by comparing the kinetics (p. 76) of the inhibited and uninhibited reactions (see C). This makes it possible to distinguish *competitive inhibitors* (left) from *noncompetitive inhibitors* (right), for example. *Allosteric inhibition* is particularly important for metabolic regulation (p. 80). Competitive inhibitors (left) are usually **substrate analogues**, that is, compounds that have properties similar to those of one of the substrates of the target enzyme. Because substrate and inhibitor compete for the same binding site on the enzyme, this type of inhibition is referred to as **competitive**. Competitive inhibitors are bound by the enzyme, but cannot be converted further and therefore *reversibly* occupy some of the enzyme molecules present. In the presence of the inhibitor, a *higher* substrate concentration is therefore needed to achieve a half-maximum rate; the Michaelis constant K_m increases (see C). High concentrations of the substrate displace the inhibitor again. The maximum rate, V_{max} , is therefore not influenced by competitive inhibitors. **Analogues of the transition state** usually also act competitively.

When an inhibitor interacts with one of the enzyme's functionally important groups, but does not affect binding of the substrate, the inhibition is **non-competitive** (right). In this case, K_m remains unchanged, but k_{cat} and thus V_{max} decrease. Irreversible inhibitors usually result in a non-competitive type of inhibition because the concentration of active enzyme $[E]$ decreases. **Mixed inhibition** occurs when the inhibitor affects both K_m and also V_{max} . A special case of mixed inhibition is termed **uncompetitive**. This type of inhibition occurs when the inhibitor decreases V_{max} and K_m by the same factor. However, purely uncompetitive inhibitors are very rare. A possible cause for this type of inhibition is selective binding of the inhibitor to the EA complex.

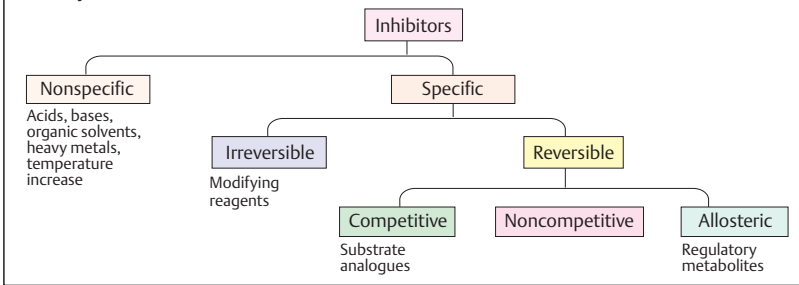
Allosteric inhibitors bind to separate binding sites outside the active center. This results in a *conformational change* in the enzyme protein that indirectly reduces its activity (p. 80). Allosteric effects usually only occur in *oligomeric enzymes*. The kinetics of this type of system cannot be described using the Michaelis–Menten model.

C. Inhibition kinetics

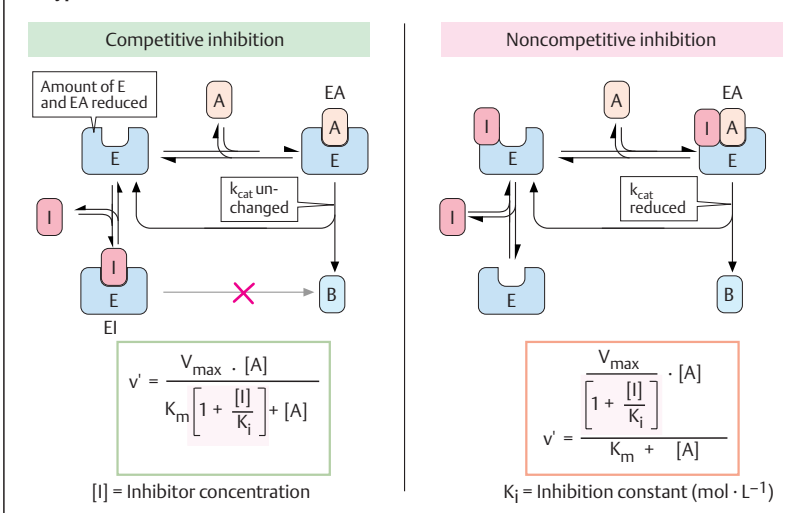
The action of inhibitors can be illustrated clearly in the **Lineweaver–Burk plot** (right). In this type of plot ($1/v$ plotted against $1/[A]$; p. 76), the intercept of the approximation lines with the y-axis corresponds to $1/V_{max}$ while the x-axis intercept gives the value of $-1/K_m$. This is why the straight lines obtained in the absence (blue) and presence of a *competitive inhibitor* (c, green) intersect on the y-axis ($1/V_{max}$ unchanged), while *non-competitive inhibitors* (nc, red) result in a straight line with a greater y-intercept but unchanged x-intercept ($1/V_{max}$ increased, K_m unchanged).

Allosteric enzymes are recognizable in Lineweaver–Burk plots because of upwardly curved lines (not shown).

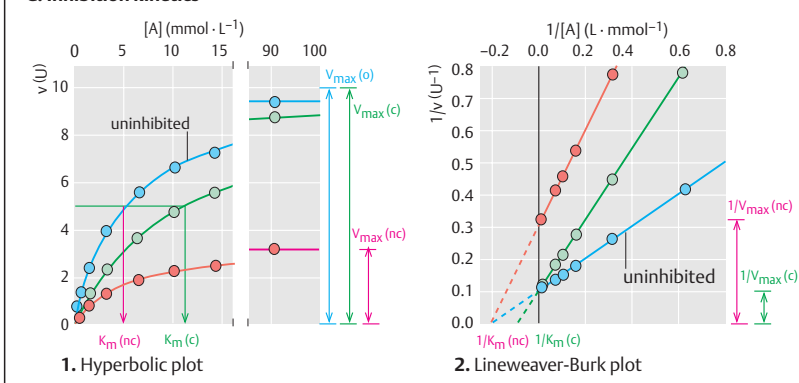
A. Enzyme inhibition



B. Types of inhibition



C. Inhibition kinetics



Enzymatic Analysis

Enzymes play an important role in *biochemical analysis*. In biological material—e.g., in body fluids—even tiny quantities of an enzyme can be detected by measuring its catalytic activity. This is the basis of serum enzyme diagnostics (pp. 94, 300). However, enzymes are also used as *reagents* to determine the concentrations of metabolites—e.g., the blood glucose level (C). Most enzymatic analysis procedures use the method of spectrophotometry (A).

A. Principle of spectrophotometry

Many substances *absorb light* in the visible or ultraviolet region of the spectrum. This property can be used to determine the concentration of such a substance. The extent of light absorption depends on the type and concentration of the substance and on the wavelength of the light used. **Monochromatic light**—i.e., light with a defined wavelength isolated from white light using a monochromator—is therefore used. Monochromatic light with an intensity of I_0 is passed through a rectangular vessel made of glass or quartz (a *cuvette*), which contains a solution of the absorbing substance.

The **absorption, A**, of the solution (previously referred to as its *extinction*) is defined as the *negative decadic logarithm of the quotient I/I_0* . The **Beer-Lambert law** states that A is proportional to the concentration, c, of the absorbing substance and the thickness, d, of the solution it passes through. As mentioned earlier, the **absorption coefficient, ϵ** , depends on the type of substance and the wavelength.

B. Measurement of lactate dehydrogenase activity

Measurement of lactate dehydrogenase (LDH, p. 72) activity takes advantage of the fact that while the reduced coenzyme $\text{NADH} + \text{H}^+$ absorbs light at 340 nm, oxidized NAD^+ does not. *Absorption spectra* (i.e., plots of A against the wavelength) for the substrates and the coenzymes of the LDH reaction are shown in 1. Differences in absorption behavior between NAD^+ and NADH between 300 nm and 400 nm result from changes in the nicotinamide ring during oxidation or reduction (p. 12). To measure the activity, a solution containing lactate and NAD^+ is placed in a cuvette, and absorption is recorded at a *constant wavelength* of 340 nm (2.). The uncatalyzed LDH reaction is extremely

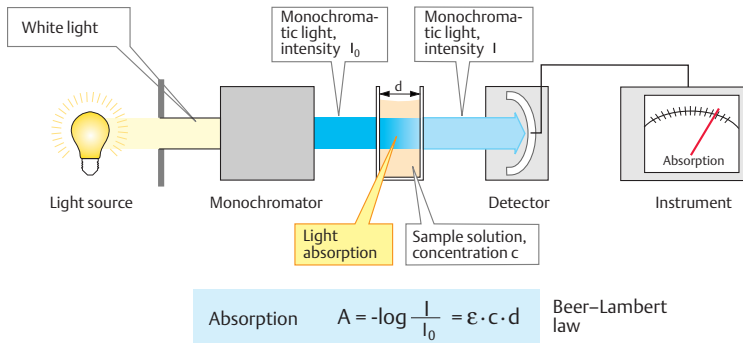
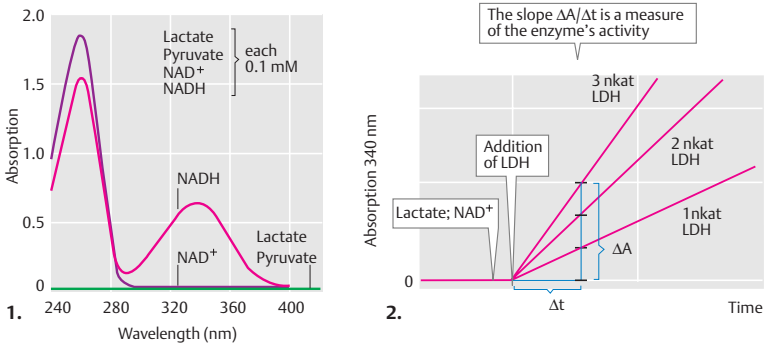
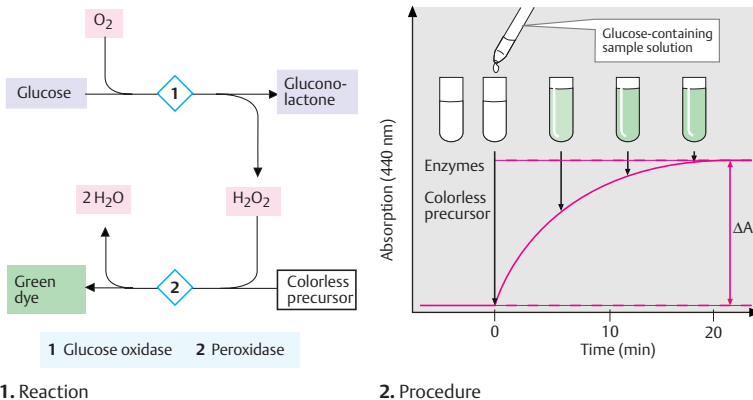
slow. It is only after addition of the enzyme that measurable quantities of NADH are formed and absorption increases. Since according to the Beer-Lambert law the rate of the increase in absorption $\Delta A/\Delta t$ is proportional to the reaction rate $\Delta c/\Delta t$, the absorption coefficient ϵ at 340 nm or comparison with a standard solution can be used to calculate LDH activity.

C. Enzymatic determination of glucose

Most biomolecules do not show any measurable absorption in the visible or ultraviolet spectrum. In addition, they are usually present in the form of mixtures with other, similar compounds that would also react to a chemical test procedure. These two problems can be avoided by using an appropriate enzyme to produce a colored dye selectively from the metabolite that is being analyzed. The absorption of the dye can then be measured.

A procedure (1) that is often used to measure glucose when monitoring blood glucose levels (p. 372) involves two successive enzymatic reactions. The glucose-specific enzyme *glucose oxidase* (obtained from fungi [1]) first produces gluconolactone and hydrogen peroxide (H_2O_2), which in the second step—catalyzed by a *peroxidase*—oxidizes a colorless precursor into a green dye [2]. When all of the glucose in the sample has been used up, the amount of dye formed—which can be measured on the basis of its light absorption—is equivalent to the quantity of glucose originally present.

The glucose oxidase-peroxidase reactions can also be used as a rapid test for glucose in the urine (p. 430). This method employs commercially available paper test strips impregnated with the necessary reagents (the two enzymes and the colorless precursor).

A. Principle of spectrophotometry**B. Measurement of lactate dehydrogenase activity****C. Enzymatic determination of glucose**

Coenzymes I

A. Redox coenzymes

All oxidoreductases are dependent on the action of coenzymes (p. 78). Coenzymes with redox function can act in *soluble form* (S) or as a *prosthetic group* (P). Depending on the compound, they transfer different types of “*reducing equivalents*” (e^- , $e^- + H^+$ or H^- , p. 12). Their normal potentials E^0 are shown on page 13.

The pyridine nucleotides **NAD⁺** and **NADP⁺** (1) are widely distributed as coenzymes of dehydrogenases. They transport *hydride ions* ($2 e^-$ and $1 H^+$, p. 12) and *always* act in dissolved form. NADH transfers reducing equivalents from catabolic pathways to the respiratory chain and thus contributes to energy metabolism. In contrast, NADPH is the most important *reductant* involved in biosynthesis (p. 96). It arises mainly in the pentose phosphate pathway (p. 132) and, for example, is necessary for the biosynthesis of fatty acids and cholesterol and also for biotransformation.

The flavin coenzymes **FMN** and **FAD** (2) contain *flavin* (isoalloxazine) as a redox-active group. This is a three-membered, N-containing ring system that can accept a maximum of two electrons and two protons during reduction. FMN and FAD carry the phosphorylated sugar alcohol *ribose* at the flavin ring. FAD arises from FMN through bonding with AMP. The two coenzymes are functionally similar. They are found in *dehydrogenases*, *oxidases*, and *monooxygenases*. In contrast to the pyridine nucleotides, flavin reactions give rise to *radical intermediates* (p. 12). To prevent damage to cell components, the flavins therefore always remain bound as prosthetic groups in the enzyme protein.

The role of **ubiquinone** (coenzyme Q, 3) in transferring reducing equivalents in the respiratory chain is discussed on p. 120ff. During reduction, *benzoquinone* (Q) is converted into the aromatic *hydroquinol* (ubiquinol, QH₂). The isoprenoid side chain of ubiquinone can have various lengths. It holds the molecule in the membrane, where it is freely mobile. Similar coenzymes (plastoquinones) are also found in photosynthesis.

L-Ascorbic acid (vitamin C, 4) is a powerful reducing agent. As an **antioxidant**, it provides nonspecific protection against oxidative damage (p. 288), but it is also an essential **cofactor** for various monooxygenases and dioxygenases. Ascorbic acid is involved in the hydroxylation of proline and lysine residues during the biosynthesis of collagen (p. 350), in the synthe-

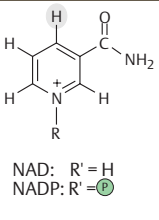
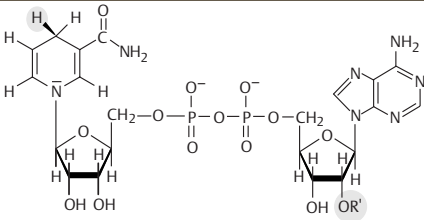
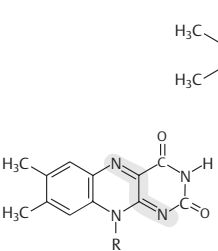
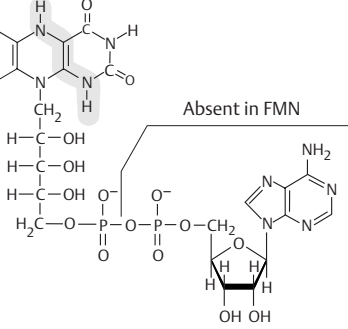
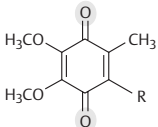
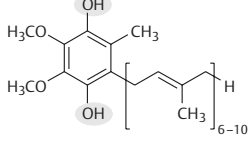
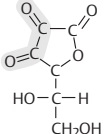
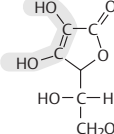
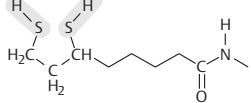
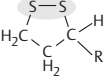
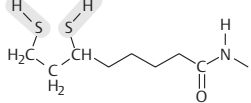
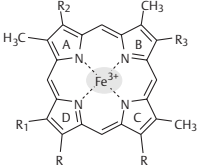
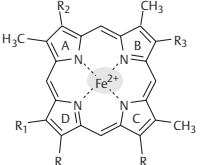
sis of catecholamines (p. 434) and bile acids (p. 320), as well as in the breakdown of tyrosine. The reduced form of the coenzyme is a relatively strong acid and forms salts, the **ascorbates**. The oxidized form is known as **dehydroascorbic acid**.

In **lipoic acid** (5), an intramolecular *disulfide bond* functions as a redox-active structure. As a result of reduction, it is converted into the corresponding *dithiol*. As a prosthetic group, lipoic acid is usually covalently bound to a lysine residue (R) of the enzyme, and it is then referred to as **lipoamide**. Lipoamide is mainly involved in oxidative decarboxylation of 2-oxoacids (p. 112). The peptide coenzyme **glutathione** is a similar disulfide/dithiol system (not shown; p. 288f.).

Heme coenzymes (6) with redox functions exist in the *respiratory chain* (p. 120), in *photosynthesis*, and in *monooxygenases* (p. 324) and *peroxidases*. Heme-containing proteins with redox functions are also referred to as **cytochromes**. In cytochromes, in contrast to hemoglobin and myoglobin, the iron changes its valence (usually between +2 and +4). There are several classes of heme (a, b, and c), which have different types of substituent—R₁ to R₃. Hemoglobin, myoglobin, and the heme enzymes contain heme b. Two heme a groups are found in cytochrome c oxidase, while heme c occurs in cytochrome c, where it is covalently bound with cysteine residues of the protein part via thioester bonds.

Iron-sulfur clusters (not shown) are described on page 122. The rarely occurring redox coenzymes include *tetrahydrobiopterin* (THB, p. 170) and *molybdopterin* (p. 180).

A. Redox coenzymes

Coenzyme	Oxidized form	Reduced form
1. NAD(P)⁺  (S, 1 H ⁺)	 NAD: R' = H NADP: R' = 	 Absent in FMN
2. Flavins (FAD, FMN)  (P, 2e ⁻ /2 H ⁺)	 Absent in FMN	 Absent in FMN
3. Ubiquinone (coenzyme Q)  (S, 2e ⁻ /2 H ⁺)		
4. Ascorbic Acid (Vitamin C)  (S, 2e ⁻ /2 H ⁺)		
5. Lipoamide (P, 2e ⁻ /2 H ⁺)		
6. Heme  (P, 1 e ⁻)		

Coenzymes II

A. Group-transferring coenzymes 1

The **nucleoside phosphates** (1) are not only *precursors* for nucleic acid biosynthesis; many of them also have coenzyme functions. They serve for *energy conservation*, and as a result of *energetic coupling* (p. 106) also allow endergonic processes to proceed. Metabolites are often made more reactive ("activated") as a result of the transfer of phosphate residues (phosphorylation). Thus, bonding with nucleoside diphosphate residues (mainly UDP and CDP) provides activated precursors for polysaccharides and lipids (p. 92). Endergonic formation of bonds by *ligases* (enzyme class 6) always depends on nucleoside triphosphates. Acyl residues are usually activated by transfer to **coenzyme A** (2, p. 8). In this coenzyme, *pantetheine* is linked to 3'-phospho-ADP by a phosphoric anhydride bond. Pantetheine consists of three components connected by amide bonds—*pantoic acid*, β -*alanine*, and *cysteamine*, the latter two of which are biogenic amines formed by the decarboxylation of aspartate and cysteine, respectively. The compound formed from pantoic acid and β -alanine (pantothenic acid) has vitamin-like characteristics for humans (p. 394). Reactions between the thiol group of the cysteamine residue and carboxylic acids give rise to **thioesters**, such as acetyl-CoA. This reaction is strongly endergonic, and it is therefore coupled to exergonic processes. Thioesters represent the activated form of carboxylic acids, because acyl residues in acyl-CoA have a high chemical potential and are easily transferred to other molecules. This property is often exploited in metabolism.

Thiamine diphosphate (TPP, 3), in cooperation with enzymes, is able to activate aldehydes or ketones as *hydroxyalkyl groups* and then to pass them on to other molecules. This type of transfer is important in the transketolase reaction, for example (p. 132). Hydroxyalkyl residues also arise in the decarboxylation of oxoacids. In this case, they are released as aldehydes or transferred to lipoamide residues of 2-oxoacid dehydrogenases (p. 112). The functional component of TPP is the sulfur- and nitrogen-containing *thiazole ring*.

Pyridoxal phosphate (4) is the most important coenzyme in amino acid metabolism. Its role in *transamination* reactions is discussed in detail on page 166. Pyridoxal phosphate is also involved regularly in other reactions involving amino acids, such as *decarboxylations* and *dehydrations*. The aldehyde form of pyridoxal

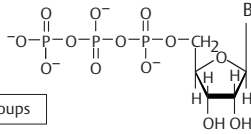
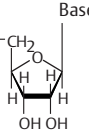
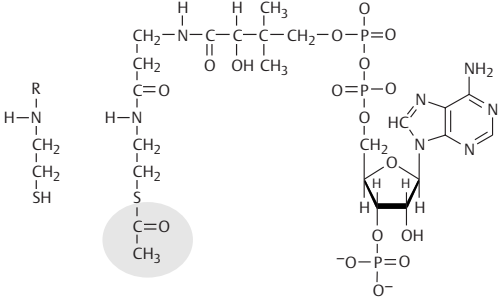
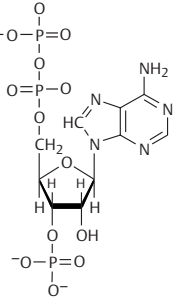
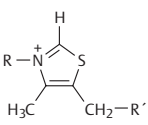
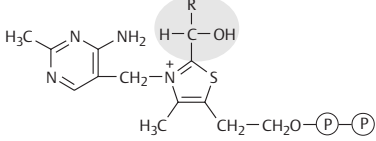
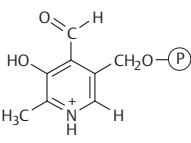
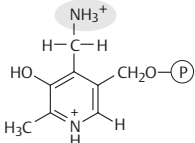
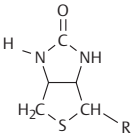
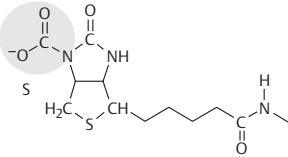
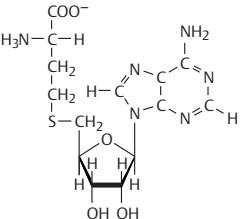
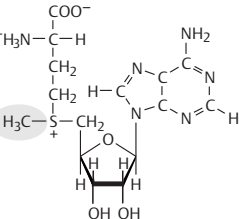
phosphate shown here (left) is not generally found in free form. In the absence of substrates, the aldehyde group is covalently bound to the ϵ -amino group of a lysine residue as *al-dimine* ("Schiff base"). **Pyridoxamine phosphate** (right) is an intermediate of transamination reactions. It reverts to the aldehyde form by reacting with 2-oxoacids.

Biotin (5) is the coenzyme of all *carboxylases*. Like pyridoxal phosphate, it forms an amide-type bond via the carboxyl group with a lysine residue of the carboxylase. This reaction is catalyzed by a specific enzyme. Using ATP, biotin reacts with hydrogen carbonate (HCO_3^-) to form **N-carboxybiotin**. From this activated form, *carbon dioxide* (CO_2) is then transferred to other molecules, into which a carboxyl group is introduced in this way. Examples of biotin-dependent reactions include the formation of oxaloacetic acid from pyruvate (p. 116) and the synthesis of malonyl-CoA from acetyl-CoA (p. 150).

"Activated methyl" in the form of **S-adenosyl methionine** (SAM, 6) is involved in many methylation reactions, e.g., in creatine synthesis (p. 344), the conversion of norepinephrine to epinephrine (p. 434), and the methylation of DNA (p. 234).

SAM is derived from degradation of the proteinogenic amino acid *methionine*, to which the adenosyl residue of an ATP molecule is transferred. After release of the activated methyl group, S-adenosyl homocysteine (SAH) is left over. This can be converted back into methionine in two further steps. First, cleavage of the adenosine residue gives rise to the nonproteinogenic amino acid *homocysteine*, to which a methyl group is transferred, once again with the help of *N*⁵-methyl-tetrahydrofolate (p. 91). Alternatively, *homocysteine* can also be broken down into propionyl-CoA.

A. Group-transferring coenzymes 1

Coenzyme	Free form	Charged form	Important enzymes
1. Nucleoside phosphates  B-Rib B-Rib-P B-Rib-P-P		Base 	Phospho- transferases Nucleotidyl- transferases Ligases
2. Coenzyme A  CoA			Acyltrans- ferases CoA trans- ferases
3. Thiamin diphosphate  TPP			Decarboxylases Oxoacid dehydrogenases Transketolase
4. Pyridoxal phosphate  PLP NH ₂ groups Amino acid residues			Transaminases Many lyases
5. Biotin CO ₂			Carboxylases
6. S-adenosyl methionine (SAM)  SAM			Methyltrans- ferases

Coenzymes III

A. Group-transferring coenzymes 2

Tetrahydrofolate (THF, **3**) is a coenzyme that can transfer C_1 residues in different oxidation states. THF arises from the vitamin *folic acid* (p. 394) by double hydrogenation of the heterocyclic *pterin* ring. The C_1 units being transferred are bound to N-5, N-10, or both atoms. THF and its derivatives play a central part in C_1 metabolism (p. 184).

The most important derivatives are:

- (a) **N^5 -formyl-THF** and (b) **N^{10} -formyl-THF**. The formyl residue of these two compounds has the oxidation state of a carboxylic acid. During the synthesis of purine bases, N^{10} -formyl-THF provides two C atoms of the purine ring (p. 182).
- (c) **N^5 , N^{10} -methenyl-THF** and (d) **N^5 , N^{10} -methylene-THF**. These have C_1 residues in the oxidation state of an aldehyde. The N^5 , N^{10} -methylene derivative arises mainly from THF and serine, which is thereby converted to glycine. It supplies the methyl group of the thymidine nucleotides and is therefore essential for DNA synthesis (p. 184). The *dihydrofolate* produced during the reaction must be regenerated to N^5 , N^{10} -methylene-THF via THF. Important cytostatic drugs target this reaction sequence (p. 454).
- (e) **N^5 -methyl-THF**. This compound, in which the methyl group has the oxidation state of an alcohol, is produced by reduction of N^5 , N^{10} -methylene-THF. It provides the methyl group of *methylcobalamin* (see below) and is therefore essential for the conversion of homocysteine back to *methionine* (pp. 88, 184).

Because of the importance of the folate coenzymes in DNA synthesis and thus for cell proliferation (p. 444f.), folate deficiency particularly affects cells that are dividing rapidly, for example, blood-forming cells in the bone marrow. On the other hand, interference with THF metabolism offers possibilities in cancer chemotherapy (p. 454). The sulfonamide antibiotics inhibit the growth of microorganisms by interfering with their folate synthesis (p. 252). The **cobalamins** (**2**) are chemically the most complex form of coenzyme. They also represent the only natural substances that contain the transition metal *cobalt* (Co) as an essential component. Higher organisms are unable to synthesize cobalamins themselves, and are therefore dependent on a supply of **vitamin B₁₂** (cobalamin) as a precursor (p. 394).

Cobalamin deficiency is often due not to an inadequate supply of the vitamin itself but to a lack of intrinsic factor, a glycoprotein produced in the stomach that is necessary for absorption of the vitamin in the small intestine. In medicine, the more stable *cyanocobalamin* is usually employed to treat vitamin B₁₂ deficiency.

The central component of the cobalamins is the **corrin ring**, a member of the tetrapyrroles, at the center of which the cobalt ion is located. The end of one of the side chains of the ring carries a nucleotide with the unusual base *dimethylbenzimidazole*. The ligands for the metal ion are the four N atoms of the pyrrole ring, a nitrogen from dimethylbenzimidazole, and a **group X**, which is organometallically bound—i.e., *mainly covalently*. Cobalamin coenzymes that are important for humans include:

Methylcobalamin with a methyl group as group X. This compound functions as a coenzyme for *methyltransferases*. In human metabolism it is required only for the resynthesis of methionine from homocysteine (“remethylation,” p. 184).

Adenosylcobalamin (coenzyme B₁₂). This derivative carries a covalently bound adenosyl residue at the metal atom. This mitochondrial compound is a coenzyme of various *isomerases*, which catalyze rearrangements following a radical mechanism. The radical arises here through *homolytic cleavage* of the bond between the metal and the adenosyl group. The only reaction of this type in animal metabolism is the rearrangement of *methylmalonyl-CoA* to form *succinyl-CoA*, which completes the breakdown of the odd-numbered fatty acids and of the branched amino acids valine and isoleucine (p. 170).

Coenzymes IV

Many coenzymes serve to *activate* molecules or groups that are poorly reactive. Activation consists in the formation of reactive intermediate compounds in which the group concerned is located at such a high chemical potential (p. 16) that it can be transferred to other molecules in an exergonic reaction (p. 106). Acetyl-CoA, “*activated acetic acid*,” is a well-known example of this type of compound (pp. 8, 44).

ATP and the other **nucleoside triphosphate coenzymes** not only transfer phosphate residues, but also provide the nucleotide components for activation reactions. On this page, we discuss metabolites or groups that are activated in metabolism by bonding with nucleosides or nucleotides. Intermediates of this type are mainly found in the metabolism of complex carbohydrates and lipids.

A. Metabolite activation by nucleotides

1. Uridine diphosphate glucose (UDP-glucose)

The inclusion of further glucose residues into polymers such as glycogen or starches is an endergonic process. The activation of the **glucose** building blocks in several steps is required for this to take place, in which two ATPs are used per glucose. After the phosphorylation of free glucose (**a**), glucose 6-phosphate is isomerized to glucose 1-phosphate (**b**); reaction with uridine triphosphate (UTP, **c**) then gives rise to **UDP-glucose** (**d**), in which the anomeric OH group at C-1 of the sugar is bound with phosphate. This “energy-rich” compound (an acetal phosphate) allows exergonic transfer of glucose residues to glycogen (p. 136) or to other acceptors.

The energetics of the partial reactions show that both production of UTP from UDP and ATP and the synthesis of UDP-glucose from UTP and glucose 1-phosphate are in equilibrium (the change in free enthalpy, ΔG° , is close to 0). The entire process is driven by the highly exergonic hydrolysis of the *diphosphate* (which was produced in reaction **d**) in reaction **e**. It is for this reason that the production of UDP-glucose becomes exergonic enough to proceed almost to completion. Shifting the reaction equilibrium by removing one of the reaction products is often used in metabolism—e.g., in the pyruvate kinase reaction (p. 130) or in the urea cycle (p. 172). Reaction **f** (transfer of a glucose residue from UDP-glucose to glycogen) is also markedly exergonic, with $\Delta G^\circ = -13.5 \text{ kJ} \cdot \text{mol}^{-1}$.

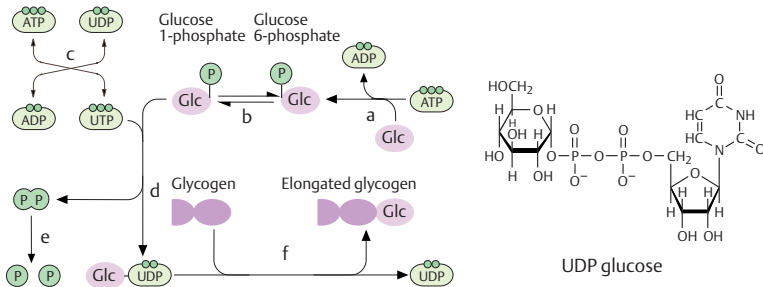
UDP-glucose (“activated glucose”) is used not only for glycogen synthesis, but is also the precursor of UDP-galactose and UDP-glucuronic acid, and thus the starting material for lactose, glycosaminoglycans and glucuronides (p. 316). Like glucose, glucuronic acid can be activated by UDP. One of the functions of **UDP-glucuronate** is to form conjugates during biotransformation (p. 322).

2. Cytidine diphosphate choline (CDP-choline)

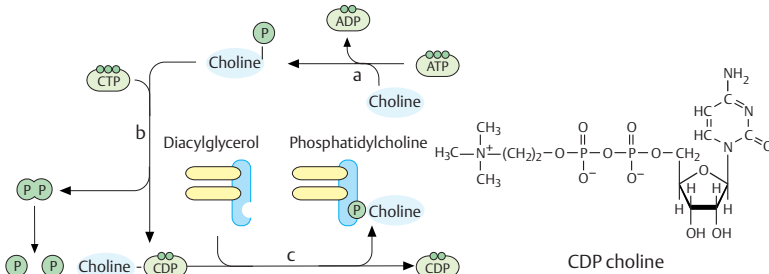
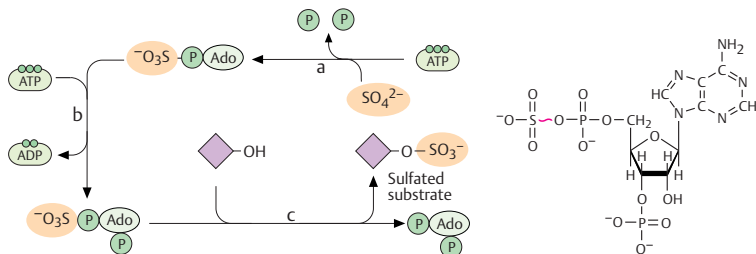
The amino alcohol **choline** is activated for inclusion in phospholipids following a similar principle (p. 154). Choline is first phosphorylated by ATP to form choline phosphate (**a**), which then reacts with CTP with cleavage of diphosphate to become **CDP-choline** (**b**). In contrast to UDP-glucose, it is not free choline that is transferred from CDP-choline, but rather choline phosphate, which with diacylglycerol yields phosphatidylcholine (lecithin) (**c**). Other alcohol components of phospholipids are activated in a similar manner to choline, e.g., ethanolamine and myo-inositol.

3. Phosphoadenosine phosphosulfate (PAPS)

Sulfate residues occur as strongly polar groups with a permanent negative charge in various biomolecules—e.g., in *glycosaminoglycans* (p. 352) and *conjugates* of steroid hormones and xenobiotics (p. 322). **Sulfate** is incorporated mainly to increase the water binding capacity or water solubility of the molecule in question. In the synthesis of the “activated sulfate” **PAPS**, ATP first reacts with inorganic sulfate to form adenosine phosphosulfate (APS, **a**). This intermediate already contains the “energy-rich” mixed anhydride bond between phosphoric acid and sulfuric acid. In the second step, the 3'-OH group of APS is then phosphorylated, with ATP being used again (**b**). After transfer of the sulfate residue to OH groups (**c**), adenosine-3',5'-bisphosphate remains.

A. Metabolite activation by nucleotides

c)	ATP + UDP	$\pm 0 \text{ kJ}\cdot\text{mol}^{-1}$	$\rightarrow$ UTP + ADP
d)	UTP + Glucose 1-phosphate	$\pm 0 \text{ kJ}\cdot\text{mol}^{-1}$	$\rightarrow$ UDP-Glucose + Diphosphate
e)	Diphosphate + H ₂ O	$-33.5 \text{ kJ}\cdot\text{mol}^{-1}$	$\rightarrow$ 2 Phosphate
d) + e)	UTP + Glucose 1-phosphate	$-33.5 \text{ kJ}\cdot\text{mol}^{-1}$	$\rightarrow$ UDP-Glucose + 2 Phosphate
f)	UDP-Glucose + Glycogen(n)	$-13.5 \text{ kJ}\cdot\text{mol}^{-1}$	$\rightarrow$ UDP + Glycogen (n+1)

1. Uridine diphosphate glucose (UDP-glucose)**2. Cytidine diphosphate choline (CDP-choline)****3. Phosphoadenosine phosphosulfate (PAPS)**

Pathobiochemistry

A. Congenital enzyme deficiencies

In view of the central importance of enzymes in the course and regulation of metabolism, it can be readily understood that inherited enzyme deficiencies or their complete absence can cause severe disease. To date, hundreds of “inborn errors of metabolism” of this nature have been described. Since maternal metabolism can initially compensate for enzyme deficiencies in the fetus, the symptoms usually become apparent only several days or weeks after birth. It is estimated that the incidence is one case for every 4000 to 5000 live births. Nowadays, neonatal screening is therefore performed in appropriately equipped hospitals; this can identify the more common metabolic defects from unusually high or low concentrations of certain metabolites in the blood determined by mass spectrometry. Early diagnosis is important because subsequent damage can be reduced or fully avoided if treatment is started immediately.

If the defective enzyme is part of a metabolic pathway and if its loss cannot be compensated, the enzyme's substrate (B) and sometimes its precursors (e.g., A) usually accumulate in the body. On the other hand, side reactions can occur, which are otherwise unimportant (in this case, conversion of B to D). Conversely, the concentration of metabolites formed *after* the defective step is diminished, or they are completely absent. The table lists characteristic changes in metabolite levels for some common enzyme deficiencies. The individual metabolic disorders are discussed in greater detail elsewhere.

B. Serum enzyme diagnostics

Normally, only low enzyme activities are found in the blood. However, when diseases occur in which cells are damaged or destroyed, intracellular enzymes enter the blood and can be detected there because of their activity. This is known as **serum enzyme diagnostics**. It not only permits conclusions about the severity of the tissue damage but also provides evidence of the site of damage because some enzymes have a characteristic organ distribution. On the left the relative concentrations of diagnostically important enzymes in the liver, skeletal muscle and cardiac muscle are shown schematically (1). For example, *aspartate aminotransferase* (glutamate oxaloacetate transaminase, GOT) is present in similar amounts in

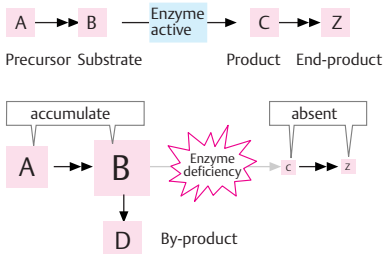
all three tissues, while there are large amounts of *glutamate dehydrogenase* (GLDH) and *alanine aminotransferase* (glutamate pyruvate transaminase, GPT) in the liver (p. 166). In contrast, *creatine kinase* (CK) occurs mainly in muscle (p. 344).

This distribution is reflected in enzyme diagnostics. After a heart attack that damages the myocardium (2a), the CK activity in the blood increases greatly within 1–2 days and then falls again rather more slowly. *Lactate dehydrogenase* (LDH) and the related *hydroxybutyrate dehydrogenase* (HBDH) are also released. Of the transaminases, the level of aspartate transaminase (GOT) normally exceeds that of alanine transaminase (GPT). In liver disease, e.g., viral hepatitis, in contrast, the liver-specific GPT activity in the serum is markedly higher than that of GOT (2b). The levels of *alkaline phosphatase* (AP) and *γ-glutamyltransferase* (γGT) change to a lesser degree. Other enzymes relevant in enzyme diagnostics are discussed on page 300.

C. Enzymes as pharmacological targets

Besides the aspects discussed above, enzymes are also of great medical interest because they are the most important **targets for drugs**. The majority of drugs used for the chemotherapy of diseases bind specifically to functional proteins and influence their function. Apart from *enzymes* (1, top), important target proteins include *membrane transporters* (top left), and especially *receptors for hormones* (bottom left) and *neurotransmitters* (bottom right). These proteins together account for over 90% of all drug targets (2). Precise knowledge of the target molecule is of crucial importance in the development of new drugs.

A. Congenital enzyme deficiencies

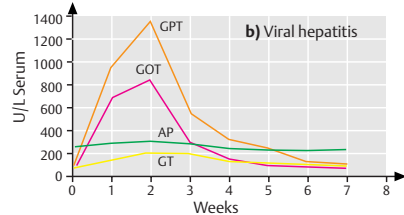
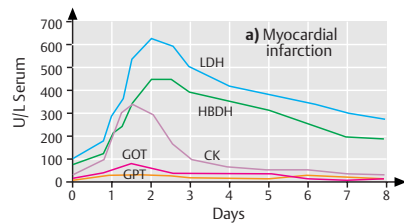


Disease	Enzyme	Effects	page
Phenylketonuria	Phenylalanine hydroxylase	Phenylalanine (B) ↑ Phenylpyruvate (D) ↑ Tyrosine (C) ↓ Melanin (Z) ↓	177
Galactosemia	Galactose 1-phosphate uridylyltransferase	Galactose (A) ↑ Galactose 1-phosphate (B) ↑ Galactitol (D) ↑	143
Propionic aciduria	Propionyl-CoA carboxylase	Propionyl-CoA (B) ↑ Propionic acid (D) ↑	177

B. Serum enzyme diagnostics

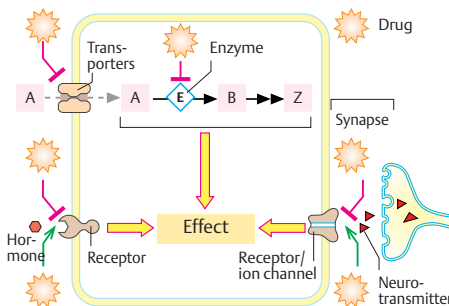
	Liver	Skeletal muscle	Cardiac muscle
LDH	■	■	■
GLDH	■	■	■
GOT	■	■	■
GPT	■	■	■
CK	■	■	■

1. Enzyme concentrations in liver and muscle

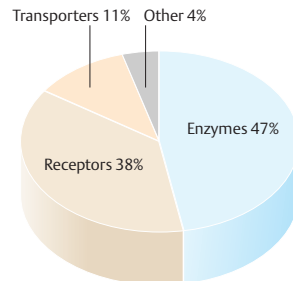


2. Serum enzyme levels in diseases

C. Enzymes as pharmacological targets



1. Target molecules of drugs



Intermediary Metabolism I

Hundreds of chemical reactions are constantly taking place in every cell, which together are referred to as **metabolism**. The chemical compounds involved in this are known as **metabolites**. Organized sequences of chemical reactions with a high rate of throughput, known as **metabolic pathways**, become possible through the existence of specific **enzymes** (p. 72f.). Several central metabolic pathways are common to most cells and organisms. These pathways, which serve for synthesis, degradation, and interconversion of important metabolites, and also for energy conservation, are referred to as **intermediary metabolism**.

A. Forms of nutrition

To survive, cells require organic and inorganic **nutrients**, as well as **energy**. Animal- and thus human-metabolism represents just one of the many **forms of nutrition** that have developed over the course of evolution. Depending on how these basic needs are met, living organisms can be classified into several major groups.

The crucial criteria are (1) the **energy source** (radiant energy or chemical energy?), (2) the **electron source** necessary for conservation of chemical energy in the form of ATP (organic or inorganic compounds?) and (3) the **source of the carbon atoms** (organic compounds or CO₂?). Organisms that can synthesize organic molecules from CO₂ are termed **autotrophs**. This group includes plants and many microorganisms. Green plants, for instance, meet their energy requirement with sunlight and use water as an electron source (see **B**). They are therefore **photo-litho-autotrophic**. Animals depend on organic substances for all three functions and are therefore called **heterotrophic** or more accurately, **chemo-organo-heterotrophic**. Some anaerobic archaeobacteria are extremely simple as their needs are satisfied by CO₂ and water alone. They are **chemo-litho-autotrophic**.

B. Autotrophic and heterotrophic organisms

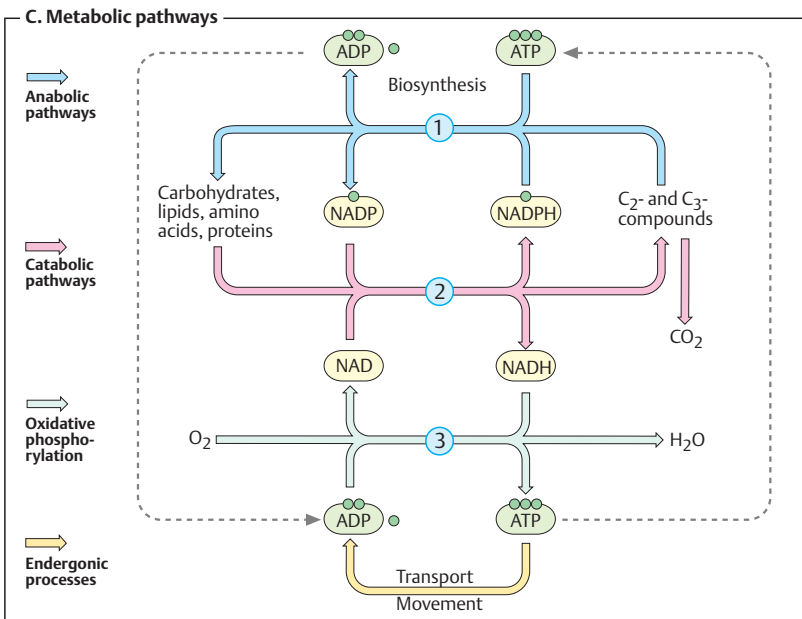
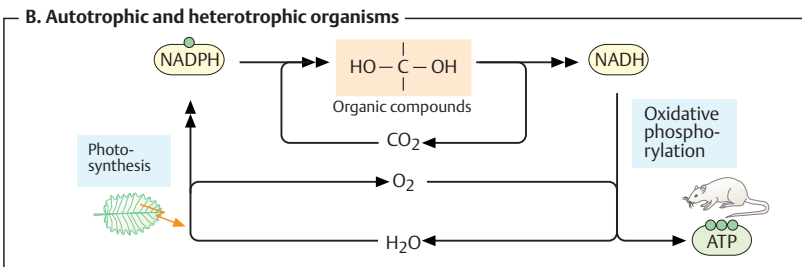
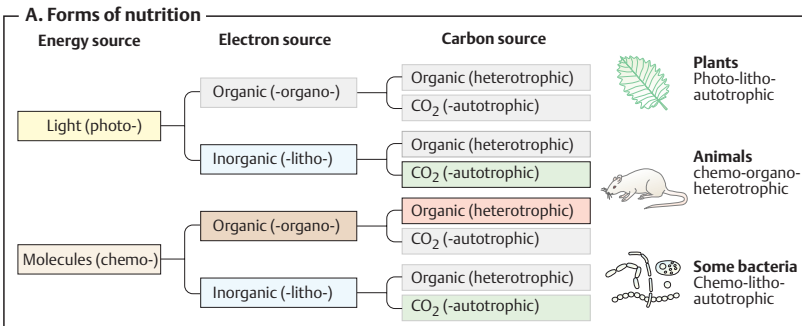
As **heterotrophic** animals can only use previously synthesized organic substances, they rely on the metabolic services of **autotrophic** plants, which supply them not only with organic molecules but also with essential **oxygen**. In fact, almost all the molecular oxygen present in the atmosphere originates from plant

photosynthesis (left). In the “**light reaction**,” water (H₂O) is converted to oxygen and “energy-rich” electrons in the form of NADPH with the aid of sunlight. The reduced coenzyme then serves for the plants to synthesize organic compounds such as glucose from CO₂ (**dark reaction**). Animals—and also plants—utilize their **catabolic metabolism** (right, see also **C**) to break glucose down again to CO₂ and H₂O, thereby obtaining useful chemical energy in the form of ATP (p. 104). The two processes can be represented as a closed circle, which serves to convert light energy to ATP.

C. Metabolic pathways

In animal metabolism, organic precursors are broken down by **catabolic pathways** (pink arrows) into smaller fragments. The resulting **metabolite pool** of C₂ and C₃ compounds is either used to obtain energy by **oxidative phosphorylation** through further catabolic conversion, or are assembled again into more complex molecules by **anabolic pathways** (blue arrows). The catabolic pathways supply reduced coenzymes, which feed both oxidative phosphorylation (in the form of NADH) and anabolic pathways (in the form of NADPH) with electrons (“**reducing equivalents**”).

In all cells, adenosine triphosphate (ATP; p. 104) is the most important form of storage for chemical energy. Exergonic cleavage of ATP, via **energetic coupling** (p. 106), makes energy-dependent (**endergonic**) processes possible (yellow arrow). Most anabolic pathways, as well as movement and transport processes, are energy-dependent.



Intermediary Metabolism II

The most important pathways in human intermediary metabolism are illustrated in the form of a flow chart which shows their interactions and the part played by central metabolites and coenzymes in metabolic events.

A. Intermediary metabolism: overview

The *polymeric substances* contained in the **diet** (proteins, carbohydrates, nucleic acids and fats [top]) cannot be used by the organism directly. Digestive enzymes (p. 268) first have to degrade them to *monomers* (amino acids, sugars, nucleotides and fatty acids). These are then absorbed by the cells of the intestinal mucosal and made available for metabolism.

Most of the metabolites obtained in the diet are broken down by **catabolic pathways** (pink arrows) into smaller fragments. The resulting products are termed the **metabolite pool**. The pool contains mainly molecules with 2 or 3 C atoms, of which only three particularly important representatives—*pyruvate*, coenzyme-bound *acetyl residues* (in the form of acetyl-CoA; p. 8), and *glycerol*—are shown here.

Depending on the metabolic situation, the molecules of the metabolite pool are broken down further to obtain energy in **oxidative phosphorylation**, or are built up again into larger molecules by **anabolic pathways** (blue arrows). The metabolites of the pool therefore represent connecting links between the metabolism of proteins, carbohydrates and lipids. For instance, the *pyruvate* formed during breakdown of glucose (3) is converted to *acetyl-CoA*, from which fatty acids (4) and isoprenoids (5) can be produced in turn. Both pathways require NADPH as reducing agent, which also derives from glucose metabolism (1). Pyruvate can also be used for glucose biosynthesis by means of gluconeogenesis (2). Precursors that arise from the breakdown of *amino acids* (7) and the *glycerol*, produced by fat metabolism, are even more important.

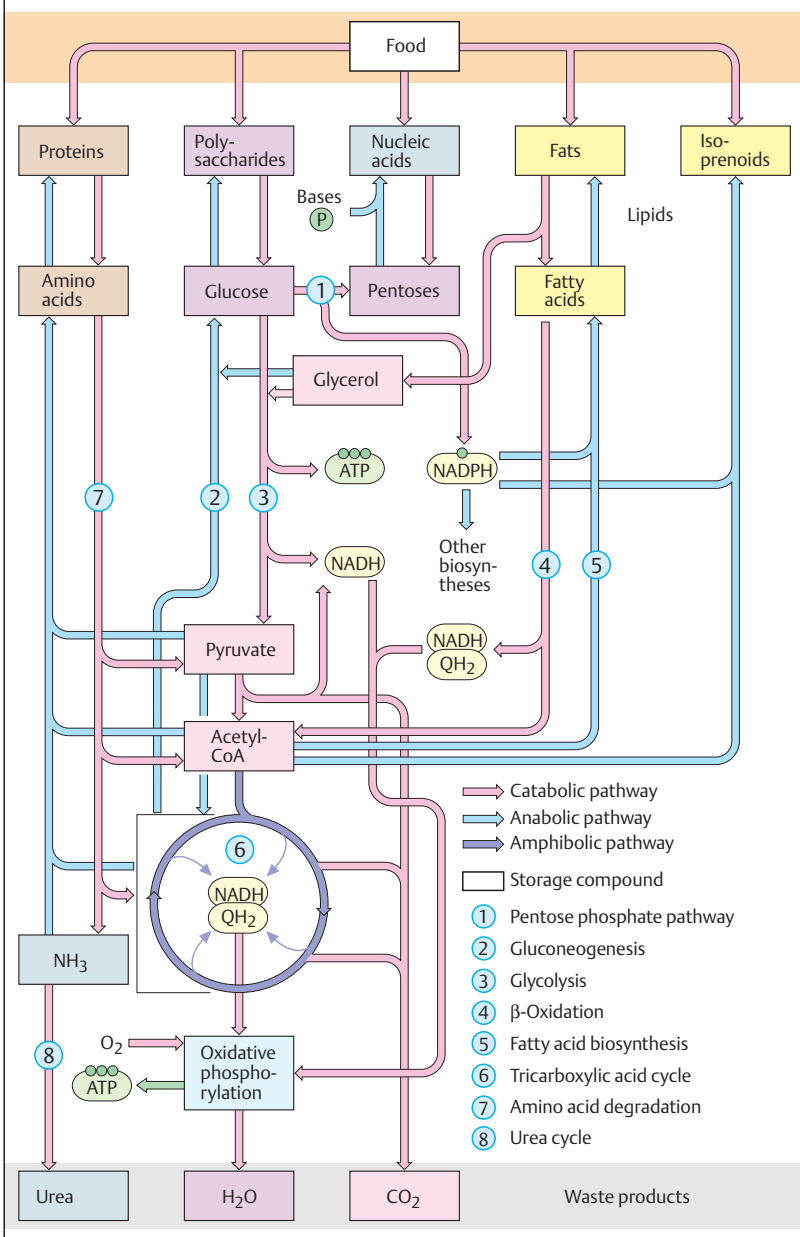
The metabolite pool also includes the intermediates of the tricarboxylic acid cycle (6). This cyclic pathway has catabolic and anabolic functions—i.e., it is **amphibolic** (violet circle; p. 116).

Waste products from the degradation of organic substances in animal metabolism (bottom) include *carbon dioxide* (CO₂), *water* (H₂O), and *ammonia* (NH₃). The toxic substance ammonia is incorporated into *urea* by the urea cycle (8) and excreted in this form (p. 172).

Chemical energy is stored by all organisms mainly in the form of **adenosine triphosphate** (ATP, p. 104). ATP *synthesis* requires energy—i.e., this reaction is *endergonic*. Conversely, cleavage of ATP into ADP and phosphate releases energy and is *exergonic*. Hydrolysis of ATP via **energetic coupling** (p. 106), makes energy-dependent (*endergonic*) processes possible. Quantitatively, the most important pathway for the synthesis of ATP in most cells is **oxidative phosphorylation** (p. 110). Catabolic pathways first form a range of different reduced coenzymes (especially NADH and reduced ubiquinone, QH₂). “Energy-rich” electrons are then transferred from these compounds to molecular oxygen. This strongly exergonic redox reaction is catalyzed by the **respiratory chain** (p. 120). The resulting chemical energy is initially used to build up an electrochemical gradient (p. 108), which in turn provides energy for the synthesis of ATP from ADP and inorganic phosphate (p. 122). In *anaerobic conditions*—i.e., in the absence of oxygen—most organisms can fall back on ATP that arises in glycolysis (3). This less efficient type of ATP synthesis is referred to as **fermentation** (p. 110).

While NADH exclusively supplies oxidative phosphorylation, NADPH—a very similar coenzyme—is the reducing agent for anabolic pathways. NADPH is mainly formed in the pentose phosphate pathway (PPP, 1; p. 132).

A. Intermediary metabolism: overview



Regulatory Mechanisms I

The activities of all metabolic pathways are subject to precise regulation to adjust the synthesis and degradation of metabolites to physiological requirements. An overview of the regulatory mechanisms is presented here.

Metabolite flow along a metabolic pathway is mainly determined by the activities of the **enzymes** involved (p. 72ff.). To control the flow, not all enzymes of a pathway have to be regulated. It is sufficient to change the activity of the enzyme that catalyzes the *slowest* step in the reaction chain. Most metabolic pathways have **key enzymes** of this type on which the regulatory mechanisms operate.

A. Key enzymes

The importance of key enzymes in regulation of metabolism can be illustrated by an example from mechanics (1). The volume of water that flows in a given period through a system of connected tanks is determined by the diameter of the narrowest connecting pipe (top). If this is widened, the water outflow volume increases to the same degree.

Many metabolites are the starting point of a catabolic pathway and at the same time an end product of an anabolic reaction chain (2). If both pathways were active at the same time, a futile cycle would occur. In these cases, reciprocal regulation is therefore often observed. If the key enzyme of the catabolic pathway is active, the anabolic pathway is inhibited and vice versa.

B. Fundamental mechanisms of metabolic regulation

Control of intermediary metabolism takes place on several levels. All regulatory mechanisms involved act on processes that influence the synthesis and/or activity of key enzymes.

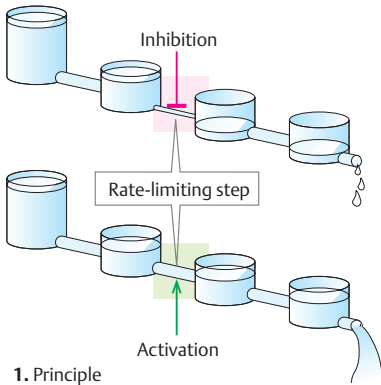
Transcriptional control. At the genetic level, the transcription of genes coding for key enzymes is usually regulated (see "Transcriptional control," p. 242). This is mediated by regulatory proteins (so-called transcription factors) that bind to DNA in the promoter region of the genes. The activity of transcription factors is, in turn, controlled by metabolites (A, Z, top) and/or hormones. These processes take some time to become effective (minutes to hours).

Interconversion. *Interconversion* of enzymes takes effect considerably faster than transcrip-

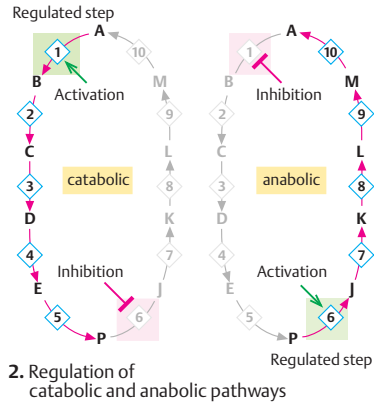
tional control. In this case, the enzyme is already present at its site of action, but it is initially still inactive. It is only when needed that it is converted into the catalytically active form, after hormonal signaling and mediation from second messengers (p. 406ff) through an *activating enzyme*. If the metabolic pathway is no longer required, an *inactivating enzyme* returns the key enzyme to its inactive resting state. Interconversion in most cases involves **ATP-dependent phosphorylation** of the enzyme protein by a *protein kinase* [1] or **dephosphorylation** of it by a *protein phosphatase* [2] (p. 410). The phosphorylated form of the key enzyme is usually the more active one, but the reverse may also occur. **Proteolytic activation** or **inactivation** [3] of enzymes is also a frequent control mechanism.

Compartmentation. Some pathways are switched off when a key enzyme is moved into a different cell compartment where it is held by an inhibitor. When necessary, it is returned to its site of action, as a result of hormone or metabolite signaling. Movement of the enzyme glucokinase between the nucleus and cytoplasm is an example (p. 140).

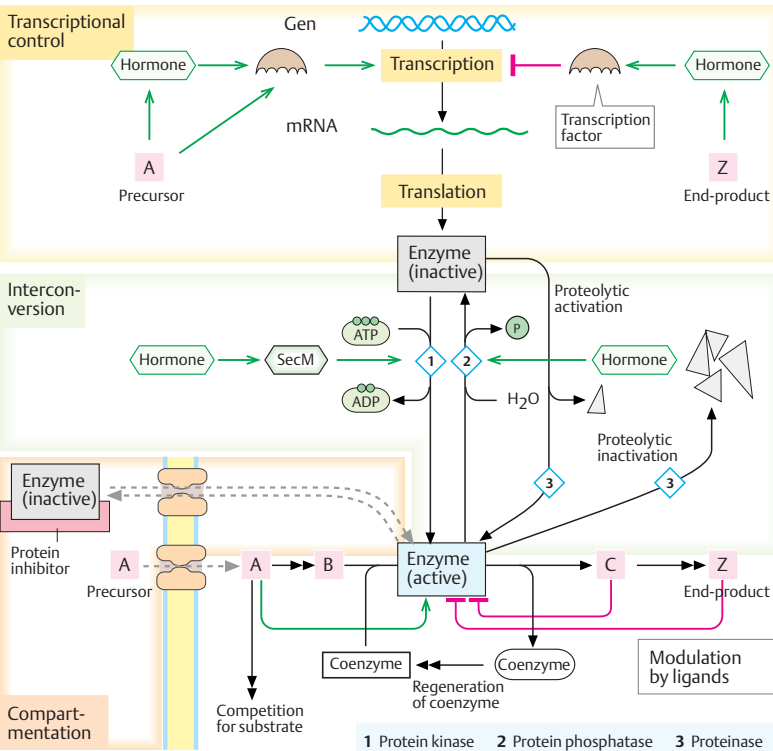
Modulation by ligands. An important variable that regulates flow through a metabolic pathway is *precursor* and *coenzyme availability*. If the coenzyme of a key enzyme is regenerated by a second, independent metabolic pathway, the speed of the second pathway can limit that of the first one (see, e.g., respiratory control, p. 124). Fine control of enzyme activity is brought about by **ligands** (substrates, products, coenzymes, etc.), which as *allosteric effectors* modulate activity (p. 80). Inhibition by immediate reaction products (here, metabolite C, *product inhibition*), by end products of the entire reaction chain (*feedback inhibition*, Z in this case), or by metabolites from completely different metabolic pathways is often encountered. The precursors for a reaction chain (A, bottom) can stimulate their own utilization through enzyme activation.

A. Key enzymes

1. Principle



2. Regulation of catabolic and anabolic pathways

B. Fundamental mechanisms of metabolic regulation

Regulatory Mechanisms II

The diagrams here illustrate the principles of metabolic regulation discussed on the previous page, using typical cases. Many other examples are discussed elsewhere in conjunction with the metabolism of individual groups of metabolites.

A. Transcriptional control

The liver is the only organ besides the kidney that not only can break down glucose by *glycolysis* but also synthesize it by *gluconeogenesis* (p. 134). If both pathways were to take place at the same time, this would create a *futile cycle*, which would consume ATP to no purpose and severely damage the hepatocytes. *Reciprocal regulation* (p. 100) of glycolysis and gluconeogenesis is controlled in part by transcription of **phenolphosphopyruvate carboxykinase** (PEP-CK, [1]).

When glucose is missing (left), synthesis of PEP-CK, a key enzyme in gluconeogenesis, is increased under the influence of the corticosteroid cortisol. The mechanism of transcriptional control by *cortisol* is discussed in detail on pages 242 and 418. When the glucose level rises again, *insulin* is secreted; this inhibits transcription of PEP-CK (and thereby gluconeogenesis) by inactivating the transcription factor Foxo (p. 428).

B. Interconversion

The regulation of **pyruvate dehydrogenase** (PDH, [2]), an enzyme at the interface between carbohydrate and lipid metabolism, is particularly complex (p. 112). PDH is inactivated by phosphorylation catalyzed by a specific *protein kinase* [3] and is reactivated by a *phosphatase* specific for PDH (4). Kinase and phosphatase in turn are regulated by hormones and metabolites. The reaction products *acetyl-CoA* and *NADH* activate the kinase and inhibit PDH activity (product inhibition). *NADH* achieves the same effect by inhibiting the phosphatase. PDH is activated by *insulin* and the precursor *pyruvate*, which inhibit the kinase, and Ca^{2+} , which activates the phosphatase. The Ca^{2+} effect is especially important in muscle.

C. Compartmentation

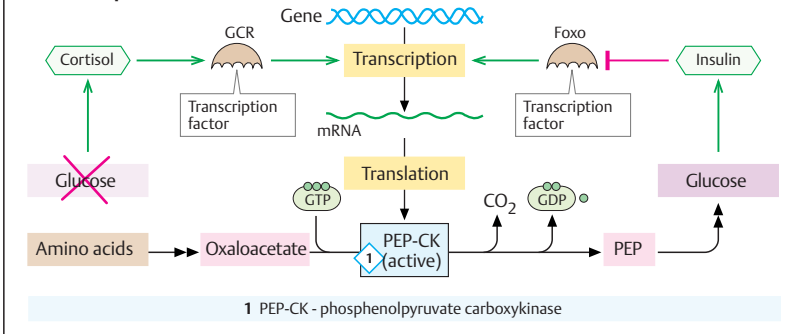
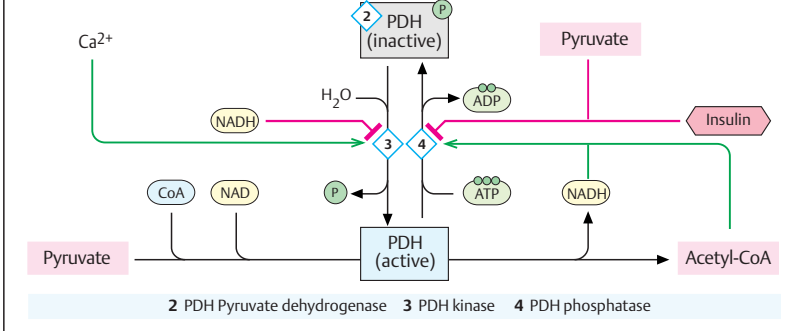
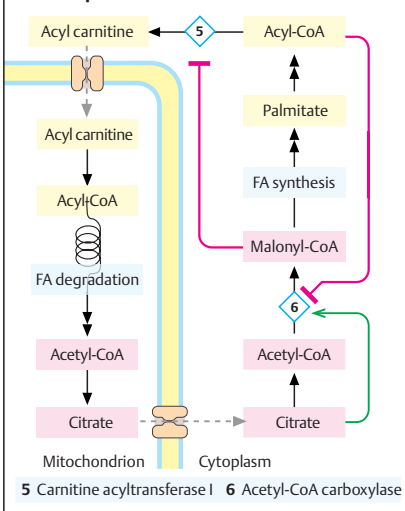
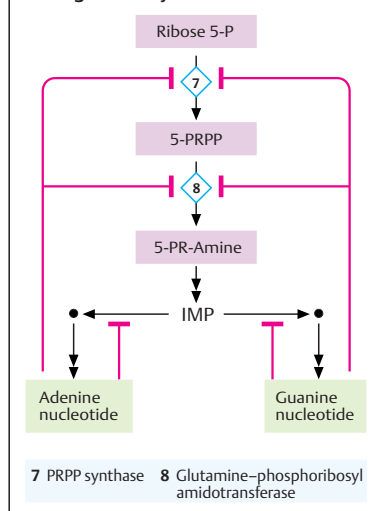
Fatty acid metabolism is divided between two cell compartments. Degradation of activated fatty acids (acyl-CoA) by β -oxidation takes

place in the matrix of mitochondria, while *fatty acid biosynthesis*, which is followed by the formation of acyl-CoA, occurs in the cytoplasm. Here, too, *reciprocal regulation* must ensure that immediate breakdown of newly formed acyl-CoA is avoided. This is achieved by *malonyl-CoA*, which occurs in higher concentrations only during current fatty acid biosynthesis, and prevents the uptake of acyl-CoA into the mitochondria by inhibiting **carnitine acyltransferase I** (5) (p. 146). Conversely, when the concentration of acyl-CoA becomes too high, this inhibits **acetyl-CoA carboxylase** (6) and thus the production of malonyl-CoA. This halts the inhibition of acyl-CoA transport into the mitochondria.

When acetyl-CoA is abundant in the mitochondrial matrix, citrate is produced from it; this leaves the mitochondria to act as a precursor of fatty acid synthesis in the cytoplasm. Citrate promotes its own use by activating acetyl-CoA carboxylase. This is a typical example of activation of a pathway by a precursor (*feed-forward activation*).

D. Regulation by metabolites

Cells normally require only small amounts of nucleotides. They are needed in large quantities as nucleic acid precursors only in the S phase of the cell cycle (p. 444) and during intensive transcription. The regulation of purine nucleotide biosynthesis is a good example of **feedback inhibition**. Several key enzymes in this pathway, including the “early” enzymes *phosphoribosyl diphosphate (PRPP) synthase* (7) and *phosphoribosyl aminotransferase* (8), are inhibited allosterically by adenine and guanine nucleotides. The synthesis of purine nucleotides therefore comes rapidly to a standstill if the products of the pathways are not immediately incorporated into nucleic acids. The biosynthesis of pyrimidine nucleotides is also controlled in this way.

A. Transcriptional control**B. Interconversion****C. Compartmentation****D. Regulation by metabolites**

ATP

The nucleotide coenzyme **adenosine triphosphate** (ATP) serves as the most important **storage form of chemical energy** in all cells. Cleavage of ATP is strongly exergonic. The released energy (ΔG) is used to drive endergonic processes (such as biosynthesis and movement and transport processes) through *energetic coupling* (p. 16). Other **nucleoside triphosphate coenzymes** (GTP, CTP, and UTP) have similar chemical properties to ATP, but they are used for different tasks in metabolism (p. 92).

A. ATP: structure

ATP is a *nucleoside triphosphate* in which a chain of three phosphate residues is linked to the 5'-OH group of the nucleoside adenosine (p. 64). These phosphate residues are termed α , β , and γ . The α phosphate is bound to ribose by a *phosphoric acid ester bond*. The linkages *between* the three phosphate residues, on the other hand, involve much more unstable *phosphoric acid anhydride bonds*. The active coenzyme is in fact generally a complex of ATP with a Mg^{2+} ion, which is coordinatively bound to the α and β phosphate residues ($Mg^{2+} \cdot ATP^{4-}$). However, the term "ATP" is usually used for the sake of simplicity.

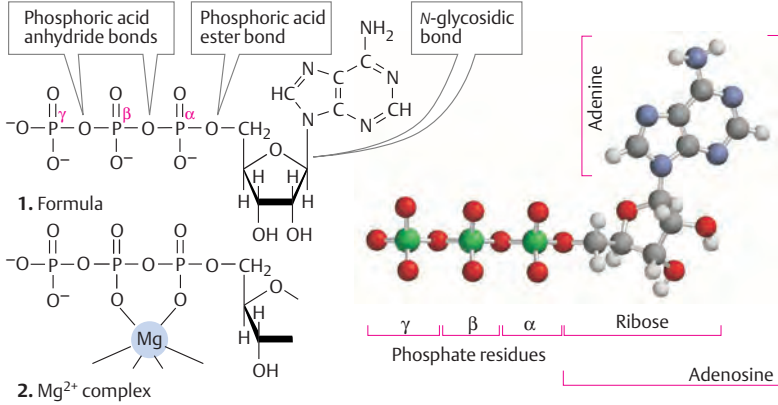
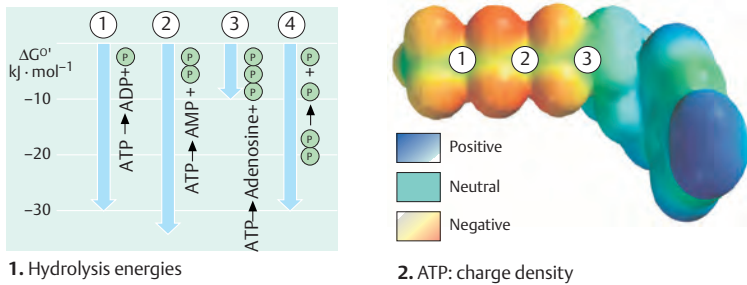
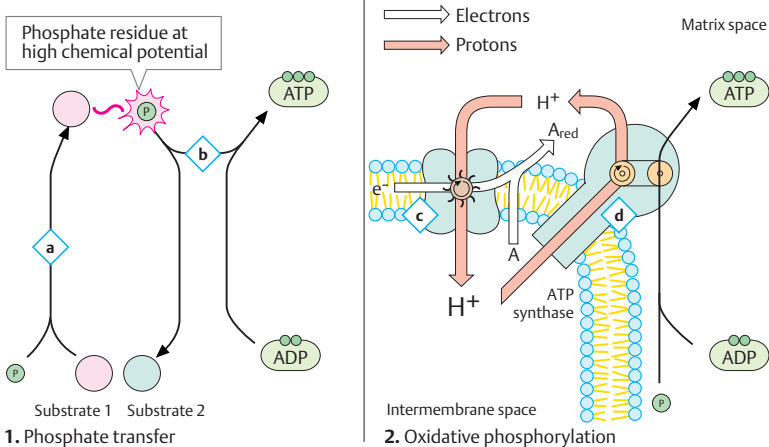
B. Energies of hydrolysis

The formula for the phosphate residues of ATP shown in **A**, with single and double bonds, is not an accurate representation of the actual charge distribution. The oxygen atoms of all three phosphate residues have similarly strong negative charges (orange), while the phosphorus atoms represent centers of positive charge (green). The main reason for the instability of phosphoric anhydride bonds is the *repulsion between these negatively charged oxygen atoms*, which is partly relieved by cleavage of a phosphate residue. In addition, the free phosphate anion formed by hydrolysis of ATP is *better hydrated* and *more strongly resonance-stabilized* than the corresponding residue in ATP. This also contributes to the strongly exergonic character of ATP hydrolysis. In *standard conditions*, the change in free enthalpy ΔG° (prime) (p. 18) that occurs in the hydrolysis of phosphoric acid anhydride bonds amounts to -30 to $-35 \text{ kJ} \cdot \text{mol}^{-1}$ at pH 7. The particular anhydride bond of ATP that is cleaved only has a minor influence on ΔG° (1, 2). Even the hydrolysis of diphosphate (4) still

yields about $-30 \text{ kJ} \cdot \text{mol}^{-1}$. By contrast, cleavage of the ester bond between ribose and phosphate (3) only provides $-9 \text{ kJ} \cdot \text{mol}^{-1}$. In the cell, the effective ΔG of ATP hydrolysis is substantially larger, because the concentrations of ATP, ADP and P_i are much lower than in standard conditions (all reactants at $1 \text{ mol} \cdot \text{L}^{-1}$) and there is an excess of ATP over ADP. The pH value and Mg^{2+} concentration also affect the value of ΔG . The *physiological energy yield* of ATP hydrolysis to ADP and inorganic phosphate (P_i) is probably around $-50 \text{ kJ} \cdot \text{mol}^{-1}$.

C. Types of ATP formation

Only a few compounds contain phosphate residues with a chemical potential (p. 18) high enough for them to be transferred to ADP and thus to allow **ATP synthesis**. Processes that raise inorganic phosphate to such a high potential are called **substrate level phosphorylations** (1.; p. 106). Reactions of this type take place only in glycolysis (p. 130) and in the tricarboxylic acid cycle (p. 114). Another "energy-rich" phosphate compound is *creatine phosphate*, which is formed from ATP in muscle and can regenerate ATP (p. 344). Most cellular ATP does not arise in the way described above (i.e., by transfer of phosphate residues from organic molecules to ADP), but rather by **oxidative phosphorylation** (2.; p. 110, 120). This process takes place in mitochondria (in plants also in chloroplasts) and is energetically coupled to a proton gradient over a membrane. These H^+ gradients are established by electron transport chains and are used by the enzyme *ATP synthase* as a source of energy for direct transfer of inorganic phosphate to ADP (p. 122). In contrast to substrate level phosphorylation, oxidative phosphorylation requires the presence of oxygen (i.e., *aerobic conditions*).

A. ATP: structure**B. Energies of hydrolysis****C. Types of ATP formation**

Energetic Coupling

Energetic coupling is a mechanism in which an energy-dependent (endergonic) process is coupled functionally to a second energy-providing (exergonic) process so that the overall process proceeds spontaneously (p. 16). For example, the endergonic production of light in a flashlight is coupled to an exergonic chemical reaction, while an electric motor uses the exergonic flow of electrons to carry out endergonic mechanical work.

A. Energetic coupling

Living cells couple all types of endergonic processes to a specific chemical reaction, the cleavage of **adenosine triphosphate** (ATP; p. 104) to ADP and phosphate or AMP and diphosphate. ATP and similar molecules are therefore often called “energy-rich” compounds. However, this expression should not be misunderstood. The binding energies between the atoms in ATP are no higher than in other molecules, but rather the amounts of energy (measured as ΔG , p. 18), which are involved when **phosphate** is transferred from ATP to other molecules.

To estimate the group transfer potential of “energy-rich” compounds, the change in free enthalpy ΔG° during *hydrolysis* (pp. 18, 104) is considered arbitrarily. However, this does not mean that ATP is in fact hydrolyzed in energetically coupled reactions. If ATP hydrolysis and an endergonic process were simply allowed to run alongside each other, the hydrolysis would only produce heat, without influencing the endergonic process. For chemical coupling, the two reactions have to be interlinked in such a way that a *common intermediate* arises. This fact is illustrated here using the example of the **glutamine synthase reaction**.

Direct transfer of NH_4^+ to glutamate is endergonic ($\Delta G^\circ = +14 \text{ kJ} \cdot \text{mol}^{-1}$), and can therefore not take place spontaneously under standard conditions (p. 18). In the cell, the reaction is divided into two exergonic steps. First, the γ -phosphate residue is transferred from ATP to glutamate. This gives rise to an “energy-rich” *mixed acid anhydride*. In the second step, the phosphate residue of the intermediate is substituted by NH_3 , and glutamine and free phosphate are produced. The energy balance of the reaction as a whole ($\Delta G^\circ = -17 \text{ kJ} \cdot \text{mol}^{-1}$) is the sum of the changes in free enthalpy of direct glutamine synthesis ($\Delta G^\circ = 14 \text{ kJ} \cdot \text{mol}^{-1}$) plus

ATP hydrolysis ($\Delta G^\circ = -31 \text{ kJ} \cdot \text{mol}^{-1}$), although in reality ATP has not been hydrolyzed at all.

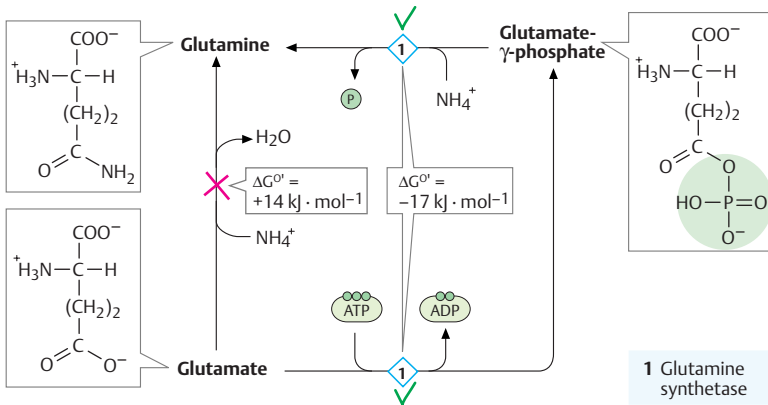
B. Substrate-level phosphorylation

In the cell, there are only a few metabolites that are able to transfer phosphate to ADP in an exergonic reaction and thereby form ATP (p. 104). In the synthesis of these metabolites, inorganic phosphate or phosphate bound in an esterlike fashion is transferred to bonds with a high phosphate transfer potential. Reactions of this type are termed “*substrate-level phosphorylations*,” as they represent individual steps within metabolic pathways.

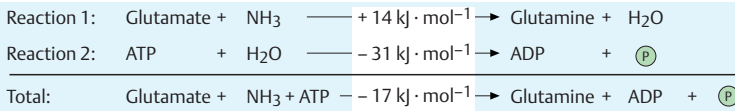
In the **glyceraldehyde 3-phosphate dehydrogenase** [1] reaction, a step involved in glycolysis (left), the aldehyde group in glyceraldehyde 3-phosphate is oxidized to a carboxyl group. During the reaction, an inorganic phosphate is also introduced into the product, producing a mixed acid anhydride—1,3-bisphosphoglycerate. **Phosphopyruvate hydratase** [2] (“enolase,” center) catalyzes the elimination of water from 2-phosphoglycerate. In the *enol phosphate* formed (phosphoenol pyruvate), the phosphate residue—in contrast to 2-phosphoglycerate—is at an extremely high potential (ΔG° of hydrolysis: $-62 \text{ kJ} \cdot \text{mol}^{-1}$). A third substrate-level phosphorylation is the formation of succinyl phosphate, which occurs in the tricarboxylic acid cycle as an individual step in the **succinyl CoA ligase** [3] (“thiokinase,” right) reaction. Here again, inorganic phosphate is introduced into a mixed acid anhydride bond to be transferred from there to ADP or GDP (p. 114). Succinyl phosphate is only an intermediate here, and is not released by the enzyme.

In the literature, the term “substrate-level phosphorylation” is used inconsistently. Some authors use it to refer to reactions in which inorganic phosphate is raised to a high potential, while others use it for the subsequent reactions, in which ATP or GTP is formed from the energy-rich intermediates.

A. Energetic coupling

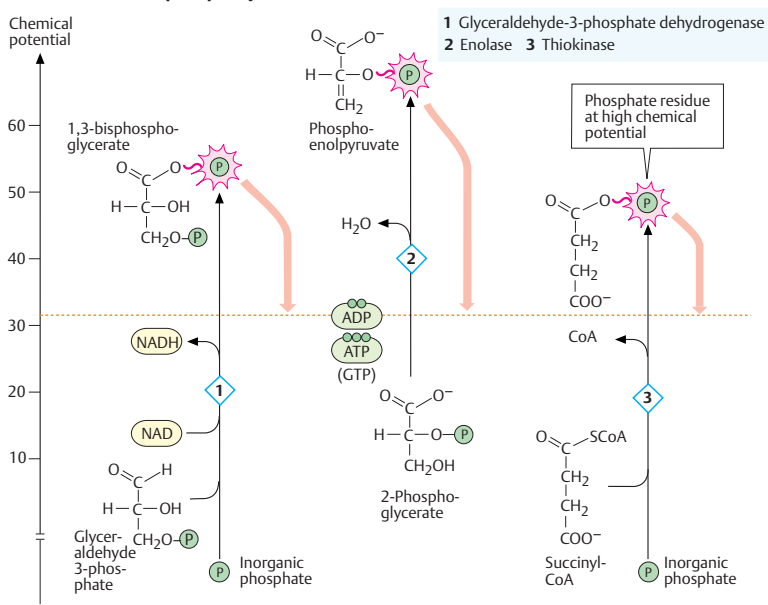


1. Glutamine synthetase reaction



2. Energy balance

B. Substrate-level phosphorylation



Energy Conservation at Membranes

Metabolic energy can be stored not only in the form of “energy-rich” bonds (p. 106), but also by separating electric charges from each other at the expense of energy, and using an insulating layer to prevent them from becoming redistributed evenly again. In the field of technology, this type of system is called a *capacitor*. Using the same principle, energy is also stored (“conserved”) at biological membranes. The membrane functions as an insulator; while electrically charged atoms and molecules (*ions*) represent the charges.

A. Electrochemical gradients

Many ions (Na^+ , K^+ , Ca^{2+} , Cl^- , etc.) are distributed unevenly inside and outside living cells (1). This is due to active transport processes (see B) and to the fact that biological membranes contain selective and partly regulated **ion channels** that control the passage of individual ion types (p. 402). Whether a given ion can cross a membrane, and if so in which direction, depends on the **electrochemical gradient**—i.e., on the concentrations of the ion on each side of the membrane (the *concentration gradient*) and on the *difference* in the electrical potential between the interior and exterior, namely the **membrane potential** of the cell. The behavior of a given type of ion is described quantitatively by the **Nernst equation**:

$$\Delta\psi_G = (R \cdot T/F \cdot n) \cdot \ln (c_{\text{outside}}/c_{\text{inside}}).$$

$\Delta\psi_G$ is the membrane potential in millivolts (mV) at which *no* net transport of the ion through the membrane takes place (**equilibrium potential**). The value of the factor $R \cdot T/F \cdot n$ is 26 mV for monovalent ions at 25°C. The table (2) shows that this gives an equilibrium potential for K^+ of about –90 mV, i.e., similar to the resting potential (see below). In contrast, $\Delta\psi_G$ is +70 mV for Na^+ ions, which is much higher than the resting potential. Na^+ ions therefore flow into the cell immediately when Na^+ channels open (p. 360).

The actual membrane potential of resting cells (**resting potential**) is –50 to –90 mV—i.e., there is an excess negative charge on the inner side of the plasma membrane. The main contributors to the resting potential are the cations Na^+ and K^+ , as well as Cl^- and organic anions (1). Data on the concentrations of these ions outside and inside animal cells, and the corre-

sponding permeability coefficients, are shown in the table (2).

B. Na^+/K^+ -ATPase

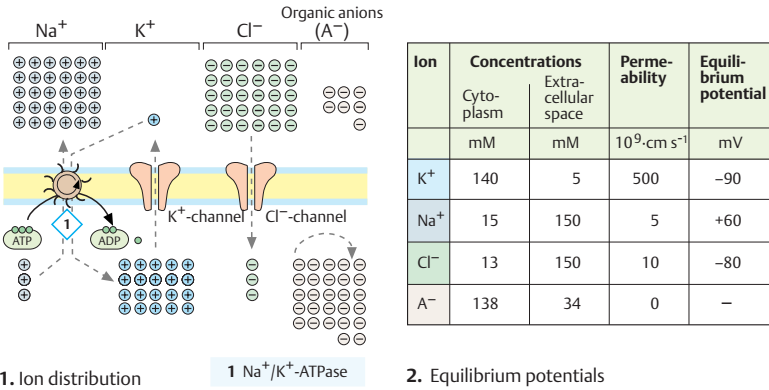
The main reason for the unequal distribution of Na^+ and K^+ ions inside and outside living cells and therefore for the membrane potential is an active *sodium–potassium pump* in the plasma membrane, an enzyme which consistently transfers 3 Na^+ ions outward in exchange for 2 K^+ ions using ATP. This **Na^+/K^+ -ATPase** [1] is present in all cells and is responsible for a considerable proportion of overall ATP consumption in the body.

The catalytic cycle of the Na^+/K^+ pump (2) involves several steps. First, the enzyme binds 3 Na^+ ions (top right) on the inside of the membrane. Phosphorylation of an aspartate residue in the α subunit then leads to a conformational change and release of the Na^+ ions on the outside. The next step is to bind 2 external K^+ ions. The enzyme returns to its initial conformation by hydrolysis of the aspartyl phosphate and, after releasing the K^+ ions on the inside, it is ready to start a new cycle.

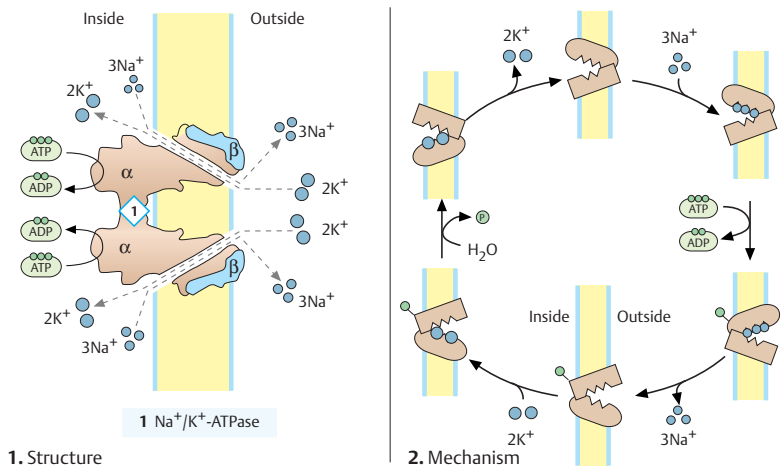
C. Proton gradients

Oxonium ions (H_3O^+ , “ H^+ ions,” p. 14) can also form electrochemical gradients. Such a **proton gradient** plays a decisive part in cellular ATP synthesis as part of *oxidative phosphorylation* (p. 120). As usual, the energy content of the gradient depends on the concentration gradient—i.e., on the **pH difference**, ΔpH , between the two sides of the membrane. In addition, the **membrane potential**, $\Delta\psi$, also makes a contribution. Together, these two values give the **proton motive force**, Δp , a measure for the chemical work that the H^+ gradient can do. For example, the proton gradient across the inner mitochondrial membrane (p. 122) thus delivers approximately 24 kJ per mol H^+ .

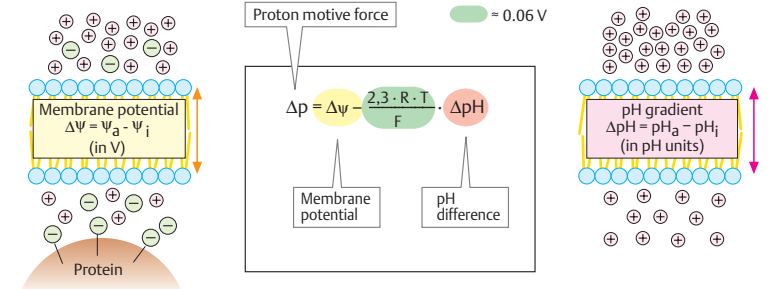
A. Electrochemical gradients



B. Na^+/K^+ -ATPase



C. Proton gradients



Energy Metabolism: Overview

A. Energy metabolism: overview

Energy metabolism refers to all the metabolic pathways and reactions that serve to provide chemical energy in the cell in the form of ATP (p. 104). As discussed on page 96, very different forms of nutrition have developed during evolution. Animals and therefore humans have purely *chemoheterotrophic* metabolism. To synthesize ATP, they require organic compounds which are ingested in the diet and then broken down by oxidation through *catabolic* pathways. The kinds of molecules that can be degraded, and whether the breakdown is partial or complete, depend on whether or not *molecular oxygen* (O_2) is available.

In the presence of adequate amounts of O_2 (**aerobic state, 1**) all types of metabolites can be used to obtain energy. These include glucose, fatty acids, amino acids and all other compounds that can enter the tricarboxylic cycle through catabolic pathways. In the absence of O_2 (**anaerobic state, 2**) or during temporary oxygen deficiency (hypoxia) the only way that mammals can produce ATP is the breakdown of *glucose* by anaerobic glycolysis (1b).

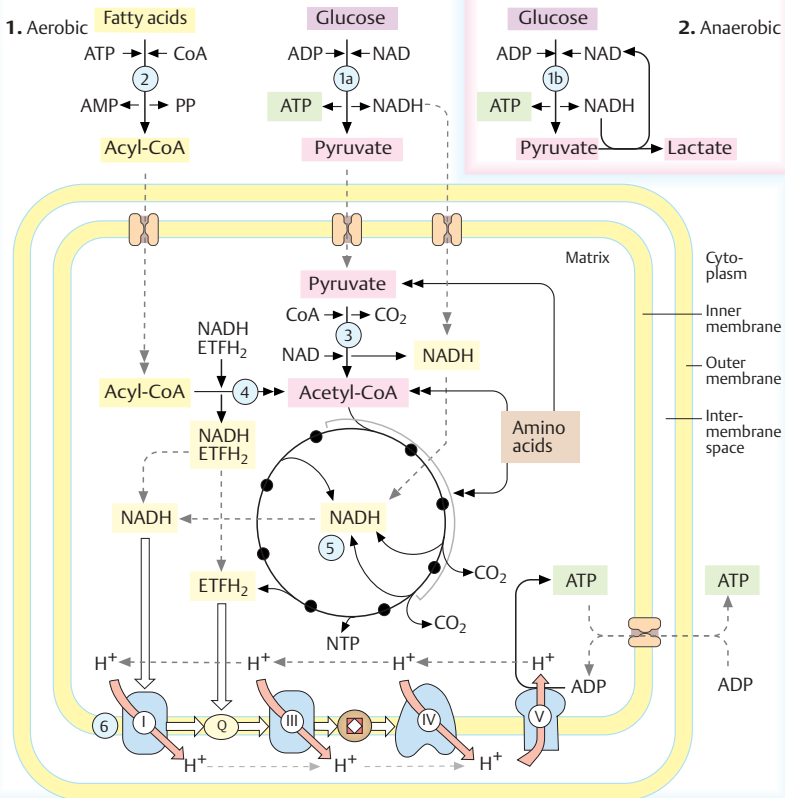
Under **aerobic conditions** (left), ATP is obtained almost entirely by oxidative phosphorylation (6). After activation to *Acyl-CoA* (2), **fatty acids** reach the mitochondria through the carnitine shuttle (p. 146) where they are broken down by β -oxidation (4) to CoA-bound acetyl residues. In the cytoplasm, **glucose** is converted by glycolysis (1a; p. 130) to pyruvate, which is broken down further to acetyl-CoA by oxidative decarboxylation (3) in the mitochondrial matrix. The NADH formed in the cytoplasm reaches the mitochondrial matrix via the malate shuttle (p. 118). The acetyl residues formed are oxidized to CO_2 in the tricarboxylic cycle (5). The degradation of **amino acids** also generates acetyl residues or products that can enter the cycle directly (p. 168f.). Electrons from reduced coenzymes (NADH, QH_2) are transferred to oxygen through the respiratory chain. This releases chemical energy, which is used through a proton gradient to form ATP (6).

In the **anaerobic state** (top right) NADH and QH_2 can no longer be reoxidized by the respiratory chain. As a result, not only mitochondrial ATP synthesis but almost the entire metabolism of the mitochondrial matrix comes to a standstill. The cell then relies completely on ATP production by anaerobic **glycolysis** (1b). If

this process is to take place continuously, the NADH produced in the cytoplasm must be constantly reoxidized. To do so, animal cells reduce pyruvate to lactate under anaerobic conditions and release this into the blood. Processes of this kind are called **fermentations**. Their ATP yield is low and only 2 ATPs arise per glucose when lactate is produced.

To estimate the number of ATP molecules formed in the aerobic state, the **P/O ratio** must be known, i.e., the molar ratio of ATP produced ("P") to water produced ("O"). According to current knowledge, about 10 protons are transported into the intermembrane space during the transport of two electrons from NADH to O_2 , and only six from ubiquinol (QH_2). ATP synthase (p. 122) requires roughly three H^+ for synthesis of one ATP so that maximum P/O ratios of **3 and 2** are possible. From this, a yield of up to 38 ATP per mol of glucose can be calculated. However, the actual value is considerably lower as the transport of some metabolites into the mitochondrial matrix and the exchange of ATP^{4-} for ADP^{3-} are driven by the H^+ gradient (p. 118). The actual P/O ratios for the oxidation of NADH and QH_2 are rather **2.5 and 1.5** respectively. On this basis, the yield is roughly **32 ATPs per glucose**. However, this figure is not constant either, as it can be adjusted by uncouplers (UCPs; p. 124) and other mechanisms as needed.

A. Energy metabolism: overview



Process	Name	Page	Reactant(s)/product(s)	Key enzyme(s)	Regulated by
1a	Aerobic glycolysis (CP)	131	Glucose $\rightarrow$ 2 Pyruvate	P. 139	P. 139
1b	Anaerobic glycolysis (CP)	131	Glucose $\rightarrow$ 2 Lactate	P. 139	P. 125
2	Fatty acid activation (OMM)	147	Fatty acid $\rightarrow$ Acyl-CoA	P. 145	P. 145
3	Oxidative decarboxylation (MM)	113	Pyruvate $\rightarrow$ Acetyl-CoA	P. 101	P. 101
4	β -oxidation (MM)	147	Acyl-CoA $\rightarrow$ n Acetyl-CoA	P. 145	P. 145
5	Tricarboxylic acid cycle (MM)	115 117	Acetyl-CoA $\rightarrow$ 2 CO_2	Citrate synthase Iso-citrate-DH Oxoglutarate-DH	Citrate $\downarrow$, NADH $\uparrow$ Succinyl-CoA $\downarrow$ ADP $\uparrow$, Ca^{2+} $\uparrow$ ATP $\downarrow$, NADH $\downarrow$ Succinyl-CoA $\downarrow$ NADH $\downarrow$
6	Oxidative phosphorylation (IMM)	121	NADH, ETFH_2 , O_2 , ADP $\rightarrow$ NAD $^+$, ETF, H_2O , ATP	-	$[\text{NAD}^+]/[\text{NADH}]$ $[\text{ADP}]/[\text{ATP}]$ S. 125

OMM: outer mitochondrial membrane, CP: cytoplasm, DH: dehydrogenase, IMM: inner mitochondrial membrane, MM: mitochondrial matrix

Oxoacid Dehydrogenases

The intermediary metabolism has *multienzyme complexes* which catalyze the **oxidative decarboxylation** of 2-oxoacids and the transfer to coenzyme A of the acyl residue produced. NAD^+ acts as the electron acceptor. Other coenzymes in the reaction are thiamine diphosphate, lipoamide and FAD. The *oxoacid dehydrogenases* include a) the *pyruvate dehydrogenase complex* (PDH, pyruvate $\rightarrow$ acetyl-CoA), b) the *2-oxoglutarate dehydrogenase complex* of the tricarboxylic acid cycle (ODH, 2-oxoglutarate $\rightarrow$ succinyl-CoA), and c) enzymes used in amino acid catabolism, e.g., the *branched chain dehydrogenase complex*, which is involved in the catabolism of valine, leucine, and isoleucine (p. 170).

A. Pyruvate dehydrogenase: reactions

The pyruvate dehydrogenase reaction takes place in the mitochondrial matrix. Three different enzymes (E1–E3) form the PDH multi-enzyme complex (see also B).

- [1] In the first step, *pyruvate dehydrogenase* (E1) catalyzes the decarboxylation of pyruvate and the transfer of the resulting hydroxyethyl residue to **thiamine diphosphate** (TPP). The same enzyme then catalyzes oxidation of the TPP-bound hydroxyethyl group to yield an acetyl residue. This residue and the reducing equivalents obtained are then transferred to **lipoamide**.
- [2] The second enzyme, *dihydrolipoamide acetyltransferase* (E2) shifts the acetyl residue from lipoamide to **coenzyme A**, with dihydrolipoamide being left over.
- [3] The third enzyme, *dihydrolipoamide dehydrogenase* (E3), reoxidizes dihydrolipoamide, with formation of **NADH**. The electrons are first taken over by enzyme-bound **FAD** and then transferred from this via a catalytically active disulfide bond in the E3 subunit (not shown) to soluble NAD^+ . This reaction pathway is possibly only because the involved FAD inside the E3 protein is in an environment that gives it an unusually low redox potential (cf. p. 13, B).

The five different **coenzymes** involved are associated with the enzyme components in different ways. Thiamine diphosphate is non-covalently bound to E1, whereas lipoamide is covalently bound to a lysine residue of E2 and FAD is bound as a *prosthetic group* to E3. NAD^+

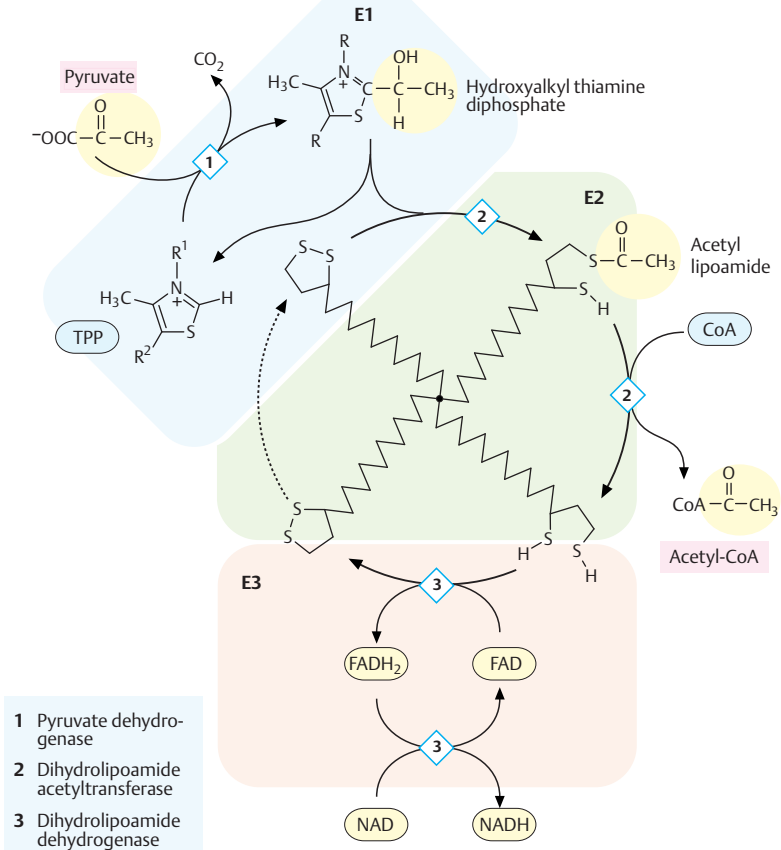
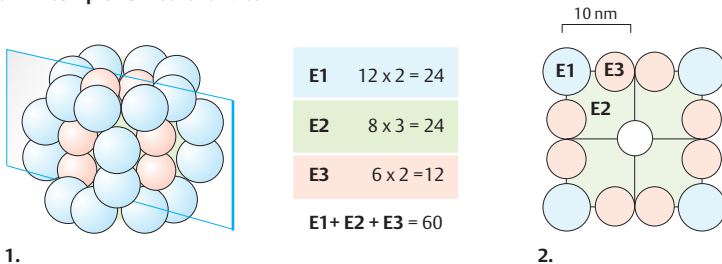
and coenzyme A, being soluble coenzymes, are only temporarily associated with the complex. An important aspect of PDH catalysis is the spatial relationships between the components of the complex. The covalently bound lipoamide coenzyme is part of a mobile domain of E2, and is therefore highly mobile. This structure is known as the *lipoamide arm*, and swings back and forth between E1 and E3 during catalysis. In this way, lipoamide can interact with the TPP bound at E1, with solute coenzyme A, and also with the FAD that serves as the electron acceptor in E3.

B. PDH complex of *Escherichia coli*

The PDH complex of the bacterium *Escherichia coli* has been particularly well studied. It has a molecular mass of 5.3×10^6 , and with a diameter of more than 30 nm it is larger than a ribosome. The complex consists of a total of 60 polypeptides (1, 2): 24 molecules of E2 (eight trimers) form the cube-shaped core of the complex. Each of the six surfaces of the cube is occupied by a dimer of E3 components, while each of the twelve edges of the cube is occupied by dimers of E1 molecules. Animal oxoacid dehydrogenases have similar structures, but differ in the numbers of subunits and their molecular masses.

■ Further information

The PDH reaction, which is practically irreversible, occupies a strategic position at the interface between carbohydrate and fatty acid metabolism, and also supplies acetyl residues to the tricarboxylic acid cycle. PDH activity is therefore strictly regulated. Interconversion is particularly important in animal cells (p. 102, B). Several PDH-specific *protein kinases* inactivate the E1 components through phosphorylation, while *protein phosphatases* reactivate them again. The binding of the kinases and phosphatases to the complex is in turn regulated by metabolites. For example, high concentrations of acetyl-CoA promote binding of kinases and thereby inhibit the reaction, while Ca^{2+} increases the activity of the phosphatase. The hormone insulin activates PDH via inhibition of phosphorylation.

A. Pyruvate dehydrogenase: reactions**B. PDH complex of *Escherichia coli***

Tricarboxylic Acid Cycle: Reactions

The **tricarboxylic acid cycle** (TCA cycle, also known as the citric acid cycle or Krebs cycle) is a cyclic metabolic pathway in the mitochondrial matrix. In eight steps, it oxidizes acetyl residues ($\text{CH}_3\text{-CO-}$) to carbon dioxide (CO_2). The reducing equivalents obtained in this process are transferred to NAD^+ or ubiquinone, and from there to the respiratory chain (p. 120). Additional metabolic functions of the cycle are discussed on page 116.

A. Tricarboxylic acid cycle

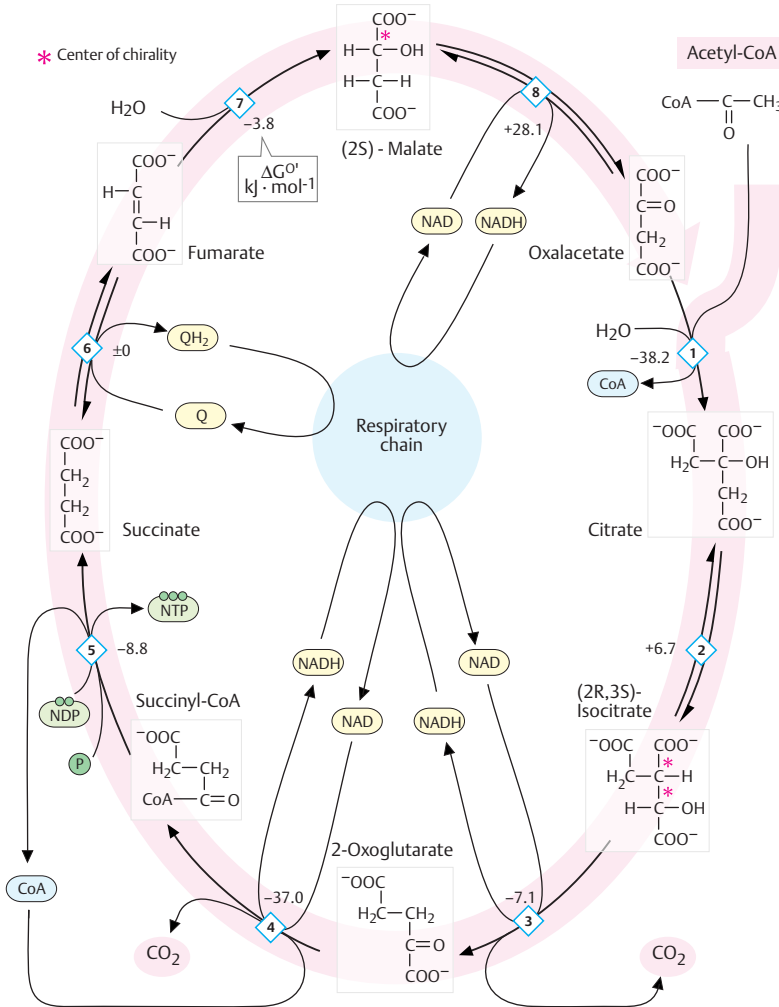
The acetyl-CoA that supplies the cycle with acetyl residues is mainly derived from the β -oxidation of fatty acids (p. 112) and from the *pyruvate dehydrogenase reaction*. Both of these processes take place in the mitochondrial matrix.

- (1) In the first step of the cycle, *citrate synthase* [1] catalyzes the transfer of an acetyl residue from **acetyl-CoA** (p. 8) to a carrier molecule, oxaloacetic acid. The product of this reaction is **citrate**, the tricarboxylic acid which gives the cycle its name.
- (2) In the next step, citrate undergoes isomerization to yield **isocitrate**. In the process, only the hydroxyl group is shifted within the molecule. The corresponding enzyme is called *aconitate hydratase* [2] ("aconitase"), because unsaturated *aconitate* arises as an enzyme-bound intermediate during the reaction (not shown). Due to the properties of aconitase, the isomerization is absolutely *stereospecific*. Although citrate is not chiral, isocitrate has two chiral centers, so that it could potentially appear in *four* isomeric forms. However, in the TCA cycle, only one of these stereoisomers, (2*R*,3*S*)-isocitrate, is produced.
- (3) The first oxidative step now follows. *Isocitrate dehydrogenase* [3] oxidizes the hydroxyl group of isocitrate into a keto group. At the same time, a carboxyl group is released as CO_2 , and **2-oxoglutarate** (also known as α -ketoglutarate) and NADH are formed.
- (4) The next step, the formation of **succinyl-CoA**, also involves one oxidation and one decarboxylation. It is catalyzed by *2-oxoglutarate dehydrogenase* [4], a multienzyme complex closely resembling the PDH complex (p. 112). NADH is once again formed in this reaction.

- (5) The subsequent cleavage of the thioester succinyl-CoA into **succinate** and coenzyme A by *thiokinase* [5] is strongly *exergonic* and is used to synthesize a phosphoric acid anhydride bond ("*substrate-level phosphorylation*," p. 104). In mammals there are two **isoenzymes** of thiokinase. One of them, prevailing in heart and skeletal muscle, produces **ATP** as usual, while the isoenzyme preferentially expressed in liver and kidney transfers the phosphate residue to GDP to yield guanosine triphosphate (**GTP**).
- (6) Via the reactions described so far, the acetyl residue has been completely oxidized to CO_2 . At the same time, however, the carrier molecule oxaloacetate has been reduced to succinate. Three further reactions in the cycle now regenerate oxaloacetate from succinate. Initially, *succinate dehydrogenase* [6] reduces succinate to **fumarate**. In contrast to the other enzymes in the cycle, succinate dehydrogenase is an integral protein of the inner mitochondrial membrane. It is therefore also assigned to the respiratory chain as complex II. Although succinate dehydrogenase contains FAD as a prosthetic group, **ubiquinone** is the final electron acceptor of the reaction.
- (7) Water is now added to the double bond of fumarate by *fumarase* [7], and chiral (2*S*)-**malate** is produced.
- (8) In the last step of the cycle, malate is again oxidized by *malate dehydrogenase* [8] into **oxaloacetate**, with NADH again being produced. With this reaction, the cycle is complete and can start again from the beginning. As the equilibrium of the reaction lies well on the side of malate, the formation of oxaloacetic acid by reaction [8] depends on the strongly exergonic reaction [1], which immediately removes it from the equilibrium.

The **net outcome** is that each rotation of the cycle converts one acetyl residue and two molecules of H_2O into two molecules of CO_2 . At the same time, one ATP or GTP, three $\text{NADH}+\text{H}^+$ and one reduced ubiquinone (QH_2) are produced. By oxidative phosphorylation, the cell obtains around nine molecules of ATP from these reduced coenzymes (p. 122). Together with the directly formed ATP or GTP, this yields a total of 10 ATPs per acetyl group.

A. Tricarboxylic acid cycle



Tricarboxylic Acid Cycle: Metabolic Functions

A. Tricarboxylic acid cycle: functions

The tricarboxylic acid cycle (p. 114) is often described as the “hub of intermediary metabolism.” It has both catabolic and anabolic functions—it is **amphibolic**.

As a **catabolic pathway**, it initiates the “**terminal oxidation**” of energy substrates. Many catabolic pathways lead to intermediates of the tricarboxylic acid cycle, or supply metabolites such as pyruvate and acetyl-CoA that can enter the cycle, where their C atoms are oxidized to CO₂. The reducing equivalents obtained in this way are then used for *oxidative phosphorylation*—i.e., to aerobically synthesize ATP (p. 104).

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

- Glucose (gluconeogenesis; precursors: oxaloacetate and malate—p. 134)
- Porphyrins (precursor: succinyl-CoA—p. 188)
- Amino acids (precursors: 2-oxoglutarate, oxaloacetate—p. 174)
- Fatty acids and isoprenoids (precursor: citrate—see below)

The intermediates of the tricarboxylic acid cycle are present in the mitochondria only in very small quantities. After the oxidation of acetyl-CoA to CO₂, they are constantly regenerated, and their concentrations therefore remain constant, averaged over time. Anabolic pathways, which remove intermediates from the cycle (e.g., gluconeogenesis) would quickly use up the small quantities present in the mitochondria if metabolites did not reenter the cycle at other sites to replace the compounds consumed. Processes that replenish the cycle in this way are called **anaplerotic reactions**. Conversely, reactions that can remove excess metabolites from the cycle are called **cataplerotic**. This latter group includes transaminations, which use oxaloacetate and 2-oxoglutarate (p. 168).

The degradation of most amino acids is anaplerotic, because it produces either intermediates of the cycle or pyruvate (*glucogenic amino acids*; p. 168). Gluconeogenesis is in fact largely sustained by the degradation of amino acids. A particularly important anaplerotic step in animal metabolism leads from pyruvate to oxaloacetate. This ATP-dependent reaction is catalyzed by *pyruvate carboxylase* [4]. It

allows pyruvate-yielding amino acids and lactate to be used for gluconeogenesis.

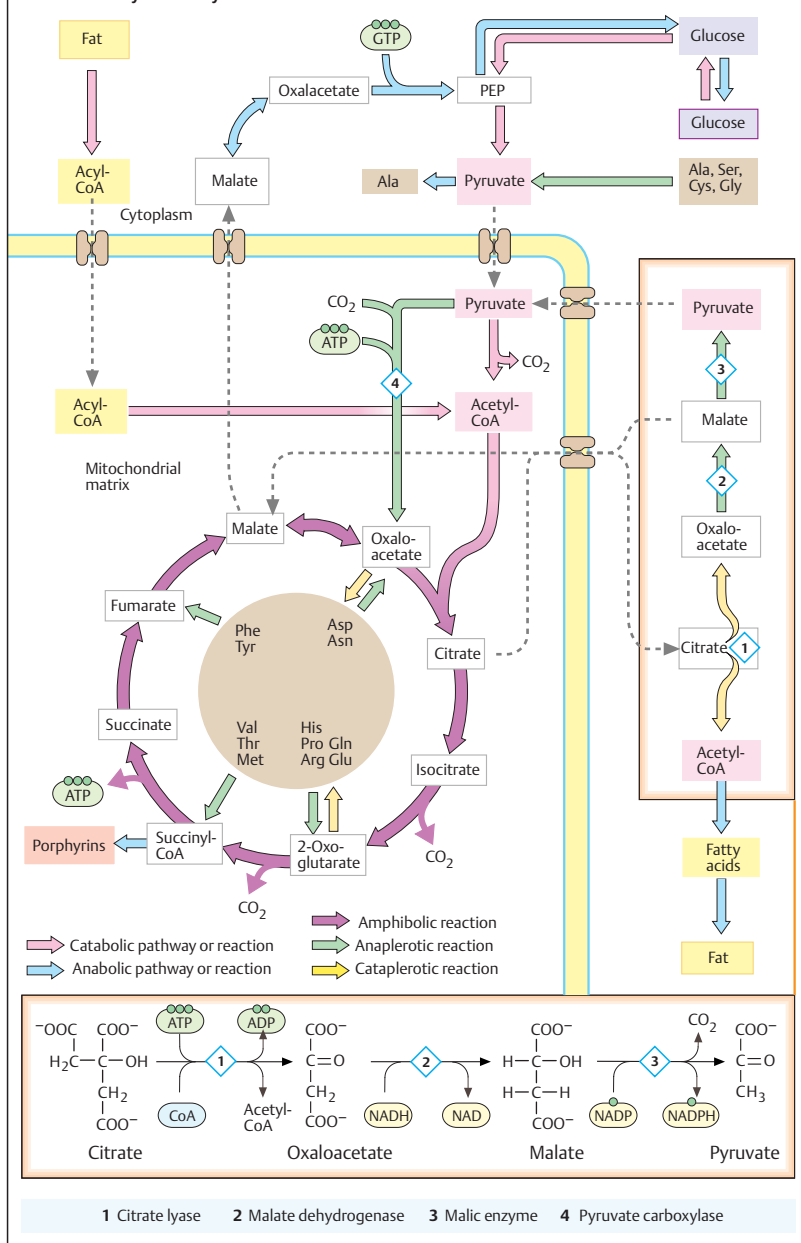
By contrast, *acetyl-CoA does not have anaplerotic effects* in animal metabolism. Its carbon skeleton is completely oxidized to CO₂ and is therefore no longer available for biosynthesis. Since fatty acid degradation only supplies acetyl-CoA, animals are unable to convert fatty acids into glucose. During periods of hunger, it is therefore not the fat reserves that are initially drawn on, but proteins. In contrast to fatty acids, the amino acids released are able to maintain the blood glucose level (p. 378).

The tricarboxylic acid cycle not only takes up acetyl-CoA from fatty acid degradation, but also supplies the material for the *biosynthesis of fatty acids* and isoprenoids. Acetyl-CoA, which is formed in the matrix space of mitochondria by pyruvate dehydrogenase (p. 112), is not capable of passing through the inner mitochondrial membrane. The acetyl residue is therefore condensed with oxaloacetate by mitochondrial *citrate synthase* to form citrate. This then leaves the mitochondria by antiport with malate (right; p. 118). In the cytoplasm, it is cleaved again by ATP-dependent *citrate lyase* [1] into acetyl-CoA and oxaloacetate. The oxaloacetate formed is reduced by a cytoplasmic *malate dehydrogenase* [2] to malate, which then returns to the mitochondrion via the antiport already mentioned. Alternatively, the malate can be oxidized by “*malic enzyme*” [3], with decarboxylation, to pyruvate. The NADPH formed in this process is also used for fatty acid biosynthesis.

■ Further information

Using the so-called **glyoxylic acid cycle**, plants and bacteria are able to convert acetyl-CoA into succinate, which then enters the tricarboxylic acid cycle. For these organisms, fat degradation therefore functions as an anaplerotic process. In plants, this pathway is located in special organelles, the *glyoxysomes*.

A. Tricarboxylic acid cycle: Metabolic functions



Mitochondrial Transport

Mitochondria are surrounded by two membranes (p. 206). The *outer membrane* contains **porins**, which allow passage of molecules with a mass of up to 10 kDa (p. 206). In contrast, the *inner membrane* is impermeable even for small molecules (gases such as O₂ and CO₂ are exceptions). All substrates of mitochondrial metabolism and also its products must therefore be moved through the inner membrane with the aid of specific **transporters**. There is also a special transport system for importing nuclear-coded proteins, which extends through both membranes (p. 218).

A. Metabolite transport

The direction and intensity of metabolite transport through the inner mitochondrial membrane depend on the metabolic situation. The diagram illustrates transport processes that link the metabolite “pools” in the cytoplasm and the mitochondrial matrix in the catabolic (left) and anabolic case (right).

Carbohydrates are degraded in the cytoplasm to **pyruvate**, which reaches the matrix through the *monocarboxylate transporter* (1). The incidentally-produced NADH is taken up indirectly through *shuttles* (3) (see C). **Fatty acids** enter the matrix in the form of acyl-CoA via the *carnitine shuttle* (2, p. 146). **Amino nitrogen** reaches the matrix mainly as glutamine and glutamate. The **ATP** produced in the matrix is exchanged for ADP to enter the cytoplasm. In anabolic metabolism, **oxaloacetate** and **citrate** are the main substances exported from the mitochondria to act as precursors of gluconeogenesis and fatty acid synthesis. The transporters involved are discussed in B.

B. Transport forms

Transport coupled to the hydrolysis of ATP (i.e., primary active transport in the narrower sense, p. 210) does not play an important part in mitochondria. The **proton gradient** and the **membrane potential** of the inner membrane are the driving force for most transport processes (p. 108). The H⁺ concentration in the intermembrane space (p. 120) is 10–100 times higher and the ion distribution is unequal so that the potential on the matrix side is 180–200 mV more negative than outside. This favors all transport processes in which a net negative charge can be transferred from the matrix to the intermembrane space or a net

positive charge into the matrix. As the charges of the transported metabolites show (red numbers), this applies, for example to *ADP/ATP translocase* and *tricarboxylate transporter*.

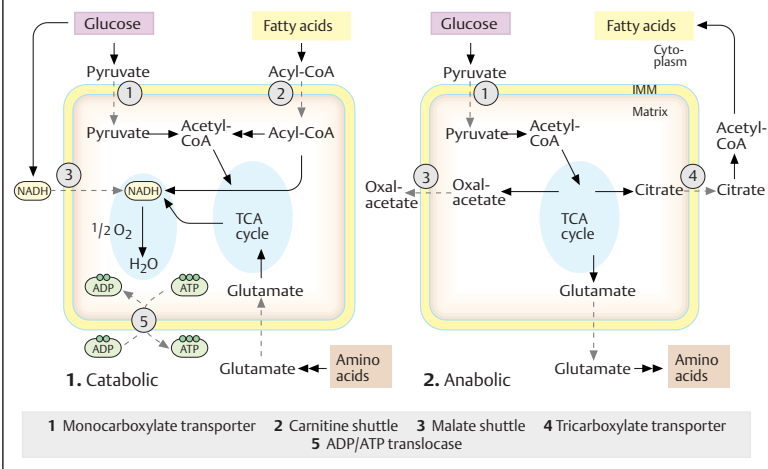
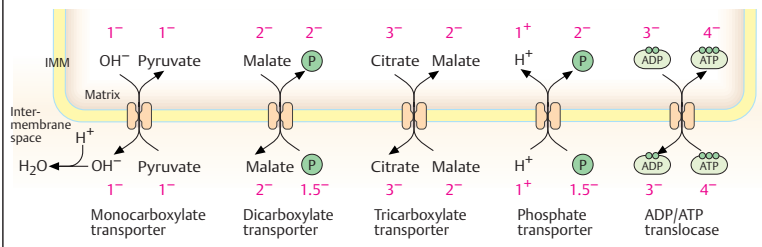
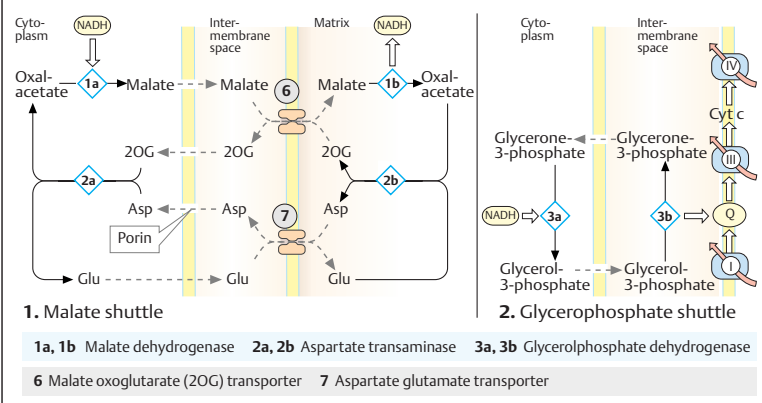
Pyruvate (left) produced in the cytoplasm is imported into the matrix via an antiport in exchange for OH⁻. Although this process is *electrically neutral*, the OH⁻ ions react irreversibly in the intermembrane space with H⁺ ions to form H₂O. This maintains an OH⁻ concentration gradient. The cotransport of **phosphate** and H⁺ through the *phosphate transporter* is also driven by the proton gradient.

C. Malate and glycerophosphate shuttle

As there is no transport system for NADH in the inner membrane, cytoplasmic NADH is imported into the matrix indirectly.

In the **malate shuttle** (left)—which is active, e.g., in the heart, liver and kidneys—oxaloacetate is reduced in the cytoplasm by *malate dehydrogenase* [1a] to malate with the aid of NADH. Malate is exchanged for 2-oxoglutarate in the antiport to reach the matrix, where the mitochondrial isoenzyme of *MDH* [1b] regenerates oxaloacetate and NADH. The latter is reoxidized by the respiratory chain, while oxaloacetate, for which no transporter is available in the inner membrane, is first transaminated to aspartate by *aspartate transaminase* [2a]. Aspartate then leaves the matrix and provides oxaloacetate again in the cytoplasm for step [1a] and glutamate for transport back into the matrix [2b]. Overall, NADH is moved from the cytoplasm into the matrix without consuming ATP.

The **glycerophosphate shuttle** (right) is active in skeletal muscle and in the brain of higher animals. In this process, cytoplasmic NADH is used to reduce glyceralone-3-phosphate, a glycolysis intermediate, to **glycerol-3-phosphate** [3a]. This passes through porins into the intermembrane space and is re-oxidized to glyceralone-3-phosphate on the outside of the inner membrane by *glycerol-3-phosphate dehydrogenase* [3b]. The reducing equivalents are passed on to the respiratory chain through **ubiquinone** (Q).

A. Metabolite transport**B. Transport forms****C. Malate and glycerophosphate shuttle**

Respiratory Chain

The *respiratory chain* is one of the pathways involved in *oxidative phosphorylation* (p. 110). It catalyzes the steps by which electrons are transported from NADH or reduced ubiquinone (QH₂) to molecular oxygen. Due to the wide difference between the redox potentials of the donor (NADH or QH₂) and the acceptor (O₂), this reaction is strongly exergonic (p. 12). Most of the energy released is used to establish a proton gradient across the inner mitochondrial membrane, which is then ultimately used to synthesize ATP with the help of *ATP synthase*.

A. Components of the respiratory chain

The **electron transport chain** consists of three large protein complexes (**complexes I, III, and IV**), which are integrated into the inner mitochondrial membrane, and two mobile carrier molecules—**ubiquinone** (coenzyme Q) and **cytochrome c**. *Succinate dehydrogenase*, which actually belongs to the tricarboxylic acid cycle, is also assigned to the respiratory chain as **complex II**. *ATP synthase* (p. 122) is sometimes referred to as **complex V**, although it is not involved in electron transport.

The complexes in the respiratory chain are made up of numerous subunits and contain protein-bound **redox cofactors** (pp. 12, 86). These include *flavins* (FMN or FAD in complexes I and II), *iron-sulfur clusters* (in I, II, and III), and *heme groups* (in II, III, and IV). Of the more than 80 polypeptides in the respiratory chain, only 13 are coded by the mitochondrial genome (see p. 210). All of the others are encoded by nuclear genes, and have to be imported into the mitochondria after being synthesized in the cytoplasm.

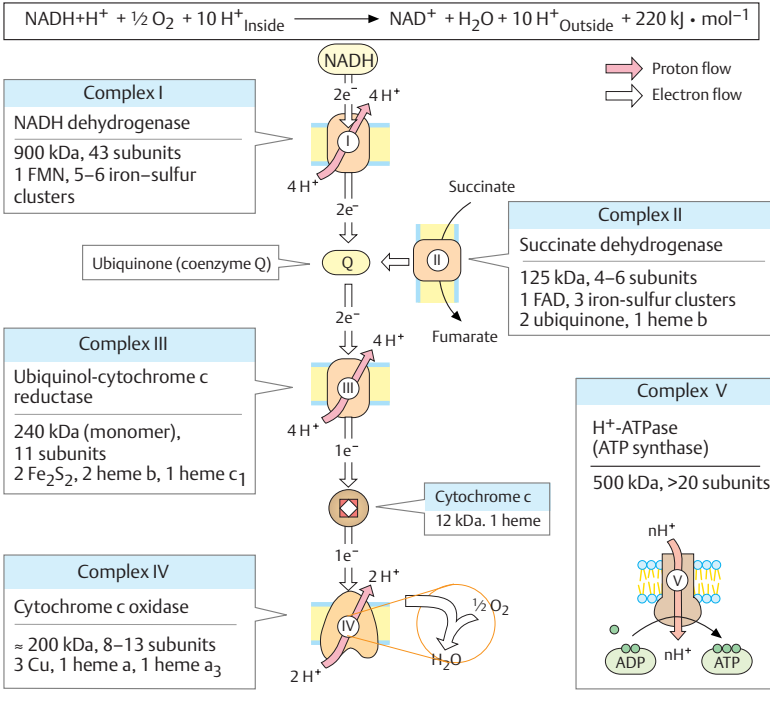
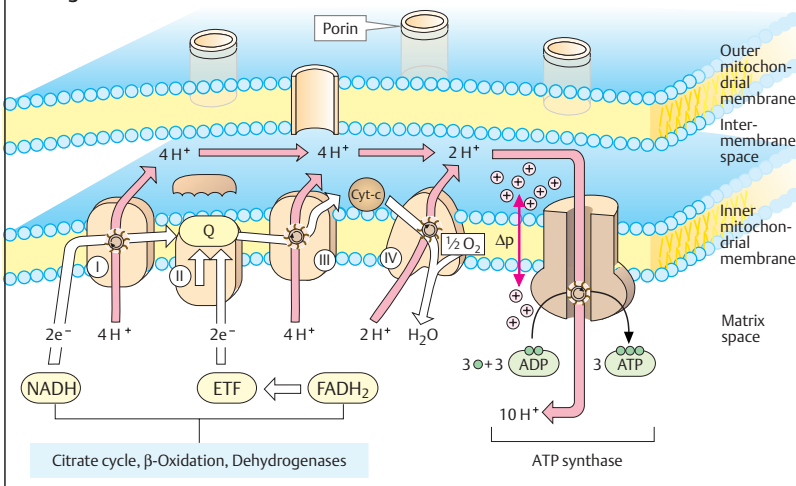
Electrons enter the respiratory chain in various different ways. In the oxidation of NADH by **complex I**, electrons pass via FMN and Fe/S clusters to ubiquinone (Q). Electrons arising during the oxidation of succinate, acyl-CoA, and other substrates are passed to ubiquinone by *succinate dehydrogenase* or other *mitochondrial dehydrogenases* via enzyme-bound FADH₂ and the electron-transporting flavoprotein (ETF; p. 146). Ubiquinol passes electrons on to **complex III**, which transfers them via two b-type heme groups, one Fe/S cluster, and heme c₁ to the small heme protein **cytochrome c**. Cytochrome c then transports the electrons to complex IV—**cytochrome c oxidase**. Cytochrome c oxidase contains redox-active

components in the form of two copper centers (Cu_A and Cu_B) and two hemes a and a₃, through which the electrons finally reach *oxygen* (see p. 122). As the result of the two-electron reduction of ½O₂, the strongly basic O²⁻ anion is produced (at least formally), and this is converted into water by binding of two protons. The electron transfer is coupled to the **formation of a proton gradient** by complexes I, III, and IV (p. 108).

B. Organization

Proton transport via complexes I, III, and IV takes place *vectorially* from the matrix into the intermembrane space. When electrons are being transported through the respiratory chain, the H⁺ concentration in this space increases—i.e., the pH value there is reduced by about one pH unit. For each H₂O molecule formed, around 10 H⁺ ions are pumped into the intermembrane space. If the inner membrane is intact, then generally only *ATP synthase* (p. 122) can allow protons to flow back into the matrix. This is the basis for the coupling of electron transport to ATP synthesis, which is important for regulation purposes (p. 124).

As mentioned, although complexes I through V are all integrated into the inner membrane of the mitochondrion, they are not usually in contact with one another, since the electrons are transferred by ubiquinone and cytochrome c. With its long nonpolar side chain, ubiquinone is freely mobile within the membrane. Cytochrome c is water-soluble and is located on the *outside* of the inner membrane. NADH oxidation via complex I takes place on the *inside* of the membrane—i.e., in the matrix space, where the tricarboxylic acid cycle and β-oxidation (the most important sources of NADH) are also located. O₂ reduction and ATP formation also take place in the matrix.

A. Components of the respiratory chain**B. Organization**

ATP Synthesis

In the **respiratory chain** (p. 120), electrons are transferred from NADH or ubiquinol (QH_2) to O_2 . The energy obtained in this process is used to establish a **proton gradient** across the inner mitochondrial membrane. **ATP synthesis** is ultimately coupled to the return of protons from the intermembrane space into the matrix.

A. Redox systems of the respiratory chain

The electrons provided by NADH do not reach oxygen directly, but instead are transferred to it in various steps. They pass through at least 10 intermediate redox systems, most of which are bound as **prosthetic groups** in complexes I, III, and IV. The large number of coenzymes involved in electron transport may initially appear surprising. However, as discussed on page 12, in redox reactions, the *change in free enthalpy* ΔG —i.e., the chemical work that is done—depends only on the difference in redox potentials, ΔE , between the donor and the acceptor. Introducing additional redox systems does not alter the reaction's overall energy yield. In the case of the respiratory chain, the difference between the normal potential of the donor (NAD^+/NADH , $E^\circ = -0.32 \text{ V}$) and that of the acceptor ($\text{O}_2/\text{H}_2\text{O}$, $E^\circ = +0.82 \text{ V}$) corresponds to an energy difference ΔG° of more than $200 \text{ kJ} \cdot \text{mol}^{-1}$. This large amount is divided into smaller, more manageable “packages,” the size of which is determined by the difference in redox potentials between the respective *intermediates*. This division is responsible for the astonishingly high energy yield (about 60%) achieved by the respiratory chain. The illustration shows the important redox systems involved in mitochondrial electron transport and their approximate redox potentials. These potentials determine the path followed by the electrons, as the members of a **redox series** have to be arranged in order of increasing redox potential if transport is to occur spontaneously (p. 12).

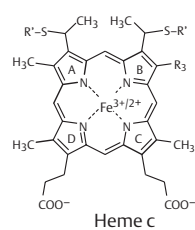
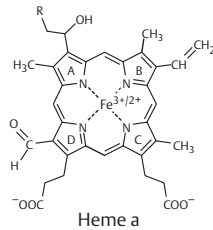
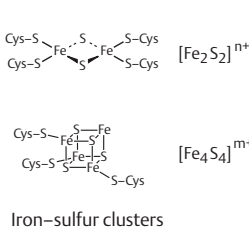
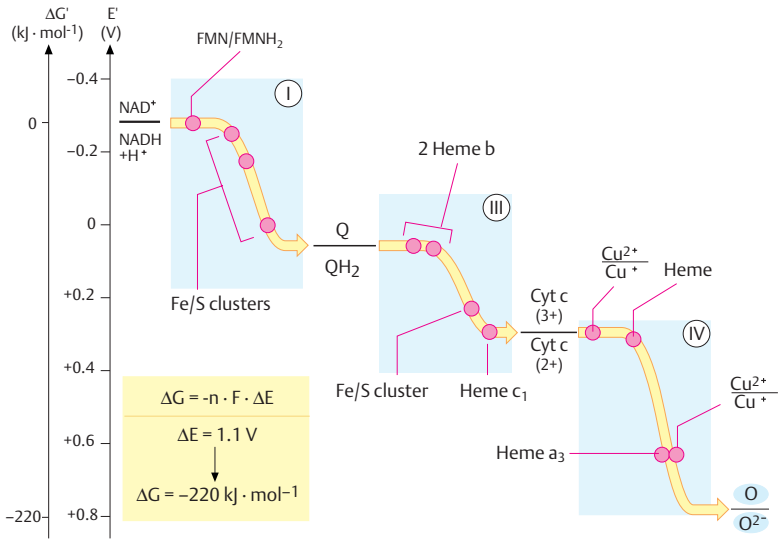
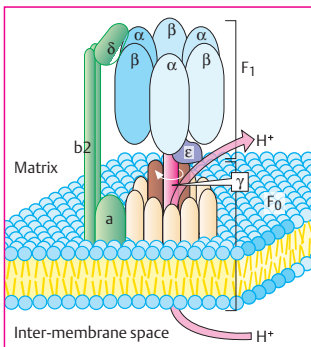
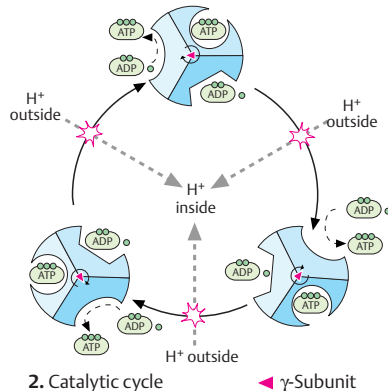
In **complex I**, the electrons are passed from $\text{NADH} + \text{H}^+$ first to **FMN** (p. 86) and then on to several **iron-sulfur** (Fe/S) clusters. These redox systems are only stable in the interior of proteins. Depending on the type, Fe/S clusters may contain two to six iron ions, which form complexes with inorganic sulfide and the SH groups of cysteine residues. **Ubiquinone** (coenzyme Q; p. 12) is a mobile carrier that takes up electrons from complexes I and II and from re-

duced ETF and passes them on to **complex III**. **Heme groups** are also involved in electron transport in a variety of ways. Type b hemes correspond to that found in hemoglobin (p. 286). Heme c in cytochrome c is covalently bound to the protein, while the tetrapyrrole ring of heme a is isoprenylated and carries a formyl group. In **complex IV**, a **copper ion** (Cu_2) and heme a_3 react directly with oxygen.

B. ATP synthase

The **ATP synthase** (**complex V**) that transports H^+ to generate ATP is a complex molecular machine. The enzyme consists of two parts—a **proton channel** (F_0 for “oligomycin-sensitive”) that is integrated into the membrane; and a **catalytic unit** (F_1) that protrudes into the matrix. The F_0 part consists of 12 membrane-spanning c-peptides and one a-subunit. The “head” of the F_1 part is composed of three α and three β subunits, between which there are three active centers. The “stem” between F_0 and F_1 consists of one γ and one ϵ subunit. Two more polypeptides, b2 and δ , form a kind of “stator,” fixing the α and β subunits relative to the F_0 part.

The catalytic cycle can be divided into three phases, through each of which the three active sites pass in sequence. First, ADP and P_i are bound (1), then the anhydride bond forms (2), and finally the product is released. Whenever protons pass through the F_0 channel protein into the matrix, all three active sites change from their current state to the next. It has been shown that the energy for proton transport is initially converted into a rotation of the γ subunit, which in turn cyclically alters the conformation of the α and β subunits, which are stationary relative to the F_0 part, and thereby drives ATP synthesis.

A. Redox systems of the respiratory chain**B. ATP synthase****1. Structure and location**

Regulation of Energy Metabolism

Nutrient degradation and ATP synthesis have to be continually adjusted to the body's changing energy requirements. The need to coordinate the production and consumption of ATP is already evident from the fact that the *total amounts* of coenzymes in the organism are low. The human body forms about 65 kg ATP per day, but only contains 3–4 g of adenine nucleotides (AMP, ADP, and ATP). Each ADP molecule therefore has to be phosphorylated to ATP and dephosphorylated again many thousand times a day.

A. Respiratory control

The simple regulatory mechanism which ensures that ATP synthesis is “automatically” coordinated with ATP consumption is known as **respiratory control**. It is based on the fact that the tricarboxylic acid (TCA) cycle (1), respiratory chain (2) and ATP synthesis (3) are *coupled* via shared coenzymes.

If a cell uses only a little ATP (1), hardly any ADP will be available in the mitochondria. Without ADP, *ATP synthase* (3) is unable to dissipate the proton gradient across the inner mitochondrial membrane. This in turn inhibits electron transport in the respiratory chain, which means that NADH can no longer be re-oxidized to NAD⁺. Finally, the resulting high NADH/NAD⁺ ratio inhibits the TCA cycle, and thus slows down the degradation of the substrates AH₂ (1). Conversely, high rates of ATP utilization (2) stimulate nutrient degradation and the respiratory chain via the same mechanism.

If the formation of a H⁺ gradient is prevented, e.g., by uncoupling (3, see also B), substrate oxidation (1) and electron transport (2) proceed much more rapidly. However, instead of ATP, only heat is produced.

B. Uncouplers

Substances that functionally separate oxidation and phosphorylation from one another are referred to as **uncouplers**. They break down the proton gradient by allowing H⁺ ions to pass from the intermembrane space back into the mitochondrial matrix without the involvement of ATP synthase. Uncoupling effects are produced by mechanical damage to the inner membrane or by lipid-soluble substances that can transport protons through the membrane, such as **2,4-dinitrophenol** (1). The protein

UCP-1 (uncoupling protein-1, Thermogenin, 2)—an ion channel in mitochondria of *brown fat* tissue—is an endogenous uncoupler. Brown fat is found, for example, in newborns and in hibernating animals, and serves exclusively to generate heat. In cold periods, norepinephrine (p. 434) activates the *hormone-sensitive lipase* [1] (p. 330). The increased lipolysis leads to the production of large quantities of free fatty acids in the adipocytes, which activate H⁺ transport via UCP-1. This makes fatty acid degradation independent of ADP availability—i.e., it takes place at maximum velocity and only releases heat. It is becoming increasingly clear that there are also UCPs in other cells, which are controlled by hormones such as thyroxine (p. 426). In this way they regulate the ATP yield and the basal metabolic rate.

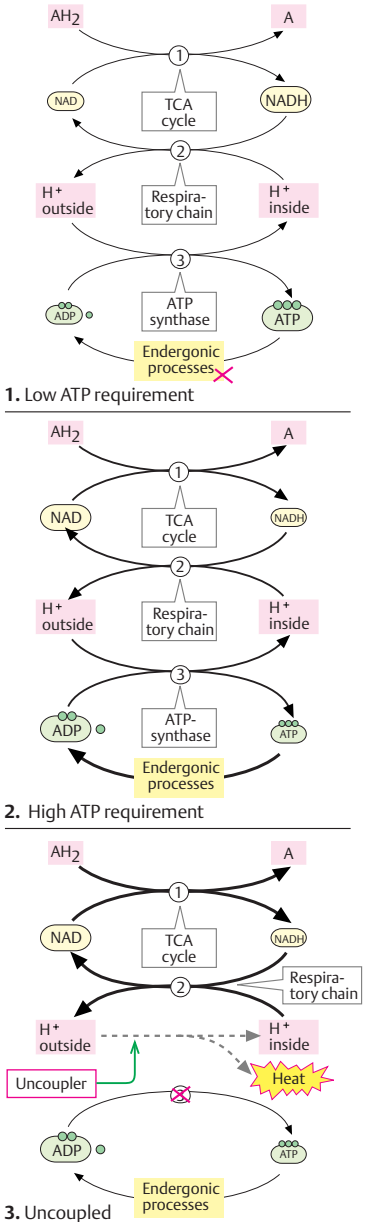
C. AMP-dependent protein kinase

Another global regulatory mechanism that controls the activity of anabolic and catabolic pathways, depending on the supply of ATP, is based on the activity of a particular protein kinase (p. 410), which is activated by adenosine monophosphate (AMP). This *AMP-dependent protein kinase* (AMP-K) acts especially in the liver, muscle and central nervous system (CNS).

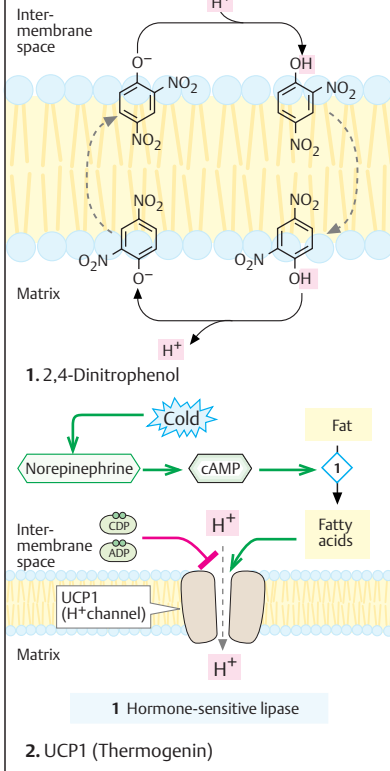
Anabolic pathways and endergonic processes such as muscle contraction use a lot of ATP and thus increase the intracellular ADP level. When this rises, *adenylate kinase* [2] (p. 344) converts more ADP to ATP and **AMP**. The latter activates AMP-K, which then phosphorylates numerous key enzymes of intermediary metabolism (p. 100). This inhibits anabolic pathways that consume ATP and activates catabolic pathways that produce ATP. When the ATP level increases again, this reduces the supply of AMP and thus the activity of the kinase.

In the liver, AMP-K promotes the β -oxidation of fatty acids and ketogenesis, for example (p. 318), while fatty acid biosynthesis is inhibited by inactivation of acetyl-CoA carboxylase (p. 150). In muscle, AMP-K promotes glucose uptake through activation of Glut-4 (p. 140) and inhibits glycogen synthesis.

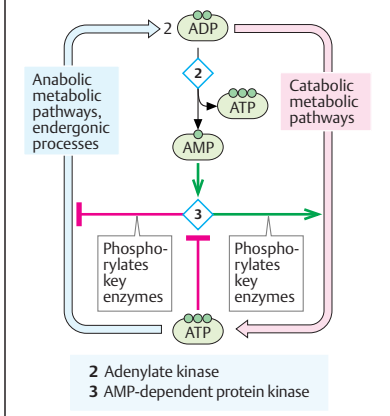
A. Respiratory control



B. Uncouplers



C. AMP-dependent protein kinase



Pathobiochemistry

Oxidative phosphorylation located in the mitochondria provides the majority of cellular ATP in the presence of molecular oxygen (O₂) (p. 110). Organs with high ATP consumption (brain, red muscle fibers, etc.) rely on this more effective way of obtaining energy. They are particularly at risk from acute or chronic oxygen deficiency (*hypoxia*, **A**) and defects in mitochondrial function (**B**). The CNS and the myocardium are damaged irreversibly by even brief interruptions of O₂ supply (e.g., in stroke and myocardial infarction). A better understanding of the biochemical processes that take place in the afflicted cells might substantially improve the treatment of these common diseases.

A. Hypoxia

When O₂ requirements are increased or the availability is reduced, the oxygen supply of organs is ensured by a range of physiological and biochemical mechanisms. A temporary O₂ deficiency can be partially or completely compensated in these ways (**1**): the *lungs* increase O₂ uptake by a greater respiratory rate and greater breath volume, while the *heart* increases the amount of blood transported per unit of time by faster and stronger contractions. In the *erythrocytes* allosteric effectors of hemoglobin such as 2,3-bisphosphoglycerate (p. 290) ensure that more O₂ is delivered to the tissues. Under hypoxic conditions, the number of erythrocytes (the hematocrit) also increases in the longer term through the action of hormones such as erythropoietin (Epo). It is now known that there are also mechanisms in the body's cells that adjust metabolism to hypoxia. Transcription factors (p. 240), known as *hypoxia-induced factors* (HIFs), play a central part.

The best investigated is the gene activator **HIF-1 α** . It binds to DNA and via the mediator CBP/p300 (p. 243) it increases the transcription of genes whose products are needed under hypoxic conditions. These include numerous enzymes of glycolysis and other metabolic pathways, and also the blood-forming hormone Epo (see above) as well as growth factors, which improve the vascular supply of the tissues in the longer term.

Why HIF-1 α is active only in hypoxia has now been explained (**2**): when the O₂ supply is normal (**normoxia**, left), *prolyl* and *asparaginyl hydroxylases* [**1**] hydroxylate proline and as-

paragine residues of HIF-1 α . After VHL-protein binds to the hydroxylated protein, the complex is degraded by the proteasome (p. 162). When O₂ is lacking (**hypoxia**, right) this hydroxylation does not occur and HIF-1 α can combine with HIF-1 β to form an active transcription factor.

B. Mitochondrial diseases

Besides the cell nucleus, mitochondria also contain functional DNA (mtDNA, p. 206). However, the **mitochondrial genome** accounts for less than 0.1% of the cell's total DNA. In humans, mtDNA consists of a circular double strand consisting of 16 569 base pairs containing 37 genes. Most of them code for RNAs needed for translation and only 13 for proteins that occur as subunits in respiratory chain complexes. All of the other mitochondrial proteins are coded by nuclear DNA and have to be imported into the mitochondria after translation.

Diseases due to mutations of mtDNA are called **mitochondrial diseases**. The table shows important disorders of this type. The clinical conditions range from mild disorders to severe organ damage, which can occur already in early childhood. Tissues with high energy requirements are affected most. Frequent symptoms include impairment of muscle function (*myopathies*) and neurological disorders.

A much-discussed but as yet insufficiently confirmed theory attributes increasing organ degeneration in **old age** to an accumulation of mutations in the mitochondrial genome. Mutations of mtDNA are in fact particularly numerous as there are high concentrations of mutagenic "reactive oxygen species" (ROS, p. 288) in the mitochondria while effective DNA repair enzymes (p. 254) are absent.

Overview

The **carbohydrates** are a large group of biomolecules, subgroups of which include the sugars (*monosaccharides*) and their polymers (*oligo- and polysaccharides*, p. 28f.). Only a few of the numerous monosaccharides (p. 30) are used in animal metabolism. These include, in particular, the aldohexoses *D-glucose* and its derivatives, along with *D-galactose*, *D-mannose* and the ketohexose *D-fructose*. Pentoses are used in animals primarily as building blocks for nucleic acids. *Oligosaccharides* occur as components of glycolipids and glycoproteins (p. 34) and in milk, while animal *polysaccharides* with a few exceptions (chitin, p. 32, proteoglycans, p. 354) function as *reserve carbohydrates*.

A. Carbohydrate metabolism: overview

At the core of carbohydrate metabolism is **glucose-6-phosphate**, a phosphoric acid ester of glucose that is the starting point or endpoint of several metabolic pathways. Glucose and the other sugars (fructose, galactose and mannose) are taken up into the cell (1) by passive or secondary active transport (p. 210) as free monosaccharides and immediately phosphorylated in the cytoplasm in the 6 or 1 position by phosphotransferases (*kinases*). Phosphorylation serves to hold the sugar in the cell, as the plasma membrane has no transporters for sugar phosphates.

Metabolic pathways. Depending on the organ and the cell's needs, glucose-6-phosphate (Glc-6-P) is metabolized in different ways:

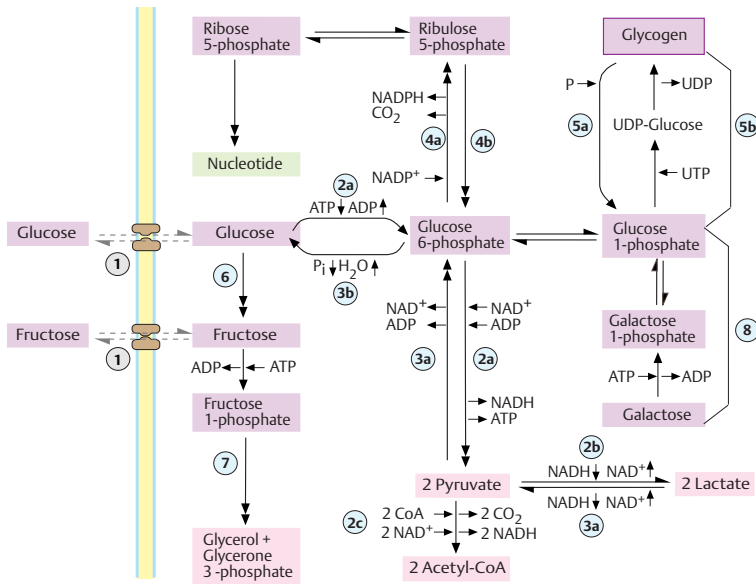
- If the cell requires ATP and/or C_2 and C_3 building blocks, Glc-6-P is metabolized to pyruvate by means of **glycolysis** (2). Glycolysis is in terms of evolution a very old metabolic pathway, which occurs in almost all cells. In the absence of O_2 glycolysis is the only pathway that can generate ATP (**anaerobic glycolysis**). In this case, fermentation products have to be produced from pyruvate—lactate in the case of animal metabolism—to regenerate NAD^+ (2b). In the presence of O_2 pyruvate is metabolized further to acetyl-CoA (**aerobic glycolysis**, 2c), while NADH is reoxidized by the respiratory chain.
- Synthesis of glucose by **gluconeogenesis** (3a, 3b) takes place almost exclusively in the liver and kidneys. The most important precursors are glucogenic amino acids (especially alanine and glutamine), and also lactate and glycerol. Gluconeogenesis can pro-

vide glucose-dependent tissue for weeks, even without an external glucose supply.

- The oxidative part of the **pentose phosphate pathway** (PPP, 4a) generates NADPH for anabolic metabolic pathways and pentoses for nucleotide synthesis. The regenerative part of the pathway (4b) converts pentoses back to hexoses or passes them to glycolysis, when NADPH is not required but pentoses are.
- When there is an abundant glucose supply, glucose-6-phosphate is converted in the liver and muscle via glucose 1-phosphate to UDP-glucose, the activated form, and used to synthesize glycogen (**glycogen synthesis**, 5b). When there is glucose deficiency, stored glycogen is broken down again to glucose (**glycogenolysis**, 5a). Glycogenolysis in the liver maintains the blood sugar level while in muscle it provides glucose for anaerobic glycolysis.
- Fructose, which is important for nourishing spermatozoa, for example, can be produced from glucose through the **polyol pathway** (6). The sugar alcohol sorbitol occurs as an intermediate. Fructose from the diet is metabolized by a special metabolic pathway to glycerol and glycerone 3-phosphate (**fructose degradation**, 7).
- After phosphorylation to galactose 1-phosphate through the isomers, galactose is converted in the liver to glucose 6-phosphate via the isomeric glucose 1-phosphate (**galactose degradation**, 8).

Regulation. The activity of the metabolic pathways that use or produce glucose 6-phosphate is controlled by a complex regulatory system involving several hormones (especially *insulin*, *glucagon* and *cortisol*). The system ensures that peripheral organs that require glucose are constantly supplied with glucose by the bloodstream. The regulation of carbohydrate metabolism is discussed in detail on pages 102, 140, and 142.

A. Carbohydrate metabolism: overview



Process	Name	Page	Reactant(s)/product(s)	Key enzyme(s)	Regulated by
1	Sugar transport (PM)	273	–	–	Ins $\uparrow$ (muscle, AT)
2a+2b	Aerobic glycolysis (CP)	131	Glucose $\rightarrow$ 2 Pyruvate	Hexokinase Glucokinase Phosphofructokinase Pyruvate kinase	Glc-6-P $\downarrow$ Ins $\downarrow$, Cor $\downarrow$, cAMP $\downarrow$ Ins $\downarrow$, AMP $\downarrow$, Fru-2,6-bP $\downarrow$ Cor $\downarrow$, ATP $\downarrow$, Citrate $\downarrow$ Fru-1,6-bP $\downarrow$, ATP $\downarrow$, cAMP $\downarrow$
2a+2c	Anaerobic glycolysis (CP)	131	Glucose $\rightarrow$ 2 Lactate	see above	Supply of pyruvate and NAD $^{+}$
3a+3b	Gluconeogenesis (MM, CP, sER)	135	Lactate, glycerol, glucogenic amino acids	PEP carboxykinase F-1,6-bisphosphatase Glc-6-phosphatase	Ins $\downarrow$, Acetyl-CoA $\downarrow$, cAMP $\downarrow$ Ins $\downarrow$, Cor $\downarrow$, cAMP $\downarrow$ Fru-2,6-bP $\downarrow$, Ins $\downarrow$, AMP $\downarrow$, Cor $\downarrow$, cAMP $\downarrow$ Ins $\downarrow$, Cor $\downarrow$, cAMP $\downarrow$
4a+4b	PPP (MM)	133	Glc-6-P $\leftrightarrow$ Ribulose-5-P	–	Ins $\downarrow$
5a	Glycogenolysis (MM)	137	Glycogen $\rightarrow$ Glc-1-P	Phosphorylase	cAMP $\downarrow$, AMP $\downarrow$, Ca $^{2+}$ $\downarrow$, Ins $\downarrow$
5b	Glycogenesis (CP)	137	Glc-1-P $\rightarrow$ Glycogen	Glycogen synthase	Citrate
6	Polyol pathway	–	Glucose $\rightarrow$ Fructose	Aldehyde reductase	Glucose $\downarrow$, NO $\downarrow$
7	Fructose degradation	317	Fru $\rightarrow$ Glycerol, Glycerone-3-P	Aldolase B	Ins $\downarrow$, cAMP $\downarrow$
8	Galactose degradation	317	Galactose $\rightarrow$ Glc-1-P		

Cor: cortisol, CP: cytoplasm, AT: adipose tissue, sER: smooth endoplasmic reticulum, PPP: pentose phosphate pathway, Ins: insulin, MM: mitochondrial matrix, PM: plasma membrane

Glycolysis

Glycolysis is a catabolic pathway in the cytoplasm that is found in almost all organisms and cells—irrespective of whether they live aerobically or anaerobically. The balance of the pathway is simple: glucose is broken down into two molecules of pyruvate, and in addition two molecules of ATP and two of NADH are formed.

In the presence of oxygen, pyruvate and NADH reach the mitochondria, where they undergo further transformation (**aerobic glycolysis**). In anaerobic conditions, *fermentation products* such as lactate or ethanol have to be formed in the cytoplasm from pyruvate and NADH, to regenerate NAD⁺ so that glycolysis can continue (**anaerobic glycolysis**). In the anaerobic state, glycolysis is the only means of obtaining ATP that animal cells have.

A. Glycolysis: reactions

Glycolysis involves 10 individual steps, including three isomerizations and four phosphate transfers. The only redox reaction in the pathway takes place in step [6].

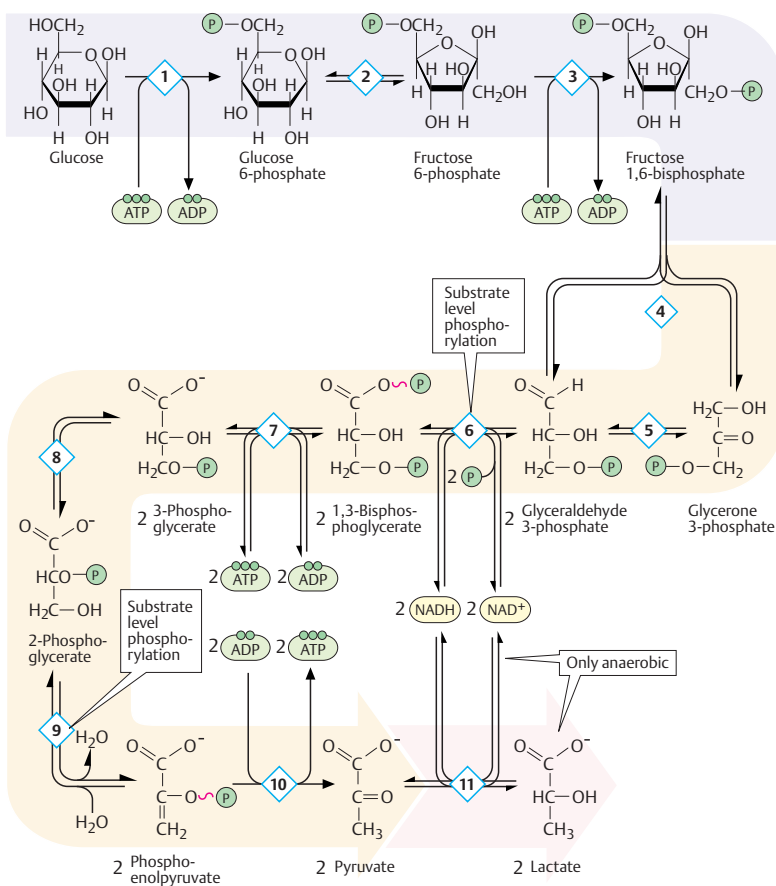
- [1] Glucose, which reaches the cells from the blood via tissue-specific glucose transporters (Glut, p. 212) is first phosphorylated to **glucose 6-phosphate**. Since the ester is not capable of leaving the cell, *hexokinase* [1] is an important key enzyme in glycolysis. *Glucokinase*, an isoenzyme in the liver, is under the control of insulin and is regulated in part by compartmentation (p. 140).
- [2] In the next step, glucose 6-phosphate is converted by *glucose-6-phosphate isomerase* [2] to **fructose 6-phosphate**.
- [3] Using ATP again, another phosphorylation takes place, giving rise to **fructose 1,6-bisphosphate**. *Phosphofructokinase* [3] is the second important key enzyme in the pathway. Its activity is regulated precisely by hormones and metabolites (p. 138).
- [4] The C₆ compound fructose 1,6-bisphosphate is now broken down by *aldolase* [4] into the C₃ compounds **glyceraldehyde 3-phosphate** (also known as glyceral 3-phosphate) and its isomer **glycerone 3-phosphate** (dihydroxyacetone phosphate).
- [5] The latter two products are placed in fast equilibrium, catalyzed by *triosephosphate isomerase* [5].

- [6] Glyceraldehyde 3-phosphate is now oxidized by *glyceraldehyde-3-phosphate dehydrogenase* [6], with NADH being formed. In this reaction, inorganic phosphate is taken up into the molecule (*substrate-level phosphorylation*; p. 104), and **1,3-bisphosphoglycerate** is produced. This intermediate contains a mixed acid–anhydride bond, in which the phosphate residue is at a high chemical potential.
- [7] Catalyzed by *phosphoglycerate kinase* [7], the “energy-rich” phosphate residue is now transferred to ADP, producing **3-phosphoglycerate** and ATP. The ATP balance is thus in equilibrium.
- [8] *Phosphoglycerate mutase* [8] then shifts the remaining phosphate residue to the 2 position, thus producing the isomer **2-phosphoglycerate**.
- [9] Elimination of water from 2-phosphoglycerate with the aid of *enolase* [9] produces the enol phosphate (p. 6) **phosphoenolpyruvate** (PEP). This reaction raises the second phosphate residue to a high chemical potential.
- [10] In the last step, *pyruvate kinase* [10] transfers this phosphate residue to ADP. The remaining enol pyruvate is immediately rearranged into **pyruvate**, which is much more stable. The pyruvate kinase reaction is also tightly regulated. Along with step [7] and the thiokinase reaction in the TCA cycle (p. 114), it is one of the three reactions in animal metabolism that are able to produce ATP independently of the respiratory chain.

In glycolysis, two molecules of ATP are initially used for activation (steps 1 and 3). Later, two ATPs are formed per C₃ fragment (steps 7 and 10). Overall, therefore, there is a small net gain of 2 mol ATP per mol of glucose.

Only three reactions (steps 1, 3, and 10) are associated with large changes in free enthalpy. In these cases, the equilibrium lies well on the side of the products (p. 18). All of the other steps are freely reversible. The same steps are also followed—in the reverse direction—in gluconeogenesis (p. 134), with the same enzymes being activated as in glucose degradation. The non-reversible steps 1, 3, and 10 are bypassed in gluconeogenesis.

A. Glycolysis: reactions



1 Hexokinase

2 Glucose-6-phosphate isomerase

3 Phosphofructokinase

4 Aldolase

5 Triose-phosphate isomerase

6 Glyceraldehyde-3-phosphate dehydrogenase

7 Phosphoglycerate kinase

8 Phosphoglycerate mutase

9 Enolase

10 Pyruvate kinase

11 Lactate dehydrogenase

Pentose Phosphate Pathway

The pentose phosphate pathway (PPP, also known as the *hexose monophosphate pathway*) is an oxidative metabolic pathway located in the cytoplasm, which, like glycolysis, starts from **glucose 6-phosphate**. It supplies two important precursors for anabolic pathways: **NADPH**, which is required for the biosynthesis of fatty acids and isoprenoids, for example (pp. 150, 158), and **ribose 5-phosphate**, a precursor in nucleotide biosynthesis (p. 182).

A. Pentose phosphate pathway: oxidative part

In the **oxidative segment** of the PPP (A, left), glucose 6-phosphate is converted to ribulose 5-phosphate. One CO₂ and two NADPH are formed in the process. Depending on the metabolic state, the more complex **regenerative part** (B, right) can convert some of the pentose phosphates back to hexose phosphates, or it can pass them on to glycolysis for further breakdown. In most cells, less than 10% of glucose 6-phosphate is degraded via the pentose phosphate pathway.

- [1] The **oxidative part** starts with the oxidation of **glucose 6-phosphate** by *glucose-6-phosphate dehydrogenase*. This forms NADPH for the first time. The second product, **6-phosphogluconolactone**, is the intramolecular ester (*lactone*) of 6-phosphogluconate.
- [2] A specific *hydrolase* then cleaves the lactone, exposing the carboxyl group of **6-phosphogluconate**.
- [3] The last enzyme in the oxidative part is *phosphogluconate dehydrogenase*, which releases the carboxylate group of 6-phosphogluconate as CO₂ and at the same time oxidizes the hydroxyl group at C₃ to an oxo group. In addition to a second NADPH, this also produces the ketopentose **ribulose 5-phosphate**. This is converted by an isomerase to ribose 5-phosphate, the initial compound for nucleotide synthesis.

The **regenerative part** of the PPP is only shown here schematically. Its function is to adjust the *net* production of NADPH and pentose phosphates to the cell's current requirements. Normally, the demand for NADPH is much higher than that for pentose phosphates. In these conditions, the reaction steps shown first convert six ribulose 5-phosphates to five molecules of fructose 6-phosphate and then, by isomerization, regenerates five glucose 6-phosphates.

These can once again supply NADPH to the oxidative part of the PPP. Repeating these reactions finally results in the oxidation of one glucose 6-phosphate into six CO₂. Twelve NADPH arise in this process. In sum, no pentose phosphates are produced via this pathway.

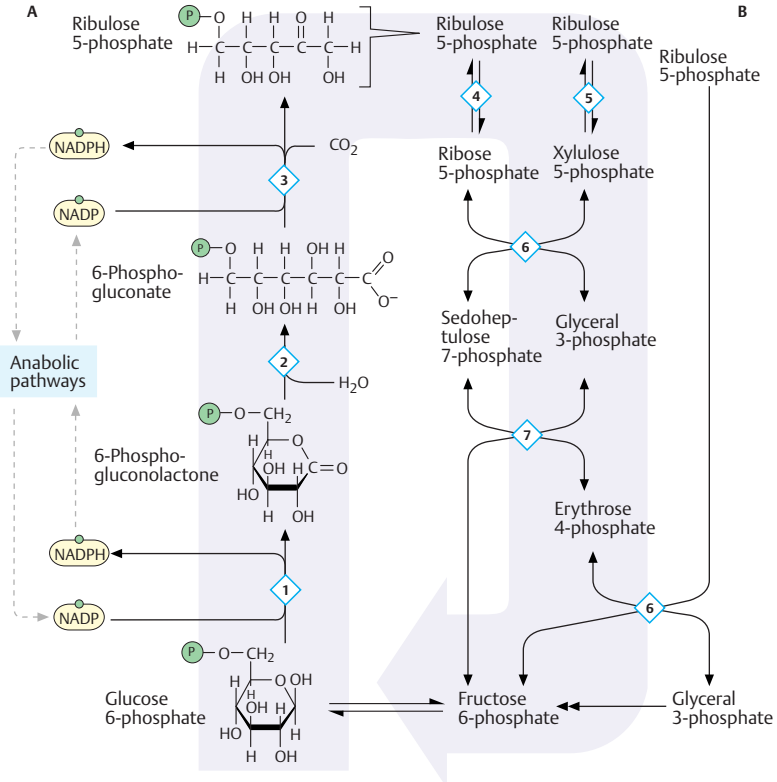
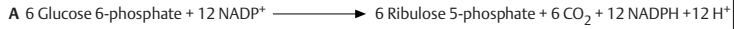
When energy in the form of ATP is required in addition to NADPH, the cell is able to channel the products of the regenerative part of the PPP (fructose 6-phosphate and glyceraldehyde 3-phosphate) into glycolysis. Further degradation is performed via the TCA cycle and the respiratory chain to CO₂ and water. Overall, 12 mol NADPH and around 150 mol ATP are obtained in this way from 6 mol glucose 6-phosphate. PPP activity is stimulated by *insulin* (p. 428). This not only increases the rate of glucose degradation, but also produces additional NADPH for fatty acid synthesis.

The reactions in the regenerative segment of the PPP are freely reversible. It is therefore easily possible to use the regenerative part of the pathway to convert hexose phosphates into pentose phosphates. This can occur when there is a high demand for pentose phosphates—e.g., during DNA replication in the S phase of the cell cycle (p. 444).

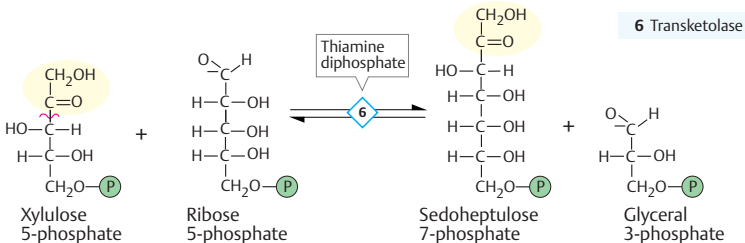
B. Transketolase reaction

In the recombination of the metabolites in the regenerative part of the PPP, two enzymes that transfer the C₂ or C₃ fragments from one sugar phosphate to another are particularly important:

Transketolase [6] transfers C₃ units. In this way, for example, two C₅ sugars are converted to the C₇ ketosugar sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate or vice versa ($5+5 = 7+3$). The coenzyme in the reaction is *thiamine diphosphate* (TPP; p. 88), which also occurs in oxoacid dehydrogenases (p. 112). In a similar way, but without the involvement of TPP, *transaldolase* ([7] see A) catalyzes the transfer of C₃ fragments, thus converting a heptose phosphate and triose phosphate to a tetrose phosphate and hexose phosphate ($7+3 = 4+6$).

A. Pentose phosphate pathway


- 1 Glucose-6-phosphate dehydrogenase 2 Gluconolactonase 3 Phosphogluconate dehydrogenase
4 Ribulose-5-phosphate isomerase 5 Ribose-5-phosphate epimerase 6 Transketolase 7 Transaldolase

B. Transketolase reaction


Gluconeogenesis

Some tissues, such as *brain* and *erythrocytes*, depend on a constant supply of glucose (p. 372). If the carbohydrates taken up in food are not sufficient, the blood sugar level can be maintained for a limited time by *degradation of hepatic glycogen* (p. 136). If these reserves are also exhausted, *de-novo* synthesis of glucose (**gluconeogenesis**) begins. The **liver** is mainly responsible for this, but the tubular cells of the **kidney** also show a high level of gluconeogenic activity (p. 338). The main precursors for gluconeogenesis are **glucogenic amino acids** (p. 168), derived mainly from proteolysis in muscle. Another important precursor is **lactate**, which is formed in erythrocytes and muscle proteins when there is oxygen deficiency. **Glycerol** produced from the degradation of fats can also be used for gluconeogenesis. However, the conversion of fatty acids into glucose is *not* possible in animal metabolism (p. 144). The human body can synthesize several hundred grams of glucose per day by gluconeogenesis.

A. Gluconeogenesis

Many of the reaction steps involved in gluconeogenesis are catalyzed by the same enzymes that are used in glycolysis (for numbering see p. 131). However, enzymes [12] to [15] are specific to gluconeogenesis and are only synthesized, under the influence of *cortisol* and *glucagon*, when needed (p. 138). Glycolysis takes place exclusively in the cytoplasm, while gluconeogenesis also involves the *mitochondria* and the *endoplasmic reticulum* (ER). Gluconeogenesis consumes 4 ATP and 2 GTP per glucose—i.e., three times as many nucleotide triphosphates as glycolysis produces.

[11] **Lactate** as a precursor for gluconeogenesis is mainly derived from muscle (Cori cycle, p. 372) and erythrocytes. *Lactate dehydrogenase* (p. 72) oxidizes it to pyruvate, with NADH formation.

[12] The first steps of actual gluconeogenesis take place in the *mitochondria*. The reason for this “detour” is the equilibrium state of the highly exergonic pyruvate kinase reaction ($\Delta G = -62 \text{ kJ} \cdot \text{mol}^{-1}$, p. 106). Coupling to ATP hydrolysis would not be sufficient to convert pyruvate *directly* into phosphoenol pyruvate (PEP). **Pyruvate** is therefore initially transported into the mitochondrial matrix via the monocarboxylate transporter (p. 118), and—in a biotin-de-

pendent reaction catalyzed by *pyruvate carboxylase*—is carboxylated there to **oxaloacetate**. Oxaloacetate is also an intermediate in the TCA cycle. *Amino acids* with breakdown products that enter the cycle or supply pyruvate are therefore *glucogenic* (p. 168).

The oxaloacetate formed in the mitochondrial matrix is exported from the mitochondria via malate shuttle reactions (p. 118).

[13] In the cytoplasm, oxaloacetate is converted into **phosphoenol pyruvate** by *PEP carboxykinase* using GTP (not ATP). The subsequent steps up to fructose 1,6-bisphosphate represent the reverse of the corresponding reactions involved in glycolysis. One additional ATP per C_3 fragment is used for the synthesis of 1,3-bisphosphoglycerate.

Two gluconeogenesis-specific phosphatases then successively cleave off the two phosphate residues from **fructose 1,6-bisphosphate**. In between these reactions lies the isomerization of fructose 6-phosphate to **glucose 6-phosphate**—another glycolytic reaction.

[14] The reaction catalyzed by *fructose 1,6-bisphosphatase* is an important regulation point in gluconeogenesis (p. 138).

[15] The last enzyme in the pathway, *glucose 6-phosphatase*, occurs in the liver, but not in muscle (p. 136). As an integral membrane protein it is located in the interior of the smooth ER. Specific transporters allow glucose 6-phosphate to enter the ER and allow the **glucose** formed there to return to the cytoplasm. From here it is ultimately released into the blood via the transporter Glut-2 (p. 212).

Free **glycerol**, derived mainly from fat degradation, initially undergoes phosphorylation at C-3 [16]. The **glycerol 3-phosphate** formed is then oxidized by an NAD⁺-dependent dehydrogenase to form **glycerone 3-phosphate** [17] and thereby channeled into gluconeogenesis. An FAD-dependent mitochondrial enzyme is also able to catalyze this reaction (known as the “glycerophosphate shuttle”; p. 118).

Glycogen Metabolism

The polysaccharide glycogen (p. 32) is used in animals as a **carbohydrate reserve**, from which glucose phosphates and glucose can be released when needed. Glucose storage itself would not make sense, as high concentrations within cells would make them strongly hypertonic and would therefore cause an influx of water. By contrast, the insoluble macromolecule glycogen has only low osmotic activity.

A. Glycogen balance

The human body can store up to 450 g of glycogen, up to 150 g in the **liver** and almost all of the remainder in **muscle** (p. 372). The glycogen content of the other organs is low. **Hepatic glycogen** is mainly used to maintain the **blood glucose level** in the post-absorptive state (p. 378). The glycogen content of the liver therefore varies widely, and can decline to almost zero in periods of extended hunger. After this, gluconeogenesis (p. 134) takes over the glucose supply for the body. **Muscle glycogen** serves as an **energy reserve** and is not involved in blood glucose regulation. Muscle does not contain any glucose 6-phosphatase and is therefore unable to release glucose into the blood. The glycogen content of muscle therefore does not fluctuate as widely as that of the liver.

B. Glycogen metabolism

Animal glycogen, like plant amylopectin (p. 32), is a **branched homopolymer of glucose**. The glucose residues are linked by $\alpha 1 \rightarrow 4$ -glycosidic bonds. Every 10th or so glucose residue has an additional $\alpha 1 \rightarrow 6$ bond to another glucose. These branches are extended by additional $\alpha 1 \rightarrow 4$ -linked glucose residues. This structure produces tree-shaped molecules consisting of up to 50 000 residues ($M > 1 \times 10^7$ Da).

Hepatic glycogen is never completely degraded. In general, only the nonreducing ends of the “tree” are shortened, or elongated when glucose is abundant.

[2] The formation of glycosidic bonds between sugars is **endergonic**. Initially, therefore, the activated form—**UDP-glucose**—is synthesized by reaction of glucose 1-phosphate with UTP (see p. 92).

[3] **Glycogen synthase** now transfers glucose residues one by one from UDP-glucose to the nonreducing ends of the available “branches.”

[4] Once the growing chain has reached a certain length (> 11 residues), the **branching enzyme** cleaves an oligosaccharide consisting of 6–7 residues from the end of it, and adds this into the interior of the same chain, or a neighboring one, with $\alpha 1 \rightarrow 6$ linkage. These **branches** are then further extended by glycogen synthase.

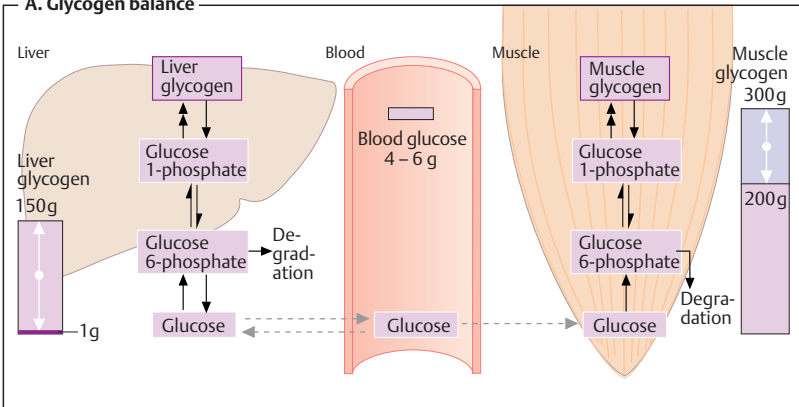
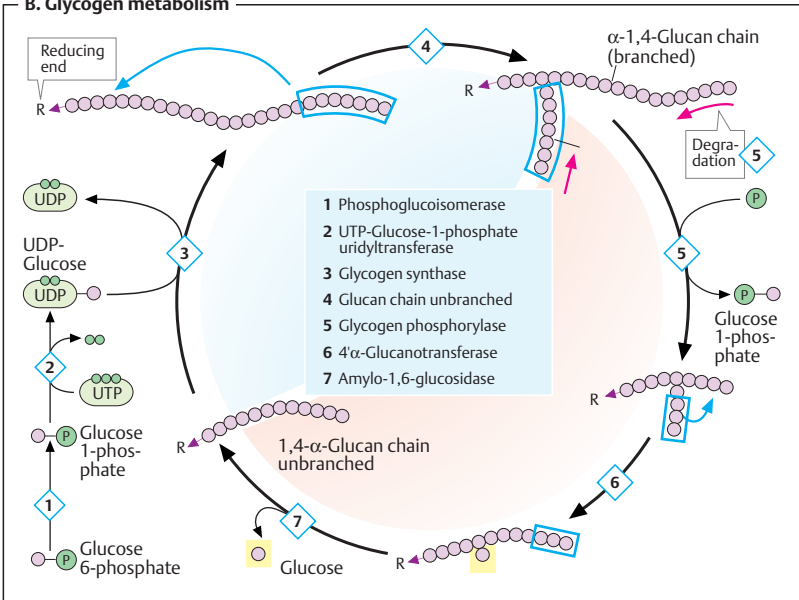
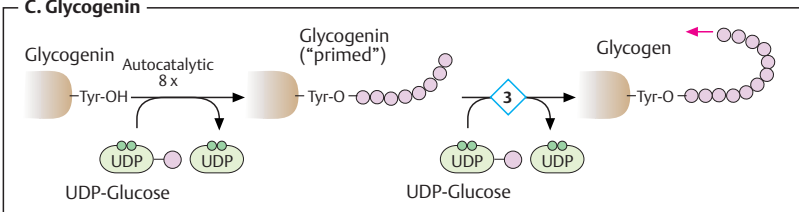
[5] The branched structure of glycogen allows rapid release of sugar residues. The most important degradative enzyme, **glycogen phosphorylase**, cleaves residues from a nonreducing end, one after another, as **glucose 1-phosphate**. The larger the number of these ends, the more phosphorylase molecules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

[6] [7] Due to the structure of glycogen phosphorylase, degradation comes to a halt four residues before branching points. Two more enzymes overcome this blockage. First, a **glucanotransferase** [6] moves a trisaccharide from the side chain to the end of the main chain. A **1,6-glucosidase** [7] then cleaves the single remaining residue as a free glucose and leaves behind an unbranched chain that is once again accessible to phosphorylase.

The **regulation of glycogen metabolism** by interconversion, and the role of hormones in these processes, are discussed on page 140ff.

C. Glycogenin

The insoluble glycogen molecules are linked through the reducing end of the tree to a special protein, **glycogenin**. Glycogenin has enzymic properties and catalyzes covalent bonding of a first glucose residue to one of its own tyrosine residues and elongation of this by up to seven additional glucose residues. It is only at this point that **glycogen synthase** becomes active to supply further elongation.

A. Glycogen balance**B. Glycogen metabolism****C. Glycogenin**

Regulation of Carbohydrate Metabolism I

A. Regulation of carbohydrate metabolism

Carbohydrate metabolism is subject to complex regulatory mechanisms involving *hormones*, *metabolites*, and *coenzymes*. The simplified scheme shown here applies to the **liver**, which has central functions in carbohydrate metabolism (p. 316). Some of the control mechanisms depicted are not effective in other tissues.

One of the liver's most important tasks is to store excess glucose in the form of glycogen and to release glucose from glycogen when required (*buffer function*). When the glycogen reserves are exhausted, the liver can provide glucose by *de-novo* synthesis (*gluconeogenesis*; p. 134). In addition, like all tissues, the liver breaks glucose down via glycolysis. These functions have to be coordinated with each other. For example, there is no point in glycolysis and gluconeogenesis taking place *simultaneously*, and glycogen synthesis and glycogen degradation should not occur simultaneously either. These futile cycles (p. 100) are prevented by the fact that two *different* enzymes exist for important steps in both pathways, which catalyze only the anabolic or the catabolic reaction. The enzymes are also regulated differently. Only these **key enzymes** are shown here.

Hormones

The hormones that influence carbohydrate metabolism include the peptides *insulin* and *glucagon* (pp. 428, 432); a glucocorticoid, *cortisol* (p. 420); and a catecholamine, *epinephrine* (p. 434).

Insulin activates *glycogen synthase* ([1]; p. 140), and induces enzymes involved in glycolysis [3, 5, 7]. At the same time, it inhibits the synthesis of several gluconeogenesis enzymes [4, 6, 8, 9]; p. 242). **Glucagon**, the most important antagonist of insulin, has the reverse effect. It induces gluconeogenesis enzymes [4, 6, 8, 9] and represses *pyruvate kinase* [7], a key enzyme of glycolysis. Additional effects of glucagon are based on the *interconversion* of enzymes and are mediated by the second messenger *cAMP*. This inhibits glycogen synthesis [1] and activates glycogenolysis [2]. **Epinephrine** has a similar effect on the liver. The inhibition of *pyruvate kinase* [7] by glucagon is also due to interconversion.

Glucocorticoids—mainly **cortisol**—induce all of the key enzymes involved in gluconeogenesis

[4, 6, 8, 9]. At the same time, they also induce enzymes involved in amino acid degradation and thereby provide precursors for gluconeogenesis. Regulation of the expression of *PEP carboxykinase*, a key enzyme in gluconeogenesis, is discussed in detail on page 242.

Metabolites

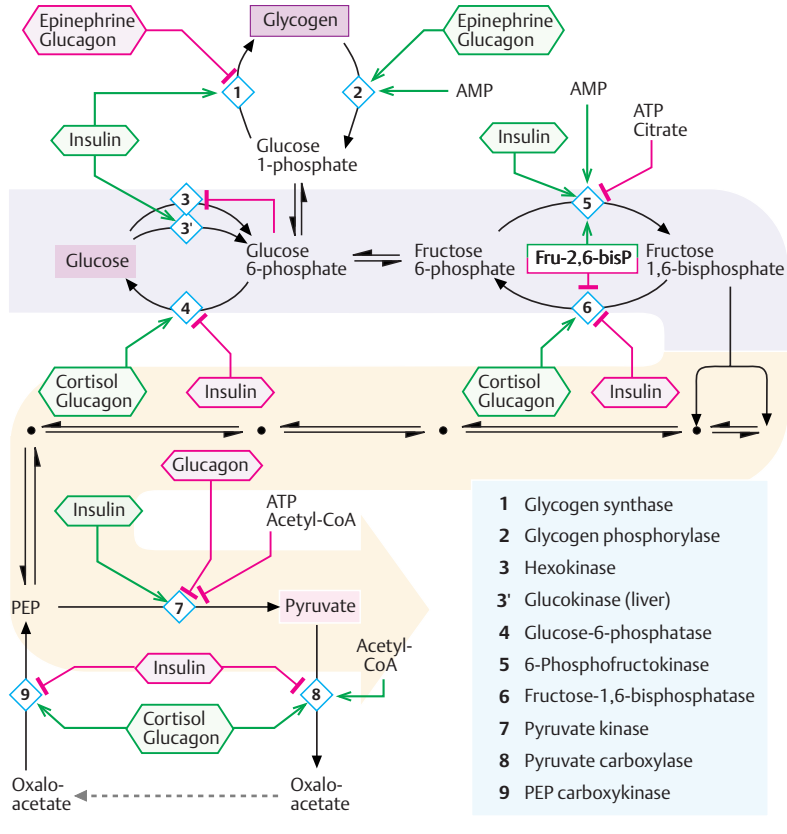
High concentrations of **ATP** and **citrate** inhibit glycolysis by allosteric regulation of *phosphofructokinase* [5]. **ATP** also inhibits *pyruvate kinase* [7]. **Acetyl-CoA** has a similar effect. All of these metabolites arise from glucose degradation (*feedback inhibition*). **ADP** and **AMP** are signals of ATP deficiency; they activate glycogen degradation and inhibit gluconeogenesis (p. 134).

B. Fructose 2,6-bisphosphate

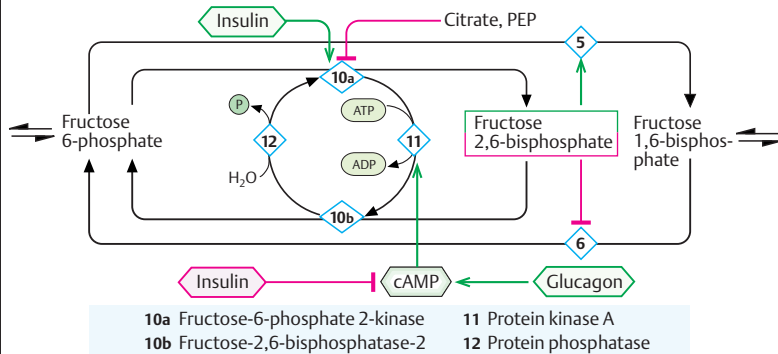
Fructose 2,6-bisphosphate (Fru-2,6-bP) plays an important part in carbohydrate metabolism. This metabolite is formed in small quantities in the hepatocytes from fructose 6-phosphate and has purely *regulatory functions*. It stimulates glycolysis by allosteric activation of *phosphofructokinase* [5] and inhibits gluconeogenesis by inhibition of *fructose 1,6-bisphosphatase* [6].

The synthesis and degradation of Fru-2,6-bP are catalyzed by one and the same protein [10a, 10b]. If the enzyme is present in an unphosphorylated form [10a], it acts as a *kinase* and leads to the *formation* of Fru-2,6-bP. After phosphorylation by cAMP-dependent protein kinase A, it acts as a *phosphatase* [10b] and now catalyzes the *degradation* of Fru-2,6-bP to fructose 6-phosphate. The equilibrium between [10a] and [10b] is regulated by hormones. Epinephrine and glucagon increase the cAMP level. As a result of increased protein kinase A activity, this reduces the Fru-2,6-bP concentration and thus inhibits glycolysis, while at the same time activating gluconeogenesis. Conversely, via [10a], insulin activates the synthesis of Fru-2,6-bP and thus glycolysis. In addition, insulin also inhibits the action of glucagon by reducing the cAMP level (p. 428).

A. Regulation of carbohydrate metabolism



B. Fructose-2,6-bisphosphate



Regulation of Carbohydrate Metabolism II

On this page we discuss other mechanisms that are important in the regulation of carbohydrate metabolism in the liver and muscle.

A. Glucokinase translocation

The liver contains several forms (*isoenzymes*) of hexokinase. The highly active isoenzyme **glucokinase** (GK, hexokinase IV) is particularly important. GK is inactive at low blood glucose levels as in these conditions it is moved into the cell nucleus by *translocation* (p. 100), where it is bound to a regulatory protein (GKRP). Under the microscope (right), it can be seen clearly that GK, which fluoresces red, is concentrated in the roundish nuclei (top). When the blood glucose rises, GK is transported back into the cytoplasm within a few minutes (bottom). “Hiding” GK in the nucleus prevents the glucose that is formed by glycogenolysis during glucose deficiency being converted into glucose 6-phosphate, which would be unable to leave the cell. Other enzymes involved in glycolysis and gluconeogenesis are also regulated by translocation.

B. Regulation of glycogen metabolism

The liver is mainly responsible for glucose homeostasis in the body (p. 316). When blood glucose levels are high, the hepatocytes absorb glucose from the blood and convert it to **glycogen** (p. 32) and other metabolites, whereas glucose obtained from the breakdown of glycogen is released into the blood during glucose deficiency. The speed of glycogen formation is determined by the activity of *glycogen synthase* (bottom right), while its breakdown is controlled by *glycogen phosphorylase* (bottom left) (p. 136). The diagram shows in simplified form the points at which the peptide hormones *insulin* (p. 428) and *glucagon* (p. 432) intervene in glycogen metabolism.

When the blood glucose level falls, **glucagon** is secreted from the pancreas. It activates glycogenolysis and inhibits glycogen synthesis. Binding of glucagon to receptors in the plasma membrane, as described on p. 406, leads to the production of the *second messenger cAMP* through activation of adenylate cyclase [1]. *Protein kinase A* (PK-A, [3]), which is activated by cAMP, has several points of attack: it inactivates glycogen synthase by *phosphorylation*, thus halting glycogen synthesis. In addition, it

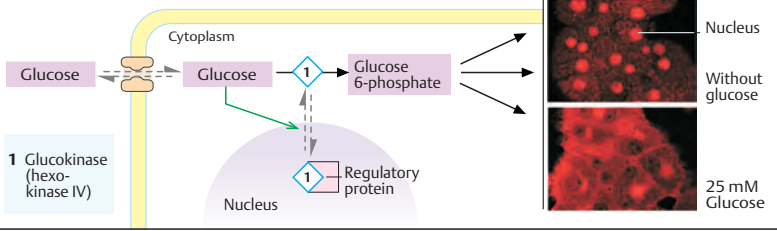
activates another protein kinase [4], which ultimately converts the inactive form of glycogen phosphorylase to the active form by phosphorylation (*interconversion*, p. 100). Active phosphorylase releases glucose from glycogen through two intermediate steps, and the glucose is released into the blood. When the cAMP levels fall again, *protein phosphatases* [6] gain the upper hand; they dephosphorylate the various phosphoproteins of the cascade, thus stopping glycogen breakdown and getting glycogen synthesis going again.

When the blood glucose level is high, **insulin** increases glycogen synthesis and stops glycogenolysis. Via intermediate products, it inhibits the *protein kinase GSK-3* ([5]; p. 428) and thus prevents inactivation of glycogen synthase. Insulin also counteracts glucagon by reducing the cAMP level by activating *cAMP phosphodiesterase* (PDE, [2]). Finally, insulin activates certain *protein phosphatases* [6], thus switching glycogen metabolism from catabolic to anabolic mode.

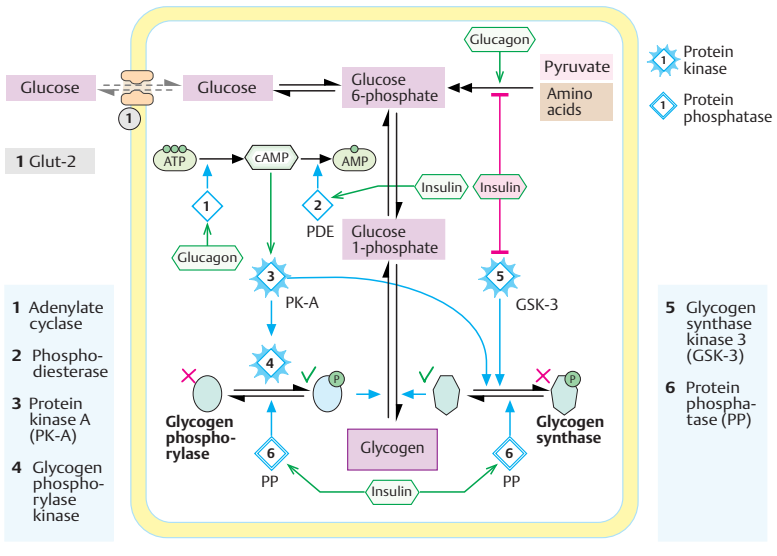
C. Anaerobic ATP production in muscle

Muscle working anaerobically relies on glycolysis to produce ATP. It takes up glucose from the blood via the insulin-dependent transporter **Glut-4** (p. 212) or obtains it from breakdown of glycogen. Different regulatory mechanisms adjust the speed of glycogen breakdown to the muscle work being performed. Ca²⁺ flowing into the sarcoplasm before the start of a contraction (p. 342) activates glycogenolysis (1) and thus helps to provide ATP. When a lot of ATP is consumed, **AMP** increases, which is a signal for energy deficiency. In muscle, it activates not only glycogenolysis (1) but also *AMP-dependent protein kinase* (p. 124), which increases glucose uptake via Glut-4 among other things (not shown). The stress hormone **epinephrine** also activates glycogenolysis via PK-A (see B). The H⁺ ions produced in glycolysis are exported into the blood in cotransport with lactate (2) so that no significant pH reduction occurs in muscle fibers even after prolonged activity.

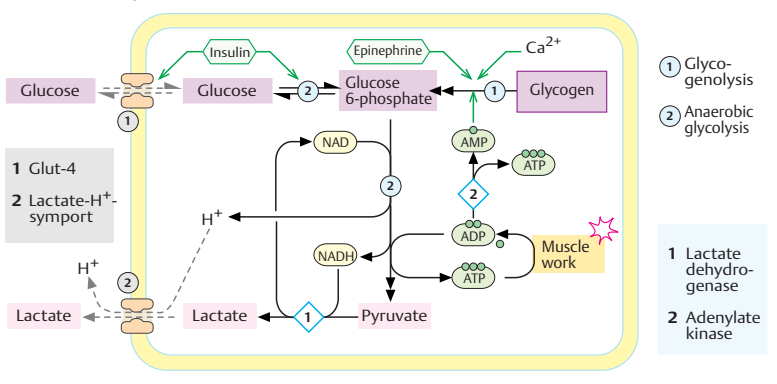
A. Glucokinase translocation



B. Regulation of glycogen metabolism



Anaerobic ATP production in muscle



Pathobiochemistry

Because of the essential role of glucose in all cells, defects in central metabolic pathways such as glycolysis or gluconeogenesis are generally incompatible with life. On the other hand, inborn deficiencies (p. 94) of enzymes involved in the metabolism of fructose, galactose and glycogen are relatively common.

A. Fructose intolerance and galactosemia

Fructose metabolism is initially independent of that of glucose. It is only in the second and third steps that products are formed that can enter glycolysis (p. 316). The most common inborn deficiency in this pathway is **fructose intolerance** (incidence in Europe 1 : 20000). This usually involves *aldolase B* [1], which converts the initially formed fructose 1-phosphate to glyceraldehyde and glyceralone 3-phosphate. Acute symptoms (nausea, digestive disorders, neurological deficits) occur only when infants ingest foods containing fructose (e.g., fruit, honey, or household sugar). Chronic ingestion can lead to irreversible liver damage. The biochemical cause of these disorders is a *depletion of cellular ATP and phosphate*, as ingested fructose is phosphorylated but cannot be broken down further. In addition, *fructose 1-phosphate* builds up, inhibiting glycolysis and glycogenolysis. Treatment is limited to keeping to a diet that is as low as possible in fructose.

The classical form of **galactosemia** (incidence 1 : 40000) is due to a deficiency of *galactose 1-phosphate uridylyltransferase* [2]. If this enzyme is lacking, galactose can no longer enter glycolysis (p. 316). When newborn babies with the disease are given milk and therefore lactose (p. 30), they react with vomiting, refusal of feeds and seizures. Hepatic and renal dysfunction also occurs, which can lead to death if the disease is not recognized. Apart from the free sugar, metabolites such as *galactose 1-phosphate* and *galactitol*, a sugar alcohol formed by reduction, accumulate in the blood; galactitol damages the lens of the eye in particular and inhibits glycogen synthesis. Most of the complications of galactosemia can be avoided by a low-galactose diet.

B. Glycogen storage diseases

"Glycogen storage diseases" or **glycogenoses** are collective terms for inborn disorders of glycogen metabolism. Their overall incidence in Germany is one case per 10000 to 20000 live births. Today, there are at least 12 known forms, many of which bear the names of the persons who first described them. Important glycogenoses, with their causes and symptoms, are summarized in the table. About 70% of all cases involve types I, II and VI, while the other forms are usually very rare.

Glycogenoses mostly affect organs where there is intensive glycogen metabolism, i.e., liver, muscle or both. Deposits of large amounts of glycogen often (but not always) lead to enlargement and/or damage of these tissues. Besides muscle weakness, the typical symptoms also include hypoglycemia (low blood glucose level).

Type I (*von Gierke disease*) can be considered as an example of the pathogenesis of a glycogenosis; this was identified as an enzyme deficiency as long ago as 1952. In this case, *glucose-6-phosphatase* [3] is damaged, with the result that the liver can no longer release glucose derived from glycogen metabolism or gluconeogenesis into the blood. Muscle, which lacks this enzyme (p. 136), is not affected.

The first symptom of the disease shortly after birth is severe *hypoglycemia*, which does not respond to glucagon. Growth disorders and gradual liver enlargement (*hepatomegaly*) then occur in addition. As glucose 6-phosphate can no longer be hydrolyzed to glucose, it is metabolized increasingly by other pathways (glycolysis, pentose phosphate pathway and glycogen synthesis). Along with the increased glycogen production, this also results in *lactic acidosis* and increased synthesis of purine bases, reflected in *hyperuricemia* (p. 186). Lipid degradation and ketone body production are also increased (*ketonemia*). To compensate for the metabolically caused hypoglycemia, patients have to ingest carbohydrates constantly. If they watch their diet strictly, they can achieve an advanced age nowadays.

Overview

The term **lipid** includes a large number of compounds of very different structure. The only thing they have in common is their markedly hydrophobic character. A summary of the most important structural classes can be found on page 36.

With the exception of the *isoprenoids* (top right), almost all lipids that are important in human metabolism contain an alcohol (*glycerol* or *sphingosine*) as the basic element, which is linked to *fatty acids* and other components. Fatty acid metabolism is therefore at the heart of lipid metabolism.

A. Lipid metabolism: overview

Because of the large number of reaction pathways in lipid metabolism, parts of the schema are shown in simplified form and not all involved cofactors are shown. While the synthesis and degradation of fatty acids are catalyzed by soluble enzymes, larger lipids are converted by enzymes that are an integral part of the membranes of the smooth endoplasmic reticulum (sER).

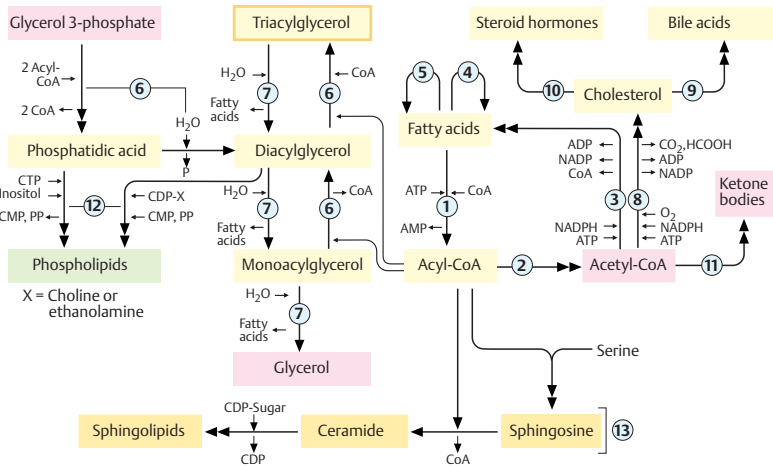
Metabolic pathways

- Before fatty acids can be broken down or incorporated into other compounds, they have to be linked with coenzyme A to form the more reactive *acyl-CoA derivatives* (**fatty acid activation, 1**)
- Degradation of activated fatty acids by **β -oxidation (2)** takes place in the mitochondrial matrix. This provides *acetyl-CoA* and reduced coenzymes (NADH and QH_2), which are used in the respiratory chain for ATP production. The *carnitine shuttle* transports *acyl-CoA* into the mitochondria (not shown, see p. 146).
- *Acetyl-CoA* is also the starting material for **fatty acid biosynthesis (3)**, which takes place in the cytoplasm, in contrast to fatty acid degradation. NADPH, derived mainly from the pentose phosphate pathway, acts as a reducing agent. The necessary *acetyl-CoA* is obtained from citrate exported from the mitochondrion (p. 118).
- Fatty acid biosynthesis provides the saturated C_{16} fatty acid *palmitic acid*. Longer fatty acids are synthesized in the sER by **fatty acid lengthening (4)**. Details of this are given on page 152.
- Human metabolism can manufacture unsaturated fatty acids from saturated ones to a limited degree (**fatty acid desaturation, 5**).

However, double bonds can be introduced only up to C-9. The most important product of the desaturases is *oleic acid*.

- *Neutral fats* (triacylglycerols) are synthesized from *acyl-CoA* and *glycerol-3-phosphate* by gradual transfer of acyl residues (**lipogenesis, 6**). *Phosphatidates* are important intermediates that can provide diacylglycerols by phosphate cleavage or be converted further to phospholipids (see below).
- To break down fats, *lipases* release the three acyl residues as fatty acid anions (**lipolysis, 7**).
- *Cholesterol* is the parent compound for all steroids. Its synthesis is a process that involves many steps in the liver and peripheral tissues (**cholesterol synthesis, 8**).
- The strongly amphipathic bile acids are the most important cholesterol derivatives in quantitative terms. They are synthesized in the liver (**bile acid synthesis, 9**) and act as emulsifiers for fats and other lipids in the bile and intestine.
- **Steroid hormone synthesis (10)** is complicated. It is performed by specialized cells in endocrine glands.
- **Ketone bodies (11)** are products of fatty acid degradation. Especially in the fasting state, they act as valuable energy substrates for muscle, the CNS and other organs (p. 378).
- In **phospholipid synthesis (12)** amino alcohols or inositol are transferred to phosphatidate and diacylglycerol, with activated CDP derivatives acting as precursors.
- **Sphingolipid synthesis (13)** takes place similarly, with *ceramide* acting as precursor. In contrast to the glycerolipids, most sphingolipids have a mono- or oligosaccharide instead of phosphate as the polar component.

A. Lipid metabolism: overview



	Name	Page	Reactant(s) - product(s)	Key enzyme(s)	Regulated by
1	FA activation (aMM, PM)	151	Fatty acid → acyl CoA	–	–
2	β-oxidation (MM)	147	Acyl CoA → n acetyl-CoA	Carnitine acyltransferase I	NAD ⁺ /NADH ratio Supply of acyl CoA (p. 103), dietary lipids ↑
3	Fatty acid synthesis (CP)	151	n acetyl CoA → acyl CoA	Citrate lyase Acetyl CoA carboxylase Fatty acid synthase	Ins ↓, Cor ↓ Ins ↑, Citrate ↓ (S. 103) Cor ↓, AMPK ↓, Acyl-CoA ↓ Ins ↓, Cor ↓
4	FA elongation (sER)	153	FA elongation (sER)	–	–
5	FA desaturation (sER)	153	Saturated FA → unsaturated FA	–	–
6	Lipogenesis (sER)	155	Glycerol, acyl CoA → triacylglycerol	–	Hormones incl. Ins. ↓ Leptin ↓, Somatotropin ↓
7	Lipolysis (PM, CP)	331	Triacylglycerol → glycerol, fatty acids	Lipoprotein lipase, hormone-sensitive lipase	Ins ↓, AT
8	Cholesterol synthesis (CP, sER)	157	Acetyl CoA → cholesterol	HMG-CoA reductase	Ins ↓, T ₃ ↓, Cholesterol ↓, cAMP ↓, AMPK ↓
9	Bile acid synthesis	321	Cholesterol → bile acids	7α-Hydroxylase (p. 320)	Bile acids ↓
10	Steroid hormone synthesis (sER)	425	Cholesterol → hormones	Cholesterol ester hydrolase, StAR protein (p. 424)	Glandotropic hormones
11	Ketone body formation	319	Acetyl CoA → ketone bodies	–	–
12	Phospholipid synthesis (sER)	155	Diacylglycerol → phospholipids	–	–
13	Sphingolipid synthesis	155	Sphingosine → sphingolipids	–	–

AT: adipose tissue, Cor: cortisol, CP: cytoplasm, FA: fatty acid, Ins: insulin, MM: mitochondrial matrix, OMM: outer mitochondrial membrane, PM: plasma membranes, sER: smooth endoplasmic reticulum

Fatty Acid Degradation: β -Oxidation

After uptake by the cell, fatty acids are activated by conversion into their CoA derivatives—**acyl-CoA** is formed. For this reaction there are several *acyl-CoA synthases* with different substrate specificity and location. The subsequent fatty acid degradation pathway also depends on the type of fatty acid. *Short-chain* (C_4 – C_6) and *medium-chain* (C_8 – C_{10}) fatty acids are taken up by means of a monocarboxylate transporter (p. 119) and activated in the matrix to acyl-CoA. *Long-chain* (C_{12} – C_{18}) fatty acids are activated to acyl-CoA at the outer mitochondrial membrane and then transported through the mitochondrial membrane by means of the **carnitine shuttle**. The short-, medium- and long-chain fatty acids are then broken down by **β -oxidation**. In contrast, very long-chain fatty acids ($> C_{18}$) and branched-chain fatty acids are transported into the peroxisomes where they are metabolized (p. 226).

A. Transport of long-chain fatty acids

There is a group-specific transport system in the inner mitochondrial membrane for long-chain fatty acids (C_{12} – C_{18}). The acyl groups are first transferred to **carnitine** by *carnitine acyltransferase I* [1a] in the outer mitochondrial membrane before they are channeled into the mitochondrial matrix as **acyl carnitine** by an *acyl carnitine / carnitine antiporter* in exchange for free carnitine. There, the mitochondrial isoenzyme, *carnitine acyltransferase II* [1b], catalyzes transfer of the acyl residue back to CoA.

This **carnitine shuttle** is the *rate-determining step* in mitochondrial fatty acid degradation. Malonyl-CoA inhibits carnitine acyltransferase (p. 144) and thus the uptake of fatty acids into the matrix. The production of malonyl-CoA by *acetyl-CoA carboxylase* is subject to careful tissue-specific control.

B. Fatty acid degradation: β -oxidation

The degradation of the fatty acids in the mitochondrial matrix occurs through a series of oxidative reactions. These form a reaction loop by which activated fatty acids are broken down gradually into C_2 units as activated acetic acid (**acetyl-CoA**). In this process, each CH_2 group of the acyl residue at C-3 (the β -C atom) is oxidized to a keto group—hence the term **β -oxidation**. The reduced coenzymes produced in the process are passed to the respiratory

chain. Both spatially and functionally, β -oxidation is closely linked to the TCA cycle (p. 114ff.) and to the respiratory chain (p. 120).

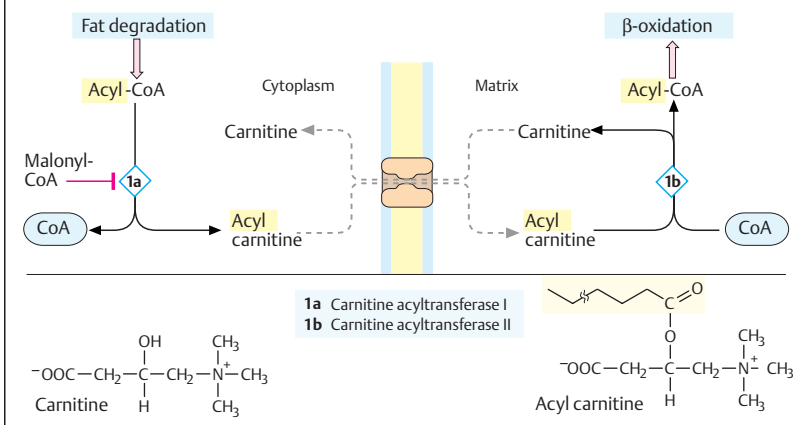
- [1] The first step in β -oxidation is dehydrogenation of **acyl-CoA** at C-2 and C-3. This yields an unsaturated **Δ^2 -enoyl-CoA** derivative with a *trans*-configured double bond. The two H atoms are initially transferred from FAD-containing *acyl-CoA dehydrogenase* to the **electron-transferring flavoprotein (ETF)**. *ETF dehydrogenase* [5] then passes them on from ETF to ubiquinone (coenzyme Q), a component of the *respiratory chain* (p. 120).
- [2] This step is followed by the addition of a water molecule to the double bond of the enoyl-CoA (*hydration*), with formation of **β -hydroxyacyl-CoA**.
- [3] In the next reaction, the OH group at C-3 is oxidized to a carbonyl group (*dehydrogenation*). This gives rise to **β -ketoacyl-CoA**, and the reduction equivalents are transferred to NAD^+ , which also passes them on to the *respiratory chain*.
- [4] β -Ketoacyl-CoA is now broken down by an *acyl transferase* into **acetyl-CoA** and an **acyl-CoA shortened by 2 C atoms** (“*thiolclastic cleavage*”).

β -Oxidation is catalyzed by various isoenzymes specialized for fatty acids of different length. Several cycles are required for complete degradation of long-chain fatty acids—eight cycles in the case of stearyl-CoA ($C_{18:0}$), for example.

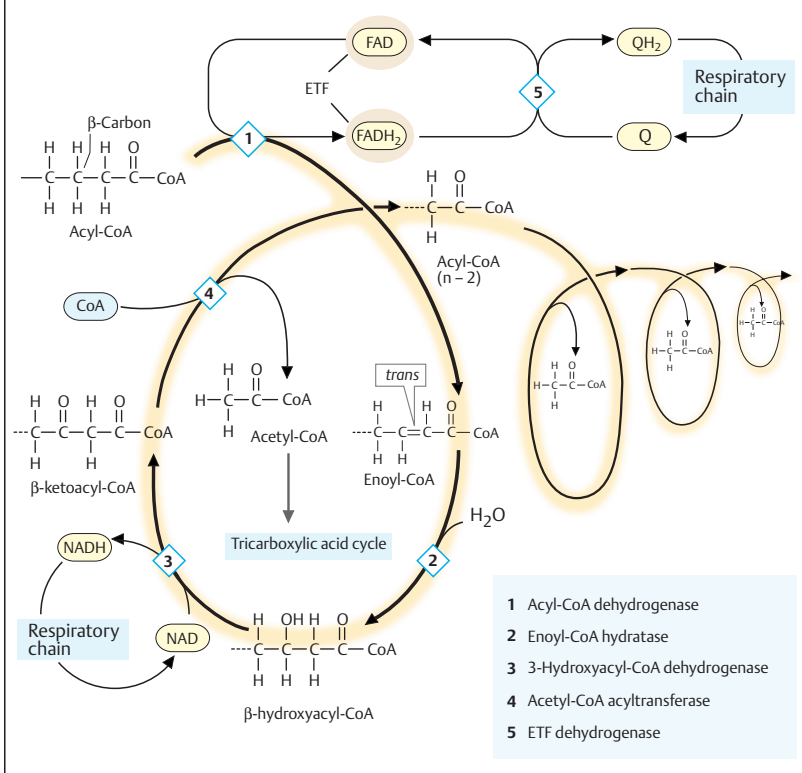
When oxidative degradation is complete, one molecule of palmitic acid supplies around 106 molecules of ATP, corresponding to an energy of $3300 \text{ kJ} \cdot \text{mol}^{-1}$. This high energy yield makes fats an ideal form of storage for metabolic energy (p. 372). Between meals, β -oxidation of fatty acids serves as a source of energy for many tissues such as muscle or kidneys, while in the liver it mainly serves to produce ketone bodies. β -Oxidation does not take place in the brain and erythrocytes.

The most important regulator of β -oxidation is the $NAD^+ / NADH$ ratio. If the respiratory chain is not using any NADH, then not only the TCA cycle but also β -oxidation come to a standstill due to the lack of NAD^+ .

A. Transport of long-chain fatty acids



A. Fatty acid degradation: β -oxidation



Minor Pathways of Fatty Acid Degradation

A. Degradation of unsaturated fatty acids

Unsaturated fatty acids usually contain a *cis* double bond at position 9 or 12—e.g., linoleic acid (18:2; 9,12). As with saturated fatty acids, degradation in this case occurs via β -oxidation until the C-9-*cis* double bond is reached. Since *enoyl-CoA hydratase* can only accept substrates with *trans* double bonds, the corresponding *enoyl-CoA* is converted by an isomerase from the *cis* isomer into the *trans* isomer [1]. Degradation by β -oxidation can now continue until a shortened *trans*- Δ^2 , *cis*- Δ^4 derivative occurs in the next cycle. This cannot be isomerized in the same way as before and instead is reduced in an NADPH-dependent way to the *trans*- Δ^3 compound [2]. After rearrangement by *enoyl-CoA isomerase* [1], degradation can finally be completed via β -oxidation.

B. Other fatty acid degradation pathways

1. Fatty acids with an odd number of C atoms are broken down in the same way as “normal” fatty acids by β -oxidation in the mitochondria

(p. 146). In the last step of β -oxidation, **propionyl-CoA** arises instead of acetyl-CoA. This activated C₃ carboxylic acid, which can also arise in amino acid metabolism, is converted into **succinyl-CoA** (p. 170).

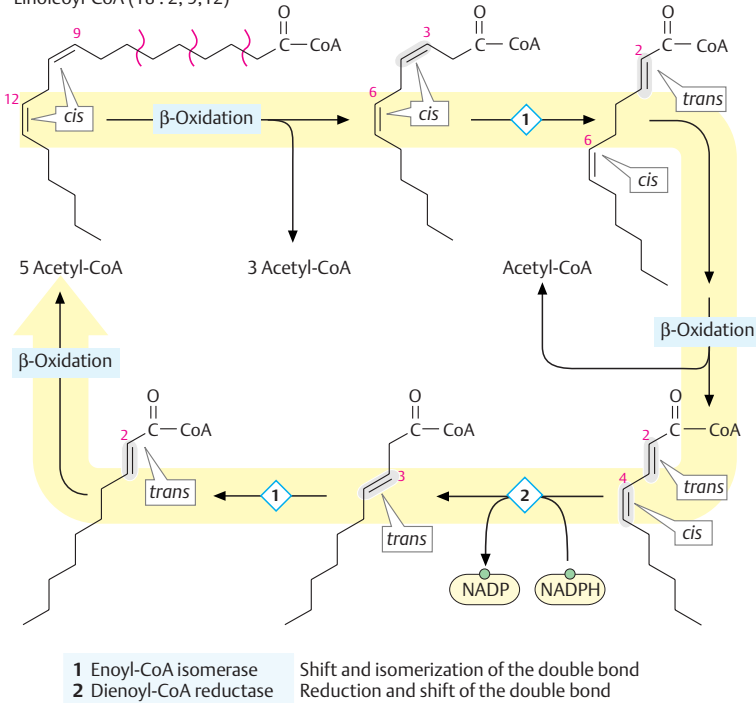
2. The degradation of **fatty acids with methyl branching**, such as phytanic acid begins in the **peroxisomes** with α -oxidation, in which an α -hydroxylase hydroxylates the α -C atom (p. 226). The fatty acid is further degraded by oxidation and decarboxylation to pristanic acid (not shown). This is followed by peroxisomal β -oxidation reactions, which release acetyl-CoA and propionyl-CoA alternately. At a chain length of eight C atoms, the fatty acid is transferred to the mitochondria where it is degraded further as a medium-chain fatty acid.

3. Fatty acids can also be broken down to a small extent in the **endoplasmic reticulum** by ω -oxidation. This involves hydroxylation of the terminal methyl group of the fatty acid by a cytochrome P450 system (p. 324) and further oxidized by dehydrogenases to a carboxylate group. The resulting **dicarboxylic acids** can be further metabolized by β -oxidation down to dicarboxylic acids with six to 10 C atoms. These are fairly water-soluble and can be broken down elsewhere like medium-chain fatty acids, or can be excreted in the urine.

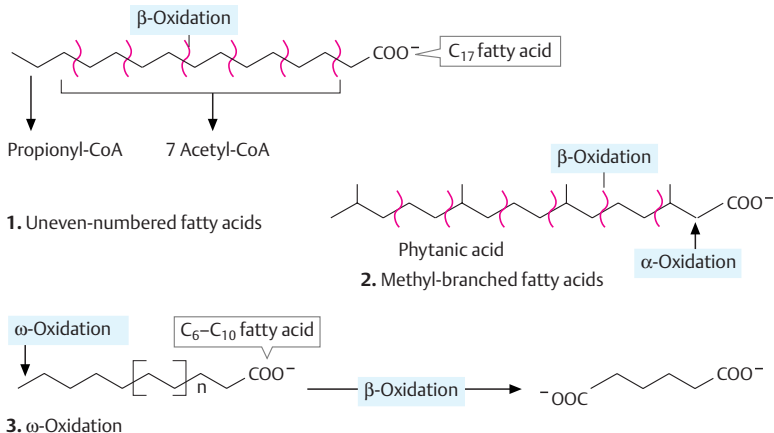
Fatty acid (FA) examples	Site of activation	Transport	Degradation	Additional reactions
Short- (C ₄ –C ₆) and medium-chain (C ₈ –C ₁₀) FAs e.g., butyric acid, capronic acid, caprylic acid, caprinic acid	Outer mitochondrial membrane	Monocarboxylate transporter into mitochondria	β-Oxidation in mitochondria	–
Long-chain (C ₁₂ –C ₂₀), saturated, even-numbered FAs e.g., palmitic acid, stearic acid	Outer mitochondrial membrane	Carnitine shuttle into mitochondria	β-Oxidation in mitochondria	–
Very long-chain FAs ($\geq$ C ₂₀), some long-chain FAs, methyl-branched FAs e.g., phytanic acid, xenobiotics	Peroxisomal membrane	Into peroxisomes	By α- and β-oxidation in peroxisomes to medium- and short-chain FAs, transfer and final breakdown in mitochondria	During β -oxidation in peroxisomes, no ATP gained but H ₂ O ₂ formed
Unsaturated, long-chain FAs (C ₁₂ –C ₂₀) e.g., oleic acid, linoleic acid, linolenic acid, arachidonic acid	Outer mitochondrial membrane	Carnitine shuttle into mitochondria	β-Oxidation in mitochondria	Relocation of double bonds (DB), reduction of <i>trans</i> DB
Odd-numbered FAs e.g., margaric acid (C ₁₇)	Outer mitochondrial membrane	Carnitine shuttle into mitochondria	β-Oxidation in mitochondria	Final product is propionyl-CoA; conversion to succinyl-CoA
Some FAs and xenobiotics	–	Into ER	ω-Oxidation in ER	End-products are C ₆ –C ₁₀ dicarboxylic acids

A. Degradation of unsaturated fatty acids

Linoleoyl-CoA (18 : 2; 9,12)



B. Other fatty acid degradation pathways



Fatty Acid Biosynthesis

Whenever the diet contains an excess of usable energy substrates, the body produces fatty acids. Next to proteins, glucose is the most important source.

The biosynthesis of fatty acids takes place mainly in the liver and also in fat tissue. The individual steps of the reaction are catalyzed by a multifunctional enzyme system, *fatty acid synthase*. This enzyme is located in the cytoplasm and requires acetyl-CoA as a starter molecule. In a cyclic reaction, this acetyl residue is then elongated by one C₂ unit at a time for seven cycles. NADPH is used as a reducing agent in the process. The end product of the reaction is the saturated C₁₆ acid, *palmitic acid*, which can then be converted to other fatty acids (p. 152).

A. Fatty acid biosynthesis

1. Reactions. The key step in fatty acid synthesis, catalyzed by *acetyl-CoA carboxylase* [1], precedes the actual synthesis. By ATP-dependent carboxylation of acetyl-CoA the carboxylase supplies the **malonyl-CoA** required by the synthase. The biotin-containing enzyme is *activated* by insulin-induced dephosphorylation and *inactivated* by the kinase AMP-K (p. 124). *Citrate* is an allosteric activator, while *palmitoyl-CoA*, the end product of synthesis, inhibits the carboxylase.

The actual assembly of fatty acids through a cyclic process is catalyzed by *fatty acid synthase*, a multifunctional enzyme (see below). The first cycle of synthesis starts with the transfer of an acetyl residue from acetyl-CoA to the peripheral cysteine group of the enzyme (Cys-SH; see below) [2]. At the same time, a malonyl residue is transferred from malonyl-CoA to its 4-phosphopantetheine group (Pan-SH; see below) [3]. The chain is then elongated by condensation of the acetyl residue—or (in later cycles) the acyl residue—with the malonyl group, with simultaneous decarboxylation [4]. The next three reactions (reduction of the 3-oxo group [5], dehydration of the 3-hydroxyl derivative [6], and another reduction [7]) correspond in principle to a reversal of β -oxidation, but they use NADPH instead of NADH as a reductant. The NADPH is supplied by reactions of the pentose phosphate pathway (p. 132) and malic enzyme (p. 116). Finally, the elongated acyl residue is transferred back to the peripheral cysteine residue [2], so that the cycle can begin again with renewed loading of the Pan-SH with a malonyl residue. After seven cycles, when the acyl residue has a

length of 16 C atoms, **palmitate** is hydrolytically released [8].

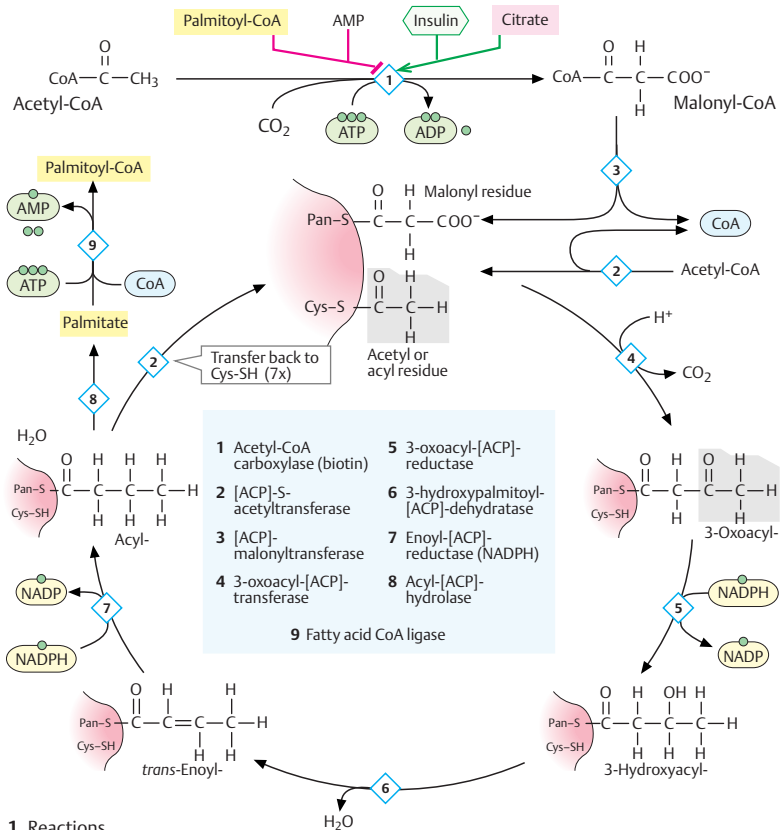
2. Structure of fatty acid synthase. Reactions [2]–[8] of fatty acid synthesis take place in *fatty acid synthase*, a compact, multifunctional enzyme system. In vertebrates it consists of two identical peptide chains that together form a roughly X-shaped dimer. Each peptide chain (M = 272 kDa) catalyzes all seven of the partial reactions required to synthesize palmitate.

Both subunits can bind acyl residues as thioesters at two different SH groups: one peripheral *cysteine residue* (Cys-SH) and one central *4'-phosphopantetheine group* (Pan-SH), which resembles coenzyme A (p. 88) Pan-SH is covalently bound to a protein segment of the synthase known as the *acyl-carrier protein* (ACP). This part functions like a long arm that passes the substrate from one reaction center to the next. The two subunits of fatty acid synthase cooperate in this process; the enzyme is therefore only capable of functioning as a dimer.

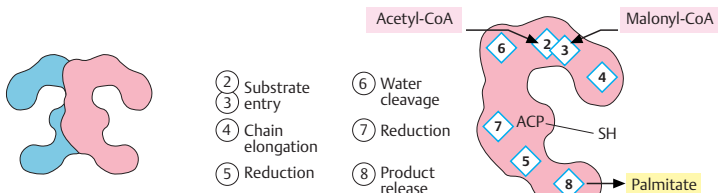
Spatially, the enzyme activities [2]–[8] are arranged in domains. Activities [2] and [3] catalyze the **entry of the substrates** acetyl-CoA/acyl-CoA and malonyl-CoA; subsequent condensation of the two partners [4] **elongates the chain**. This is followed by **reduction** of the oxo group, **water cleavage** and **reduction** of the C=C double bond. In the last step of the cycle, the growing fatty acid chain is either transferred back to the starting position (peripheral Cys-SH) [2], or **released as a finished product** when the length is correct [8].

The spatial integration of successive reactions into a single multifunctional enzyme has several advantages in comparison with separate enzymes. Competing reactions are prevented, the individual reactions proceed in a coordinated way as if on a production line, and due to low diffusion losses they are particularly efficient.

A. Fatty acid biosynthesis



1. Reactions



2. Structure of fatty acid synthase

Fatty Acid Metabolism: Other Reactions

Fatty acids synthesized in the body (p. 144) or obtained from the diet (p. 274) can be converted into other fatty acids by shortening, lengthening or introduction of double bonds. For instance, palmitate, the product of fatty acid biosynthesis (p. 150), can be elongated to stearate and even longer fatty acids or dehydrogenized to oleic acid.

A. Fatty acid elongation

Following biosynthesis, the C₁₆ carboxylic acid, palmitic acid, is first linked to coenzyme A and thereby activated in an ATP-dependent reaction [1]. Elongation of the resulting palmitoyl-CoA and other activated fatty acids then takes place in the *endoplasmic reticulum*. There, the acyl-CoA can be extended by two C atoms with the aid of malonyl-CoA. Palmitoyl-CoA can thus be converted to stearyl-CoA (C₁₈) and then to arachinic acid (C₂₀). Other reactions lead to **very long C₂₂ and C₂₄ fatty acids**. The synthesis of these acids is particularly important in the brain, where they are incorporated into cerebral lipids.

In contrast to fatty acid biosynthesis catalyzed by fatty acid synthase (p. 150), the elongation reactions proceed in separate steps involving fatty acid CoAs instead of acyl residues bound to the acyl carrier protein (ACP). One malonyl-CoA and two NADPH are required per C₂ unit.

B. Synthesis of unsaturated fatty acids

The introduction of double bonds into fatty acids by desaturation requires molecular oxygen, NADPH and cytochrome b₅. Human *fatty acid desaturases* [7] can only introduce double bonds between C-9 and C-10 or below. The example shows the conversion of stearic acid to **oleic acid**. In a similar way (not shown), palmitic acid yields **palmitoleic acid**. Other positions where double bonds can be introduced are C-4, C-5, and C-6, whereas dehydrogenation above C-9 is not possible in humans.

C. Synthesis of arachidonic acid from linoleic acid

Because of the inability to introduce double bonds into fatty acids beyond C-9, humans are unable to synthesize **polyunsaturated fatty acids** such as linoleic acid, linolenic acid and arachidonic acid from acetyl residues. The **ω-6**

fatty acid, linoleic acid, and **ω-3 fatty acid**, linolenic acid, are therefore essential components of the nutrition. (The way that C atoms of fatty acids are numbered is described on p.38.)

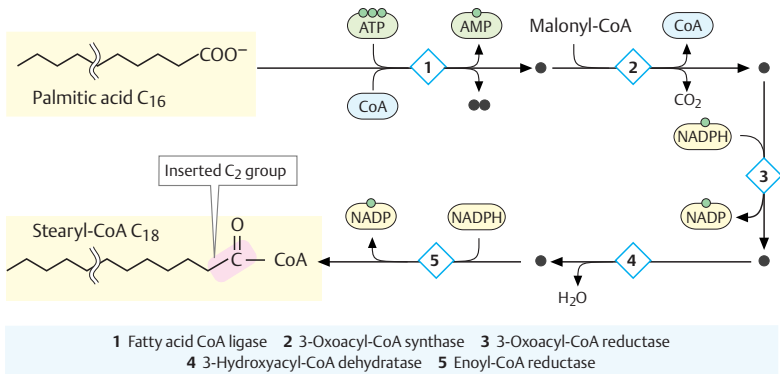
The ω-6 fatty acid, **arachidonic acid**, is synthesized from the ω-6 linoleic acid by elongation with one C₂ unit [9] and introduction of two double bonds [8 and 10]. Therefore arachidonic acid is essential only when linoleic acid is lacking in the diet.

The polyunsaturated fatty acids (PUFAs) are needed for the synthesis of signaling substances belonging to the **eicosanoid** group (p. 438). Linoleic acid is the precursor of longer polyunsaturated fatty acids in the brain and is also an important component of skin lipids.

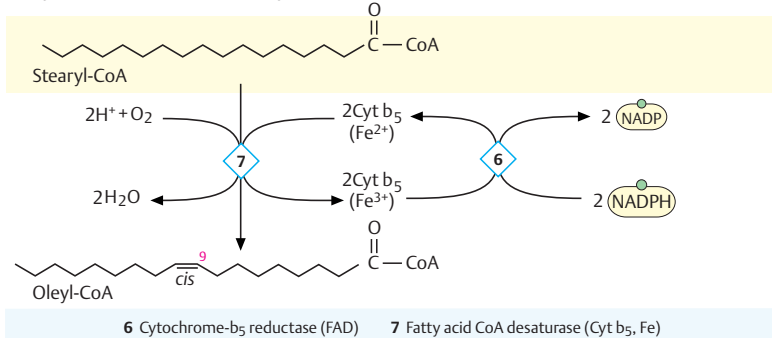
Vegetable oils and fish are the most important dietary source of polyunsaturated fatty acids. Plants can incorporate double bonds into fatty acids beyond C-9. Their ω-3 and ω-6 fatty acids are therefore a valuable part of the diet. Fish cannot synthesize any polyunsaturated fatty acids themselves but they accumulate them from their diet. Ultimately, their polyunsaturated fatty acids are also derived from plants (plankton).

The polyunsaturated fatty acids produced by animal and plant desaturases always have the double bond in *cis* configuration. Fatty acids with *trans* double bonds (**trans fatty acids**) are largely produced by microbial digestive processes in ruminants and during the refinement of edible fats. Whether *trans* fatty acids represent a health risk for cardiovascular disease is as yet unclear.

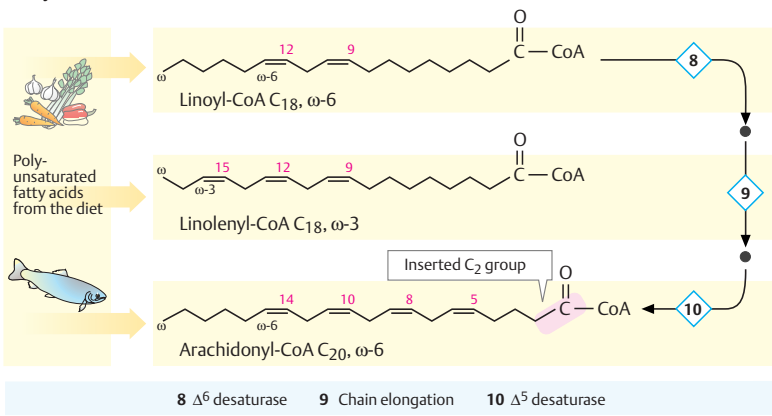
A. Fatty acid elongation



B. Synthesis of unsaturated fatty acids



C. Synthesis of arachidonic acid from linoleic acid



Biosynthesis of Complex Lipids

Complex lipids, such as neutral fats (triacylglycerols), phospholipids, and glycolipids, are synthesized via common reaction pathways. Most of the enzymes involved are associated with the membranes of the *smooth endoplasmic reticulum* (sER).

A. Biosynthesis of fats and phosphoglycerides

The biosynthesis of glycerolipids, i.e., fats and phosphoglycerides, starts with **glycerol 3-phosphate** (bottom left). This compound can arise via two pathways: it can be produced in the liver by reduction of **glycerone 3-phosphate** or by phosphorylation of **glycerol**, which the liver takes up from the blood. The enzyme involved, *glycerol kinase*, is not present in fat tissue, so glycerol 3-phosphate is produced there from glycolysis intermediates or via *glyceroneogenesis* (p. 330).

The course of glycerolipid synthesis is the same in the liver, adipose tissue and the intestine:

- [1] Esterification of glycerol 3-phosphate with a long-chain fatty acid produces a strongly amphipathic **lysophosphatidate**. In this reaction, an acyl residue is transferred from the activated precursor **acyl-CoA** to the hydroxyl group at C-1.
- [2] A second esterification of this type leads to a **phosphatidate**. Unsaturated acyl residues are usually incorporated at C-2 of the glycerol. Phosphatidates (anions of phosphatidic acids) are the key molecules in the biosynthesis of fats and phosphoglycerolipids.
- [3] In the pathway leading to fats (triacylglycerols), the phosphate residue is removed by hydrolysis. This produces **diacylglycerol (DAG)**.
- [4] Transfer of an additional acyl residue to DAG forms a **triacylglycerol**. This completes the biosynthesis of neutral fats. They are packaged into very low-density lipoproteins (VLDLs) by the liver and released into the blood. In adipocytes they are stored in the form of insoluble fat droplets.
- [5] As described on page 274, in the lumen of the intestine, ingested fats are mainly broken down into **monoacylglycerols**. The cells of the intestinal mucosa re-synthesize these into neutral fats. This pathway also proceeds via **DAGs**. Finally, the fats are packaged into chylomicrons in the en-

terocytes and released into the lymph. The biosynthesis of **phosphoglycerolipids** also starts from DAGs. The lipid head group (phosphate with alcohol) can be attached in two ways: either the head group (pathway due to [6]) or the DAG group (pathway due to [7]–[10]) is activated.

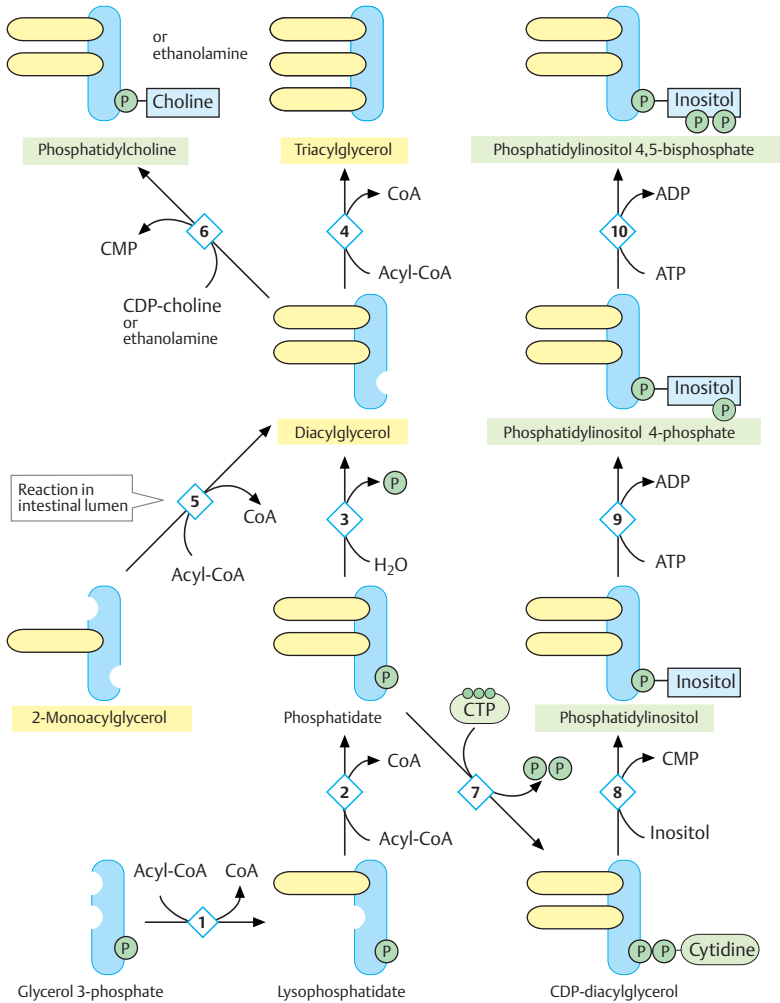
- [6] Transfer of a phosphocholine residue to the free OH group gives rise to **phosphatidylcholine** (lecithin). The phosphocholine residue is derived from the activated precursor CDP-choline (p. 92). **Phosphatidylethanolamine** is formed from CDP-ethanolamine and DAG in a similar way. By contrast, **phosphatidylserine** is derived from phosphatidylethanolamine by an exchange of the amino alcohol (not shown).
- [7] The biosynthesis of **phosphatidylinositol** starts from phosphatidate rather than DAG. In a first step, the activated intermediate, CDP-diacylglycerol, is produced by transfer of a CMP residue.
- [8] Substitution of the CMP residue by inositol then provides **phosphatidylinositol** (PtdIns).
- [9] PtdIns occurs in the plasma membrane, where it can be phosphorylated to **phosphatidylinositol 4-phosphate** (PtdInsP).
- [10] An additional phosphorylation finally gives **phosphatidylinositol 4,5-bisphosphate** (PtdInsP₂). PtdInsP₂ is the precursor for the second messengers **2,3-diacylglycerol (DAG)** and **inositol 1,4,5-trisphosphate** (InsP₃; p. 406).

Cardiolipin, a characteristic lipid of the inner mitochondrial membrane (p. 40), also arises from CDP-diacylglycerol by transfer of a phosphatidyl residue to a phosphatidylglycerol (not shown).

Further reactions serve to interconvert the phospholipids—e.g., phosphatidylserine can be transformed into phosphatidylethanolamine by decarboxylation, and the latter can then be converted into phosphatidylcholine by methylation with S-adenosyl methionine.

The biosynthesis of the **sphingolipids** is not shown here. It starts with the formation of ceramide (p. 42). **Serine** and **palmitoyl-CoA** are linked with decarboxylation, then reduced with NADPH, bound to a very-long fatty acid by an amide bond, and converted to an unsaturated compound by dehydrogenation. Ceramide resembles a diacylglycerol (p. 42). It can be converted to **cerebrosides** and **gangliosides** by transfer of activated sugars, or to **sphingomyelin** by transfer of activated head groups [6].

A. Biosynthesis of fats and phosphoglycerides



- 1 Glycerol-3-phosphate acyltransferase
- 2 1-Acylglycerol-3-phosphate acyltransferase
- 3 Phosphatidate phosphatase
- 4 Acylglycerol palmitoyltransferase
- 5 Diacylglycerol acyltransferase
- 6 1-Alkyl-2-acetylglcerol cholinphosphotransferase
- 7 Phosphatidate cytidyltransferase
- 8 CDP-diacylglycerol-inositol 3-phosphatidyltransferase
- 9 Phosphatidylinositol kinase
- 10 Phosphatidylinositol-4-phosphate kinase

Biosynthesis of Cholesterol

Cholesterol is a major constituent of animal *cell membranes* (p. 208). The body can provide for its full daily cholesterol requirement (ca. 1 g) by biosynthesis. However, with a mixed diet, only about half of the cholesterol is derived from *endogenous biosynthesis*, which takes place in many cells in the body, but mainly in the liver (~50%). The rest is obtained from *food*. Most of the cholesterol is incorporated into the lipid layer of plasma membranes, or converted into **bile acids** (p. 320). A very small amount of cholesterol is used for biosynthesis of the **steroid hormones** (p. 424). In addition, up to 1 g cholesterol per day is released into the *bile* and thus excreted. The absorption of cholesterol and plant sterols (phytosterols; p. 46) from the intestinal lumen into the enterocytes is by an incompletely understood transport mechanism. The phytosterols and some of the cholesterol are transported back into the intestinal lumen, with consumption of ATP.

A. Cholesterol biosynthesis

Cholesterol is one of the isoprenoids, the synthesis of which starts from **acetyl-CoA** (p. 44). In a long and complex reaction chain, the C_{27} sterol is built up from C_5 components. The biosynthesis of cholesterol can be divided into four parts. In the first (1), **mevalonate**, a C_6 compound, arises in the cytosol from three molecules of **acetyl-CoA**. In the second part (2), mevalonate is converted into **isopentenyl diphosphate**, the “activated isoprene.” In the third part (3), six of these C_5 molecules are linked to produce **squalene**, a C_{30} compound. Finally, squalene undergoes cyclization, with three C atoms being removed, to yield cholesterol (4).

1. Formation of mevalonate. The conversion of acetyl-CoA to acetoacetyl-CoA and then to **3-hydroxy-3-methylglutaryl-CoA** (3-HMG-CoA) corresponds to the biosynthetic pathway for *ketone bodies* (details on p. 318). In this case, however, the synthesis occurs not in the mitochondria as in ketone body synthesis, but in the smooth endoplasmic reticulum. In the next step, the 3-HMG group is cleaved from the CoA and at the same time reduced to mevalonate with the help of NADPH. *3-HMG CoA reductase* is the *key enzyme* in cholesterol biosynthesis. It is regulated by *repression* of transcription (effectors: sterols), by proteolytic degradation (effectors: cholesterol and bile acids), and by *interconversion* (effectors: hormones). Insulin

and thyroxine stimulate the enzyme and glucagon inhibits it by cAMP-dependent phosphorylation. A large supply of cholesterol from food also inhibits 3-HMG-CoA reductase.

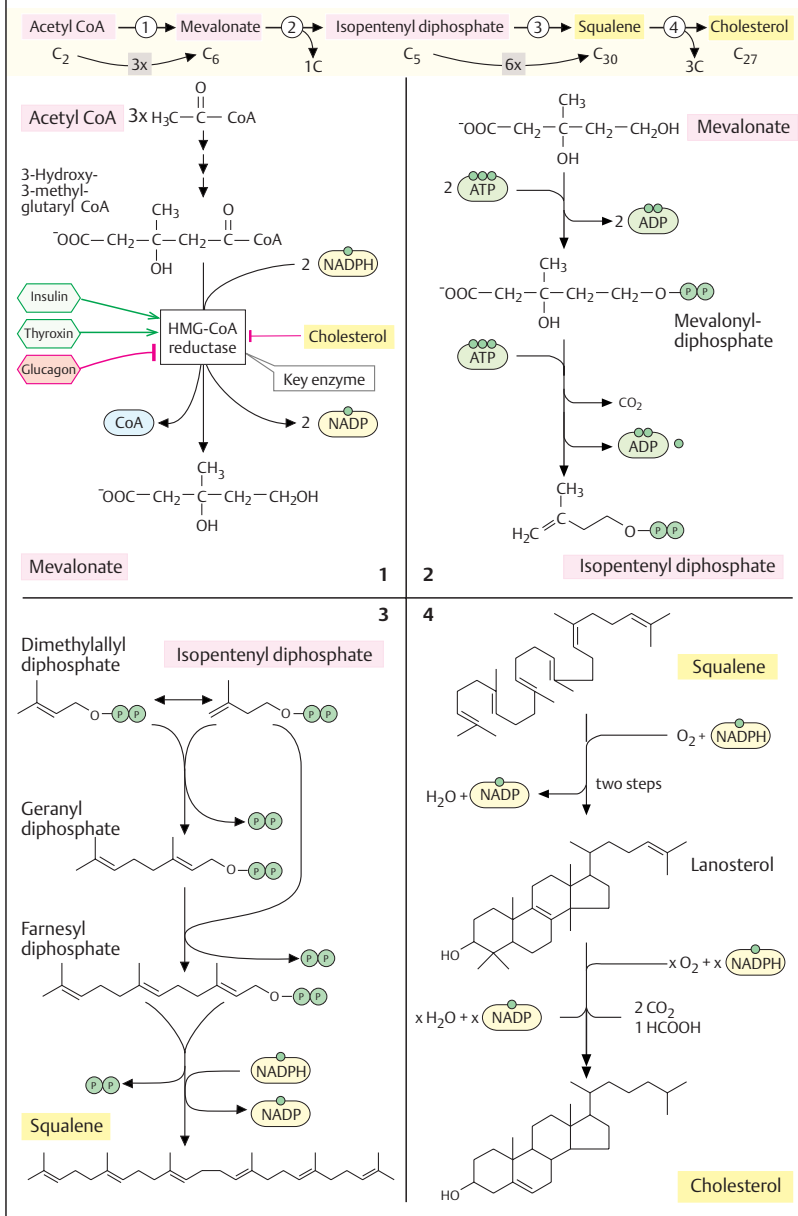
2. Formation of isopentenyl diphosphate. Mevalonate is decarboxylated to isopentenyl diphosphate, with consumption of ATP. All of the isoprenoids are built from this component (p. 44).

3. Formation of squalene. Isopentenyl diphosphate undergoes isomerization to form dimethylallyl diphosphate. The two C_5 molecules condense to yield geranyl diphosphate, and the addition of another isopentenyl diphosphate produces farnesyl diphosphate. This can then undergo dimerization, in a *head-to-head reaction*, to yield squalene. Farnesyl diphosphate is also the starting-point for other polyisoprenoids, such as dolichol and ubiquinone (p. 44).

4. Formation of cholesterol. Squalene, a linear isoprenoid, is cyclized, with O_2 being consumed, to form lanosterol, a C_{30} sterol. Three methyl groups are cleaved from this in the subsequent reaction steps, to yield the end-product cholesterol. Some of these reactions are catalyzed by *cytochrome P450 systems* (p. 324). The biosynthetic pathway described above is located entirely in the *smooth endoplasmic reticulum*. The energy needed comes from the CoA derivatives used and from ATP. The reducing agent in the formation of mevalonate and squalene, as well as in the final steps of cholesterol biosynthesis, is NADPH.

The division of the intermediates of the reaction pathway into three groups is characteristic: CoA compounds, diphosphates, and highly lipophilic, poorly water-soluble compounds (squalene to cholesterol), which are bound to sterol carriers in the cell.

A. Cholesterol biosynthesis



Pathobiochemistry

Defects of lipid metabolism are common. In many cases, they are a concomitant manifestation of other diseases, e.g., *obesity* (p. 332), *diabetes mellitus* (p. 430) or *dyslipidemia* (p. 300) and will be discussed there.

A. Disorders of lipid metabolism (examples)

Primary disorders are due to genetic defects in lipid metabolism, whereas the causes of secondary disorders lie outside lipid metabolism. Several examples are shown.

Fatty liver, an example of a secondary disorder, can be caused by products resulting from the *degradation of ethanol*. So *NADH* and *acetate* inhibit gluconeogenesis and fatty acid degradation. At the same time, the synthesis of ketone bodies and fatty acids is stimulated. The additional fats produced cannot be released into the blood efficiently because *acetaldehyde* interferes with VLDL formation.

Gaucher disease is the most common congenital *lipid storage disease*, with an incidence of roughly 1:40 000 in Central Europe. It is an example of lysosomal storage diseases (p. 224). Gaucher disease is an inherited autosomal recessive disorder which is due to a deficiency of functional *glucocerebrosidase* in the lysosomes of macrophages. The enzyme cleaves glucocerebrosides derived mainly from erythrocytes and leukocytes into glucose and ceramide (p. 42). Its deficiency in the macrophages of the liver, spleen, and bone marrow in particular leads to an accumulation there of nondegraded *cerebrosides* (p. 42). The macrophages then swell and become *Gaucher cells*. The result is enlargement and dysfunction of the affected organs. Treatment is possible by means of infusion of genetically engineered glucocerebrosidase. Apart from Gaucher disease, other diseases of sphingosine metabolism occur (*sphingolipidoses*), all of which affect the breakdown of sphingolipids and lead to deposits.

Refsum syndrome is a rare disorder of isoprenoid degradation characterized by various forms of neurological disorders. *Phytol* (p. 44), a component of chlorophyll and other substances of plant origin, is normally oxidized in animals to *phytanic acid* and this branched-chain fatty acid is broken down by α -oxidation and subsequent β -oxidation in the *peroxisomes* (p. 148). The disorder usually affects *phy-*

tanoyl-CoA hydroxylase, an enzyme involved in α -oxidation (p. 148).

Respiratory distress syndrome occurs in neonates who do not produce enough *surfactant* in their lungs. This fluid is a mixture of different surface-active substances, especially the phospholipid *lecithin* (p. 40). It ensures that the alveoli develop and maintain their shape so that they can perform their work.

The **transport of fatty acids into the mitochondria** (p. 144) can be impaired at different sites. *Carnitine* itself (p. 146), the two *carnitine-palmitoyl transferases* or *acylcarnitine translocase* can be affected. The deficiencies occur primarily in muscle and lead to muscle weakness, myoglobinuria, and fat deposition.

MCAD deficiency is an example of an inherited disorder of an enzyme involved in fatty acid degradation. In this disease, the *acyl-CoA dehydrogenase* (p. 146) for medium-chain fatty acids is nonfunctional. Fatty acids with about 6–12 C atoms accumulate in the mitochondria and interfere with metabolism, especially the TCA cycle.

B. Statins

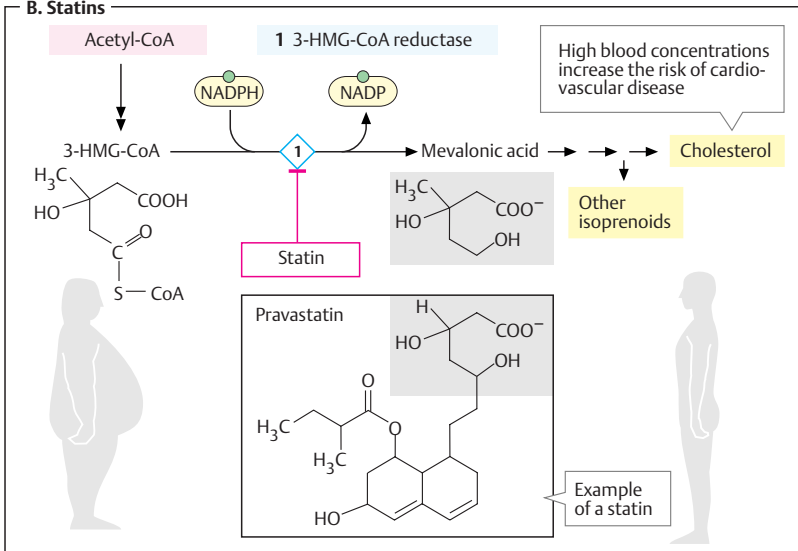
The **statins** are a group of drugs that can lower the blood levels of cholesterol. If this is over $200 \text{ mg} \cdot \text{dL}^{-1}$ or $5.2 \text{ mmol} \cdot \text{L}^{-1}$, it is regarded as raised ($\rightarrow$ increased risk of cardiovascular diseases). The statins intervene in cholesterol biosynthesis by inhibiting *3-hydroxy-3-methylglutaryl-CoA reductase* (HMG-CoA reductase; p. 156), the rate-limiting enzyme of this pathway. Statins are used widely to reduce the risk of myocardial infarction.

The formula of **pravastatin** shows that a part of the inhibitor is very similar to the reaction's substrate. The statins therefore bind to the active center of HMG-CoA reductase and block the synthesis of mevalonate.

A. Disorders of lipid metabolism (examples)

Defects	Cause	Result
Fatty liver	Excessive ethanol consumption (p. 326)	Inhibition of fatty acid degradation due to deficiency of NAD ⁺ and accumulation of NADH
Gaucher disease	Glucocerebrosidase in macrophages is absent or nonfunctioning (p. 42)	Glucocerebrosides accumulate in macrophages
Refsum syndrome	Inability to degrade phytol and its derivatives, deficiency of phytanoyl-CoA hydroxylase (first step in α -oxidation; p. 148)	Different neuropathies
Respiratory distress syndrome	Inability to produce lung surfactant (p. 40)	The deficiency of surfactant (incl. lecithin) leads to severe dyspnea, especially in newborn babies
Defect in fatty acid transport into mitochondria	Defect of the carnitine transport system, carnitine palmitoyl transferase I or II (CPT I or II) or acylcarnitine translocase (p. 144)	Hypoglycemia, hypoketosis, fat deposits, cardiomyopathy
Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	Impaired oxidation of medium-chain fatty acids with approximately 6–12 C atoms (p. 148)	Hypoglycemia because of impaired energy production from fatty acids, lethargy and coma

B. Statins



Overview

Quantitatively, proteins are the most important group of endogenous macromolecules. A person weighing 70 kg contains about 10 kg protein, with most of it located in muscle. By comparison, the proportion made up by other nitrogen-containing compounds is minor.

Protein metabolism involves two major areas: 1) synthesis and degradation of proteins themselves, and 2) synthesis and degradation of their building blocks, the *proteinogenic amino acids* (p. 50). The biosynthesis of proteins by translation on the ribosome and subsequent maturation in the ER are complex processes, which are discussed in the context of molecular and cell biology (pp. 220f., 248f.). However, amino acid biosynthesis and degradation are parts of normal intermediary metabolism that are closely linked with the TCA cycle.

A. Amino acid metabolism: overview

Despite the large number of biochemically important amino acids, their catabolic and anabolic pathways share a common range of features which will be discussed on the following pages. We will concentrate on the *non-essential amino acids*, i.e., amino acids that humans can synthesize themselves. A further important distinction is made between *glucogenic* and *ketogenic* amino acids. Glucogenic amino acids (highlighted green) can act as precursors of gluconeogenesis, while ketogenic amino acids (orange) yield ketone bodies or acetyl-CoA, which cannot be converted to glucose.

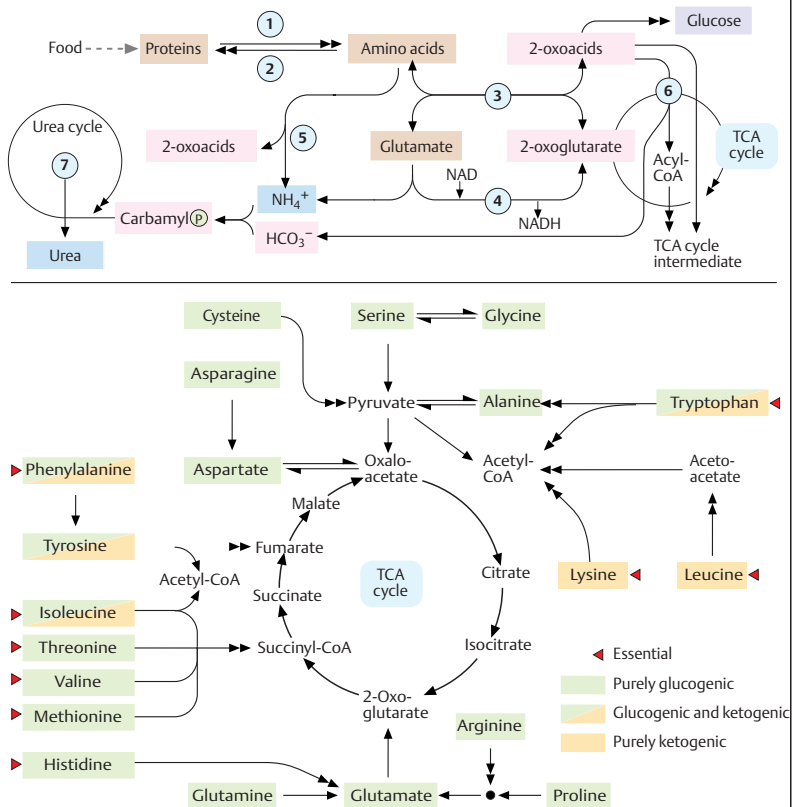
Metabolic pathways

- It is thought that humans break down 300–400 g of protein per day into amino acids (**proteolysis**, 1). On the other hand, approximately the same amount of amino acids is reincorporated into proteins (**protein biosynthesis**, 2). The body's high level of protein turnover is due to the fact that many proteins are relatively *short-lived* with half-lives of two to eight days. The *key enzymes* of intermediary metabolism are particularly short-lived and are sometimes broken down within a few hours and replaced by new molecules. This constant process of synthesis and degradation makes it possible for the cells to quickly adjust the quantities, and therefore the activity, of important enzymes to meet current requirements. By contrast, structural proteins such as the histones or hemoglobin are particularly long-

lived. Some *intracellular proteolysis* takes place in lysosomes (p. 224). In addition, there are so-called proteasomes in the cytoplasm, which break down incorrectly folded or old proteins (p. 162).

- Degradation of most amino acids begins with a **transamination** (3). The α -amino group of the amino acid is transferred to 2-oxoglutarate, which thereby becomes *glutamate*, while another 2-oxoacid is produced from the amino acid, which is degraded further. Conversely, the last step in the biosynthesis of many amino acids consists of transamination. In this case, an amino group is transferred from glutamate to the corresponding 2-oxoacid.
- **Oxidative deamination** (4) is used to release ammonium ions (NH_4^+) from glutamate and to regenerate 2-oxoglutarate. This step takes place mainly in the liver and is an important entry reaction for the urea cycle.
- Besides oxidative deamination, there are **other forms of deamination** (5). These also provide NH_4^+ and a 2-oxoacid, e.g., pyruvate.
- Further degradation of the 2-oxoacids formed by transamination or deamination often starts with an **oxidative decarboxylation** (6). CO_2 is cleaved and the remaining acyl residue is transferred to coenzyme A, and NADH also arises. Acyl-CoA derivatives produced in this manner are further degraded in various ways to final products that are intermediates of the TCA cycle or can enter this cycle.
- Ammonia (NH_3) and the ammonium ions (NH_4^+) that are in equilibrium with it are neurotoxic and must therefore be converted to less harmful products before excretion. This takes place in the **urea cycle** (7), a metabolic pathway in the liver. It converts hydrogen carbonate (HCO_3^-) and two NH_4^+ ions into urea at the expense of energy. The urea is then excreted in the urine.

A. Amino acid metabolism: overview



Process	Name	Page	Reactant(s) → product(s)	Catalyzed by	Comment
1	Proteolysis	163 269	Protein → amino acids	Proteinases, peptidases	Intracellular and extracellular
2	Protein biosynthesis (translation)	249 251	Amino acids → protein	Aminoacyl-tRNA synthetases, ribosome	–
3	Transamination	167	AA(1) + OA(2) → OA(1) + AA(2)	Many aminotransferases	Involved in degradation and biosynthesis
4	Oxidative deamination	167	Glu + NAD ⁺ → 2-OG + NADH + NH ₄ ⁺	Glutamate dehydrogenase	ATP1, GTP1 ADP1, leucine 1
5	Other deaminations	167	AA + H ₂ O → OA + NH ₄ ⁺ AA(1) + H ₂ O → AA(2) + NH ₄ ⁺	Deaminases	eliminating (Ser,Thr) hydrolytic (Gln,Asn)
6	Oxidative decarboxylation	169 171	2-OA + NAD ⁺ + CoA → Ac-CoA + NADH + HCO ₃ ⁻	Keto acid dehydrogenases	In the degradation of Ala, Thr, Met, Val, Leu, Ile, Asp, Glu, Lys
7	Urea cycle (liver)	173	HCO ₃ ⁻ + NH ₄ ⁺ + aspartate → urea + fumarate	Several enzymes	Arg 1, N-Acetyl-Glu 1, COR 1, glucagon 1

AA: amino acid, COR: cortisol, 2-OG: 2-oxoglutarate, OA: 2-oxoacid

Proteolysis

Enzymes that catalyze the hydrolysis of peptide bonds (*proteinases* and *peptidases*) are present not only in the lumen of the gastrointestinal tract (p. 268), but also on the surface of cells and inside the cells. Besides the degradation of proteins and peptides, they have other specialized functions, e.g., in metabolic regulation (p. 100), blood coagulation (p. 294ff.), apoptosis (p. 448), and in the complement system (p. 308).

A. Proteolytic enzymes

The proteolytic enzymes are classified into **endopeptidases** and **exopeptidases**, according to their site of attack in the substrate molecule. The *endopeptidases* or *proteinases* cleave peptide bonds *inside* peptide chains. They “recognize” and bind to amino acid sequences of the protein, and then hydrolyze bonds between particular amino acid residues in a more or less specific way. The **proteinases** are classified according to their reaction mechanism. In *serine proteinases*, for example (see **C**), a serine residue in the enzyme is important for catalysis, while in *cysteine proteinases*, it is a cysteine residue, and so on. The table (right) gives examples of each class.

The **exopeptidases** attack peptides from their termini. Peptidases that act at the *N*-terminus are known as **aminopeptidases**, while **carboxypeptidases** recognize the *C*-terminus. The **di-peptidases** only hydrolyze dipeptides.

B. Proteasomes

The functional proteins in the cell have to be protected from proteolysis to prevent premature degradation. Some of the intracellularly active proteolytic enzymes are therefore enclosed in lysosomes (p. 224). The proteinases that act there are also known as **cathepsins**. Another carefully regulated system for protein degradation is located in the cytoplasm. This consists of large protein complexes ($M = 2 \times 10^6$ Da), the **proteasomes**. Proteasomes contain a barrel-shaped core consisting of 28 subunits that has a sedimentation coefficient (p. 248) of 20 S. Proteolytic activity (shown here by the scissors) is localized in the interior of the barrel-like 20-S complex and is therefore protected. The openings in the barrel are sealed by 19-S particles with a complex structure that controls access to the core.

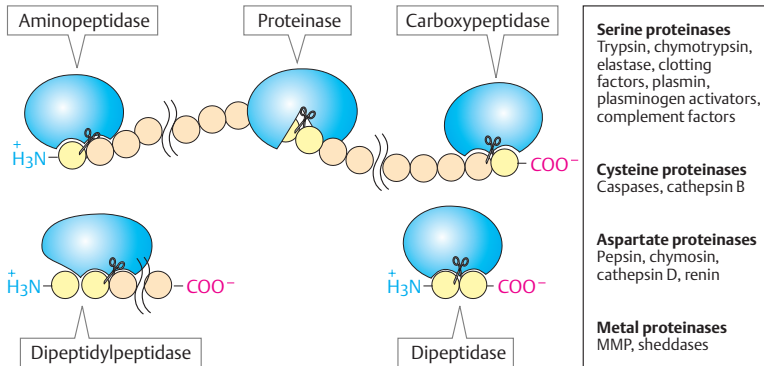
Proteins destined for degradation in the proteasome (e.g., incorrectly folded or outdated molecules) are marked by covalent linkage with chains of a small protein, **ubiquitin**. The ubiquitin is previously activated by the introduction of reactive thioester groups. Molecules marked with ubiquitin (“ubiquitinated”) are recognized by the 19-S particle, unfolded using ATP, and then shifted into the interior of the nucleus, where degradation takes place. Ubiquitin is not degraded, but is reused after renewed activation.

C. Serine proteases

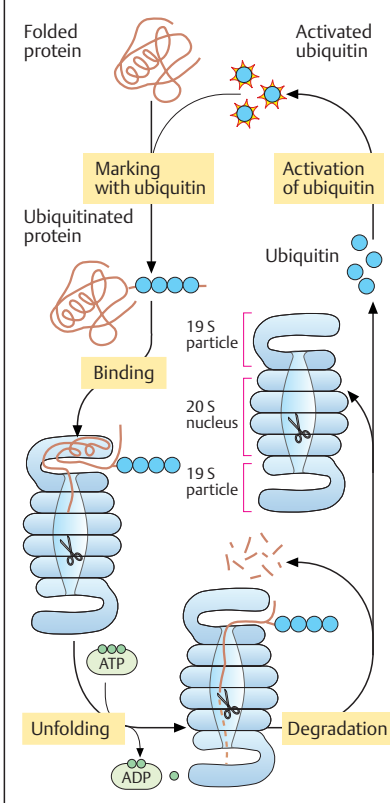
A large group of proteinases contain serine residues in their active center. The serine proteases include, for example, the digestive enzymes *trypsin*, *chymotrypsin*, and *elastase*, many *coagulation factors*, and the fibrinolytic enzyme *plasmin* and its *activators*.

As described on page 270, pancreatic proteinases are secreted as **proenzymes** (zymogens). Activation of these is also based on proteolytic cleavages. This is illustrated here in detail using the example of **trypsinogen**, the precursor of trypsin (**1**). Activation of trypsinogen starts with cleavage of an *N*-terminal hexapeptide by *enteropeptidase* (enterokinase), a specific serine proteinase that is located in the membrane of the intestinal epithelium. The cleavage product (β -trypsin) is already catalytically active, and it cleaves additional trypsinogen molecules at the sites marked in red in the illustration (autocatalytic cleavage). The precursors of chymotrypsin, elastase, and carboxypeptidase A, among others, are also activated by trypsin. The active center of trypsin is shown in the lower panel (**2**). A serine residue in the enzyme (Ser-195), assisted by a histidine residue and an aspartate residue (His-57, Asp-102), nucleophilically attacks the bond that is to be cleaved (red arrow). The cleavage site in the substrate peptide is located on the *C*-terminal side of a lysine residue, the side chain of which is fixed in a special “binding pocket” of the enzyme (left) during catalysis.

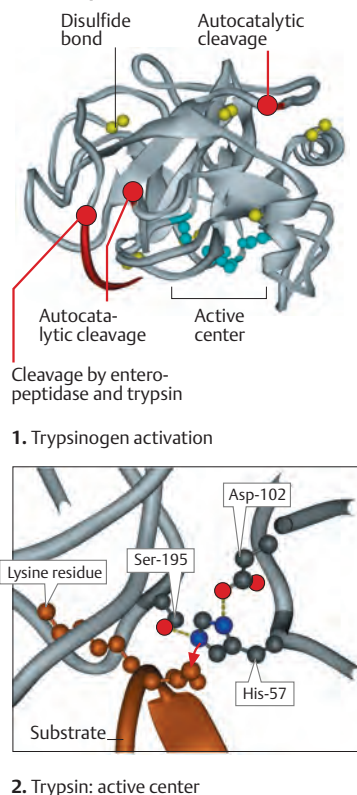
A. Proteolytic enzymes



B. Proteasomes



C. Serine proteinases



Pathways of Nitrogen

A. Nitrogen compounds

The nitrogen atoms in biologically important nitrogen compounds can adopt oxidation levels between -3 (ammonia, NH_3) and $+5$ (nitrate, NO_3^-). While plants and microorganisms also use the more highly oxidized nitrogen compounds (B), nitrogen-containing molecules in animal metabolism are mainly at the oxidation level of ammonia, with the exception of nitric oxide (NO ; p. 408).

Gaseous **ammonia** (NH_3), a moderately strong base, is in equilibrium with its conjugated acid, the **ammonium ion** (NH_4^+). Since the pK_a of the system is 9.2, the acid NH_4^+ predominates at the physiological pH of 7.4, to almost 98% (right). Therefore, we only speak of NH_3 in this book when the base is meant. While the charged ammonium ion cannot permeate biological membranes, the neutral NH_3 passes membranes readily. This is important in connection with the excretion of $\text{NH}_3/\text{NH}_4^+$ (p. 338).

B. Nitrogen cycle in nature

The atmosphere contains unlimited amounts of elemental nitrogen (N_2). Before it can enter the biological cycle, however, it has to be reduced to NH_3 (**nitrogen fixation**, 4). Only very few types of bacteria and blue-green algae are capable of this (see C).

Most bacteria and plants can meet their nitrogen requirement by **ammonium assimilation**. NH_4^+ is incorporated in glutamate by *glutamate dehydrogenase* (GLDH, [1]) as the α -amino group or by *glutamine synthase* [2] into the amide group of glutamine. Glutamate plays a central part in amino acid metabolism, because it acts as a universal donor of NH_2 groups in transaminations (p. 166) and in this way it enables synthesis of other amino acids.

Humans cannot meet their nitrogen requirement by NH_4^+ assimilation because their GLDH has different properties from that of plants and microorganisms. In addition, the NH_4^+ concentration in the mitochondrial matrix, where the GLDH is located, is kept so low by *carbamyl phosphate synthase* [5], that no effective synthesis of glutamate is possible. Humans therefore rely completely on previously synthesized amino acids, which are obtained from the proteins in their animal and vegetable diet.

Other reactions in the nitrogen cycle that produce or consume NH_4^+ are also reserved to microorganisms and plants. **Nitrate reduction** produces NH_4^+ from NO_3^- , while **nitrifying** bacteria perform the reverse reaction. From the agricultural aspect, **denitrification** is undesirable as it converts valuable nitrate into elemental nitrogen.

Higher animals require special metabolic pathways for the **excretion of nitrogen**. *Ureotelic organisms*, which include humans, produce *urea* (p. 172) and excrete it in the urine. As this is associated with substantial water loss, *uricotelic animals* (e.g., birds and reptiles) convert glutamine to *uric acid*, which is excreted in solid form, to save water.

C. Symbiotic nitrogen fixation

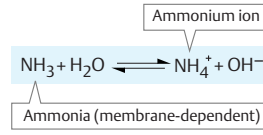
Nitrogen-fixing organisms live in the soil freely or in **symbiosis** with plants. Symbiosis between bacteria of the *Rhizobium* genus and legumes (*Fabales*) such as clover, beans, and peas, is of particular economic significance. Through symbiosis, these plants attain a high protein content and are therefore valuable nutritionally.

Bacteria in symbiosis with *Fabales* live as so-called **bacteroids** in **root nodules** inside plant cells. The plant supplies the bacteroids with nutrients while benefiting from the fixed nitrogen provided by the symbionts. *Nitrogenase* is the N_2 -fixing enzyme in the bacteria. It consists of two components: a *Fe protein*, containing a $[\text{Fe}_4\text{S}_4]$ cluster (p. 120) as redox system, accepts electrons from *ferredoxin* and passes them on to the second component, *FeMo protein*. This molybdenum-containing protein transfers the electrons to N_2 and so produces ammonium ions (NH_4^+) via various intermediate steps. Some of the reducing equivalents are transferred to H^+ in a subsidiary reaction. This is why hydrogen is always produced along with NH_3 .

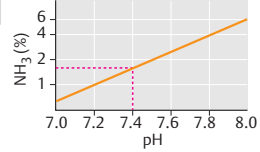
A. Nitrogen compounds

Oxidation ↓	-3	NH ₃	↑ Reduction
	0	N ₂	
	+2	NO	
	+3	NO ₂ ⁻	
	+5	NO ₃ ⁻	

1. Oxidation stages



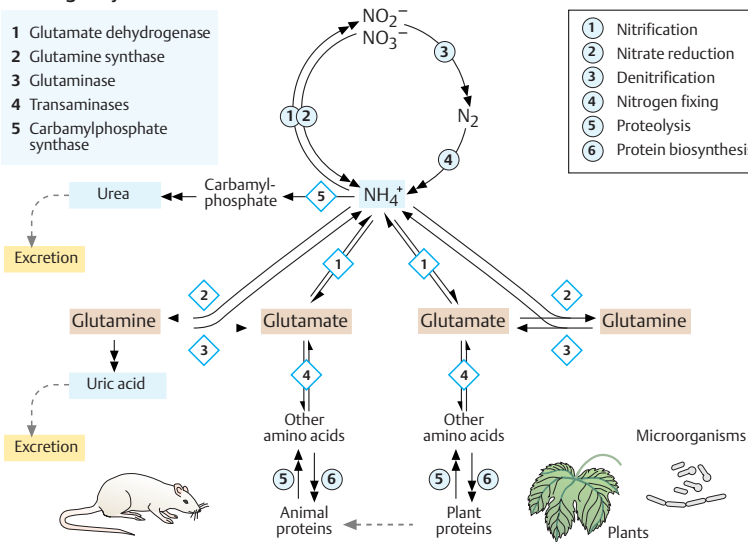
2. Ammonia equilibrium



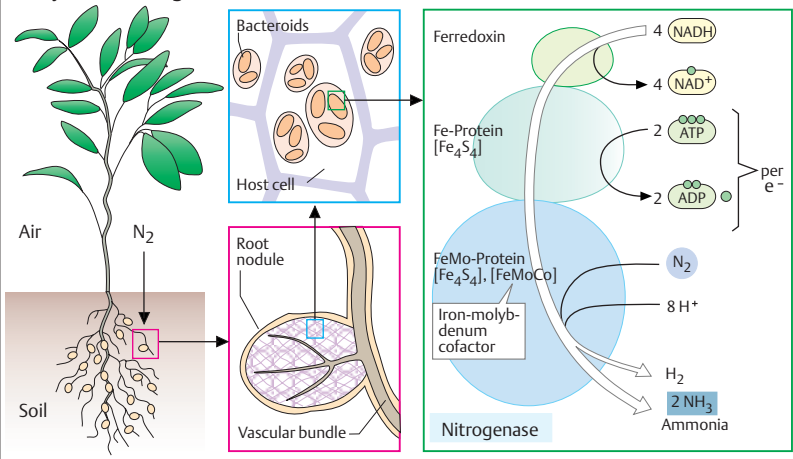
B. Nitrogen cycle in nature

- 1 Glutamate dehydrogenase
- 2 Glutamine synthase
- 3 Glutaminase
- 4 Transaminases
- 5 Carbamylphosphate synthase

- 1 Nitrification
- 2 Nitrate reduction
- 3 Denitrification
- 4 Nitrogen fixing
- 5 Proteolysis
- 6 Protein biosynthesis



C. Symbiotic nitrogen fixation



Transamination and Deamination

During protein degradation amino nitrogen accumulates which, in contrast to carbon, is not suitable for oxidative energy production. If they are not being reused for biosynthesis, the amino groups of most amino acids are therefore transferred initially to 2-oxoglutarate by transamination. In a second step, NH_4^+ is released by deamination from the glutamate produced in this way. The NH_4^+ is ultimately incorporated into urea (p. 172) and excreted in this form.

A. Transamination

Among the NH_2 transfer reactions, **transaminations** (1) are particularly important. They are catalyzed by *aminotransferases* ("transaminases", [1]) and occur in both catabolic and anabolic amino acid metabolism. During transamination, the amino group of an amino acid (amino acid 1) is transferred to a 2-oxoacid (oxoacid 2). From the amino acid, this produces a 2-oxoacid (oxoacid 1), while from the original oxoacid, an amino acid is formed (amino acid 2). The NH_2 group is temporarily taken over by enzyme-bound coenzyme **pyridoxal phosphate** (PLP; p. 88), which thus becomes *pyridoxamine phosphate*.

The table gives examples of aminotransferases and substrates involved in the respective reaction. Using the example of γ -aminobutyric acid (GABA) aminotransferase, it also shows that aldehydes can be converted to amines or amines to aldehydes by transamination.

Next the function of the coenzyme in transamination is illustrated in detail in (3). In the absence of substrates, the aldehyde group of pyridoxal phosphate is covalently bound to a lysine residue of the transaminase (not shown). This type of compound is known as an **aldimine** or "Schiff base." During the reaction, amino acid 1 (AA1) displaces the lysine residue and a new aldimine is formed. The double bond is then shifted by isomerization. The resulting **ketimine** is finally hydrolyzed to yield the 2-oxoacid and **pyridoxamine phosphate**.

In the second part of the reaction, these steps take place in the *opposite direction*: pyridoxamine phosphate and the second 2-oxoacid (OA2) form a ketimine, which is isomerized to aldimine. Finally, the second amino acid (AA2) is released and the coenzyme is regenerated.

B. Deamination

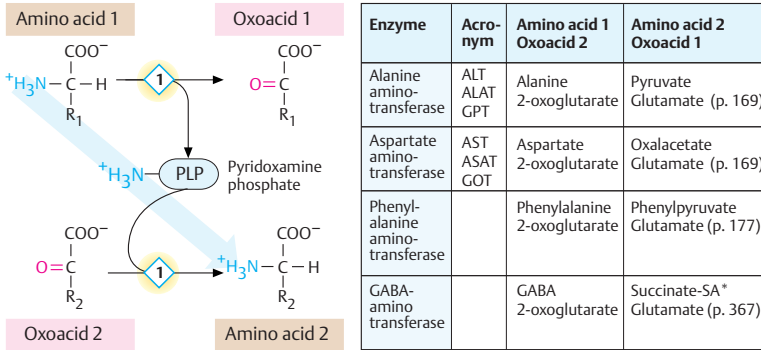
If the NH_2 group of an amino acid is released as ammonia, the process is referred to as **deamination**. There are several different mechanisms for this.

In **hydrolytic deamination** the amide group in the side chain of glutamine is hydrolyzed by a *glutaminase* [2], producing glutamate and an ammonium ion NH_4^+ . In an analogous reaction, *asparaginases* convert asparagine to aspartate and NH_4^+ . **Oxidative deamination**, which in human metabolism involves only glutamate, is a particularly important mechanism. In this reaction, the α -amino group of the amino acid is initially *oxidized* to an imino group, and the reducing equivalents are transferred to NAD or NADP. In the second step, the imino group is then cleaved by *hydrolysis*. As in transamination, this produces a 2-oxoacid. Oxidative deamination of glutamate mainly takes place in the liver, where 2-oxoglutarate and NH_4^+ are produced from glutamate in this way, catalyzed by *glutamate dehydrogenase* [3].

Serine and *threonine* are broken down to NH_4^+ plus pyruvate and 2-oxobutyrate, respectively by **eliminating deamination**. In this reaction, which is catalyzed by *serine (threonine) dehydratase* [4], a H_2O molecule is first eliminated from the side chain of the amino acid (hence "dehydratase"). This produces an unsaturated intermediate which spontaneously forms a ketimine. In the second step, this is hydrolyzed to NH_4^+ and pyruvate (or 2-oxobutyrate) with uptake of H_2O , as described above.

Histidine also undergoes eliminating deamination as the first reaction in its degradation. However, this reaction follows a different mechanism from deamination of serine and threonine.

A. Transamination

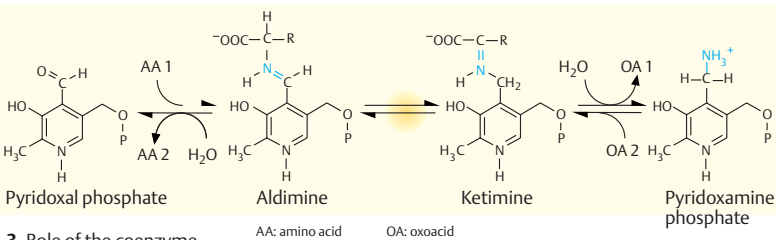


*Succinate-4-semialdehyde

1 Aminotransferase (transaminase)

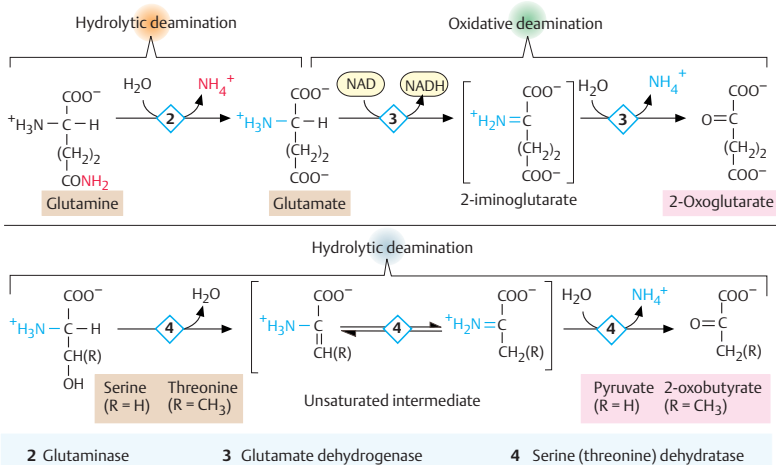
1. Principle of transamination

2. Important transaminases



3. Role of the coenzyme

B. Deamination



Amino Acid Degradation I

Despite the large number of proteinogenic amino acids, the number of end products produced during their degradation is relatively small. These compounds include four intermediates of the TCA cycle (2-oxoglutarate, succinyl CoA, fumarate, oxaloacetate) and also pyruvate, acetoacetate, and acetyl-CoA (p. 170).

A. Glucogenic and ketogenic amino acids

Since oxaloacetate is the starting point for gluconeogenesis (p. 134), amino acids whose degradation yields a TCA cycle metabolite or pyruvate are called **glucogenic** (glucoplastic) **amino acids**.

With two exceptions (lysine and leucine; see below), all of the proteinogenic amino acids are also glucogenic. Quantitatively, glucogenic amino acids represent the most important precursors for gluconeogenesis. At the same time, they also have an **anaplerotic** effect—i.e., they replenish the TCA cycle to feed the anabolic reactions that originate in it (p. 116).

Amino acids that yield acetyl-CoA or acetoacetate during degradation are called **ketogenic** (ketoplastic) **amino acids**. Their degradation products cannot be channeled into gluconeogenesis in animal metabolism, as there is no means of converting them into oxaloacetate or other precursors of gluconeogenesis. The C atoms of acetyl-CoA are oxidized to CO₂ in the TCA cycle and are therefore no longer available for gluconeogenesis. Ketogenic amino acids can be used to synthesize ketone bodies, fatty acids, and isoprenoids (p. 144). Several amino acids yield degradation products that are both *glucogenic* and *ketogenic*. This group includes phenylalanine, tyrosine, tryptophan, and isoleucine. Only *leucine* and *lysine* are *purely* ketogenic.

B. Degradation of nonessential amino acids

Nonessential amino acids are amino acids that the human body can manufacture itself (p. 174). On degradation, all of the amino acids in this group are channeled into the TCA cycle in no more than three reactions steps by deamination and/or transamination (p. 166). *Glutamate* and *aspartate* can be converted directly by transamination into metabolites of the cycle (2-oxoglutarate or oxaloacetate). The degradation pathways of *glutamine*, *arginine*,

and *proline* merge in glutamate (see C), while *cysteine*, *serine*, *glycine*, and *alanine* are degraded through pyruvate.

C. Glutamate metabolism

Glutamate is at the heart of metabolic pathways involved in the synthesis and degradation of the amino acids *alanine*, *aspartate*, *proline*, and *arginine*. Alanine and aspartate are directly connected to glutamate via transamination reactions. The aminotransferases involved—*alanine transaminase* [2] and *aspartate transaminase* [3]—have important functions in nitrogen metabolism in the liver (p. 172), while isoenzymes of aspartate transaminase also take part in the *malate shuttle* (p. 118). The activities of the two transaminases in the blood are measured in *serum enzyme diagnostics* (p. 300) to diagnose various diseases.

The degradation and biosynthesis of proline and arginine take place through the same intermediate—cyclic Δ^1 -pyrroline carboxylate. This compound arises from the reduction of glutamate by an NADH-dependent dehydrogenase [4] and is in chemical equilibrium with its open-chain form, γ -glutamate semialdehyde. Further reduction of Δ^1 -pyrroline carboxylate [5] results in **proline**, a proteinogenic amino acid with a ring structure. By contrast, the semialdehyde form can be converted to the nonproteinogenic **ornithine** by transamination of the aldehyde group [6]. This reaction shows that not only 2-oxoacids but also aldehydes can be transaminated. Another example of this type of reaction is transamination of GABA to succinate semialdehyde in the “GABA shunt” (p. 366).

Since ornithine is an intermediate of the urea cycle (p. 172), it can be converted to **arginine** by reactions in this cycle. However, this occurs in the kidney and not in the liver (p. 338). If major amounts of arginine were to be removed from the urea cycle in the liver, the cycle and thus NH₄⁺ detoxification would come to a standstill.

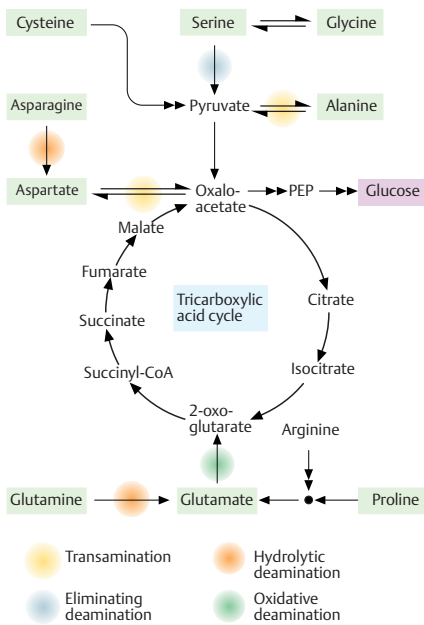
Degradation of the aforementioned amino acids takes place through the same reactions, all of which are reversible.

A. Glucogenic and ketogenic amino acids

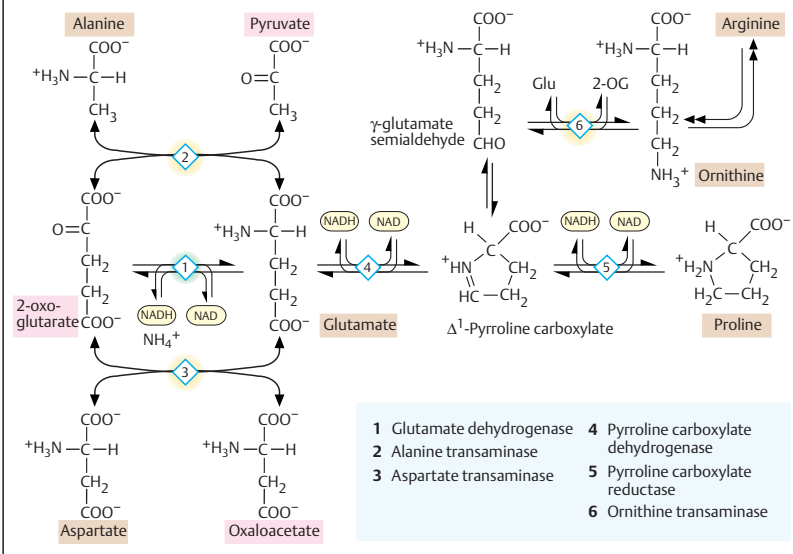
Amino acid	Degradation product(s)
Purely glucogenic	
Alanine	Pyruvate
Arginine	2-oxoglutarate
Asparagine	Oxaloacetate
Aspartate	Oxaloacetate
Cysteine	Pyruvate
Glutamate	2-oxoglutarate
Glutamine	2-oxoglutarate
Glycine	Pyruvate
Histidine	2-oxoglutarate
Methionine	Pyruvate, succinyl-CoA
Proline	2-oxoglutarate
Serine	Pyruvate
Threonine	Succinyl-CoA
Valine	Succinyl-CoA
Glucogenic and ketogenic	
Isoleucine	Succinyl-CoA, acetyl-CoA
Phenylalanine	Fumarate, acetoacetate
Tryptophan	Pyruvate, acetyl-CoA
Tyrosine	Fumarate, acetoacetate
Purely ketogenic	
Leucine	Acetoacetate, acetyl-CoA
Lysine	Acetyl-CoA

■ purely glucogenic amino acids
■ purely ketogenic amino acids
■ glucogenic and ketogenic amino acids

B. Degradation of nonessential amino acids



C. Glutamate metabolism



Amino Acid Degradation II

Essential amino acids cannot be synthesized by metabolic processes in humans (p. 174). Since their degradation is considerably more complicated than that of the nonessential amino acids, their degradation pathways are presented only schematically here. Glucogenic amino acids have a green background. Purely ketogenic amino acids are identified by an orange background, while amino acids with a glucogenic *and* ketogenic action are marked with both colors.

A. Degradation of essential amino acids

The degradation pathways of most of the essential amino acids start with *transamination*, in which the α -amino group is transferred to 2-oxoglutarate (yellow circles) with the formation of glutamate. The resulting 2-oxoacids (OA1–OA5) are converted to CoA derivatives by *oxidative decarboxylation* with elimination of CO_2 ; these are then degraded further in different pathways. The principle of oxidative decarboxylation is discussed on page 112 using the example of pyruvate dehydrogenase (PDH). The *oxoacid dehydrogenases* involved in amino acid degradation work like PDH. For example, there is a “*branched chain dehydrogenase complex*,” which catalyzes the conversion of the 2-oxoacids formed from valine and isoleucine. *Threonine* is converted by eliminating deamination (p. 166) to 2-oxobutyrate (OA6). This metabolite is also produced during degradation of *methionine* after demethylation of the thioether group and transfer of the thiol group to serine with formation of cysteine. The further degradation of 2-oxobutyrate to succinyl-CoA is shown in detail in **B**.

There are special degradation pathways for *tryptophan* and *histidine*. The aliphatic part of the tryptophan molecule is cleaved off as alanine, while degradation of the indole ring yields acetyl-CoA. Histidine first undergoes eliminating deamination and is then further broken down to glutamate in several steps. The degradation of the purely ketogenic amino acids *lysine* and *leucine* yield acetoacetate and/or acetyl-CoA exclusively. On the right of the illustration, red arrows mark the reactions involved in the oxidation of acetoacetate or acetyl-CoA to CO_2 . The degradation of acetoacetate to acetyl CoA is described on page 374.

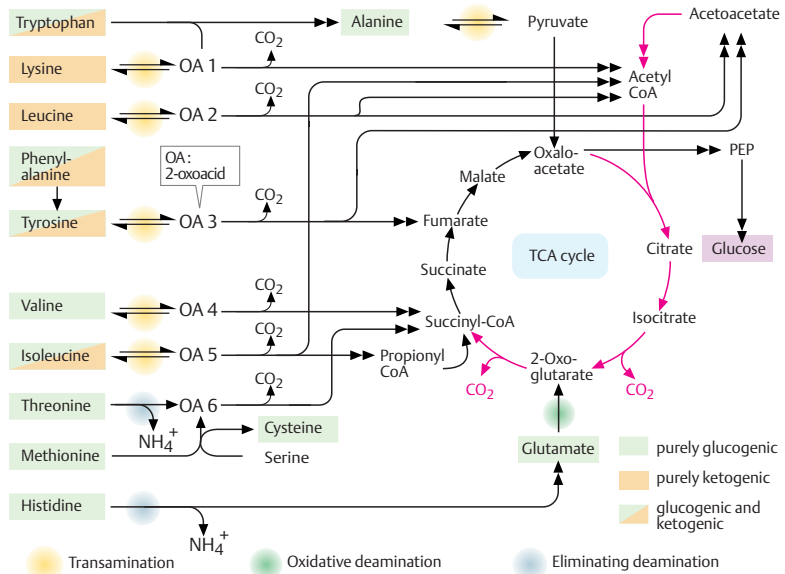
B. Degradation to succinyl-CoA

The catabolic pathways of threonine, methionine, isoleucine and valine merge in a series of reactions that convert 2-oxobutyrate to succinyl-CoA. The degradation of the nucleobase thymine also ends here (not shown, see p. 180). 2-Oxobutyrate gives rise first to propionyl-CoA through *oxidative decarboxylation* [1]; in the next step [2] this is carboxylated to methylmalonyl-CoA. The final conversion to succinyl-CoA is catalyzed by *methylmalonyl-CoA mutase* [3]. Apart from the remethylation of homocysteine to methionine (p. 184), this step is the only reaction in animal metabolism that requires *cobalamin* (p. 90), derived from vitamin B_{12} , as a coenzyme.

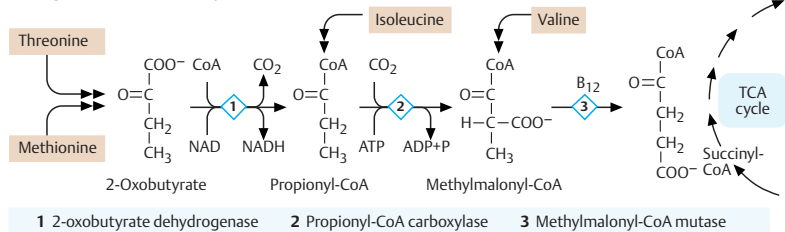
C. Tyrosine metabolism

In animal metabolism, the amino acid tyrosine is not only a component of proteins, but also the precursor of several hormones and the basic building block of the brown pigment *melanin*. Tyrosine is one of the partially essential amino acids (p. 174), as it can be formed from the essential phenylalanine by hydroxylation. *Phenylalanine hydroxylase* [4] and *tyrosine hydroxylase* [6] are among the few enzymes that use *tetrahydrobiopterin* (THB) as coenzyme. Inborn deficiencies of phenylalanine hydroxylase lead to *phenylketonuria* (p. 176). A second hydroxylation of the aromatic ring in tyrosine results in *L*-dihydroxyphenylalanine (*L*-dopa). Other reactions, which are described in detail on p. 434, lead to the *catecholamines*, a group of signaling substances that includes *epinephrine*. In the oxidation of tyrosine by *tyrosinase* [5], *L*-dopa is only an intermediate that is immediately oxidized further by the same enzyme to the *o*-diquinone, *dopachrome*. Dopachrome, assisted by other enzymes, is then polymerized to the brown pigment *melanin*, which is deposited in the skin, hair and iris, where it protects against light and acts as an antioxidant. The dark color of the *Substantia nigra* in the brain (p. 370) also comes from melanin.

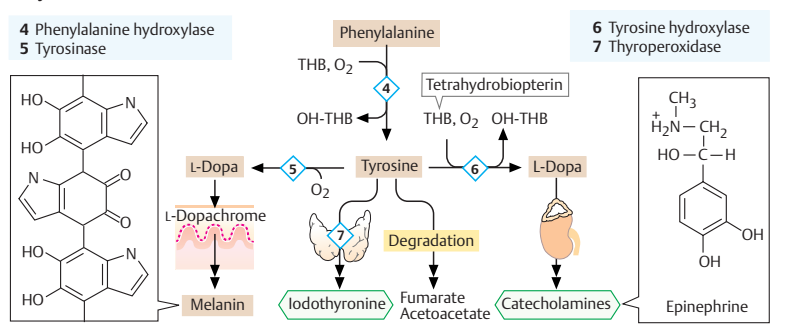
A. Degradation of essential amino acids



B. Degradation to succinyl CoA



C. Tyrosine metabolism



Urea Cycle

Ammonium ions (NH_4^+) are toxic and damage the brain above all. Their concentration in the blood is therefore normally kept below $40 \mu\text{mol} \cdot \text{L}^{-1}$. This is achieved by transporting amino nitrogen as a component of amino acids and not as free NH_4^+ ion in the plasma (p. 374). In the liver, NH_4^+ is released again (**A**) from these amino acids and incorporated in the nontoxic urea (**B**).

A. Amino acid metabolism in the liver

Apart from urea synthesis itself (**B**), the production of its precursors (NH_4^+ and aspartate; see below) also takes place in the liver. The amino nitrogen that arises in the tissues is transported in the blood to the liver, mainly in the form of **glutamine** (Gln) and **alanine** (Ala). There, Gln undergoes hydrolytic deamination by **glutaminase** [3] to **glutamate** (Glu) and NH_4^+ . The amino group in alanine is transferred to **2-oxoglutarate** (2-OG) by **alanine aminotransferase** (transaminase) [1]. This transamination also produces glutamate. Finally, NH_4^+ is released from glutamate by oxidative deamination. This reaction is catalyzed by **glutamate dehydrogenase** [4], a typical liver enzyme. **Aspartate** (Asp), the second donor of amino groups in the urea cycle, is also produced from glutamate. The **aspartate aminotransferase** (transaminase) responsible [2], like **alanine aminotransferase** [1], possesses high activity in the liver.

B. Urea cycle

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

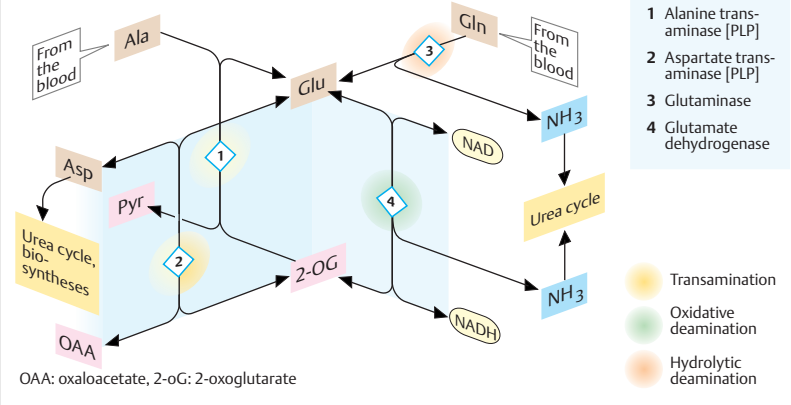
Urea is produced *only in the liver*, in a cyclic reaction sequence (the **urea cycle**). The cycle starts in the mitochondria and continues in the cytoplasm. The two nitrogen atoms in urea are derived from NH_4^+ and aspartate. The keto group is provided by **hydrogen carbonate** (HCO_3^-), or CO_2 that is in equilibrium with HCO_3^- .

- [5] In the first step, **carbamyl phosphate** is formed in the mitochondrion from hydrogen carbonate (HCO_3^-) and NH_4^+ , with two ATP molecules being consumed. In this compound, the carbamyl residue ($-\text{O}-\text{CO}-\text{NH}_2$) is at a high chemical potential. In hepatic mitochondria, enzyme [5] makes up about 20% of the matrix proteins.
- [6] In the next step, the carbamyl residue is transferred to **ornithine**, a nonproteinogenic amino acid, converting it into **citrulline**, which is also nonproteinogenic. This is passed into the cytoplasm via a transporter (ORNT1) in exchange for ornithine.
- [7] The second NH_2 group of the later urea molecule is provided by **aspartate**, which condenses with citrulline into **argininosuccinate**. ATP is cleaved into AMP and diphosphate (PP_i) to drive this endergonic reaction. To shift the equilibrium of the reaction to the side of the product, PP_i is removed from the equilibrium by hydrolysis.
- [8] Cleavage of fumarate from argininosuccinate leads to the proteinogenic amino acid **arginine**, which is synthesized in this way in animal metabolism.
- [9] In the final step, isourea is released from the guanidinium group of the arginine by hydrolysis (not shown), and is immediately rearranged into **urea**. In addition, ornithine is regenerated and returns via the ORNT1 into the mitochondria, where it becomes available for the cycle once again.

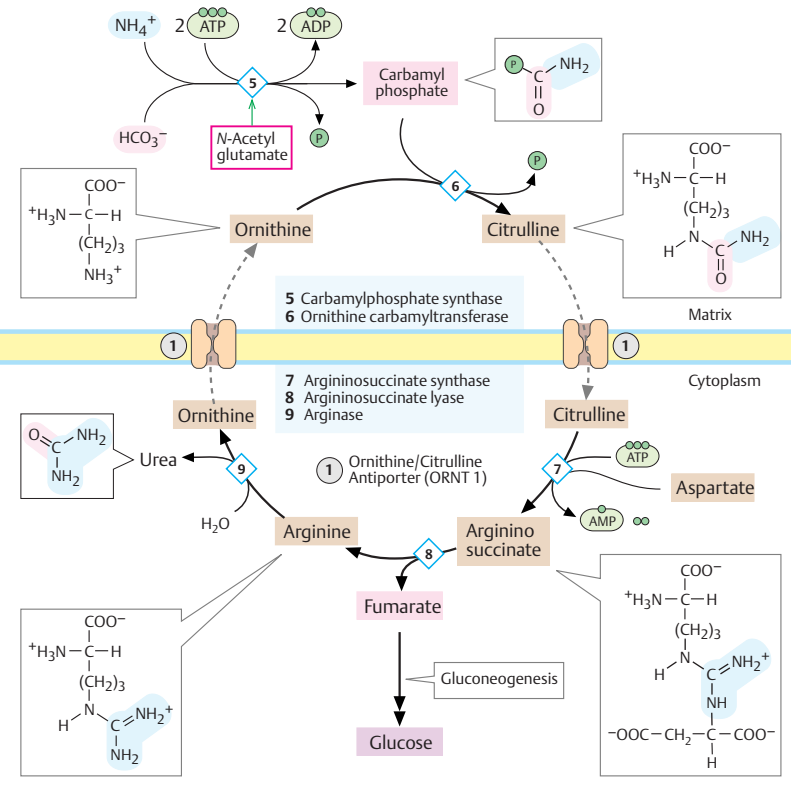
The **fumarate** produced in step [8] could be converted back to aspartate via cytoplasmic enzymes (reaction sequence: fumarate $\rightarrow$ malate $\rightarrow$ oxaloacetate $\rightarrow$ aspartate). However, recent studies indicate that it is used mainly for gluconeogenesis.

The rate of urea formation is controlled almost exclusively by reaction [1] with **N-acetyl glutamate** activating **carbamylphosphate synthase** as an allosteric effector. In turn, the concentration of acetyl glutamate depends on arginine and ATP levels, as well as other factors.

A. Amino acid metabolism in the liver



B. Urea cycle



Amino Acid Biosynthesis

A. Synthesis of nonessential amino acids

All amino acids that can be produced by transamination from the 2-oxoacids of intermediary metabolism, as well as their amides, can be synthesized by humans themselves. These are the members of the **glutamate family** (Glu, Gln, Pro, Arg, derived from 2-oxoglutarate), the **aspartate family** (including only Asp and Asn, derived from oxaloacetate) and **alanine** (Ala, derived from pyruvate). The amino acids of the **serine family** (Ser, Gly, Cys) can also be synthesized by humans.

Comparison of the diagram with page 169, **B** shows that many non-essential amino acids are synthesized by reversing reactions that are involved in their degradation. Deamination of serine to pyruvate (p. 166) cannot be reversed. Serine is therefore produced in two steps from 3-phosphoglycerate, an intermediate of glycolysis.

B. Amino acid requirements

Plants and microorganisms are able to synthesize all of the amino acids in full, but during the course of evolution, mammals have lost the ability to synthesize approximately half of the 20 proteinogenic amino acids. These **essential amino acids** therefore have to be supplied in food as components of proteins. For example, animal metabolism is no longer capable of synthesizing the *aromatic amino acids* (tyrosine is only nonessential because it can be formed from phenylalanine when there is an adequate supply available; p. 170). The *branched-chain amino acids* (valine, leucine, isoleucine) as well as *threonine*, *methionine*, and *lysine*, also belong to the essential amino acids. Whether *histidine* is essential for adults is still a matter of debate. A supply of these amino acids in food appears to be essential, at least during growth.

The *daily essential amino acid requirement* depends greatly on age and lifestyle. The table shows the average values for a healthy adult weighing 70 kg. **Partially essential amino acids** are those that can be synthesized from another amino acid when enough of this is available. The table gives their names and the corresponding precursors.

The nutritional value of proteins is decisively dependent on their essential amino acid content. Vegetable proteins—e.g., those from cereals—are low in lysine and methionine, while animal proteins contain all the amino acids in

balanced proportions (except collagens). However, plants can also provide high-value protein. These include the soy bean and other legumes, which are supplied with NH_4^+ by symbiotic N_2 fixers (p. 164).

C. Synthesis of essential amino acids

The biosynthetic pathways of the essential amino acids in microorganisms and plants are shown here only in rough outline. Lysine, methionine, threonine, and isoleucine are synthesized from *aspartate*. Another important precursor is *pyruvate*, from which valine and leucine are produced. The synthesis of the aromatic amino acids and histidine starts from *ribose 5-phosphate*. All of these pathways are complex and involve up to 11 reactions.

D. Cysteine metabolism

Cysteine is the only proteinogenic amino acid with a **thiol group** ($-\text{SH}$) in the side chain. Two cysteine molecules can be linked oxidatively through their SH groups to form the disulfide **cystine**. The same reaction between two cysteine residues of a protein leads to an intramolecular **disulfide bond**. In proteins, these bonds represent the only **covalent** interaction that stabilizes the conformation (p. 60). However, with few exceptions, disulfide bonds are found only in extracellular proteins, because reducing compounds such as glutathione (see below) are present inside the cell in such a high concentration that disulfides would be cleaved again by reduction.

Enzymatic oxidation of cysteine's thiol group produces the nonproteinogenic amino acid **taurine**. This occurs in bile salts (p. 320), as a neurotransmitter, and also free in the interior of the cell. A cysteine residue is a central component of the antioxidant tripeptide **glutathione** (p. 290). To synthesize glutathione, cysteine is linked enzymatically with glutamate and then with glycine, while consuming ATP.

Pathobiochemistry

Most disorders of amino acid metabolism involve enzymes concerned in the degradation of essential amino acids or amino acid transporters. Deficiencies of enzymes of the urea cycle are also relatively common (B).

A. Defects in amino acid degradation

Phenylketonuria. In Europe, the incidence of phenylketonuria (PKU) is about 1 : 10 000 (i.e., one case in 10 000 live births), making it one of the most common enzyme deficiencies overall. It is therefore one of the metabolic disorders sought routinely as part of *neonatal screening*. Most cases of PKU are due to a deficiency of *phenylalanine hydroxylase* [1]. Since phenylalanine can no longer be hydroxylated to tyrosine, it accumulates in the blood and cells and is converted by subsidiary reactions into products that normally are of little significance. Transamination of phenylalanine yields the ketone *phenylpyruvate*, which is excreted in the urine (hence the name phenylketonuria), or reduced further to *phenyl lactate*. Another subsidiary pathway leads to *phenylacetate*. The presence of PKU can be detected from the greatly elevated phenylalanine level in the blood. If the disorder is not identified in time, intellectual development of the newborn is delayed and ultimately there is irreversible brain damage. The main cause is the increased transamination of phenylalanine in the brain. This consumes so much 2-oxoglutarate that the TCA cycle, and thus the energy production of the neurons, is impaired. Treatment consists of a special low-phenylalanine diet, which has to be started immediately and continued for years.

Deficiencies of *homogentisate dioxygenase* [3] in the tyrosine degradation pathway are the cause of **alkaptonuria**. With time, the excess substrate, homogentisate, is oxidized nonenzymatically to dark-brown pigments, which are deposited in the tissues and turn the urine dark. **Albinism**, which is usually due to *tyrosinase* ([2]; p. 170) deficiencies, is more harmless. The lack of melanin results in very light colored skin with increased photosensitivity and white-blond hair.

In the relatively rare **homocystinuria**, excess homocysteine in the blood leads to an increased tendency to thrombosis through damage to vascular endothelia. Many patients therefore suffer from strokes and coronary heart disease, even in youth. Other symptoms

include disorders of intellectual development and eye damage. The biochemical cause is the impaired utilization of *homocysteine*, an intermediate of methionine degradation. Homocysteine is degraded further either by *cystathionine β -synthase* [4] or remethylated to methionine by *methionine synthase* [5]. Deficiencies of the two enzymes and also disorders of C₁ metabolism (p. 184) can cause the disease.

Two disorders of branched-chain amino acid degradation are also rare. In **"maple syrup urine disease,"** branched-chain dehydrogenase is deficient (p. 170). The name comes from the characteristic maple syrup odor of the urine. When methylmalonyl-CoA can no longer be isomerized to succinyl-CoA (p. 170), the free acid accumulates, causing metabolic acidosis and other symptoms (**methylmalonate acidemia**).

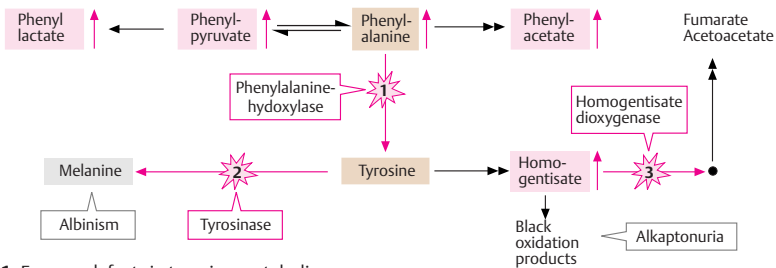
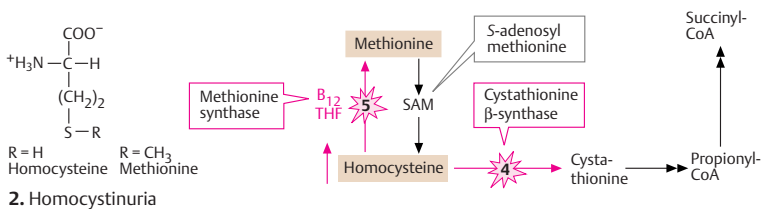
B. Defects in the urea cycle

Complete absence of one of the enzymes of the urea cycle (p. 172) would not be compatible with life. However, there is a range of partial defects that can affect any enzyme in the cycle and also the *ornithine/citrulline antiporter* (ORNT1) or the accessory enzyme *acetylglutamate synthase*.

Disorders of this nature become apparent due to a more or less marked rise in the blood NH_4^+ concentration (*hyperammonemia*), which begins 1–2 days after birth. Since NH_4^+ is highly neurotoxic, symptoms are severe, with vomiting, refusal to feed, and seizures. Untreated, these can lead to coma and death. Long-term treatment consists of a lifelong low-protein diet. In some cases, it may be helpful to administer the metabolites that can no longer be produced because of the deficiency.

A. Defects in amino acid degradation

Name (frequency)	Affected enzyme(s)	Symptoms
Phenylketonuria (1:10 000)	Phenylalanine hydroxylase	Delayed intellectual development, neurological defects
Alkaptonuria (1:25 000)	Homogentisate dioxygenase	Dark urine, arthritis
Albinism (1:20 000)	Tyrosinase	Absent melanization
Homocystinuria (1:150 000)	Cystathionine β -synthase methionine synthase	Thrombosis tendency, lens ectopia, intellectual retardation
Maple syrup urine disease (1:100 000)	Branched-chain dehydrogenase	Failure to thrive, lethargy, neurological defects
Methylmalonate acidemia (1:30 000)	Methylmalonyl-CoA mutase	Acidosis, failure to thrive


1. Enzyme defects in tyrosine metabolism

2. Homocystinuria
B. Defects in the urea cycle

Name	Affected protein(s)	Symptoms
Hyperammonemia type I	Carbamylphosphate synthase	Severe hyperammonemia from the second day of life
Hyperammonemia type II	Ornithine carbamyltransferase	Seizures, hyperventilation, lethargy, anorexia, vomiting
Citrullinemia type I	Argininosuccinate synthase	Coma and death if untreated
Argininosuccinemia	Argininosuccinate lyase	
N-acetylglutamate synthetase deficiency	N-Acetylglutamate synthase	
HHH syndrome (very rare)	ORNT 1	Sporadic developmental disorders

Overview

Nucleotides consist of a *pentose* (ribose or deoxyribose), a heterocyclic *base*, and at least one *phosphate residue*. They have a variety of functions in the cell. Nucleotides act not only as the building blocks of nucleic acids but also as coenzymes (p. 92) and as signaling molecules (p. 406).

A. Nucleotide metabolism: overview

Almost all cells perform replication and transcription and they also require numerous nucleotide coenzymes for their metabolism, so the metabolic pathways for synthesizing nucleobases and incorporating them in nucleotides and nucleic acids are present in all tissues. There are marked differences in the metabolism of purines and pyrimidines.

The diagram shows important intermediate stages in the synthesis of purine and pyrimidine nucleotides. The first complete nucleotide provided by purine biosynthesis is *inosine monophosphate* (IMP) with the base *hypoxanthine*. IMP only acts as a precursor for AMP and GMP and as an intermediate in purine degradation. It does not occur in nucleic acids and does not act as a coenzyme either.

The first complete pyrimidine nucleotide is *uridine monophosphate* (UMP). It is a component of RNA and, after twofold phosphorylation to UTP, it acts as a coenzyme for activation of sugars (p. 92).

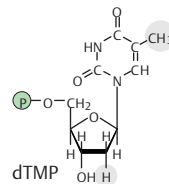
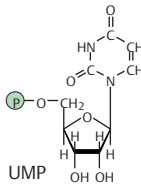
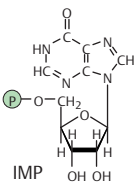
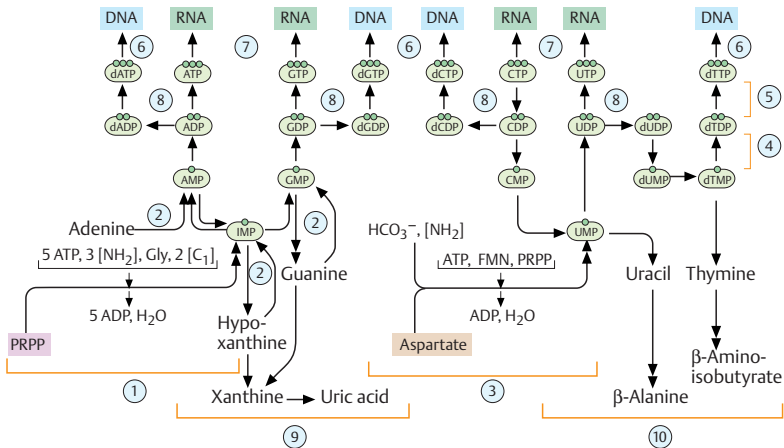
Methylation of uracil in the 5' position yields thymidine monophosphate (TMP). This nucleotide is a component of tRNAs (p. 66). *Deoxythymidine monophosphate* (dTMP) is much more common as a component of DNA. Conversion of uracil to thymine takes place at the deoxyuridine monophosphate (dUMP) stage.

The question of why DNA contains thymine instead of uracil cannot be answered conclusively. However, the following explanation is plausible: mutagens like nitrite can deaminate cytosine to uracil (p. 254), which pairs with A instead of C (p. 68). If uracil were a component of DNA instead of thymine, it would not be possible to distinguish deaminated guanine from "correct" uracil. This would make repair of the damage impossible so the rate of mutation would be increased enormously.

Metabolic pathways

- **Purine biosynthesis** (1) is a lengthy metabolic pathway with ten intermediate stages. It starts with 6'-phosphoribosyl diphosphate (PRPP), at which the purine ring is constructed piece by piece. The purines are among the few aromatic compounds that can be synthesized from scratch in animal metabolism.
- In view of the elaborate synthesis of the purine ring, it is not surprising that the purine bases are generally not degraded but about 90% of them are recycled for nucleotide synthesis (**recycling of purine bases**, 2).
- **Pyrimidine biosynthesis** (3) differs from purine synthesis. Initially, the precursor of the six-membered ring is formed from only two components (carbamyl phosphate and aspartate). The carbohydrate component is linked to the ring only shortly before the end of the pathway.
- To synthesize the nucleoside di- and triphosphates, further phosphate residues are added to the mononucleotides by ATP-dependent *kinases* (**phosphorylation**, 4 and 5).
- **Replication** (6) and **transcription** (7) of DNA are extremely complex processes involving dozens of proteins. They are described in detail from page 238 onward.
- An important reaction in the synthesis of DNA components is **ribonucleotide reduction** (8). This takes place at the diphosphate stage and converts ribose and deoxyribose residues. *Ribonucleotide reductase* employs a radical mechanism and is tightly regulated.
- **Purine degradation** (9) ends with uric acid in humans and some groups of animals, which is a compound with an intact purine ring that can precipitate in the body because of its poor solubility. In other organisms, degradation results in products that are more soluble in water.
- **Pyrimidine degradation** (10) yields end products that can enter intermediary metabolism without any problem.

A. Nucleotide metabolism: overview



No.	Name	S.	Reactants → products	Catalyzed by	Regulated by
1	Purine biosynthesis	183	PRPP → IMP → AMP, GMP	Several enzymes	Nucleotide 1 (P.103)
2	Purine base recycling	181	Base + PRPP → Nucleoside monophosphate + PP	APRT, HGPRT	–
3	Pyrimidine biosynthesis	183	Asp, HCO ₃ ⁻ , [NH ₂] → UMP	Several enzymes	CMP1, CTP1, UTP1, PRPPA
4	Phosphorylation	185	NMP + ATP → NDP + ADP	Nucleoside monophosphate kinase	–
5	Phosphorylation	185	NDP + ATP → NTP + ADP	Nucleoside diphosphate kinase	–
6	Replication	239	dsDNA → 2 dsDNA	DNA polymerases	–
7	Transcription	241	dsDNA → hnRNA, rRNA, tRNA	RNA polymerase	Transcription factors
8	Ribonucleotide reduction	185	NDP + NADPH → dNDP + NADP	Ribonucleotide reductase	ATPA, dATP1
9	Purine degradation	181	AMP, GMP → Rib-P + NH ₄ ⁺ + uric acid	Several enzymes	–
10	Pyrimidine degradation	181	CMP → UMP → uracil → β-alanine dTMP → thymine → β-aminoisobutyrate	Several enzymes	–

APRT: adenine phosphoribosyltransferase; HGPRT: hypoxanthine-guanine phosphoribosyltransferase;
NMP: nucleoside monophosphate; NDP: nucleoside diphosphate; dNDP: deoxynucleoside diphosphate; Rib-P: ribose-5-phosphate

Nucleotide Degradation

The principles underlying the degradation of purines and pyrimidines differ. In the human organism, purines are degraded into uric acid and excreted in this form. The purine ring remains intact in this process. In contrast, the ring of the pyrimidine bases (uracil, thymine, and cytosine) is broken down into small fragments, which can be returned to the metabolism or excreted.

A. Degradation of nucleotides

Purines (left)

The purine nucleotide **guanosine monophosphate (GMP)** is degraded by hydrolytic dephosphorylation to **guanosine (G)** and then to the free base **guanine (Gua)** by cleavage of ribose 5-phosphate. In the next reaction, guanine is converted by deamination into the purine base, **xanthine**.

In the most important degradative pathway for **adenosine monophosphate (AMP)**, it is the nucleotide that is deaminated by **adenosine deaminase [1]**, producing **inosine monophosphate (IMP)**. In the same way as in GMP, the purine base **hypoxanthine** is released from IMP. A single enzyme, the molybdenum-containing **xanthine dehydrogenase [2]**, then converts both hypoxanthine into xanthine and xanthine into **uric acid**. An oxo group is introduced into the substrate in each of these reaction steps. The oxo group is derived from water, with NADH acting as a reducing agent. Xanthine dehydrogenase was formerly regarded as an oxidase ("xanthine oxidase"), which uses molecular oxygen O₂ as a cosubstrate and releases hydrogen peroxide (H₂O₂). This was due to artifacts that occur during the isolation of xanthine dehydrogenase from tissues, which in the test tube confer the properties of an oxidase on the enzyme because of oxidative and proteolytic changes.

Almost all mammals continue purine degradation by converting uric acid to the more soluble **allantoin**, with further opening of the ring by means of **uricase**; the allantoin is then excreted. However, the primates, including humans, are not capable of synthesizing allantoin. **Uric acid** is therefore the form of the purines excreted in these species. The same applies to birds and many reptiles (uricotelic animals; p. 164). Other animals degrade allantoin further to allantoinic acid or urea and glyoxylate. The poor water solubility of uric acid can lead to problems in humans when the uric acid

level is pathologically elevated (hyperuricemia; p. 186).

Pyrimidines (right)

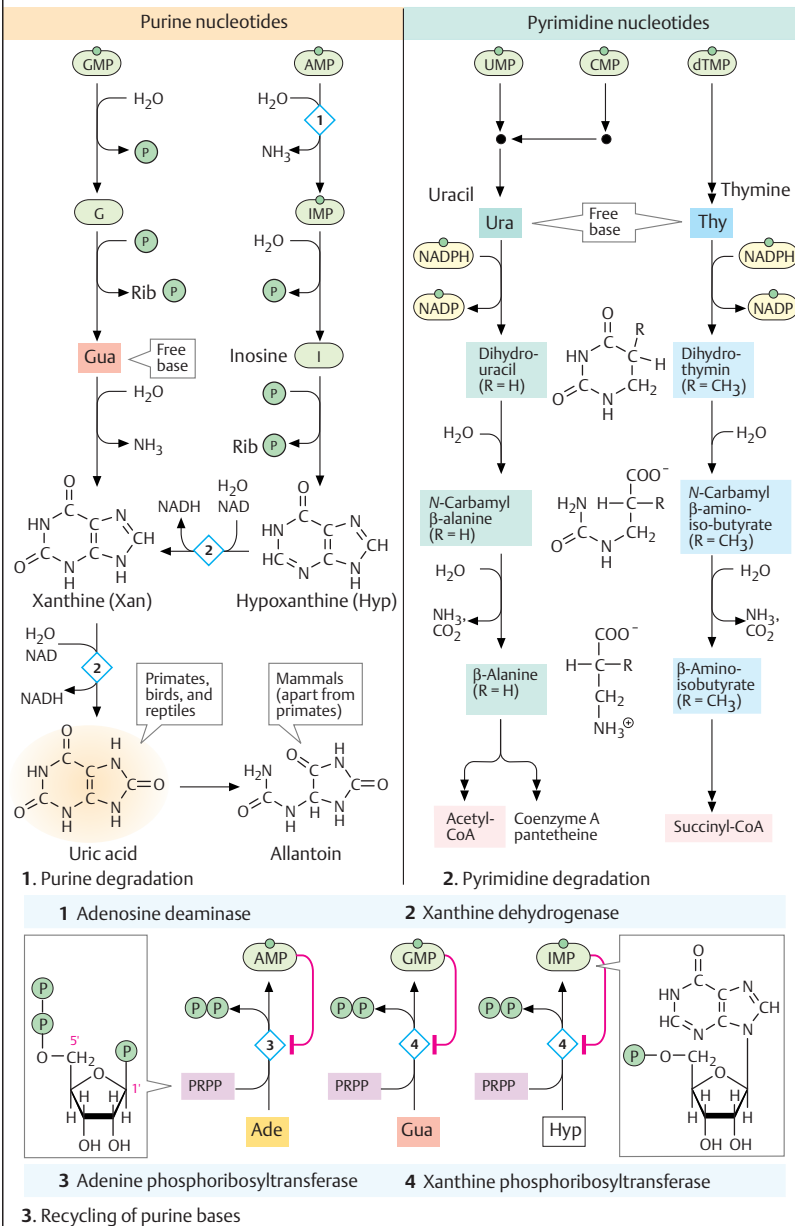
In the degradation of pyrimidine nucleotides (2), the free bases **uracil (Ura)** and **thymine (Thy)** are initially released as important intermediates. Both are further metabolized in similar ways. The pyrimidine ring is first reduced and then hydrolytically cleaved. In the next step, **β -alanine** arises by cleavage of CO₂ and NH₃ as the degradation product of uracil. This can be broken down further to yield acetate, CO₂, and NH₃. From **β -aminoisobutyrate**, the degradation product of thymine, succinyl-CoA, is ultimately produced (via methylmalonyl-CoA, p. 170, B) which can then enter the TCA cycle.

Recycling reactions (bottom)

The nucleotides are among the most complex of all metabolites. Nucleotide biosynthesis is elaborate and requires a high energy input (p. 182). It is therefore advantageous for cells if nucleobases are not completely degraded, but instead recycled for nucleotide biosynthesis. This is particularly true of the purine bases adenine and guanine. In the animal organism, some 90% of these bases are converted back into nucleoside monophosphates by linkage with phosphoribosyl diphosphate (PRPP). The proportion of pyrimidine bases that are recycled is much smaller. These recycling reactions are also called salvage pathways.

The most important enzymes in the recycling of purine bases are **adenine phosphoribosyltransferase [3]**, which is specific for adenine, and **hypoxanthine-guanine phosphoribosyltransferase (HGPRT, [4])**, which converts hypoxanthine to IMP and guanine to GMP. Inborn deficiencies of these two enzymes can cause severe hyperuricemia (p. 186).

A. Degradation of nucleotides



Purine and Pyrimidine Biosynthesis

The bases occurring in nucleic acids are derivatives of the aromatic heterocyclic compounds *purine* and *pyrimidine* (p. 64). The biosynthesis of these molecules is lengthy, but is vital for almost all cells.

A. Building blocks of nucleobases

The **pyrimidine ring** (left) is assembled from three components: the nitrogen atom N-1 and carbon atoms C-4 to C-6 are derived from *aspartate*, carbon C-2 comes from HCO_3^- , and the second nitrogen, N-3, is taken from the amide group of *glutamine*.

The synthesis of the **purine ring** is more complex. The only major component is *glycine*, which donates C-4 and C-5, as well as N-7. All of the other atoms in the ring are incorporated individually. C-6 comes from HCO_3^- . Amide groups from *glutamine* provide the atoms N-3 and N-9. The amino group donor for the inclusion of N-1 is *aspartate*, which is converted into fumarate in the process, in the same way as in the urea cycle (p. 172). Finally, the carbon atoms C-2 and C-8 are derived from formyl groups in *N¹⁰-formyl-tetrahydrofolate* (THF; p. 184).

B. Pyrimidine and purine synthesis

The key intermediates in the biosynthesis of nucleic acid components are the mononucleotides *uridine monophosphate* (UMP) in the pyrimidine series and *inosine monophosphate* (IMP, base: hypoxanthine) in the purines. The synthetic pathways for pyrimidines and purines are fundamentally different. For the pyrimidines, the pyrimidine ring is first assembled and then linked to ribose 5'-phosphate to form a nucleotide. By contrast, synthesis of the purines starts directly from ribose 5'-phosphate. The ring is then built-up step by step on this carrier molecule.

The precursors for the synthesis of the **pyrimidine ring** are *carbamyl phosphate*, which arises from glutamate and HCO_3^- (**1a**) and the amino acid *aspartate*. These two components are linked to *N*-carbamyl aspartate (**1b**) and then converted into *dihydroorotate* by closure of the ring (**1c**). In mammals, steps **1a** to **1c** take place in the cytoplasm, and are catalyzed by a single multifunctional enzyme. In the next step (**1d**), *dihydroorotate* is oxidized to *orotate* by an FMN-dependent dehydrogenase. *Orotate* is then linked with *phosphoribosyl diphosphate*

(PRPP) to form the nucleotide *orotidine 5'-monophosphate* (OMP). Finally, decarboxylation yields **uridine 5'-monophosphate** (UMP). The last two steps are catalyzed by the same enzyme, bifunctional *UMP synthase*.

Purine biosynthesis starts with PRPP (the names of the intermediates are given in the table below). Formation of the ring starts with transfer of an amino group, from which the later N-9 is derived (**2a**). Glycine and a formyl group from *N¹⁰-formyl-THF* then supply the remaining atoms of the five-membered ring (**2b**, **2c**). Before the five-membered ring is closed (in step **2f**), atoms N-3 and C-6 of the later six-membered ring are attached (**2d**, **2e**). Synthesis of the ring then continues with N-1 and C-2 (**2g**, **2i**). In the final step (**2j**), the six-membered ring is also closed, and **inosine 5'-monophosphate** (IMP) arises.

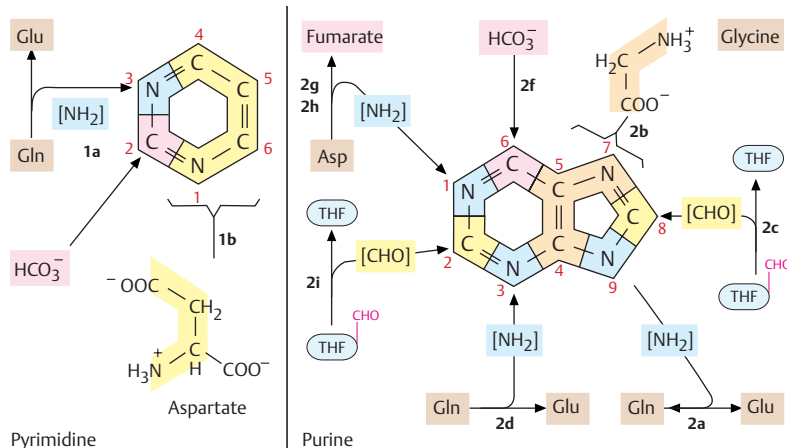
Reaction	Product
2a	Phosphoribosylamine
2b	Glycinamide ribonucleotide
2c	Formylglycinamide ribonucleotide
2d	Formylglycinamide ribonucleotide
2e, f	4-Carboxy-5-aminoimidazole ribonucleotide
2g, h	5-Aminoimidazole-4-carboxamide ribonucleotide
2i	5-Formamidoimidazole-4-carboxamide ribonucleotide
2j	Inosine monophosphate

■ Further information

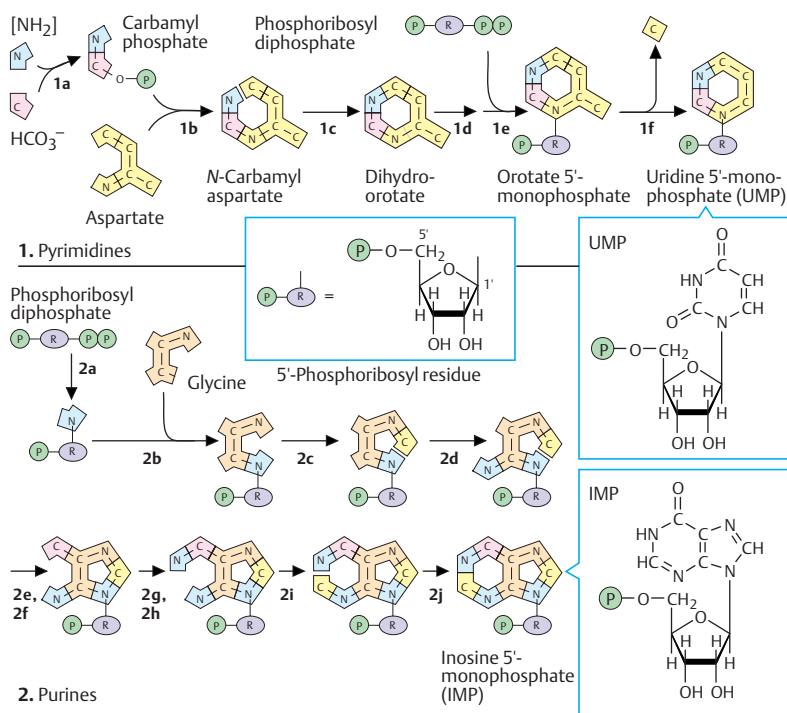
The regulation of bacterial *aspartate carbamyl-transferase* (ACTase) by ATP and CTP has been particularly well studied, and is discussed on page 80. In animals, it is not ACTase but *carbamylphosphate synthase* that is the key enzyme in pyrimidine synthesis. It is activated by ATP and PRPP and inhibited by UTP.

The biosynthesis of the purines is also regulated by *feedback inhibition*. ADP and GDP inhibit the formation of PRPP from ribose 5'-phosphate. Similarly, step **2a** is inhibited by AMP and GMP (p. 102).

A. Building blocks of nucleobases



B. Pyrimidine and purine synthesis



Nucleotide Biosynthesis

De-novo synthesis of purines and pyrimidines yields the monophosphates IMP and UMP, respectively (p. 182). All other nucleotides and deoxynucleotides are synthesized from these precursors. Nucleotide synthesis by recycling of bases is discussed on page 180.

A. Nucleotide synthesis: overview

The synthesis of **purine nucleotides** (1) starts from inosine monophosphate (**IMP**). The base it contains, *hypoxanthine*, is converted in two steps each into adenine or guanine. The nucleoside monophosphates **AMP** and **GMP** that are formed are then phosphorylated by *nucleoside phosphate kinases* [4] to yield the diphosphates **ADP** and **GDP**, and these are finally phosphorylated into the triphosphates **ATP** and **GTP** [5]. The nucleoside triphosphates serve as components for RNA, or function as coenzymes (p. 88). Conversion of the ribonucleotides into deoxyribonucleotides occurs at the level of the *diphosphates* and is catalyzed by *ribonucleotide reductase* (see [1] in C).

The biosynthetic pathways for the **pyrimidine nucleotides** (2) are more complicated. The first product, **UMP**, is phosphorylated first to the diphosphate and then to the triphosphate, **UTP**. *CTP synthase* then converts UTP into **CTP** [2]. Since pyrimidine nucleotides are also reduced to deoxyribonucleotides at the diphosphate level, CTP first has to be hydrolyzed by a *phosphatase* to yield **CDP** before **dCDP** and **dCTP** can be produced.

The DNA component deoxythymidine triphosphate (**dTTP**) is synthesized from UDP in several steps. The base thymine is formed by methylation of **dUMP** at the nucleoside monophosphate level. *Thymidylate synthase* [3] and its helper enzyme *dihydrofolate reductase* (see [8] in B) are important target enzymes for cytostatic drugs (p. 454).

B. C₁ metabolism

Derivatives of the coenzyme **tetrahydrofolate** (THF; p. 90) function by transferring C₁ groups in nucleotide metabolism and many other reactions. So two carbon atoms of the purine ring come from *N¹⁰-formyl-THF*. In the methylation of dUMP to dTMP by *thymidylate synthase* [7], the methyl group originates from *N⁵,N¹⁰-methylene-THF*. The most important reaction for regenerating this coenzyme is catalyzed by *serine hydroxymethyltransferase* [6],

which converts serine to glycine. *Remethylation* of homocysteine to methionine by *methionine synthase* ([10]; p. 176) also depends on C₁ metabolism. The methyl group is transferred from *N⁵-methyl-THF* via *methylcobalamin* (p. 90) to homocysteine.

C. Ribonucleotide reductase

The DNA component 2'-deoxyribose is not synthesized as a free sugar, but arises at the diphosphate level by reduction of ribonucleoside diphosphates. This ribonucleoside reduction is a complex process in which several proteins are involved. The reducing equivalents needed come from **NADPH**. However, they are not transferred directly from the coenzyme to the substrate, but first pass through a *redox series* that has several steps.

In the first step, *thioredoxin reductase* [11] reduces a small redox protein, **thioredoxin**, via enzyme-bound FAD. This involves cleavage of a disulfide bond in thioredoxin. The resulting SH groups in turn reduce a catalytically active disulfide bond in *ribonucleotide reductase* [1]. The free SH groups formed in this way are the actual electron donors for the reduction of ribonucleotide diphosphates.

In addition, a **tyrosine radical** in the enzyme also participates in the reaction. It initially produces a substrate radical (1). This eliminates a water molecule and thereby becomes a radical cation (2). Finally, the deoxyribose residue is produced by reduction, and the tyrosine radical is regenerated (3).

The regulation of ribonucleotide reductase is complex. The substrate specificity and activity of the enzyme are controlled by two allosteric binding sites (a and b) in the R1 subunits. ATP and dATP increase or reduce the activity of the reductase by binding at site a. Other nucleotides interact with site b, and thereby alter the enzyme's specificity.

Pathobiochemistry

Disorders of nucleotide metabolism can affect the biosynthesis and degradation of the nucleotides (**A**). Diseases associated with a raised uric acid level are particularly common. The causes and treatment of hyperuricemia are discussed separately in section **B**.

A. Pathobiochemistry of nucleotide metabolism

Orotic aciduria is the most common hereditary disorder of anabolic pyrimidine metabolism. It is due to defects of *UMP synthase*, a bifunctional enzyme that catalyzes the last two steps in pyrimidine biosynthesis in animal metabolism (p. 183, steps **1e** and **1f**). The disorder is characterized by impaired growth, anemia, and excessive urinary excretion of orotic acid. It is treated with injections of uridine or cytidine to compensate for the absence of pyrimidine synthesis.

Mutations in the *adenosine deaminase* (ADA) gene (p. 180) can cause an immune deficiency known as **SCID** (severe combined immunodeficiency). ADA deaminates not only adenosine, but also *deoxyadenosine* derived from DNA degradation. If ADA is lacking, the accumulated *deoxyadenosine* is phosphorylated via dAMP to dATP, which inhibits the biosynthesis of the deoxynucleotides. Loss of lymphocytes and therefore immune weakness occur; many children succumb to this if they are not protected from pathogens by a sterile environment. SCID is so far one of the few diseases for which gene therapy (p. 262) has been successful.

Xanthinuria is due to deficiencies of *xanthine dehydrogenase* (p. 180). Large amounts of xanthine appear in the blood and urine as a result, and this can lead to the formation of kidney and urinary tract stones.

B. Hyperuricemia

The fact that purine degradation in humans stops at the uric acid stage (p. 180) can lead to problems, since uric acid and its salts, the urates, are *poorly soluble in water*. When large amounts of uric acid are formed or uric acid excretion is disturbed, the concentrations of uric acid can increase so much in the blood (*hyperuricemia*) that uric acid crystals are deposited in the body.

The acid character of uric acid is not apparent from its conventional formula (**1**). Like all purines, uric acid occurs in two **tautomeric forms**, the *lactam form* (top) and the *lactim form* (center, *tautomers* are isomers that differ only in the position of H atoms and double bonds). The OH group at C-8 of the lactim form is relatively acidic with a pK_a of 5.8 and after dissociation it can form salts with metal ions like Na^+ ; these are **urates**. Deposition of urate crystals in the joints is the cause of very painful attacks of acute **gout**, which often start in the joint at the base of the big toe (**3**). Chronic inflammation of affected joints and development of gouty nodules (tophi) under the skin occur later.

Hyperuricemia has numerous causes (**2**). Most cases are due to disturbed *uric acid excretion* via the kidneys, which is hereditary and can be worsened by alcohol or other toxic substances. Excessive production of uric acid is also a common cause. This can be due to a high-purine diet (liver, fish, meat broth) or to excessive purine degradation, which occurs during cancer treatment, for example ("*tumor lysis syndrome*"). Type I glycogenosis (p. 142) can also cause hyperuricemia. The rare hereditary *Lesch-Nyhan syndrome* is usually due to a deficiency of *hypoxanthine phosphoribosyltransferase* (HGPRT, p. 180). The impaired recycling of the purine bases leads in this case to hyperuricemia accompanied by severe neurological disorders with self-destructive behavior.

Apart from dietary measures, hyperuricemia can be treated with *allopurinol*, a competitive inhibitor of *xanthine dehydrogenase*. This substrate analogue differs from the substrate hypoxanthine only in the arrangement of the atoms in the 5-ring. *Colchicine*, a drug obtained from meadow saffron, prevents leukocytes from taking up urate crystals (p. 199) and alleviates chronic arthritis in this way (**4**).

A. Pathobiochemistry of nucleotide metabolism

Disease	Cause	Symptoms	Therapy
Orotic aciduria	OPRT/ODC deficiency ^{a)}	Orotate in the urine, growth disorders, anemia	Administration of uridine and cytidine
Hyperuricemia			
1. Gout	Incorrect diet, enzyme deficiencies, renal disease	Accumulation of urate crystals, chronic arthritis	Diet, allopurinol, anti-inflammatory drugs
2. Lesch-Nyhan syndrome	HGPRT deficiency ^{b)}	Severe hyperuricemia, neurological disorders, autoaggression	Allopurinol, supportive measures
3. Tumor lysis syndrome	Major nucleic acid degradation after chemotherapy	Extreme hyperuricemia	Administration of urate oxidase
SCID ^{c)}	Adenosine deaminase (ADA) deficiency	Immunodeficiency due to lymphocyte loss	ADA replacement
Xanthinuria	Xanthine dehydrogenase deficiency	Xanthinemia, kidney stones	–

a) OPRT: orotate phosphoribosyltransferase; ODC: orotate decarboxylase

b) HGPRT: hypoxanthine-guanine phosphoribosyltransferase c) SCID: severe combined immunodeficiency disease

B. Hyperuricemia

1. Forms of uric acid

Keto form (Lactam)

Enol form (Lactim)

Sodium urate

2. Causes of hyperuricemia

1 Disturbed uric acid excretion

2 Increased uric acid production

a) purine-rich diet

b) disturbed purine base recycling

c) tumor lysis syndrome, Gierke disease

3. Gout in the big toe

4. Drugs for gout chemotherapy

Allopurinol

Colchicine

Heme Biosynthesis

Heme, an iron-containing tetrapyrrole pigment, is a component of O₂-binding proteins (p. 286) and a coenzyme of various oxidoreductases (p. 86). Heme biosynthesis is possible in all cells but occurs predominantly in the bone marrow (around 85%), with a much smaller percentage being formed in the liver. Both mitochondria and cytoplasm are involved in heme synthesis.

A. Biosynthesis of heme

Synthesis of the tetrapyrrole ring starts in the mitochondrion.

- [1] **Succinyl-CoA** (upper left), an intermediate in the TCA cycle, undergoes condensation with **glycine** and subsequent decarboxylation to yield **5-aminolevulinate (ALA)**. The *ALA synthase* responsible for this step is the key enzyme of the whole pathway. Synthesis of ALA synthase is *repressed* and existing enzyme is inhibited by heme, the end product of the pathway. This is a typical example of *end-product* or *feedback inhibition*.
- [2] 5-Aminolevulinate now leaves the mitochondria. In the cytoplasm, two molecules condense to form **porphobilinogen**, a compound that already contains the pyrrole ring. *Porphobilinogen synthase* is inhibited by lead ions. This is why acute lead poisoning is associated with increased concentrations of ALA in the blood and urine.
- [3] The tetrapyrrole structure characteristic of the porphyrins is produced in the next steps of the synthetic pathway. *Hydroxymethylbilane synthase* catalyzes the linkage of four porphobilinogen molecules and cleavage of an NH₂ group to yield **uroporphyrinogen III**.
- [4] Formation of this intermediate step requires a second enzyme, *uroporphyrinogen-III synthase*. If this enzyme is lacking, the “wrong” isomer, uroporphyrinogen I, is formed.

The tetrapyrrole structure of uroporphyrinogen III is still very different from that of heme. For example, the central iron atom is missing, and the ring contains only 8 of the 11 double bonds. In addition, the ring system only carries charged R side chains (four acetate and four propionate residues). As heme groups have to act in the nonpolar interior of proteins, most

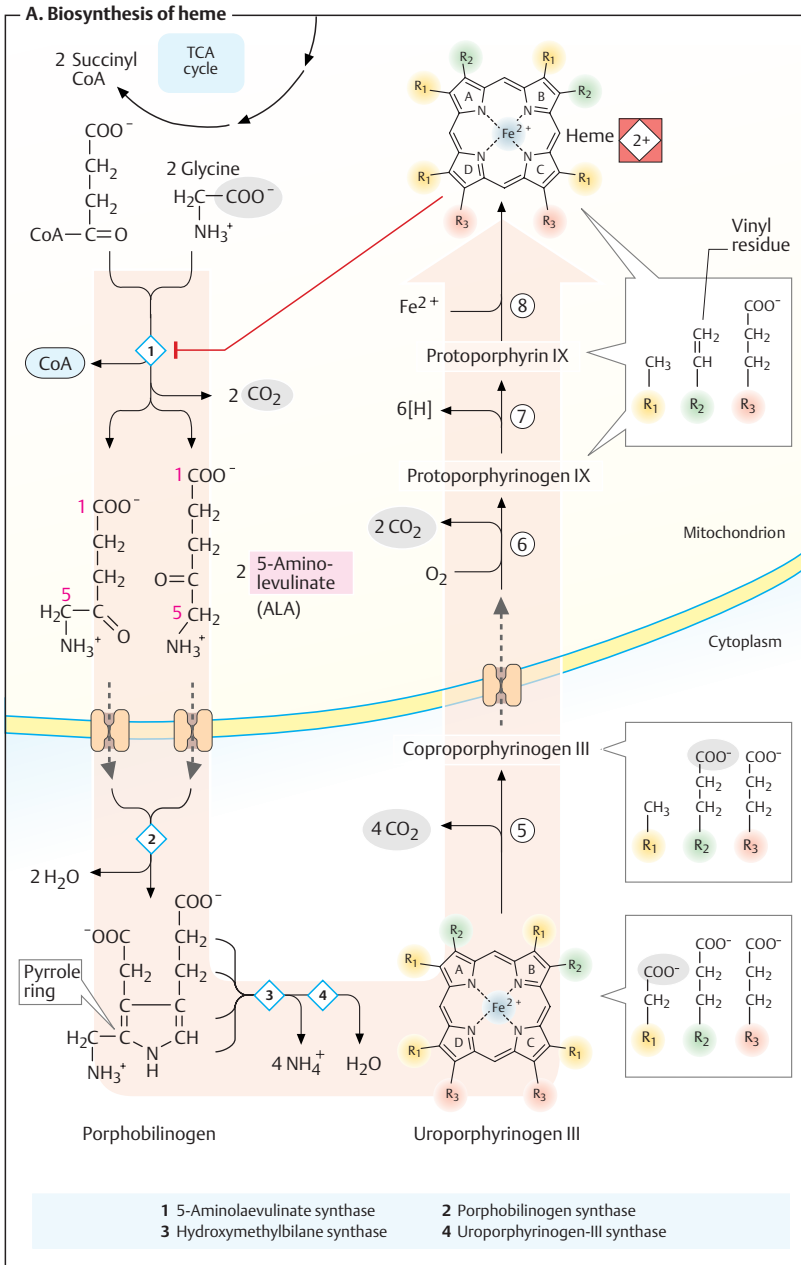
of the polar side chains have to be converted into less polar groups.

- [5] Initially, the four acetate residues (R₁) are decarboxylated into methyl groups. The resulting **coproporphyrinogen III** returns to the mitochondria again. The subsequent steps are catalyzed by enzymes located either on or inside the *inner mitochondrial membrane*.
- [6] Two of the propionate groups (R₂) are first converted by an oxidase into vinyl residues. The formation of **protoporphyrinogen IX** completes the modification of the side chains.
- [7] In the next step, another oxidation produces the conjugated π -electron system of **protoporphyrin IX**.
- [8] Finally, a divalent iron is incorporated into the ring. This step also requires a specific enzyme, *ferrochelatase*. The **heme** or **Feprotoporphyrin IX** formed in this way is found in hemoglobin and myoglobin, for example (p. 344), where it is noncovalently bound, and also in various oxidoreductases (p. 226).

■ Further information

There are a large number of hereditary or acquired disturbances of porphyrin synthesis, known as **porphyrias**, some of which can cause severe clinical disorders. Several of these diseases lead to the excretion of heme precursors in feces or urine, giving them a dark red color. Accumulation of porphyrins in the skin can also occur, and exposure to light then causes disfiguring, poorly healing blisters. Neurological disturbances are also common in the porphyrias.

It is possible that the medieval legends about human vampires (“Dracula”) originated in the behavior of porphyria sufferers (avoidance of light, behavioral disturbances, and drinking of blood in order to obtain heme—which markedly improves some forms of porphyria).



Heme Degradation

A. Degradation of heme groups

In the human body, **heme** groups (p. 86) are mainly found as a prosthetic group in erythrocyte hemoglobin. Around 100–200 million aged erythrocytes per hour are broken down in the human body. The degradation process starts in the mononuclear phagocyte system (MPS) in the spleen, liver, and bone marrow.

- [1] After the protein part (globin) has been removed, the tetrapyrrole ring of heme is oxidatively cleaved between rings A and B by **heme oxygenase**. This reaction requires molecular oxygen and NADPH, and produces green **biliverdin**, as well as CO (carbon monoxide) and Fe²⁺. The iron remains available for further use (p. 388).
- [2] In another redox reaction, biliverdin is reduced by **biliverdin reductase** to the orange-colored **bilirubin**.

The color of heme and other **porphyrin systems** results from their numerous conjugated double bonds. Heme contains a cyclic conjugation (see formula; highlighted in pink) that is removed by reaction [1]. Reaction [2] breaks the π system down into two smaller separate systems (see bilirubin formula; highlighted in yellow). The gradual color change from purple to green to yellow during heme degradation can be easily observed in a bruise or hematoma beneath the skin.

For further degradation, bilirubin is transported to the liver via the blood. As bilirubin is poorly soluble, it is bound to **albumin** for transport. Some drugs that also bind to albumin can lead to an increase in free bilirubin.

- [3] The hepatocytes take up bilirubin from the blood and conjugate it in the endoplasmic reticulum with the help of **UDP-glucuronic acid** into the more easily soluble **bilirubin monoglucuronides** and **diglucuronides**. To do this, **UDP-glucuronyl-transferase** forms ester-type bonds between the OH group at C-1 of glucuronic acid and the carboxyl groups in bilirubin (cf. p. 316). The glucuronides are then excreted by active transport into the **bile**, where they form what are known as the **bile pigments**.

Glucuronide synthesis is the rate-determining step in hepatic bilirubin metabolism. Drugs such as **phenobarbital**, for example, can induce both conjugate formation and the transport process.

Some of the bilirubin conjugates are broken down further in the intestine by bacterial

β -glucuronidases. The bilirubin released is then reduced further via intermediate steps into colorless **stercobilinogen**, some of which is oxidized again into orange- to yellow-colored stercobilin. The end products of bile pigment metabolism in the intestine are mostly excreted in feces, but a small proportion is reabsorbed (**enterohepatic circulation**). When high levels of heme degradation are taking place, stercobilinogen appears as **urobilinogen** in the urine, where oxidative processes darken it to form **urobilin**.

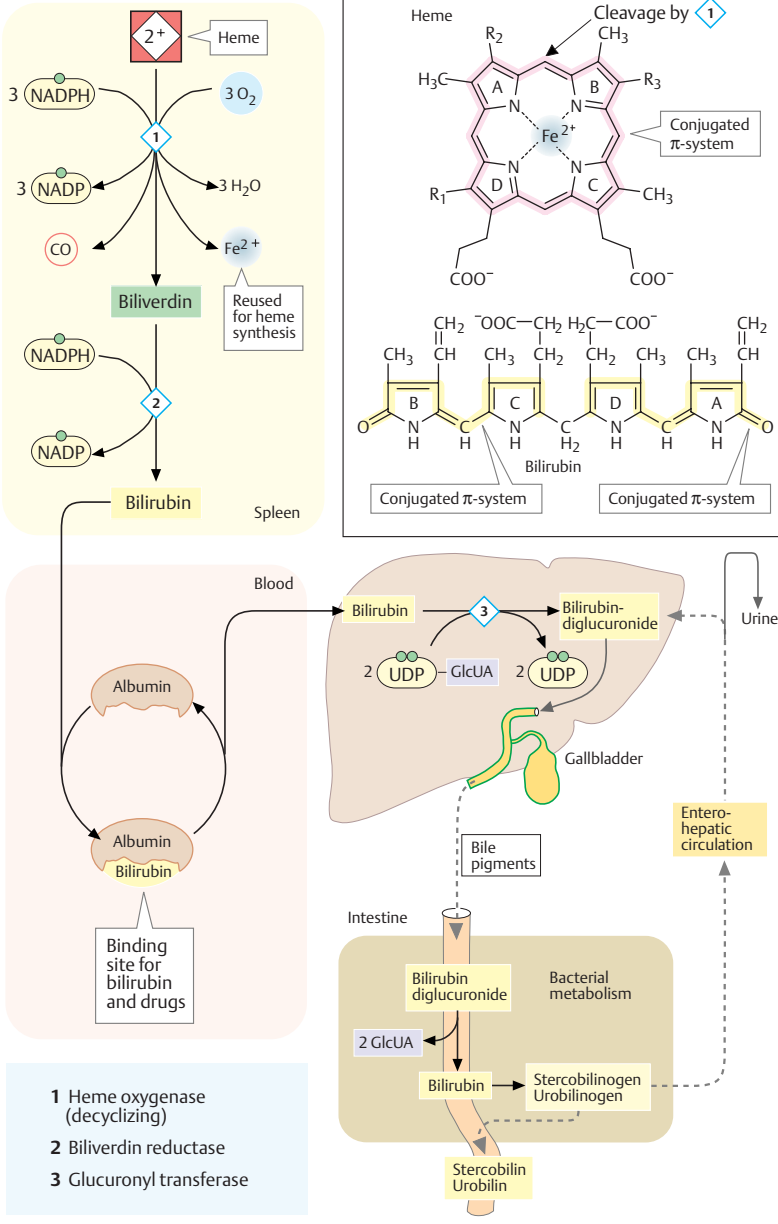
In addition to hemoglobin, other **heme proteins** (myoglobin, cytochromes, catalases, and peroxidases) also supply heme groups that are degraded via the same pathway. However, these contribute only about 10–15% to a total of ca. 250 mg of bile pigment formed per day.

■ Further information

Hyperbilirubinemias. An elevated bilirubin level ($> 10 \text{ mg} \cdot \text{L}^{-1}$) is known as **hyperbilirubinemia**. When this is present, bilirubin diffuses from the blood into peripheral tissue and gives it a yellow color (**jaundice**). The easiest way of observing this is in the white conjunctiva of the eyes.

Jaundice can have various causes. If increased erythrocyte degradation (hemolysis) produces more bilirubin, it causes **hemolytic jaundice**. If bilirubin conjugation in the liver is impaired—e.g., due to hepatitis or liver cirrhosis—it leads to **hepatocellular jaundice**, which is associated with an increase in unconjugated (“**indirect**”) bilirubin in the blood. By contrast, if there is a disturbance of bile drainage (**obstructive jaundice**, due to gallstones or pancreatic tumors), then conjugated (“**direct**”) bilirubin in the blood increases. **Neonatal jaundice** (physiologic jaundice) usually resolves after a few days by itself. In severe cases, however, unconjugated bilirubin can cross the blood–brain barrier and lead to brain damage (**kernicterus**).

A. Degradation of heme groups



Cell Organelles

Structure of Cells

The cell is the basic unit of organisms.

A. Comparison of prokaryotes and eukaryotes

Present-day living organisms can be divided into two large groups—the prokaryotes and eukaryotes. The **prokaryotes** are represented by bacteria (*eubacteria* and *archaea*). These are almost all small unicellular organisms only a few micrometers (1 micrometer = 10^{-6} m) in size. The **eukaryotes** include *fungi*, *plants*, and *animals* and comprise both unicellular and multicellular organisms. Multicellular eukaryotes are made up of a wide variety of cell types that are specialized for different tasks. The cells of eukaryotes are much larger than those of prokaryotes (volume ratio ~2000:1). The most important distinguishing feature of these cells compared to those of prokaryotes is that they have a **nucleus**.

Compared to the prokaryotes, eukaryotic cells have greater specialization and complexity in their structure and functioning. Eukaryotic cells are structured into *compartments* (see below). The metabolism and synthesis of macromolecules are distributed through these reaction spaces and are separately regulated. In prokaryotes, by contrast, these functions are organized in a simpler fashion and are spatially closely related.

Although the storage and transfer of genetic information function according to the same principles in the prokaryotes and eukaryotes, there are also differences. Eukaryotic DNA consists of very long, linear molecules with a total of 10^7 to more than 10^{10} base pairs (bp), only a small fraction of which are used for genetic information. In eukaryotes, the genes (20000–50000 per genome) are usually interrupted by noncoding regions (*introns*). Eukaryotic DNA is located in the nucleus, where together with histones and other proteins it forms the chromatin (p. 234).

In prokaryotes, DNA is ring-shaped, much shorter (up to 6×10^6 bp), and located in the cytoplasm. Almost all of it is used for information storage, and it does not contain any introns.

B. Structure of an animal cell

In the human body, there are at least 200 different cell types. The illustration outlines the basic structures of an animal cell in an extremely simplified way. The details given regarding the proportion of the compartments relative to cell volume (highlighted in yellow) and their numbers per cell (blue) refer to mammalian hepatocytes (liver cells).

The eukaryotic cell is subdivided by membranes. On the outside, it is enclosed by a **plasma membrane**. Inside the cell, there is a large space containing numerous components in solution—the **cytoplasm**. Additional membranes divide the internal space into *compartments* (confined reaction spaces). Membrane-enclosed reaction spaces in the cell with special functions are known as **organelles**.

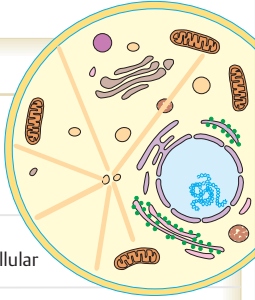
The largest organelle is the **nucleus** (p. 204). It is easily recognized using the light microscope. The **endoplasmic reticulum** (ER), a closed network of shallow sacs and tubules (p. 216ff.), is linked with the outer membrane of the nucleus. Another membrane-bound organelle is the **Golgi apparatus** (p. 216ff.), which resembles a bundle of layered slices. The **endosomes** and **exosomes** are bubble-shaped compartments (vesicles) that are involved in the exchange of substances between the cell and its surroundings (p. 214). Probably the most important organelles in the cell's metabolism are the **mitochondria**, which are around the same size as bacteria (p. 206). The **lysosomes** (p. 224) and **peroxisomes** (p. 226) are small, globular organelles that carry out specific tasks. The whole cell is traversed by a framework of proteins known as the **cytoskeleton** (p. 198ff.).

In addition to these organelles, plant cells also have plastids—e.g., **chloroplasts** in which photosynthesis takes place. In the interior of plant cells, there is a large, fluid-filled **vacuole**. Like bacteria and fungi, plant cells have a rigid **cell wall** consisting of polysaccharides and proteins.

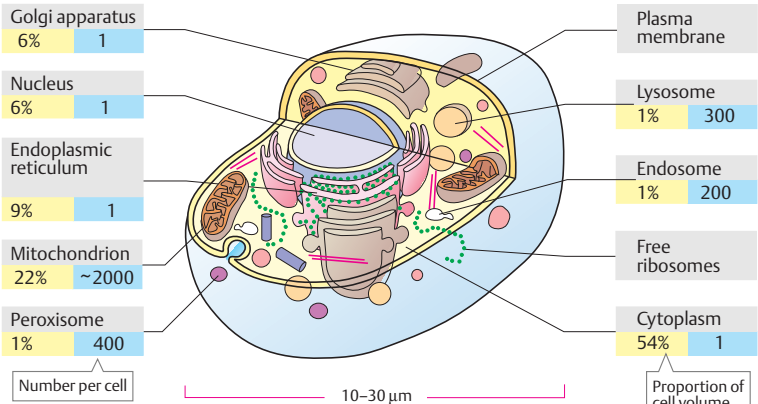
The cytoplasm is the part of the cell between the plasma membrane and nucleus. When the plasma membrane is removed by gentle treatment with *detergents* or *shear forces*, the organelles can be obtained by stepwise centrifugation. The remaining clear solution is called the **cytosol**.

A. Comparison of prokaryotes and eukaryotes

Prokaryotes	Eukaryotes
Organisms Eubacteria Archaeobacteria 1–10 μm	Organisms Fungi Plants Animals 10–100 μm
Form Single-celled	Form Single or multi cellular
Organelles, cytoskeleton, cell division apparatus Missing	Organelles, cytoskeleton, cell division apparatus Present, complicated, specialized
DNA Small, circular, no introns, plasmids	DNA Large, in nucleus, many introns
RNA: Synthesis and maturation Simple, in cytoplasm	RNA: Synthesis and maturation Complicated, in nucleus
Protein: Synthesis and maturation Simple, coupled with RNA synthesis	Protein: Synthesis and maturation Complicated, in the cytoplasm and the rough endoplasmic reticulum
Metabolism Anaerobic or aerobic very flexible	Metabolism Mostly aerobic, compartmented
Endocytosis and Exocytosis No	Endocytosis and Exocytosis Yes



B. Structure of an animal cell



Cell Components and Cytoplasm

The Gram-negative bacterium *Escherichia coli* (*E. coli*) is a symbiont, usually a harmless one, in the intestine of mammals. The structure and characteristics of this organism have been particularly well characterized. *E. coli* is also frequently used as a tool in genetic engineering (pp. 256ff.).

A. Components of a bacterial cell

A single *E. coli* cell has a **volume** of about $0.88 \mu\text{m}^3$. One-sixth of this consists of membranes and one-sixth is DNA (known as the “nucleoid”). The rest of the internal space of the cell is known as **cytoplasm** (not “cytosol”; p. 194).

The main component of *E. coli*—as in all cells—is **water**, which accounts for 70% by weight. The other components are **macromolecules** (proteins, nucleic acids, polysaccharides), **small organic molecules**, and **inorganic ions**. The majority of the macromolecules are proteins, which represent ca. 55% of the dry mass of the cell. When several assumptions are made about the distribution and size (average mass 40 kDa) of proteins, it can be estimated that there are about 250 000 protein molecules in the cytoplasm of an *E. coli* cell. In eukaryotic cells, which are about a thousand times larger, it is estimated that the number of protein molecules is in the order of several billion.

B. View inside a bacterial cell

The illustration shows a schematic view inside the **cytoplasm** of *E. coli*, magnified approximately 1 million times. At this magnification, a single carbon atom would be the size of a grain of salt, and an ATP molecule would be as large as a grain of rice. The section shown corresponds to a cube with sides 100 nm long, corresponding to about 1/600th the volume of a cell of *E. coli*. To make the macromolecules clearer, small molecules such as water, cofactors, and metabolites have all been omitted from the illustration. The section of the cytoplasm shown contains:

- Several hundred **macromolecules**, which are needed for protein biosynthesis—i.e., 30 ribosomes, more than 100 protein factors, 30 aminoacyl-tRNA synthases, 340 tRNA molecules, 2–3 mRNAs (each of which is 10 times the length of the section shown), and 6 molecules of RNA polymerase.

- About 330 other enzyme molecules, including 130 glycolytic enzymes and 100 enzymes from the tricarboxylic acid cycle.
- 30 000 **small organic molecules** with masses of 100–1000 Da—e.g., metabolites of the intermediary metabolism and coenzymes. These are shown at a magnification 10 times higher in the bottom right corner.
- And finally, 50 000 **inorganic ions**. The rest consists of water.

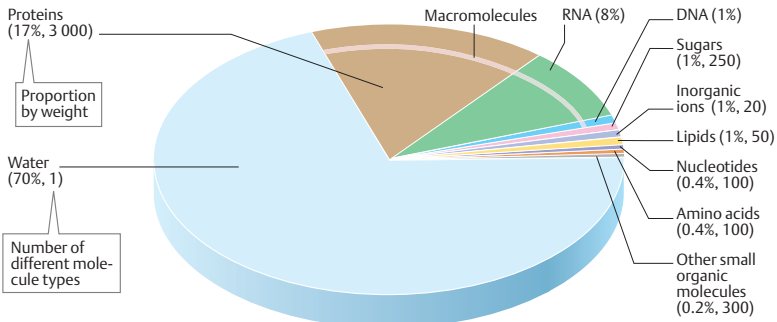
The illustration shows that the cytoplasm of cells is a compartment densely packed with macromolecules and smaller organic molecules. Because of the high concentration of macromolecules, it has a gel-like consistency. The distances between organic molecules are small. They are only separated by a few water molecules.

All of the molecules are in motion. Due to constant collisions, however, they do not advance in a straight path but move in zigzags. Due to their large mass, proteins are particularly slow. However, they do cover an average of 5 nm in 1 millisecond—a distance approximately equal to their own length. Statistically, a protein is capable of reaching any point in a bacterial cell in less than a second.

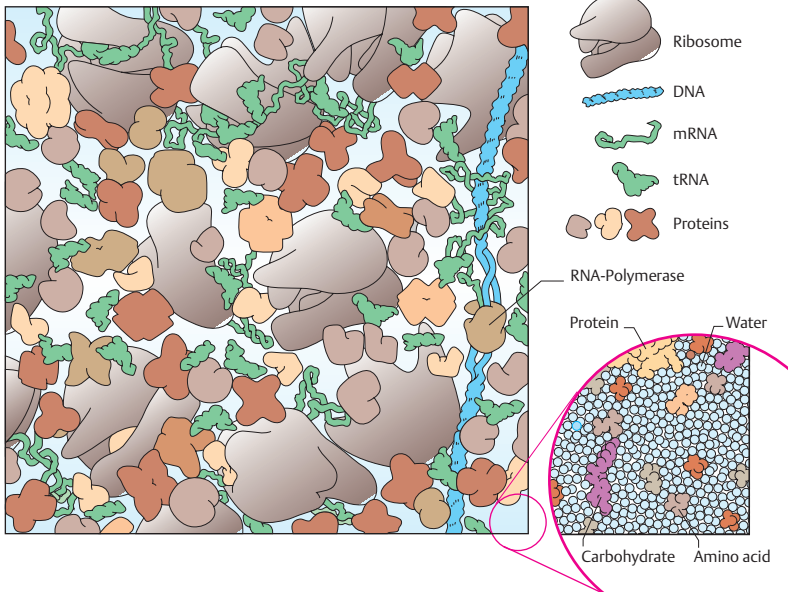
C. Important functions of the cytoplasm

In eukaryotes, the cytoplasm, representing slightly more than 50% of the cell volume, is the most important cellular compartment. It is the *central reaction space of the cell*. This is where many important pathways of the intermediary metabolism take place, often in interaction with other compartments—e.g., **glycolysis**, the **pentose phosphate pathway**, the majority of **gluconeogenesis**, **glycogen metabolism**, and **fatty acid biosynthesis**. Protein biosynthesis (**translation**; p. 248ff.) also takes place in the cytoplasm. By contrast, catabolic fatty acid degradation pathways, the tricarboxylic acid cycle, and oxidative phosphorylation are located in the mitochondria (p. 206). This spatial separation avoids futile cycles between synthetic and degradation pathways, and separate regulation is possible.

A. Components of a bacterial cell



B. View inside a bacterial cell



C. Important functions of the cytoplasm

Reaction pathway	Function
Translation	Biosynthesis of proteins on ribosomes
Glycolysis	Degradation of glucose to pyruvate and lactate
Gluconeogenesis	Glucose synthesis
Fatty acid biosynthesis	Synthesis of palmitate from acetyl-CoA
Pentose phosphate pathway	Production of NADPH, interconversion of hexoses and pentoses
Glycogen metabolism	Storage of glucose as glycogen

Components

The cytoplasm of eukaryotic cells is traversed by a three-dimensional scaffolding structure consisting of filaments (fibers). This **cytoskeleton** serves to maintain the shape of the cell, fix the position of the organelles, transport molecules and aggregates in the cell, and move the cell.

The filaments are divided into three groups, based on their *diameters*: **thin microfilaments** (5–8 nm), **intermediate filaments** (10–12 nm; IF), and **thick microtubules** (~25 nm). All of these filaments are polymers assembled from typical protein components. They are associated with other proteins.

A. Actin

Actin, the most abundant protein in eukaryotic cells, is the protein component of the **microfilaments** (actin filaments). Actin occurs as the monomolecular form, globular **G-actin**, and as a polymer, filamentous **F-actin**. G-actin is a relatively small, asymmetrical protein (42 kDa). It can aggregate reversibly to form F-actin, a helical homopolymer. This process is assisted by ATP as G-actin carries an ATP molecule that is slowly hydrolyzed in F-actin to form ADP. This ATPase activity gives actin enzymatic properties.

As individual G-actin molecules are always oriented in the same direction relative to one another, F-actin consequently has *polarity* also. It has two ends, at which polymerization takes place at different rates. If the ends are not stabilized by special proteins (as in muscle cells), then at a critical concentration of G-actin the **(+) end** of F-actin will constantly grow, while the **(-) end** simultaneously decays (known as *treadmilling*).

These partial processes can be blocked by fungal toxins experimentally. **Phalloidin**, a toxin contained in the *Amanita phalloides* mushroom, inhibits decay by binding to the **(-) end**. By contrast, **cytochalasins**, mold toxins with cytostatic effects, block polymerization by binding to the **(+) end**.

Actin-associated proteins. The cytoplasm contains more than 50 different proteins that bind specifically to G-actin and F-actin. Their interaction with actin has various functions. It can serve to regulate the G-actin pool (e.g., **profilin**), influence the polymerization rate of G-actin (e.g., **villin**), stabilize the chain ends of F-actin (**fragin**, **β -actinin**), attach filaments to one another or to other cell components (**villin**,

α -actinin, **spectrin**), or disrupt the helical structure of F-actin (**gelsolin**). These proteins thus control actin's various functions and are themselves regulated by protein kinases (p. 410).

B. Intermediate filaments

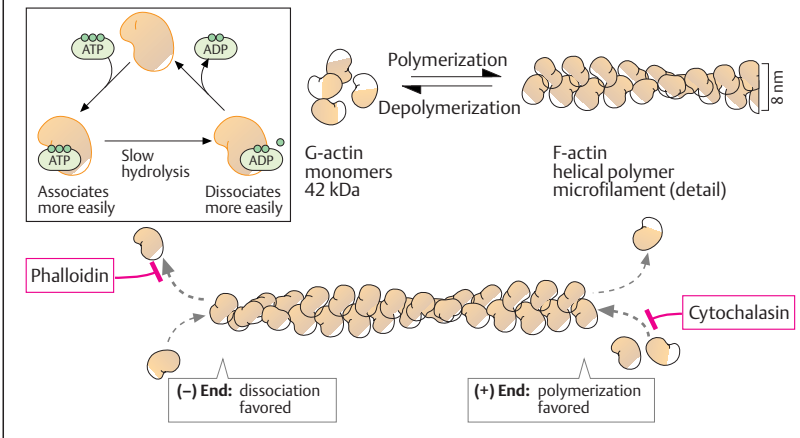
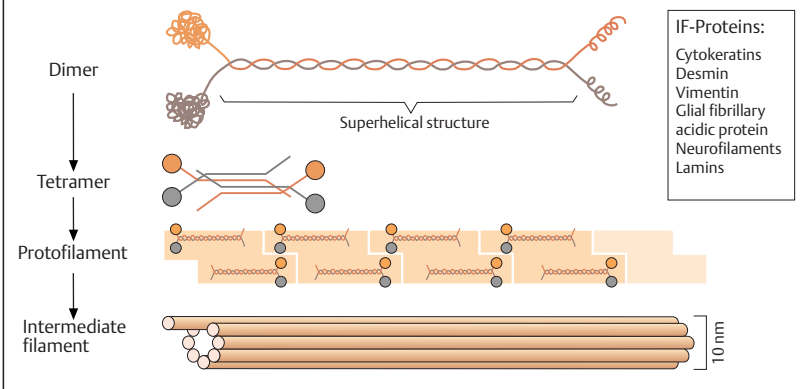
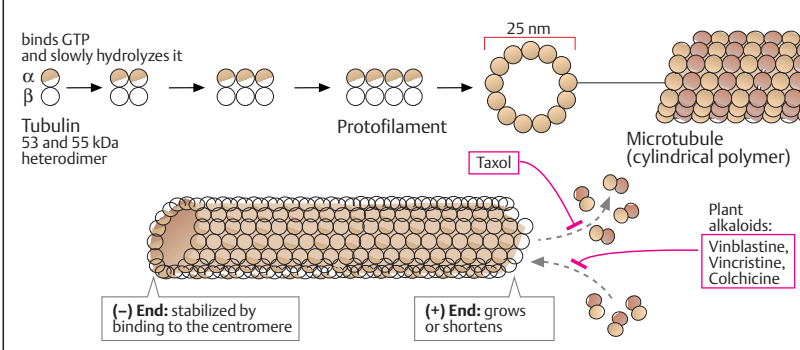
The proteins of the intermediate filaments (IFs) belong to five related protein families with tissue-specific expression. Typical representatives include the **cytokeratins**, **desmin**, **vimentin**, **glial fibrillary acidic protein** (GFAP), **neurofilament**, and **nuclear laminins**. These IF proteins have a rod-shaped basic structure in the center, which is known as a *superhelix* ("coiled coil"; p. 58). The dimers are arranged in an antiparallel fashion to form tetramers and **protofilaments** are produced by a staggered head-to-head arrangement. Eight protofilaments form an IF.

Protein monomers of IFs rarely occur in the cytoplasm, in contrast to those of microfilaments and microtubules. Their polymerization leads to stable filaments that have no polarity. IF formation is controlled by phosphorylation/dephosphorylation.

C. Tubulins

The basic components of the tube-shaped **microtubules** are **α -** and **β -tubulin**, two globular proteins (53 and 55 kDa). These combine into α,β -heterodimers, which polymerize to form linear protofilaments and a ring-shaped complex. This grows into a long tube as a result of further polymerization, each turn of which requires 13 tubulin dimers.

Like the microfilaments, microtubules are polar, dynamic structures with **(+)** and **(-)** ends. The **(-) end** is usually stabilized by bonding to the **centrosome**. The **(+) end** shows **dynamic instability**. It can either grow slowly or shorten rapidly. **GTP**, which is bound by the tubulins and hydrolyzed to GDP, plays a role in this. Various **microtubule-associated proteins** (MAPs) can interact with the microtubules, thus controlling their structure and function.

A. Actin**B. Intermediate filaments****C. Tubulins**

Structure and Functions

The cytoskeleton with its associated proteins (p. 198) carries out three major tasks:

- It represents the cell's **mechanical scaffolding**, which gives it its typical shape and connects membranes and organelles to each other. This scaffolding has dynamic properties; it is constantly being synthesized and broken down to meet the cell's requirements and changing conditions.
- It acts as the **motor for movement** of animal cells. Not only muscle cells (p. 340ff.), but also cells of noncontractile tissues contain different *motor proteins* (p. 202), which they use to achieve coordinated and directed movement. Cell movement, shape-changes during growth, cytoplasmic streaming, and cell division are all made possible by components of the cytoskeleton.
- It serves as a **transport track** within the cell. Organelles and other large protein complexes can move along the filaments with the help of the motor proteins (p. 202).

A. Microfilaments and intermediate filaments

The illustration schematically shows detail of the **microvilli** of an intestinal epithelial cell as an example of the structure and function of the components of the cytoskeleton.

Microfilaments of *F-actin* traverse the microvilli in ordered bundles. The microfilaments are attached to each other by actin-associated proteins (p. 198), particularly *fimbrin* and *villin*. *Calmodulin* and a myosinlike *ATPase* connect the microfilaments laterally to the plasma membrane. *Fodrin*, another microfilament-associated protein, anchors the actin fibers to each other at the base, as well as attaching them to the cytoplasmic membrane and to a network of **intermediate filaments**. In this example, the microfilaments have a mainly static function. In other cases, actin is also involved in dynamic processes. These include muscle contraction (p. 340ff.), cell movement, phagocytosis by immune cells, the formation of microspikes and lamellipodia (cellular extensions), and the acrosomal process during the fusion of sperm with the egg cell.

B. Microtubules

The **microtubules** of a cell are shown here. They radiate outward in all directions from a center near the nucleus, the **centrosome**. The

tube-shaped microtubules are constantly being synthesized and broken down at their (+) ends. In the centriole, the (–) end is blocked by **MAPs** (p. 198). The (+) end can also be stabilized by associated proteins—e.g., when the microtubules have reached the cytoplasmic membrane.

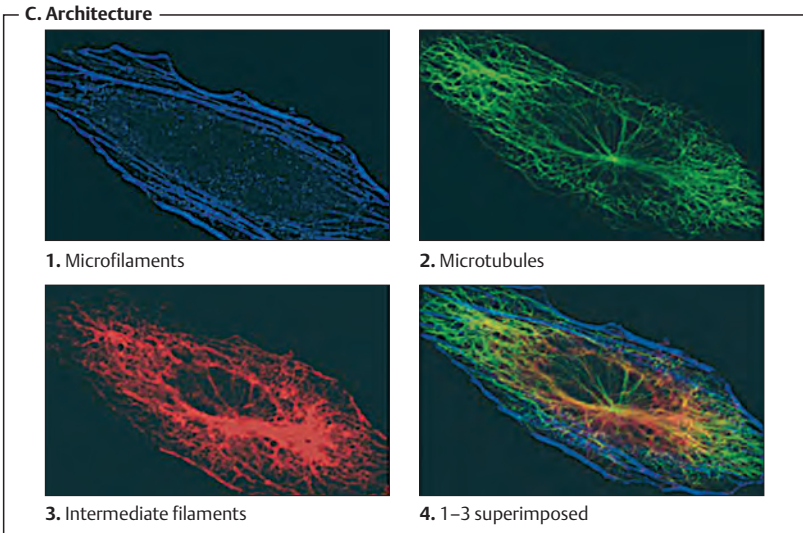
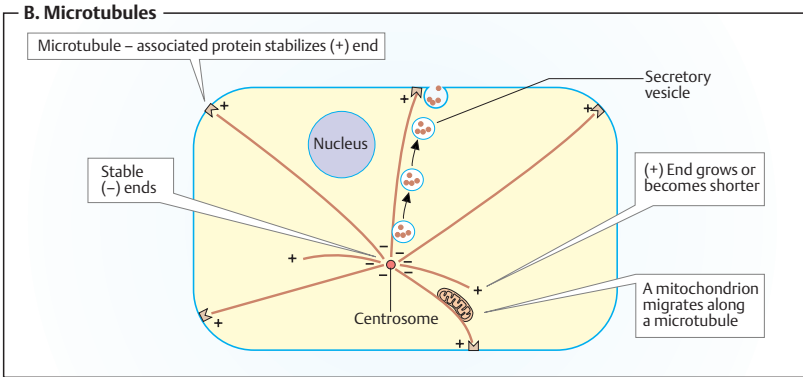
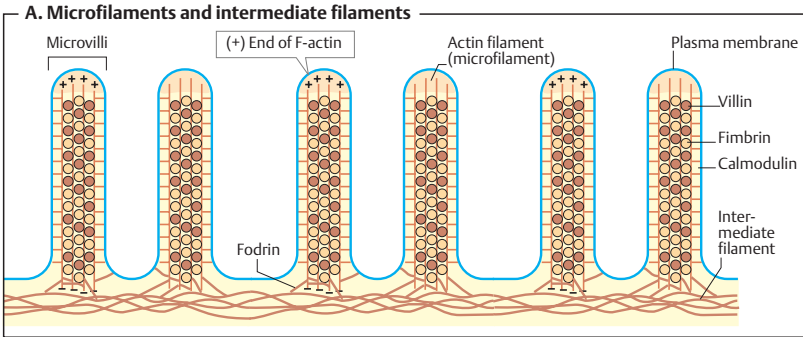
The microtubules are involved in defining the shape of the cell and also serve as guiding tracks for the transport of organelles. Together with the MAPs *dynein* and *kinesin*, microtubules are able to carry out mechanical work (p. 202)—e.g., during the transport of mitochondria, the movement of cilia (hairlike cell protrusions in the lungs, intestinal epithelium, and oviduct) and the beating of the flagella of sperm. Microtubules also play a special role in the mitotic period of cell division. In nerve cells, they provide a track system for axons in which **anterograde transport** to the synapses at the periphery and **retrograde transport** to the nucleus can take place.

C. Architecture

The complex structure and netlike density of the cytoskeleton is illustrated here using the example of a human hepatocyte in which the three typical cytoskeletal components are visualized with the help of fluorescent reagents.

1. **Microfilaments** consisting of F-actin fluoresce *blue*.
2. The **microtubules** formed of α - and β -tubulin fluoresce *green*.
3. The **intermediate filaments** fluoresce *red*.
4. When the images are superimposed, the different distribution of the cytoskeleton components can be seen especially clearly.

The majority of the microtubules extend from a central core ([–] end) in the vicinity of the nucleus (dark center) to the periphery ([+] end).



Motor Proteins

Molecular motors (“*nanomotors*”) are proteins that convert chemical energy to mechanical work. The hydrolysis energy of the chemical bonds of nucleoside triphosphates or the energy of electrochemical gradients is used to perform *mechanical work* in the form of *conformational changes* of the motor proteins.

Besides *rotary* proteins, as in **ATP synthase** (p. 122), molecular motors are known to include a whole range of enzyme systems that convert chemical energy into *linear work*, e.g., **ribosomal elongation factors** (p. 250) and enzymes that manipulate RNA and DNA, such as RNA and DNA polymerases (p. 236ff.). **Myosins**, **kinesins**, and **dyneins** are motor proteins that occur in the cytoskeleton (see below). Only these three protein families are called “*molecular motors*” in the narrow sense, because they are responsible for muscle contraction and ciliary movement, or to transport **cargo**. They use the energy released by the hydrolysis of ATP to move along filaments of the cytoskeleton by means of conformational changes.

These transport processes of the cytoskeleton's motor proteins play a part in *muscle contraction*, *cell division*, *intracellular transport of cargo*—i.e., of organelles or other cell components—for *endo-* and *exocytosis*, and other processes.

A. Myosins

The classical motor of the cytoskeleton is muscle fiber **myosin** (p. 340). Myosin polymers form thick **myosin filaments** which work jointly with thin **actin filaments** (microfilaments) to bring about *muscle contraction*.

There are over 40 other **unconventional myosins** which occur in nonmuscle cells. As *individual* motor proteins, they transport cargo along actin filaments. A **type V** myosin is shown here. It consists of two heavy and two light chains. Unconventional myosins are involved in cell motility, endocytosis, vesicle transport, and cytokinesis.

B. Kinesins

Kinesins have many representatives, which are divided into 14 classes. Their structure resembles that of the myosins. They consist of two heavy and two light chains. ATP can be bound in the globular head of the two heavy chains. The cargo to be transported is bound to the

other end of the kinesins by means of the light chains.

Kinesins work as isolated molecules. They transport their cargo—vesicles, organelles, parts of the mitotic apparatus, chromosomes, mRNA, proteins, and other cell components—along the **microtubules**, usually toward the (+) end.

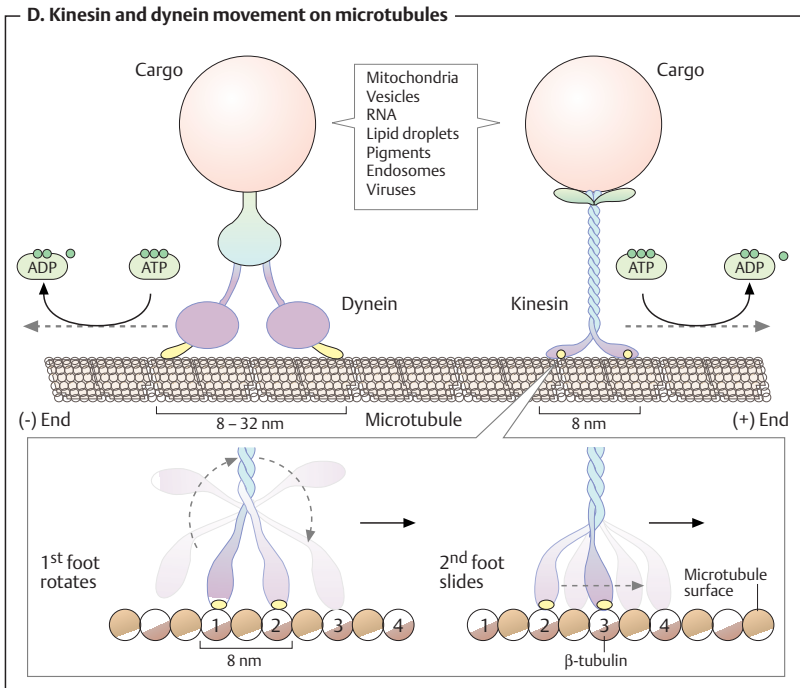
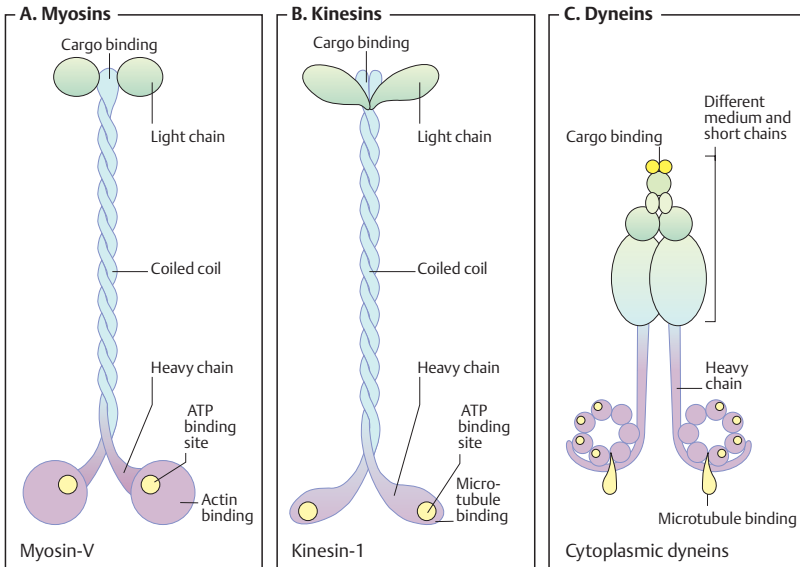
C. Dyneins

Two types of dyneins occur: 1) in the **axo-*nemes*** of cells that produce movement with the aid of flagella and cilia (e.g., pulmonary epithelium, spermatozoa), and 2) in the **cytoplasm**. Dyneins have a more complex structure than kinesins and myosins. They consist of about 12 polypeptides (total mass 1.5 MDa). Their two heavy chains contain a planar ring formed from seven modules. Binding and cleavage of ATP in this region leads to movement along the **microtubules**. The ATPase of dyneins contains a so-called **AAA motif** (ATPase associated with different cellular activities). The extent of dynein movement depends on the cargo and is controlled by **dynactin** as well as other proteins.

Dyneins are responsible for retrograde transport of cell components from the cell membrane toward the microtubule-organizing center (MTOC).

D. Kinesin and dynein movement on microtubules

The illustration shows how the two motor proteins **kinesin** and **dynein** transport cargo along a **microtubule**—kinesin toward the (+) end at the cell periphery, and dynein to the (–) end in the center. The movement is powered by ATP. Kinesins move along the microtubules in a *hand over hand mechanism* (bottom). One molecule of ATP is hydrolyzed to ADP for each 8-nm step. The length of the cytoplasmic dynein “steps” is between 8 nm and 32 nm depending on the cargo.



Nucleus

The nucleus is the largest organelle in the eukaryotic cell. With a diameter of 10–20 μm , it is easily recognizable with the light microscope. The nucleus is the location for *storage*, *replication*, and *expression* of genetic information.

Each cell usually has a nucleus. However, erythrocytes do not have one, while myocytes have a large number of nuclei.

The nucleus is separated from the cytoplasm by the **nuclear envelope**, which consists of the **outer** and **inner nuclear membranes**. The two nuclear membranes are separated from each other by the **perinuclear space**. The outer nuclear membrane is continuous with the rough endoplasmic reticulum and is covered with ribosomes. The inner side of the membrane is covered with a protein layer (the nuclear lamina; p. 352), in which the nuclear structures are anchored.

The nucleus is filled with **nucleoplasm**. It contains almost all of the cell's **DNA** (around 1% of which is mitochondrial DNA). Together with histones and structural proteins, the nuclear DNA forms the **chromatin** (p. 234). It is only during cell division that chromatin condenses into **chromosomes**. During this phase, the nuclear membrane also temporarily disintegrates.

During the phase between cell divisions, the *interphase*, it is possible to distinguish between the more densely packed **heterochromatin** and loose **euchromatin** using an electron microscope. Active *transcription* of DNA into mRNA takes place in the region of the euchromatin. A particularly electron-dense region is noticeable in many nuclei—the **nucleolus**. The DNA in the nucleolus contains numerous copies of the genes for rRNAs.

A. Functions of the nucleus

Almost all of the RNA in the cell is synthesized in the nucleus. In this process, known as **transcription**, the information stored in DNA is transcribed into RNA (p. 240). rRNA is produced mainly in the nucleolus, while mRNA and tRNA are formed in the region of the euchromatin. Enzymatic duplication of DNA—**replication**—also only takes place in the nucleus (p. 238).

The nucleotide components required for transcription and replication have to be imported into the nucleus from the cytoplasm. Incorporation of these components into RNA leads to

primary products, which are then altered by the addition of extra nucleotides, cleavage, and excision of introns (**RNA maturation**; p. 244). It is only when these processes have been completed that the RNA molecules formed in the nucleus can be exported into the cytoplasm for protein synthesis (**translation**; p. 248ff.).

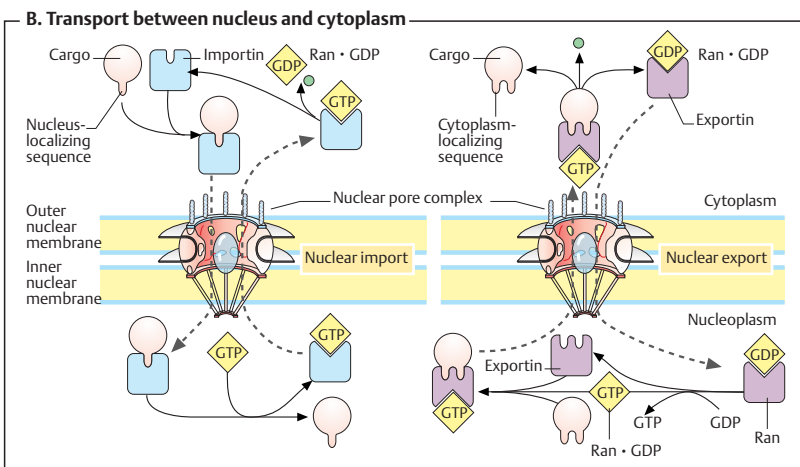
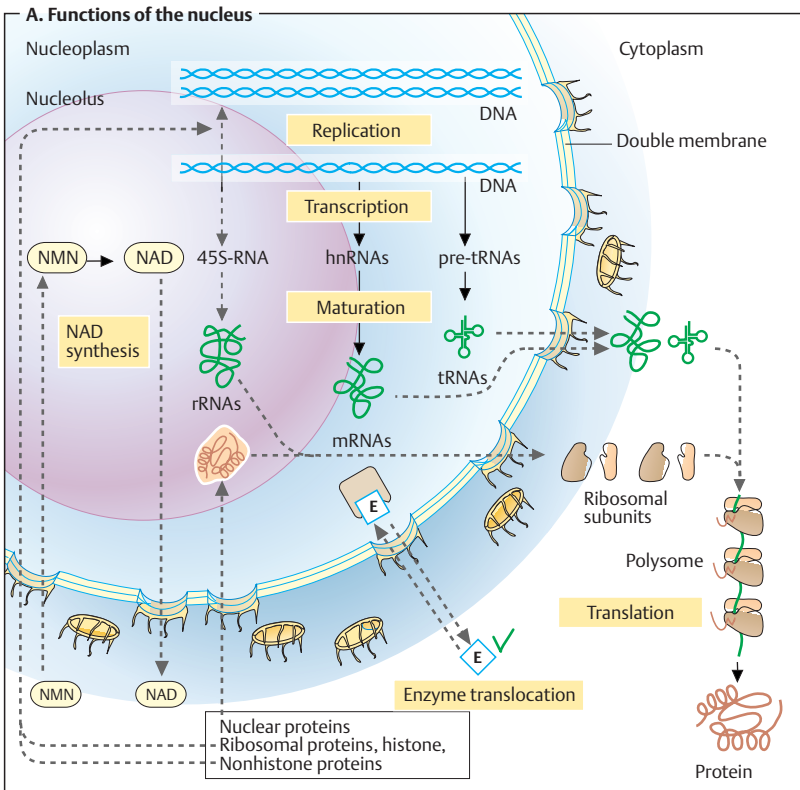
The nucleus is not capable of synthesizing proteins. All of the **nuclear proteins** therefore have to be imported—the *histones* with which DNA is associated in chromatin, and also the so-called *nonhistone proteins* (DNA polymerases and RNA polymerases, auxiliary and structural proteins, transcription factors, and ribosomal proteins). rRNA already associates with proteins in the nucleolus to form ribosome precursors.

Key enzymes in metabolism can be transferred reversibly from the cytoplasm into the nucleus (translocation, p. 140).

A special metabolic task performed by the nucleus is **biosynthesis of NAD⁺**. The immediate precursor, *nicotinamide mononucleotide* (NMN⁺), is produced in the cytoplasm and then transported into the nucleolus, where it is enzymatically converted into the dinucleotide NAD⁺. Finally, NAD⁺ then returns to the cytoplasm.

B. Transport between nucleus and cytoplasm

The exchange of substances between the nucleus and the cytoplasm is energy-dependent (consumption of GTP), selective, directed and regulated. It is mediated by about 1000 to 10000 **nuclear pore complexes** per nucleus, which are located in the nuclear membrane. The nuclear pores consist of numerous proteins (nucleoporins) that form several connected rings of varying diameter. Low-molecular weight structures and small proteins traverse the pores without difficulty. By contrast, larger proteins (> 40 kDa) can only pass through if they carry a **nuclear localization sequence** (p. 204, 218). In the cytoplasm, they are bound by **importins** and escorted through the pore complex. mRNAs, tRNAs and ribosome subunits formed in the nucleus cross the pores into the cytoplasm as complexes with **exportins**. The *G-protein Ran* is involved in loading and unloading the *importins* and *exportins* (p. 404).



Structure and Functions

Mitochondria are bacteria-sized organelles ($\sim 1 \times 2 \mu\text{m}$ in size), which are found in large numbers in almost all eukaryotic cells. Typically, there are about 2000 mitochondria per cell, representing around 25% of the cell volume. Mitochondria serve for energy production in the cells and perform a variety of other metabolic tasks.

A. Mitochondrial structure

Mitochondria are enclosed by two membranes—a smooth **outer** membrane and a markedly folded or tubular **inner mitochondrial membrane**, which has a large surface and encloses the **matrix space**. The folds of the inner membrane are known as **cristae**, and tube-like protrusions are called **tubules**. The **intermembrane space** is located between the inner and the outer membranes.

The number and shape of the mitochondria, as well as the numbers of cristae they have, can differ widely from cell type to cell type. Tissues with intensive oxidative metabolism—e.g., heart muscle—have mitochondria with particularly large numbers of cristae. Even within one type of tissue, the shape of the mitochondria can vary depending on their functional status.

Mitochondria probably developed during an early phase of evolution from aerobic bacteria that entered into symbiosis with primeval anaerobic eukaryotes. This **endosymbiont theory** is supported by many findings. For example, mitochondria have a ring-shaped **DNA** (four DNA molecules per mitochondrion) and have their own **ribosomes**. The mitochondrial genome became smaller and smaller during the course of evolution. In humans, it still contains 16 569 base pairs, which code for two rRNAs, 22 tRNAs, and 13 proteins (p. 126). Only these 13 proteins (mostly subunits of respiratory chain complexes) are produced in the mitochondrion. All of the other mitochondrial proteins are coded by the nuclear genome and have to be imported into the mitochondria after translation in the cytoplasm (p. 218). The fact that the mitochondrial envelope consists of two membranes also supports the endosymbiont theory. The inner membrane, derived from the former symbiont, has a structure reminiscent of prokaryotes. It is the only membrane to contain the unusual lipid **cardiolipin** (p. 40), but has hardly any cholesterol (p. 46).

Both mitochondrial membranes are very rich in proteins (p. 212). **Porins** (see p. 118) in the outer membrane allow small molecules ($< 10 \text{ kDa}$) to be exchanged between the cytoplasm and the intermembrane space. By contrast, the inner mitochondrial membrane is particularly impermeable to molecules (with the exception of O_2 , CO_2 , and H_2O). Numerous **transporters** in the inner membrane ensure the import and export of important metabolites (p. 118).

Mitochondria multiply only by duplicating their DNA and dividing the organelle. However, for this to happen, many proteins have to be imported from the cytosol.

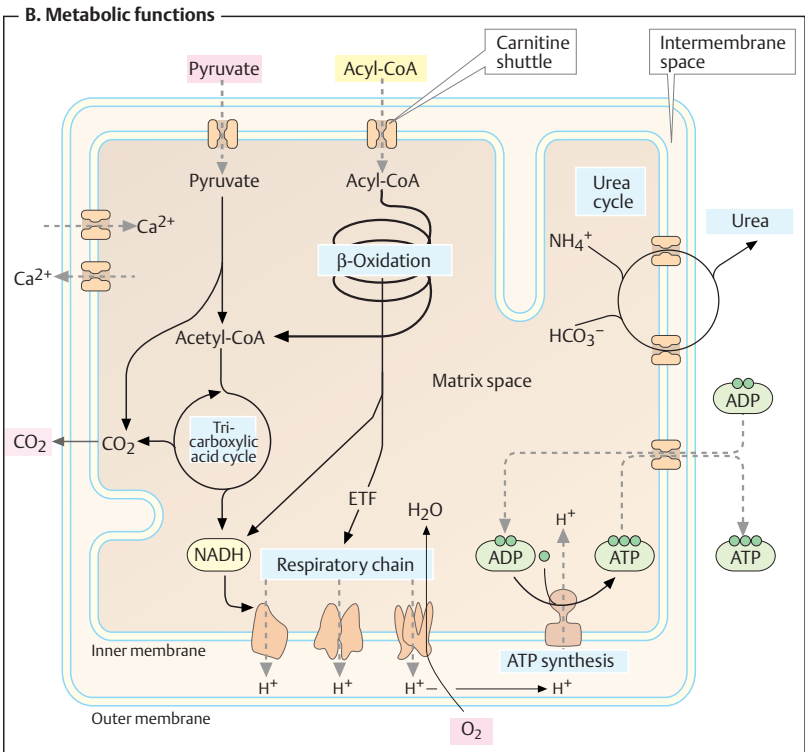
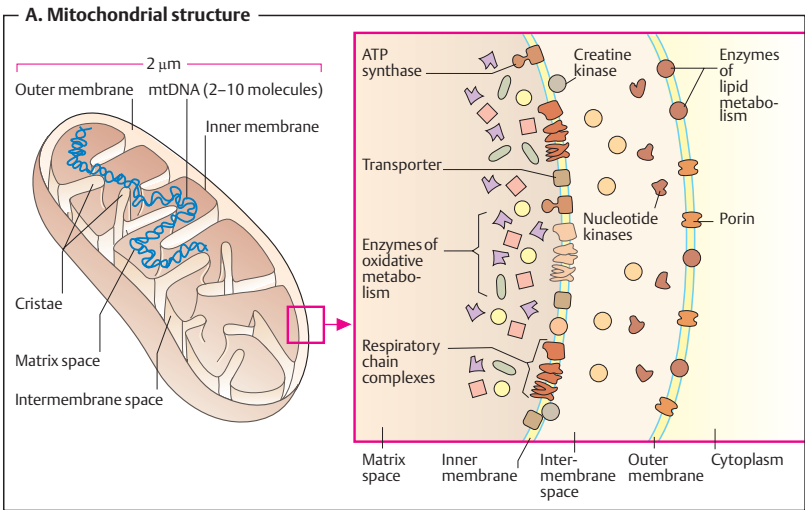
B. Metabolic functions

Mitochondria are also described as being the cell's *biochemical powerhouse*, since—through **oxidative phosphorylation** (p. 120)—they produce the majority of cellular ATP. **Pyruvate dehydrogenase** (PDH), the **tricarboxylic acid cycle**, β -oxidation of fatty acids, and parts of the **urea cycle** are located in the matrix. The **respiratory chain**, **ATP synthesis**, and enzymes involved in **heme biosynthesis** (p. 188) are associated with the inner membrane.

The inner membrane itself plays an important part in oxidative phosphorylation. As it is impermeable to protons, the respiratory chain—which pumps protons from the matrix into the intermembrane space via complexes I, III, and IV—establishes a **proton gradient** across the inner membrane. In it, the chemical energy released during NADH oxidation is conserved (p. 118). ATP synthase then uses the energy stored in the gradient to form ATP from ADP and inorganic phosphate. Several of the **transport systems** are also dependent on the H^+ gradient (p. 118).

The impermeable inner mitochondrial membrane ensures strict compartmentalization of coenzymes (e.g., NAD^+ , NADH, and CoA) and metabolites between the matrix space and the cytoplasmic space, i.e., there are separate *pools* for these metabolites.

In addition to the endoplasmic reticulum, the mitochondria also function as an intracellular **calcium reservoir**. They also play an important role in “programmed cell death”—**apoptosis** (p. 448).



Structure and Components

A. Structure of the plasma membrane

All biological membranes are constructed according to a standard pattern. They consist of a continuous **bilayer of amphipathic lipids** about 5 nm thick, into which **proteins** are embedded. In addition, some membranes also carry **carbohydrates** on their exterior, which are bound to lipids and proteins. The proportions of lipids, proteins, and carbohydrates differ markedly depending on the type of cell and membrane.

Membrane lipids are strongly *amphipathic molecules* with a polar, hydrophilic “head group” and a nonpolar, hydrophobic “tail.” In membranes, they are held together by the hydrophobic effect (p. 24) and weak van der Waals forces. They are therefore mobile relative to each other. This gives membranes a more or less fluid quality.

This **fluidity** of membranes primarily depends on their lipid composition and on temperature. Membrane fluidity is *higher* the greater the proportion of unsaturated lipids present. The **cholesterol** content also influences membrane fluidity. While cholesterol increases the fluidity of semicrystalline, closely-packed membranes, it stabilizes fluid membranes that contain a high proportion of unsaturated lipids.

Like lipids, proteins are also mobile within the membrane. If they are not fixed in place by special mechanisms, they float within the lipid layer as if in a two-dimensional liquid; biological membranes are therefore also described as being a “fluid mosaic.”

Lipids and proteins can shift easily *within* one layer of a membrane, but switching between the two layers (“*flip/flop*”) is not possible for proteins and is only possible with difficulty for lipids (with the exception of cholesterol). To move to the other side, they require special auxiliary proteins (translocators, “flipases”).

B. Membrane lipids

The illustration shows a model of a small section of a membrane. The **phospholipids** are the most important group of membrane lipids. They include *phosphatidylcholine* (Ptd-choline, lecithin), *Ptd-ethanolamine*, *Ptd-serine*, *Ptd-inositol*, and *sphingomyelin* (for their structures, see p. 40f.). In addition, membranes in animal cells also contain **cholesterol** (with the exception of inner mitochondrial membranes).

Glycolipids such as the cerebrosides and gangliosides, together with the *glycoproteins*, form the exterior coating of the cell (the *glycocalyx*).

Membrane lipids are distributed asymmetrically. More Ptd-choline and sphingomyelin are found in the outer layer. By contrast, more Ptd-serine and Ptd-ethanolamine occur in the inner layer. Ptd-inositol occurs only in the inside and the glycolipids only on the outside.

C. Functions of membranes

Enclosure and insulation of cells and organelles

(1) by membranes provides physical and chemical protection from their environment. An intact plasma membrane is essential for maintaining differences in the concentration of many substances between the intra- and extracellular compartments.

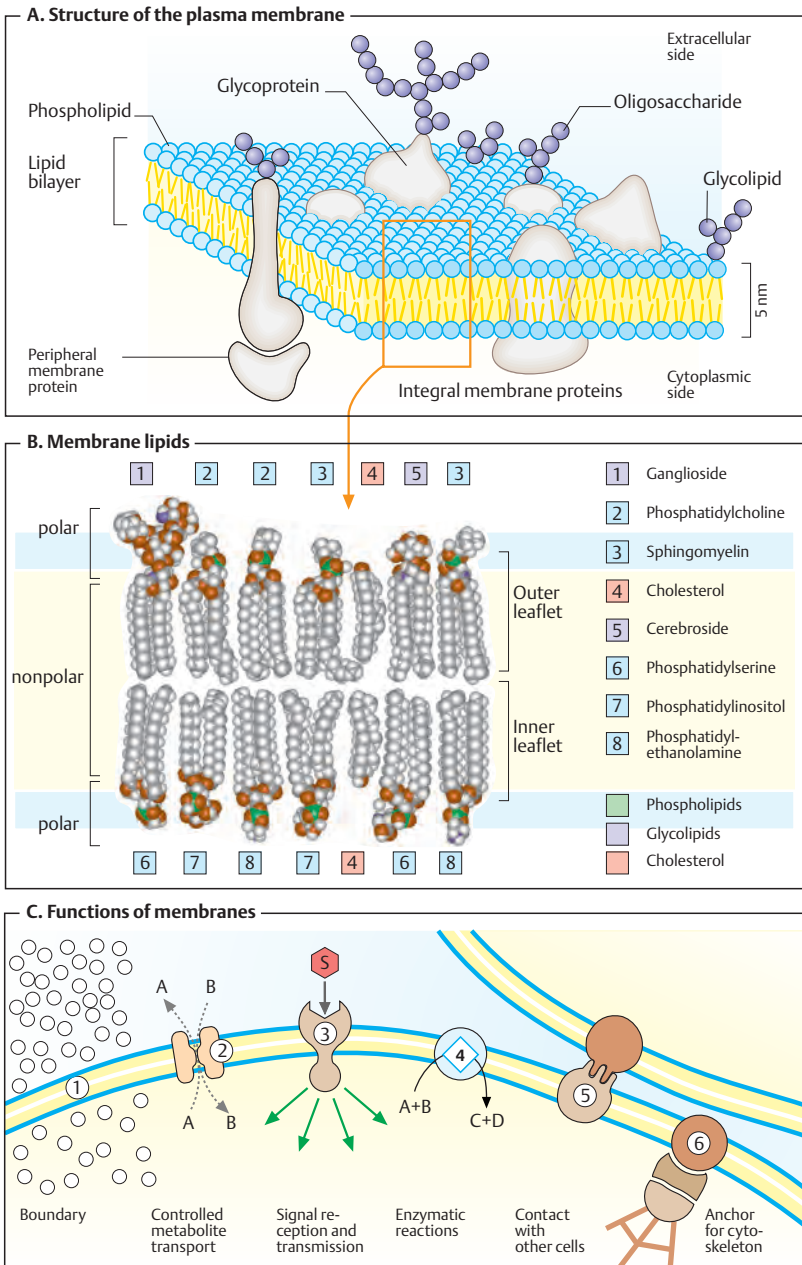
Regulated transport of substances (2), which determines the *internal milieu* and is a precondition for *homeostasis*, i.e., keeping the concentrations of substances and physiological parameters constant. Selective transport of substances through pores, channels and transporters (p. 212) is necessary because the cells and organelles are enclosed by membrane systems.

Reception of extracellular signals (3) and transfer of these signals to the interior of the cell (p. 398ff.), as well as the production of signals.

Enzymatic catalysis [4]. Important enzymes are localized at membranes, i.e., at the interface between the lipid and aqueous phases. This is where reactions with nonpolar substrates take place. Examples include *lipid biosynthesis* (p. 154) and the *metabolism of nonpolar xenobiotics* (p. 322). *Oxidative phosphorylation* (p. 110) and *photosynthesis*, the most important reactions in energy conversion, also take place at membranes.

Interaction with other cells (5) for the purpose of fusion and formation of tissues as well as communication with the extracellular matrix (p. 214).

Anchoring the cytoskeleton (6) (p. 198) to maintain the shape of cells and organelles and to provide the basis for movement processes.



Transport Processes

Membranes contain proteins as well as lipids. These **membrane proteins** (MPs) fulfill different functions; they act as *transporters*, *channels*, *receptors*, *enzymes*, or *structural proteins* and are either a proper component of the membrane (*integral MP*) or else are loosely associated with them (*peripheral MP*).

A. Types of membrane protein

Integral membrane proteins cross right through the lipid bilayer. The sections of the peptide chain that lie within the bilayer usually consist of 20 to 25 mainly hydrophobic amino acid residues that form a *right-handed α -helix*. **Type I** and **II** membrane proteins only contain *one transmembrane helix*, while **type III** proteins contain several. More rarely, different type I and II polypeptides can aggregate to form a **type IV** transmembrane protein. **Type V** and **VI** proteins carry **lipid anchors**. These are fatty acids (palmitic acid, myristic acid), isoprenoids (farnesol, geranylgeraniol, dolichol), or glycolipids such as glycosyl phosphatidylinositol (GPI) that are covalently bound to the peptide chain. Some integral membrane proteins, e.g., the porins (p. 212), traverse the membrane with antiparallel folded sheet structures. Because of their shape, this tertiary structure is called a **β -barrel**.

Peripheral membrane proteins (not shown) are attached to the membrane only from the outside. They can bind to the head groups of the phospholipids via Ca^{2+} ions or be associated with an integral membrane protein via protein–protein interaction.

Many membrane proteins on the outside of cells have covalently bound, branched carbohydrate chains with about 15 sugar residues, so they are **glycoproteins**.

B. Passive transport

Membranes enclose reaction spaces. They are impermeable for most molecules. Only gases such as O_2 , N_2 , and NH_3 and small, uncharged molecules such as glycerol or urea and lipid-soluble substances such as steroids are able to pass through biological membranes by *free diffusion*. This is the simplest form of membrane transport. When diffusion is supported by integral membrane proteins such as channel proteins or transporters, it is known as **facilitated diffusion** (or facilitated transport).

1. **Channel proteins** have a polar pore through which ions and other hydrophilic compounds can pass. For example, there are channels that allow selected ions to pass (**ion channels**; p. 402) and **porins** that allow molecules up to a specific size to pass in a rather nonspecific fashion (p. 212).

2. **Transporters** recognize and bind the molecules to be transported and help them to pass through the membrane as a result of a conformational change of the transporter. These proteins (permeases) are thus comparable with enzymes—although with the difference that they “catalyze” vectorial transport rather than an enzymatic reaction. Like enzymes, they show a certain *affinity* for each molecule transported (expressed as the dissociation constant, K_d , in $\text{mol} \cdot \text{L}^{-1}$) and a *maximum transport capacity* (V).

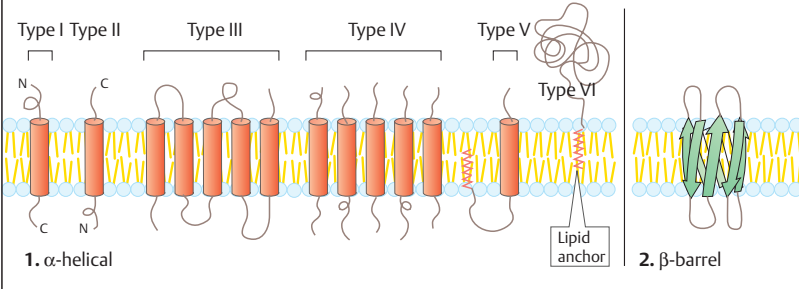
Free diffusion and transport processes facilitated by ion channels and transport proteins always follow a *concentration gradient*—i.e., the transport takes place from the site of higher concentration to the site of lower concentration. With ions, the *membrane potential* also plays a role; the processes are summed up by the term **electrochemical gradient** (p. 108). These processes therefore involve **passive transport**, which runs “downhill” on the slope of a gradient.

C. Active transport

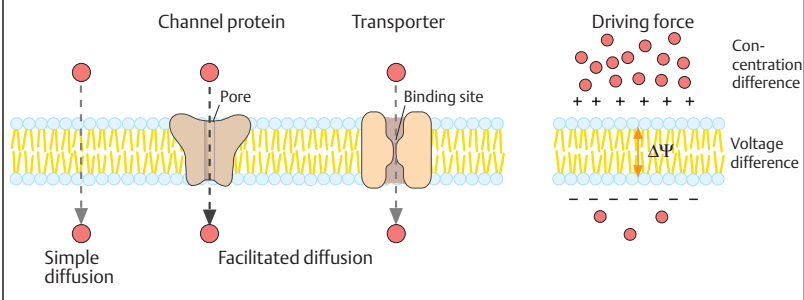
By contrast, **active transport** can also run “uphill”—i.e., against a concentration or charge gradient. It therefore requires an input of **energy**, which is usually supplied by the hydrolysis of **ATP**. **Transporters** are enzymes that first bind their “cargo” on one side of the membrane. ATP-dependent phosphorylation then causes a *conformation change* that releases the cargo on the other side of the membrane.

A nonspontaneous transport process can also become possible by **coupling** to another, active transport process (*secondary active transport*). Some important membrane transporters (both passive and active), are discussed in more detail on page 212, while endocytosis and exocytosis are described on page 214.

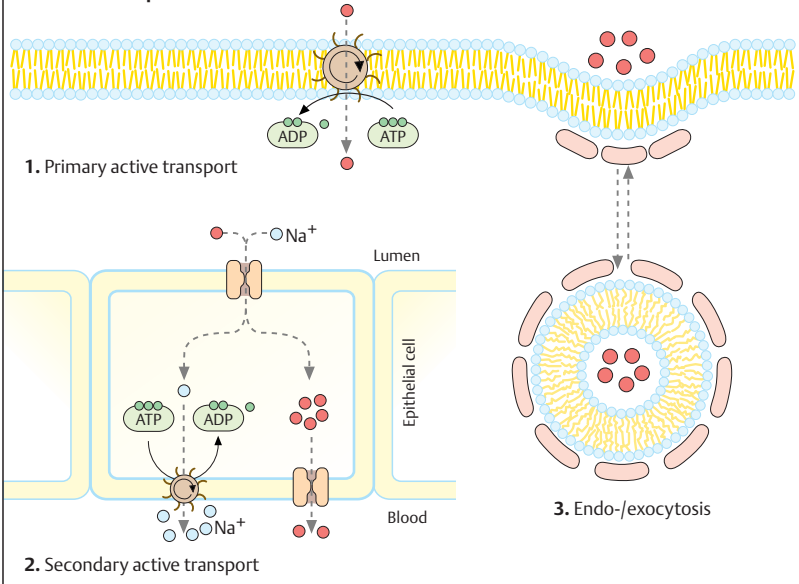
A. Types of membrane protein



B. Passive transport



C. Active transport



Transport Proteins

Membrane proteins that function as passive or active **transporters** are presented on this page. **Ion channels** are discussed on p. 402.

A. Glucose transporters

The glucose transporters (**Glut**) allow the passage of glucose through the cell membrane. Transport is driven by a difference in the concentration of glucose between inside and outside (*facilitated diffusion*; p. 210). The Glut is a family of related membrane proteins with varying organ distribution, substrate specificity, and kinetic properties. Of the 13 different isoforms, **Glut-1** and **Glut-3** have a relatively high affinity for glucose ($K_d = 1$ mM). Glut-1 occurs in the plasma membrane of *nearly all cells*, and ensures a basic glucose supply. It is also found in *erythrocytes* and the *blood–brain barrier*. Glut-3 supplies *neurons* with glucose.

Glut-2 is responsible for glucose transport in the *liver* and *pancreas* and is also present in the *kidney* and intestinal *mucosal cells*. This form has a high capacity and a lower affinity ($K_d = 15$ – 20 mM). The rate of glucose uptake by Glut-2 is therefore strongly dependent on the blood glucose level (normally ~ 4 – 8 mM). Transport by **Glut-4** ($K_d = 5$ mM), which is mainly expressed in *muscle*, *fat*, and *cardiac cells*, is controlled by **insulin**, which increases the number of transporters on the cell surface by 20- to 30-fold within a few minutes (p. 428).

Glut-5 transports fructose preferentially. The significance of the other isoforms is as yet not well understood. **Sodium-dependent glucose transporters** (SGLTs) on the luminal side of glucose-absorbing cells in the intestine and kidneys are not related to the Glut (pp. 272, 336).

The illustration shows the structure of **Glut-1**. It consists of a single peptide chain that spans the membrane with 12 α -helices of different lengths. The glucose is bound by the peptide loops that project on each side of the membrane.

B. Aquaporins

Aquaporins help **water** to pass through biological membranes. They form *hydrophilic pores* that allow H_2O molecules, but not hydrated ions or larger molecules, to pass through. Aquaporins are present in particularly high concentrations in *erythrocytes* and in the *kidney*. Aquaporin-2 in the renal

collecting ducts is regulated by **antidiuretic hormone** (ADH, vasopressin), which via cAMP causes shifting of the channels from the ER into the plasma membrane (pp. 336f.).

Aquaporin-1, shown here, occurs in the *proximal tubule* and in the *Henle loop*, where it promotes water reabsorption (p. 336). It contains eight transmembrane helices with different lengths and orientations. In the center of the protein, there is a narrowing that only H_2O molecules can overcome.

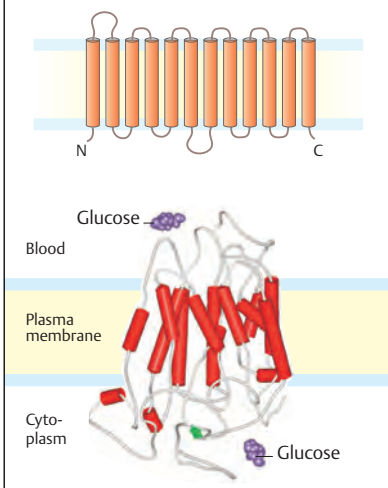
C. Sarcoplasmic Ca^{2+} pump

Transport ATPases transport cations—they are “ion pumps.” ATPases of the **F type** such as mitochondrial ATP synthase (p. 122) use H^+ transport for *ATP synthesis*. Enzymes of the **V type** consume ATP to “pump” protons into lysosomes and other acidic cell compartments (p. 224). **P type** transport ATPases are particularly numerous. These are ATP-driven cation transporters that undergo phosphorylation during the transport cycle.

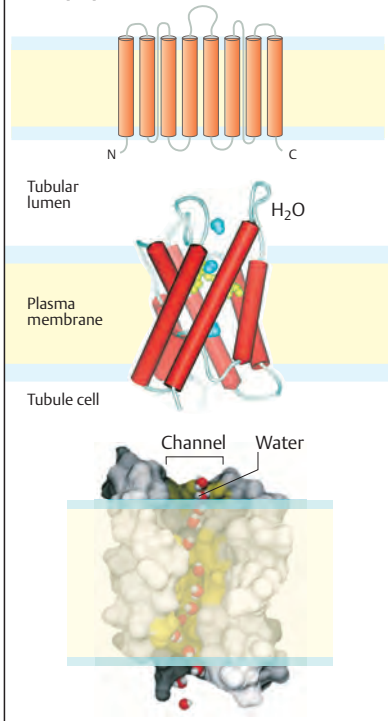
The **Ca^{2+} -ATPase** shown also belongs to the P type. In *muscle*, its task is to pump the Ca^{2+} released into the cytoplasm to trigger muscle contraction back into the sarcoplasmic reticulum (SR; p. 342). The molecule (**1**) consists of a single peptide chain that is folded into various **domains**. In the transmembrane part, which is formed by numerous α -helices, there are binding sites for two Ca^{2+} ions (blue). ATP is bound to the cytoplasmic N domain (green).

Four different stages can be distinguished in the enzyme's catalytic cycle (**2, 3**). First, binding of ATP to the N-domain leads to the uptake of two Ca^{2+} into the transmembrane part (**a**). Phosphorylation of an aspartate residue in the P-domain (**b**) and dissociation of ADP then causes a conformational change that releases the Ca^{2+} ions into the SR (**c**). Finally, dephosphorylation of the aspartate residue restores the initial conditions (**d**).

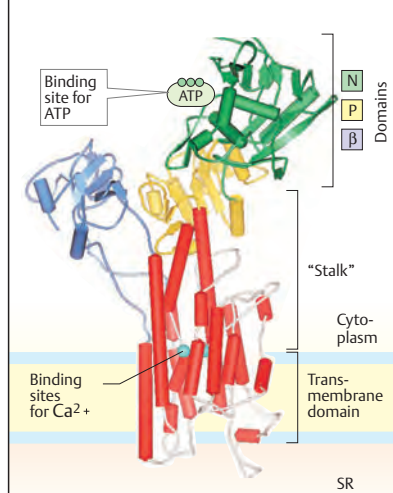
A. Glucose transporters



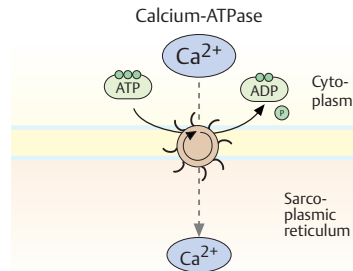
B. Aquaporins



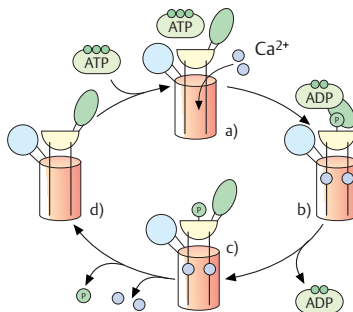
C. Sarcoplasmic Ca²⁺ pump



1. Structure



2. Location



3. Catalytic cycle

Endocytosis and Exocytosis

Vesicular transport through membranes is possible because membranes can completely enclose *molecules, particles*, or an entire *cell*. These so-called **vesicles** transport their contents and release them again by fusion with another membrane. This process is called **endocytosis** when the vesicle contents are transported into a cell, and **exocytosis** when the cell contents are expelled. Endocytosis and exocytosis are closely coupled so that the total surface area of the cell membranes remains constant.

A. Endocytosis

Cells can take up material from the extracellular space by endocytosis. The substances transported include *nutrients, receptors* with their *ligands*, immunological *signaling substances*, and also *pathogens and toxins*.

(1) Large solid objects are taken up by **phagocytosis**, e.g., other *cells* that have died because of apoptosis, *bacteria, viruses*, and *foreign bodies*. The plasma membrane enfolds the object and encloses it in a large vacuole called a **phagosome**. Final degradation of the contents takes place in lysosomes (p. 224).

(2) *Extracellular fluid* and the *molecules dissolved* in it, e.g., other proteins, are taken up by **pinocytosis**. First the plasma membrane is invaginated. This fluid-filled structure then buds off to form a vesicle, moves into the cytosol, and fuses there with endosomes or lysosomes.

(3) **Receptor-mediated endocytosis** gives the process of molecule uptake special specificity. The plasma membrane first bulges inward (forming a *coated pit*), and buds off to form a cytoplasmic **vesicle** (*coated vesicle*). **Coat proteins** are involved, the best investigated of which is **clathrin** (see below). Instead of clathrin, **caveolin** can also cause parts of the plasma membrane to bud off. The resulting bottle-shaped invaginations are called **caveolae** (not shown). For example, *folic acid* is taken up into cells by means of caveolae, whereas *low-density lipoprotein* (LDL) (p. 284), *transferrin* (p. 388), and various *proteohormones* such as epidermal growth factor (EGF), are taken up by receptor-mediated endocytosis.

B. Receptor-mediated endocytosis of LDL

First, the formation of a pit in the cytoplasm membrane is triggered by **adapter protein AP2**, which has an affinity for phospholipids and membrane proteins with special signal sequences (1). Then **clathrin** molecules attach to AP2 and support invagination of the membrane, i.e., the formation of a *coated pit* (2). The G-protein **dynamin** ensures that the vesicle buds off from the plasma membrane to become a *coated vesicle* (3). This vesicle eventually fuses with endosomes, releasing clathrin, AP2 and dynamin (4).

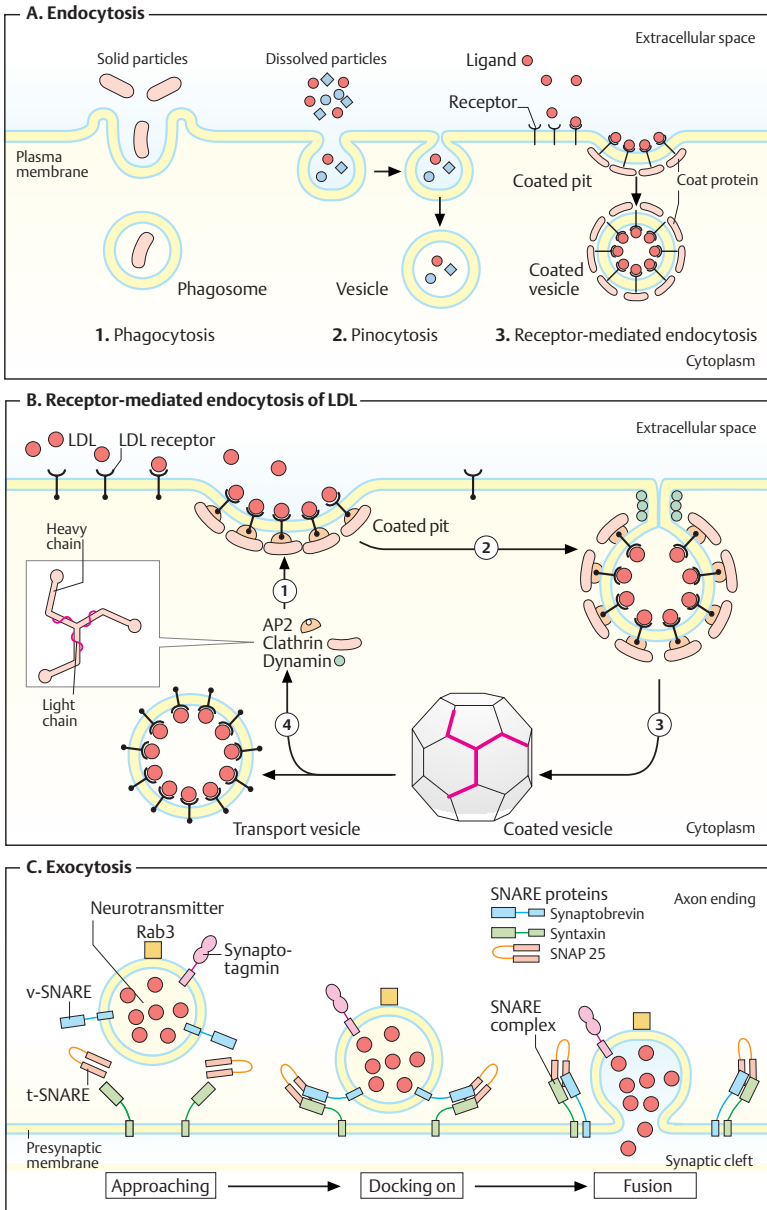
Clathrin is a three-armed protein ("*triskelion*"). Many clathrin molecules associate via these arms to form a three-dimensional lattice that encloses the membrane vesicles with their contents (1).

C. Exocytosis

Cells can expel the contents of vesicles into the extracellular space by means of exocytosis. Thus, *hepatocytes* secrete different plasma proteins into the blood; *plasma cells* secrete antibodies and *glandular cells* secrete hormones; *neurons* release neurotransmitters into the synaptic cleft by exocytosis (C).

So-called **SNARE proteins** (acronym for "Soluble NSF attachment protein receptor") are involved in exocytosis. They mediate vesicle docking and membrane fusion. To release neurotransmitters from neurons into the synaptic gap, the **v-SNARE** (vesicular) **synaptobrevin**, on the synaptic vesicle binds to the **t-SNAREs** (target), **syntaxin** and **SNAP-25**, on the target membrane. The G-protein **Rab3** and Ca^{2+} -binding protein **synaptotagmin** are also involved in this exocytosis. Ca^{2+} binding to synaptotagmin triggers a conformational change, which allows formation of the SNARE fusion complex.

The **toxins** of the bacteria *Clostridium tetani* and *C. botulinum*, which are found in infected wounds and poisoned food, respectively, cause **tetanus** and **botulism**. Both toxins inhibit neurotransmitter release at synapses. Their specific action is due to a *zinc protease* activity that cleaves SNARE proteins. Recombinant **botulinus toxin** ("**Botox**") is used to smooth skin wrinkles, among other things.



Structure and Function

The endoplasmic reticulum (ER) is an extensive closed membrane system consisting of tubular and saccular structures. In the area of the nucleus, the ER turns into the external nuclear membrane. Morphologically, a distinction is made between the **rough ER** (rER) and the **smooth ER** (sER). **Ribosomes** are only found on the membranes of the rER, while they are lacking on the sER. On the other hand, the sER is rich in membrane-bound **enzymes**, which catalyze partial reactions in **lipid metabolism** as well as **biotransformations**.

A. Rough endoplasmic reticulum and Golgi apparatus

The **rER** (1) is a site of active **protein biosynthesis** (p. 248ff.). This is where proteins destined for membranes, endosomes, lysosomes, and export from the cell are synthesized. The remaining proteins are produced in the cytoplasm on ribosomes that are not bound to membranes.

Proteins synthesized on the rER are folded and modified after translation (protein maturation; p. 222). They remain either in the rER as membrane proteins, or pass with the help of **transport vesicles** (2) to the **Golgi apparatus** (3). Transport vesicles are formed by budding from existing membranes, and they disappear again by fusing with them (p. 214). **G-proteins** of the **Rab** family are involved in these transport processes (p. 404).

The **Golgi apparatus** (3) is a complex closed network consisting of flattened membrane saccules ("cisterns"), which are stacked on top of each other in layers. Proteins mature here and are sorted and packed. A distinction is made between the **cis**, **medial**, and **trans Golgi regions**, as well as a **trans Golgi network** (tGN). The **post-translational modification of proteins**, which starts in the ER, continues in these sections.

From the Golgi apparatus, newly synthesized proteins are transported by vesicles to various targets in the cells—e.g., to lysosomes (4) and peroxisomes, to secretory vesicles (5), to the plasma membrane (6)—and release their contents into the extracellular space by fusion with the plasma membrane (**exocytosis**; p. 214). Protein transport can either proceed continuously (**constitutive**), or it can be **regulated** by chemical signals. The decision as to which pathway a protein will take and whether its transport will be constitutive or regulated

depends on the **signal sequences** or **signal structures** that the protein carries like an address label (p. 218). In addition to proteins, the Golgi apparatus also transports membrane lipids to their targets.

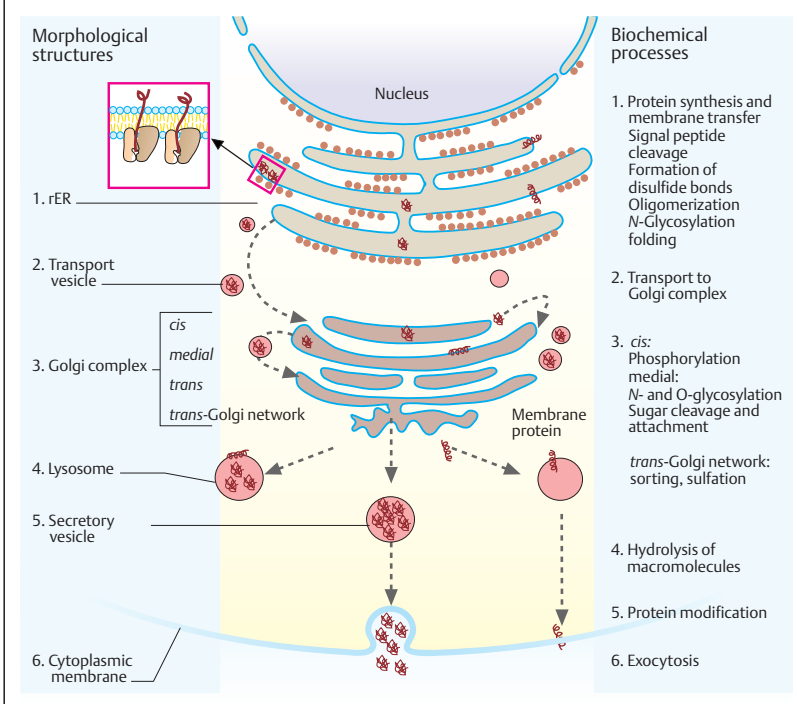
B. Smooth endoplasmic reticulum

Regions of the ER lacking bound ribosomes are known as the **smooth endoplasmic reticulum** (sER). In most cells, the proportion represented by the sER is small. A distinct sER is seen only in cells that have an active **lipid metabolism**, such as hepatocytes and Leydig cells. The sER is usually made up of branching, closed tubules. Membrane-located enzymes in the sER catalyze reactions of **lipid synthesis**, such as the formation of phospholipids (p. 154) and several steps in cholesterol biosynthesis (p. 156). In endocrine cells that produce **steroid hormones**, a large proportion of the reaction steps involved also take place in the sER (p. 424).

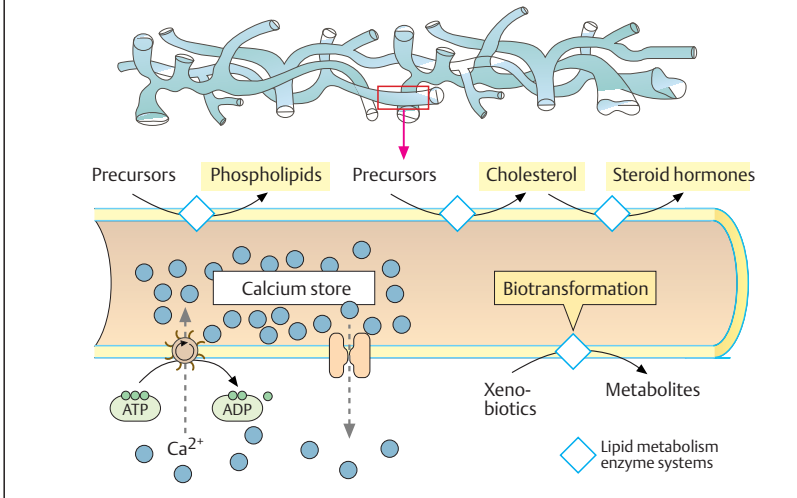
In the liver's hepatocytes, the proportion represented by the sER is particularly high. It contains enzymes that catalyze **biotransformations**. These are reactions in which nonpolar foreign substances (xenobiotics), as well as endogenous substances—e.g., steroids—are chemically altered to inactivate them and/or prepare them for conjugation with polar substances (phase I reactions; p. 322). Numerous **cytochrome P450** enzymes are involved in these conversions (p. 324) and can therefore be regarded as the major molecules of the sER.

The sER also functions as an intracellular **calcium store**, which helps to keep the Ca^{2+} level in the cytoplasm low. This function is particularly marked in the **sarcoplasmic reticulum**, a specialized form of the sER in muscle cells (p. 342). For release and uptake of Ca^{2+} , the membranes of the sER contain signal-controlled Ca^{2+} channels and energy-dependent Ca^{2+} -ATPases (p. 212). Hydrolysis of glucose 6-phosphate to glucose by **glucose-6-phosphatase** (p. 134) also takes place in the sER of the liver. Deficiencies of this enzyme affect the degradation of glycogen (type I glycogenosis; p. 143).

A. Rough endoplasmic reticulum and Golgi apparatus



B. Smooth endoplasmic reticulum



Protein Sorting

A eukaryotic cell contains roughly 10^{10} protein molecules of at least 10000 different types, which are distributed specifically in the various compartments of the cell such as the cytoplasm, membranes, and organelles, where they carry out their functions.

A. Protein transport

The **transport** of proteins across membranes from their site of synthesis on the ribosome to their site of function can involve *pores* (e.g., in the nucleus; p. 204), *transport complexes* (in mitochondria and peroxisomes) or may rely on *transport vesicles* (ER, Golgi apparatus, lysosomes, endosomes and exosomes, plasma membrane, extracellular space; p. 214). To find their way, the proteins carry *sorting signals*, which are read by receptors present in the transport machinery. Proteins that lack a signal remain in the cytosol after synthesis.

B. Protein sorting

The biosynthesis of all proteins (with the exception of the few proteins formed in the mitochondria) starts on free ribosomes in the cytoplasm (top). However, the paths that the proteins then follow soon diverge, depending on which target they are destined for. Proteins that carry a **signal peptide** for the ER (1) follow the *secretory pathway* (right). Proteins that do not have this signal follow the *cytoplasmic pathway* (left).

Secretory pathway. Proteins with a signal peptide for the ER are transferred through the membrane of the rER into the lumen (p. 220). The presence or absence of additional signal sequences and signal regions determines the subsequent transport pathway.

Proteins that have *stop-transfer sequences* (4) remain as integral membrane proteins in the ER membrane. They then pass into other membranes via vesicular transport. From the rER, their pathway first leads to the Golgi apparatus and then on to the plasma membrane. Proteins destined to remain in the rER—e.g., enzymes—find their way back from the Golgi apparatus to the rER with the help of a *retention signal* (2). Other proteins move from the Golgi apparatus to the lysosomes (3; p. 224), to the cell membrane (integral membrane proteins or constitutive exocytosis), or are transported out of the cell (9; signal-regulated exocytosis) by secretory vesicles (8).

Cytoplasmic pathway. Proteins that lack a signal peptide for the ER are synthesized in the cytoplasm on free ribosomes, and remain in that compartment. Special translocation signals, if present, mediate further transport into the mitochondria (5; p. 206), the nucleus (6; p. 204) or peroxisomes (7; p. 226).

C. Translocation signals

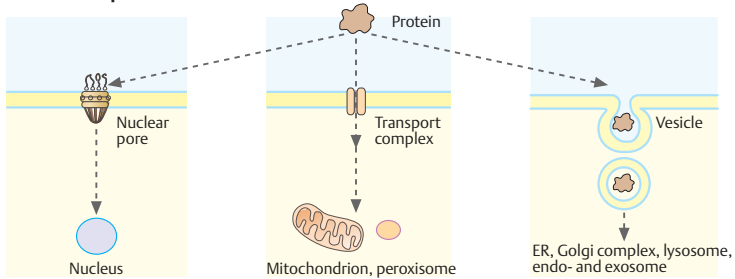
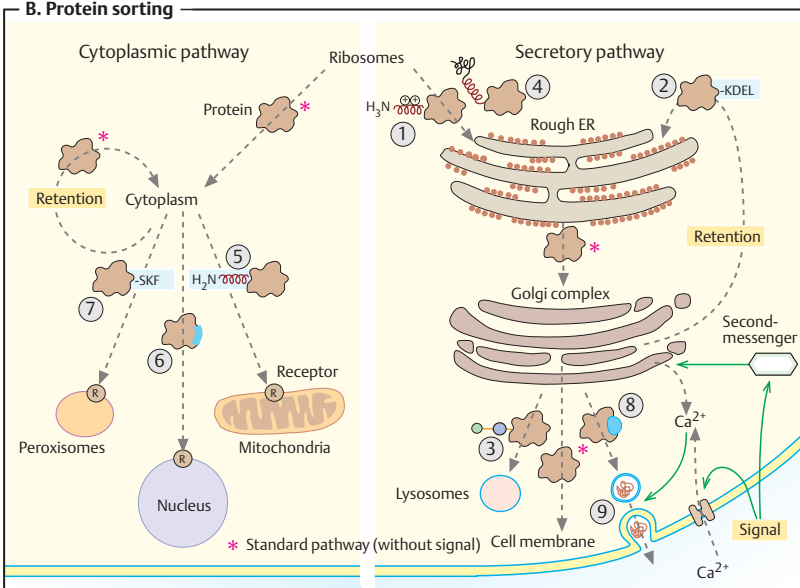
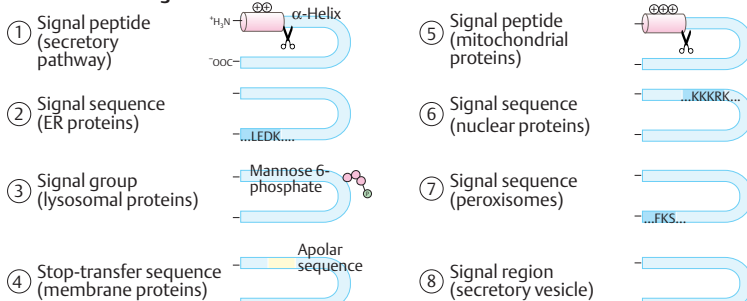
Signal peptides are short amino acid sequences at the N-terminus or C-terminus, or—more rarely—within the peptide chain. Areas on the protein surface formed by various sections of the peptide chain or by different chains are known as **signal regions**. Signal peptides and signal regions are *structural signals* that are usually recognized by *receptors* on organelles. They help to move the proteins, with the aid of additional proteins, into the organelles (*selective protein transfer*). Structural signals can also activate *enzymes* that modify the proteins and thereby determine their subsequent fate. Examples include lysosomal proteins (p. 224) and membrane proteins with lipid anchors (p. 210).

After they have been used, signal peptides at the N-terminus are cleaved off by specific *peptidases* (symbol: scissors). In proteins that contain several successive signal sequences, this process can expose the subsequent signals. By contrast, signal peptides that have to be read several times are not cleaved.

Protein transport is made possible by the interaction of transporters, receptors, translocators, G-proteins and enzymes. Proteins that are to be imported into mitochondria are kept in the unfolded state by **chaperones** of the hsp70 family (p. 222).

Protein biosynthesis, maturation, and sorting is associated with **quality control**. Proteins that are incorrectly folded are labeled with ubiquitin and degraded in proteasomes (p. 162).

Defects of the import systems in organelles can lead to diseases. In **Zellweger syndrome**, for example, *peroxisomal* import proteins are deficient (p. 226). In **I-cell disease** the import of certain hydrolases into the *lysosomes* is disturbed (p. 224).

A. Protein transport**B. Protein sorting****C. Translocation signals**

Protein Synthesis in the rER

A. Protein synthesis in the rough endoplasmic reticulum

With all proteins, protein biosynthesis (**translation**; p. 248f.) starts on free ribosomes in the cytoplasm (1). Proteins that are exported out of the cell or into lysosomes, and membrane proteins of the ER, the Golgi apparatus, and the plasma membrane, carry a **signal peptide for the ER** at their N-terminus. This is a section of 15–60 amino acids in which one or two strongly basic residues (Lys, Arg) near the N-terminus are followed by a strongly hydrophobic sequence of 10–15 residues (p. 218).

As soon as the signal peptide (red) appears on the surface of the ribosome (2), an RNA-containing **signal recognition particle (SRP)**, green) binds to the sequence and initially interrupts translation (3). The SRP then binds to an **SRP receptor** in the rER membrane, and in this way attaches the ribosome to the ER (4). After this, the SRP dissociates from the signal peptide and from the SRP receptor and is available again for step 3. This endergonic process is driven by GTP hydrolysis (5). Translation now resumes. The remainder of the protein, still unfolded, is gradually introduced into a protein channel (the **translocon**) in the lumen of the rER (6), where a **signal peptidase** located in the inner ER membrane cleaves the signal peptide while translation is still taking place (7). This converts the **preprotein** into a **proprotein**, from which the mature protein finally arises after folding and additional **post-translational modifications** (8) in the ER and in the Golgi apparatus.

If the growing polypeptide contains a **stop-transfer signal** (p. 218), then the corresponding hydrophobic section of the chain remains stuck in the membrane outside the translocon, and an **integral membrane protein** arises. In the course of translation, an additional signal sequence can re-start the transfer of the chain through the translocon. Several repetitions of this process produce integral membrane proteins with several transmembrane helices (p. 210).

B. Protein glycosylation

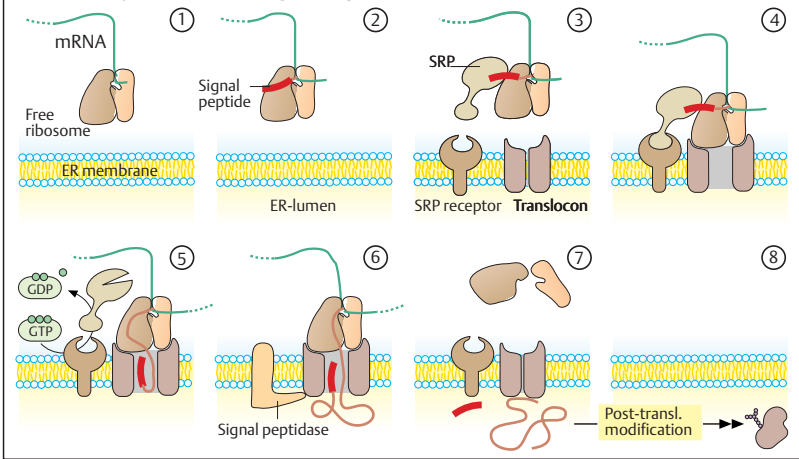
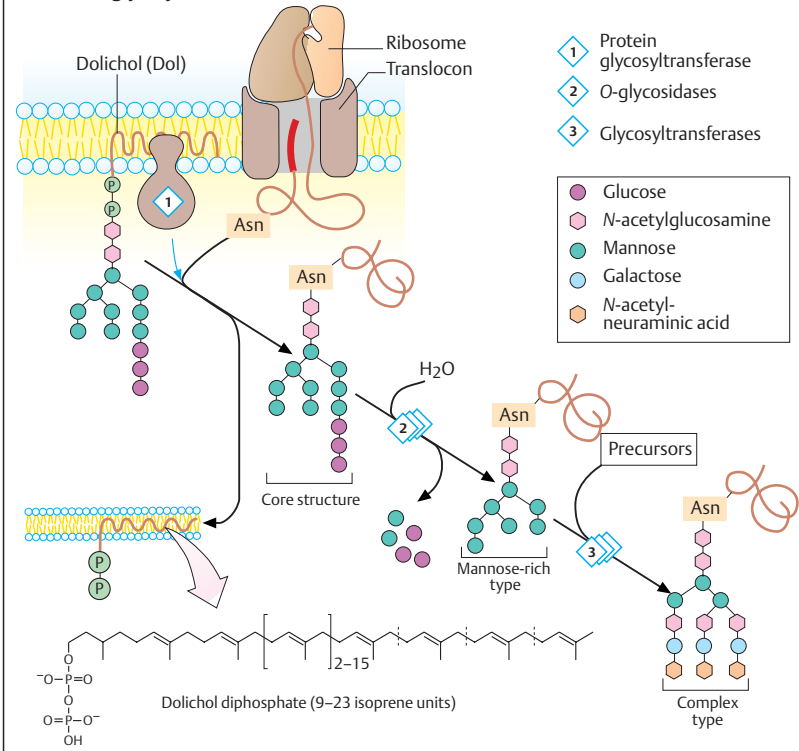
Most extracellular proteins contain covalently bound oligosaccharide residues (p. 34). For example, all plasma proteins with the exception of albumin are glycosylated. Together with glycolipids, numerous **glycoproteins** on the cell surface form the **glycocalyx**. Inside the ER, the

carbohydrate parts of the glycoproteins are co-translationally transferred to the growing chain, and are then converted into their final form while passing through the ER and the Golgi apparatus. Enzymes that modify the sugar moieties of proteins are typical components of the Golgi apparatus (p. 216).

N-linked oligosaccharides (p. 34) are always bound to the acid-amide group of asparagine residues. If a **glycosylation sequence** (–Asn–X–Ser(Thr)–, where X can be any amino acid) appears in the growing peptide chain, then a **transglycosylase** in the ER membrane [1] transfers a previously produced **core oligosaccharide** consisting of 14 hexose residues *en-bloc* from the carrier molecule **dolichol diphosphate** to the peptide.

Dolichol is a long-chain isoprenoid (p. 44) consisting of 10–20 isoprene units, which is embedded in the ER membrane. A hydroxyl group at the end of the molecule is bound to diphosphate, on which the nuclear oligosaccharide is built up in an extended reaction sequence (not shown here in detail). The core structure consists of two residues of *N*-acetylglucosamine (GlcNAc), a branched group of nine mannose residues (Man) and three terminal glucose residues (Glc).

As the proprotein passes through the ER and the Golgi apparatus, **glycosidases** [2] remove the glucose residues completely and the mannoses partially (“trimming”), thereby producing the **mannose-rich type** of oligosaccharide residues (p. 34). Subsequently, various **glycosyltransferases** [3] transfer additional monosaccharides (e.g., GlcNAc, galactose, fucose, and *N*-acetylneuraminic acid) to the mannose-rich intermediate and thereby produce the **complex type** of oligosaccharide residues (p. 34). The structure of the final oligosaccharide is cell-specific and depends on the type and activity of the glycosyltransferases present.

A. Protein synthesis in the rough endoplasmic reticulum**B. Protein glycosylation**

Protein Maturation

After translation, proteins destined for the secretory pathway (p. 218) first have to fold into their native conformation in the rER. During this process they are supported by various *auxiliary proteins*.

A. Protein folding in the rER

To prevent incorrect folding of the growing proteins during protein biosynthesis, **chaperones** (see **B**) in the lumen of the ER bind to the peptide chain and stabilize it until translation has been completed. *Binding protein* (BiP) is an important chaperone in the ER.

Many secretory proteins—e.g., pancreatic ribonuclease (RNase; p. 60)—contain several **disulfide bonds** that are formed oxidatively from SH groups only after translation. The eight cysteine residues of RNase, for instance, can in principle form 105 different pairings, but only the combination of the four disulfide bonds shown on page 61 provides an active enzyme. By contrast, incorrect pairings can block further folding or lead to unstable or insoluble conformations. The enzyme *protein disulfide isomerase* [1] accelerates the equilibration between paired and unpaired cysteine residues, so that incorrect pairs can be quickly split before the protein finds its final conformation.

Most peptide bonds in proteins take on the *trans* conformation (p. 4). Only bonds with proline residues (–X–Pro–) can be present in both *cis* and *trans* forms. In the protein's native conformation, every X–Pro bond has to have the correct conformation (*cis* or *trans*). As the uncatalyzed transition between the two forms is slow, there is a *proline cis–trans isomerase* [2] in the ER that accelerates the conversion.

B. Chaperones and chaperonins

Most proteins fold spontaneously into their native conformation, even in the test tube. In the cell, where there are very high concentrations of proteins (around $350 \text{ g} \cdot \text{L}^{-1}$), this is more difficult. In the unfolded state, the non-polar regions of the peptide chain (yellow) tend to aggregate—due to the hydrophobic effect (p. 24)—with other proteins or with each other to form insoluble products (2). In addition, unfolded proteins are readily susceptible to proteinases. To protect partly folded proteins, there are auxiliary proteins, called **chaperones** because they guard immature proteins against damaging contacts. Some chaperones

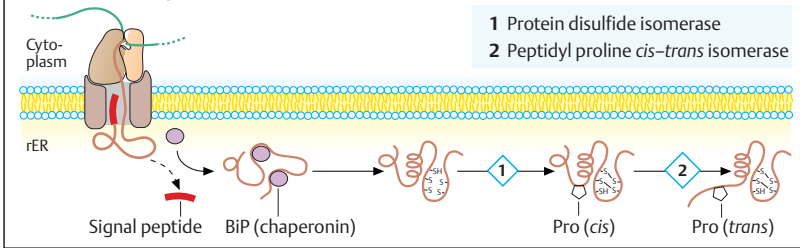
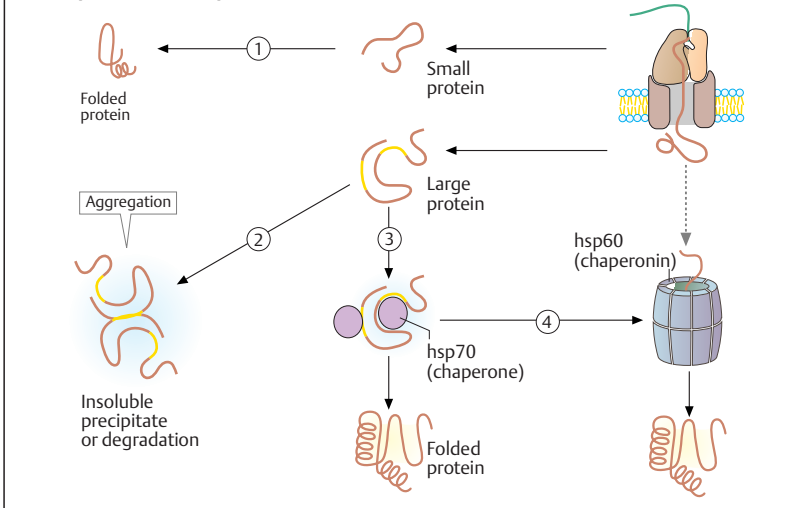
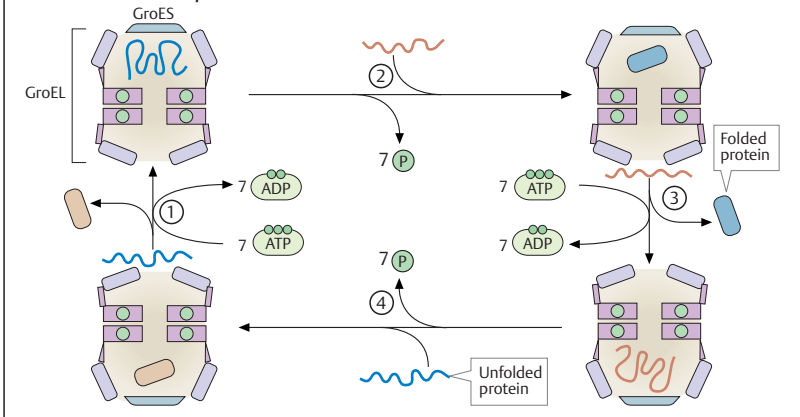
are formed increasingly during temperature stress and are therefore also known as **heat-shock proteins** (hsp). Several classes of hsp are distinguished. Chaperones of the hsp70 type are common, as are type hsp60 **chaperonins** (GroEL/ES in bacteria). Class hsp90 chaperones have special protective tasks for steroid hormone receptors (p. 418).

While small proteins can often reach their native conformation without any help (1), larger molecules require hsp70 proteins (e.g., BiP) for protection against aggregation, which bind as monomers and can dissociate again, dependent on ATP (3). By contrast, chaperonins, a special group of chaperones, form large, barrel-shaped complexes with 14 subunits in which proteins can fold independently while shielded from their environment (4; see C).

C. Function of GroEL/ES

The function of hsp60 has been investigated in detail in the bacterial chaperonin **GroEL**. The barrel has two chambers, which are closed with a lid (**GroES**) during folding of the guest protein in the chamber. Driven by ATP hydrolysis, the chambers open and close alternately—i.e., the release of the folded protein from one chamber is coupled to the uptake of an unfolded peptide in the second chamber.

Accumulation of incorrectly folded proteins can cause disease. For example, in **sickle cell anemia**, a mutation causes the accumulation of insoluble *hemoglobin* aggregates in erythrocytes (p. 300). In **prion diseases**, such as Creutzfeldt–Jakob disease and “mad cow disease” (bovine spongiform encephalitis, BSE), the native *protein PrP* alters its conformation to a self-polymerizing form, which triggers the alteration of further PrP. The development of **Alzheimer disease** involves the *amyloid- β -peptide* $\text{A}\beta$. Because of incorrect folding, this polymerizes into long fibrils which are deposited as plaques in the brain where they interfere with neuronal functions (p. 370).

A. Protein folding in the rER**B. Chaperones and chaperonins****C. Function of GroEL/ES**

Lysosomes

A. Structure and contents

Animal **lysosomes** are organelles with various shapes and a diameter of 0.2–2.0 μm that are surrounded by a single membrane. There are several hundred lysosomes per cell. ATP-driven V-type **proton “pumps”** are active in their membranes (p. 212). As these accumulate H^+ in the lysosomes, the content of lysosomes, with pH values of 4.5–5, is much more acidic than the cytoplasm (pH 7.0–7.3).

The lysosomes are the cell’s “*stomach*,” serving to break down various cell components. For this purpose, they contain some 40 different types of **hydrolases**, which are capable of degrading every type of macromolecule. The marker enzyme of lysosomes is *acid phosphatase*. The pH optimum of lysosomal enzymes is in the region of pH 5. At neutral pH, as in the cytoplasm, lysosomal enzymes only have low levels of activity. This appears to be a mechanism for protecting the cells from digesting themselves in case lysosomal enzymes enter the cytoplasm at any time.

B. Functions

Lysosomes serve for enzymatic degradation of macromolecules and cell organelles, which are supplied in various ways. The example shows the degradation of an overaged mitochondrion by *autophagy*. To accomplish this, the lysosome encloses the organelle (1). During this process, the **primary lysosome** converts into a **secondary lysosome**, in which the hydrolytic degradation takes place (2). The cleavage products are returned to the cytoplasm. Finally, **residual bodies** contain the indigestible residues of the lysosomal degradation process. They are either disposed of by exocytosis or remain in the cell as *lipofuscin* granules, which accumulate with age. Lysosomes are also responsible for the degradation of macromolecules taken up by cells via *endocytosis* and *phagocytosis* (p. 214)—e.g., lipoproteins, pro-teohormones, and bacteria (*heterophagy*). In the process, lysosomes fuse with the **endosomes** (3) in which the endocytosed substances are supplied.

C. Synthesis and transport of lysosomal proteins

Primary lysosomes arise in the region of the Golgi apparatus. Lysosomal proteins are synthesized in the rER and are glycosylated there (1; p. 216ff.). The next steps (2) are specific for lysosomal proteins (see also right part of the illustration). In a two-step reaction, terminal **mannose** residues (Man) are phosphorylated at the C-6 position. First, *N*-acetylglucosamine 1-phosphate is transferred to the OH group at C-6 in a terminal mannose residue, and *N*-acetylglucosamine is then cleaved again. Lysosomal proteins therefore carry a terminal **mannose 6-phosphate** (Man-6-P).

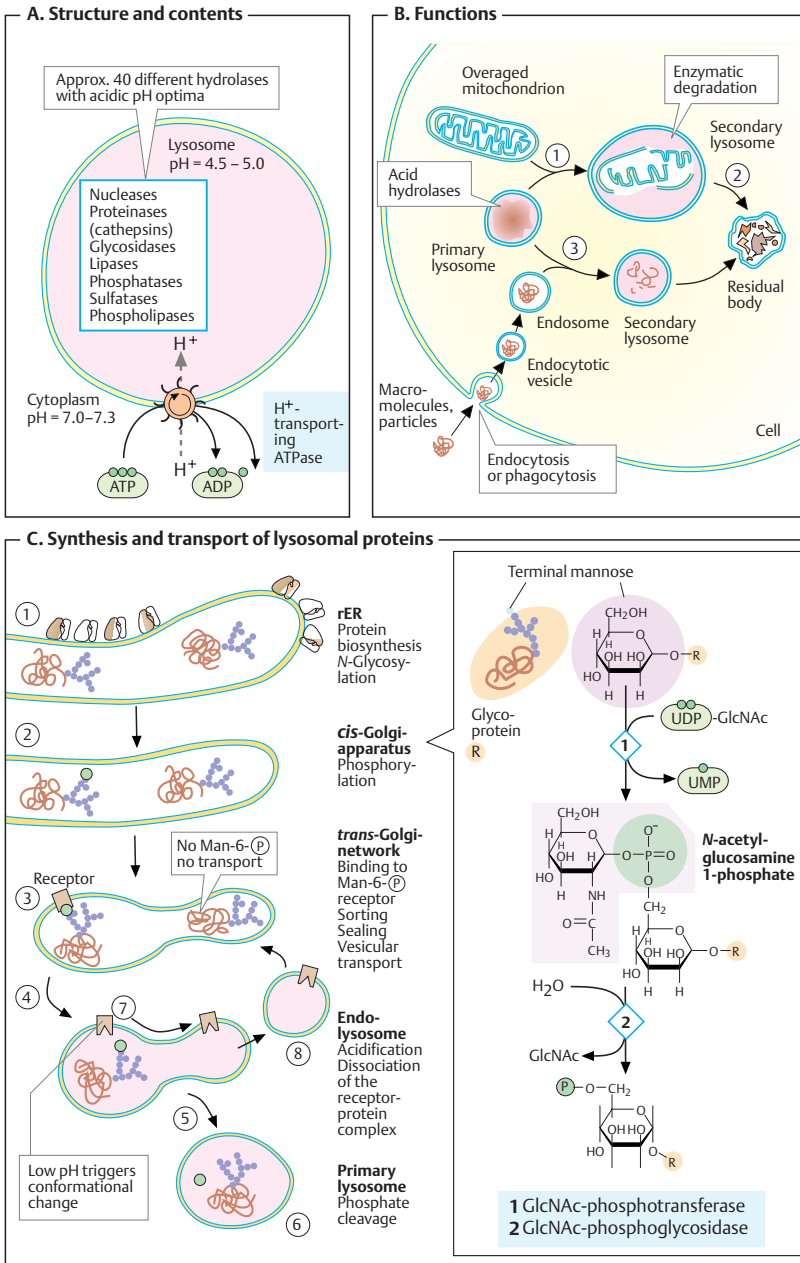
The membranes of the Golgi apparatus contain **receptor molecules** that bind Man-6-P. They recognize lysosomal proproteins by this residue and bind them (3). With the help of *clathrin*, the receptors are concentrated locally. This allows the appropriate membrane sections to be pinched off and transported to the endolysosomes with the help of transport vesicles (4), from which primary lysosomes arise through maturation (5). Finally, the phosphate groups are removed from Man-6-P (6).

The fall in the pH in the endolysosomes releases the Man-6-P receptors from the bound proteins (7) which are then transported back to the Golgi apparatus with the help of transport vesicles (8).

■ Further information

A range of hereditary diseases are due to genetic defects in lysosomal enzymes. These are known as **lysosomal storage diseases**. They particularly affect the degradation of glycogen ($\rightarrow$ *glycogenoses*; p. 142), lipids ($\rightarrow$ *lipidoses*), and proteoglycans ($\rightarrow$ *mucopolysaccharidoses*). In these diseases, unmetabolized macromolecules or degradation products accumulate in the lysosomes and lead to irreversible cell damage over time. In the longer term, organ enlargement takes place, which in severe cases may cause failure of the organ affected.

Typical lysosomal storage diseases include **Gaucher disease** (p. 158), which involves glucocerebroside degradation, **Tay–Sachs syndrome** (affecting ganglioside degradation; p. 42), and **Pompe disease** (which affects glycogen degradation; p. 142).



Peroxisomes

Peroxisomes are cytoplasmic organelles similar in size (0.2–0.5 μm) to lysosomes. They are present in all cells. Hepatocytes contain about 400 peroxisomes, which account for ca. 1% of the cell volume. Peroxisomes are surrounded by a simple membrane and contain neither DNA nor ribosomes. They are produced on the endoplasmic reticulum and can divide.

A. Oxidative metabolism

Peroxisomes contain *oxidases* [1], which reduce oxygen to hydrogen peroxide and can oxidize various substrates at the same time. Some peroxisomal enzymes use hydrogen peroxide for oxidation of substrates ($\text{H}_2\text{O}_2 + \text{SH}_2 \rightarrow 2\text{H}_2\text{O} + \text{S}$). **Catalase** [2] can also convert it to water and oxygen ($2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$; p. 20). The oxidation of *ethanol* to ethanal by catalase (p. 326) is an example of the role of the peroxisomes in oxidative metabolism. In the same way *methanol*, *formaldehyde*, *formic acid* and *nitrite* are oxidized and thereby detoxified by peroxisomes.

The illustrated catalase molecule is a tetrameric enzyme. Two of the four heme groups are visible with an iron atom (green) at the center.

B. Functions

Peroxisomes are involved in the oxidative degradation of a large variety of lipid metabolites and some amino acids. For this, they use molecular oxygen, which is thereby reduced to toxic **hydrogen peroxide** (H_2O_2). Important peroxisome functions include shortening of **very long** ($> \text{C}_{18}$) and **methyl-branched fatty acids** (FAs, p. 148), conversion of cholesterol to **bile acids** (p. 320), and the synthesis of **plasmalogens** (etherphospholipids, p. 40). Other peroxisome functions are detoxification of **glyoxylate** by converting it to glycine by means of a *glyoxylate aminotransferase*, degradation of neutral and basic **D-amino acids** by H_2O_2 -producing *D-amino acid oxidases*, hydrolysis of **lipophilic epoxides**, and oxidative disposal of acetylated **spermine** and **spermidine**.

C. Degradation of very long-chain and methyl-branched fatty acids

An alternative form of mitochondrial β -oxidation (p. 146) occurs in peroxisomes. This peroxisomal process serves to shorten **very long-chain fatty acids** ($> \text{C}_{18}$) and not to obtain energy. In peroxisomes, in the first dehydrogenation reaction of β -oxidation, the reducing equivalents are transferred directly to oxygen instead of being passed to the respiratory chain. This produces hydrogen peroxide, which is detoxified by *catalase*. The subsequent reactions of β -oxidation in peroxisomes resemble those in mitochondria, even if the enzymes differ significantly.

Shortening of very long-chain fatty acids by acetyl units comes to a halt at a length of C_4 – C_6 . These short-chain fatty acids, like the acetyl groups, are transferred into the mitochondria as *carnitine* derivatives (p. 146), where they are degraded further.

Methyl-branched fatty acids, which are often isoprenoid derivatives, are shortened by C_1 by means of α -oxidation (p. 148), so that they can be broken down further by peroxisomal β -oxidation. Products with a length of about 8 C-atoms are then also transferred into the mitochondria.

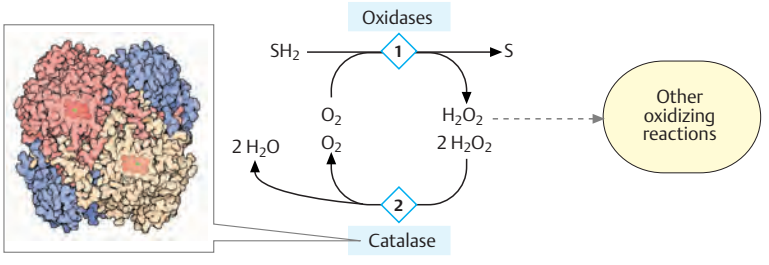
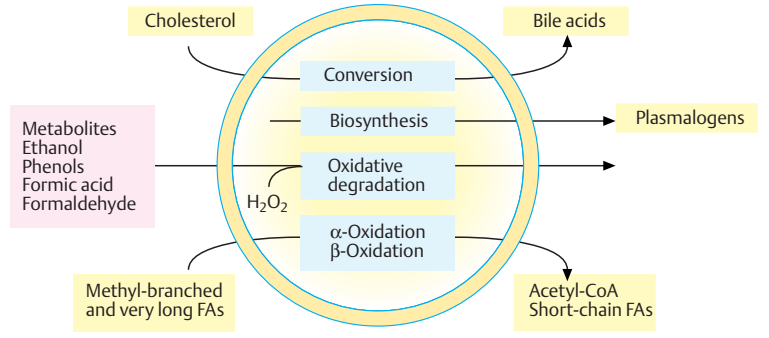
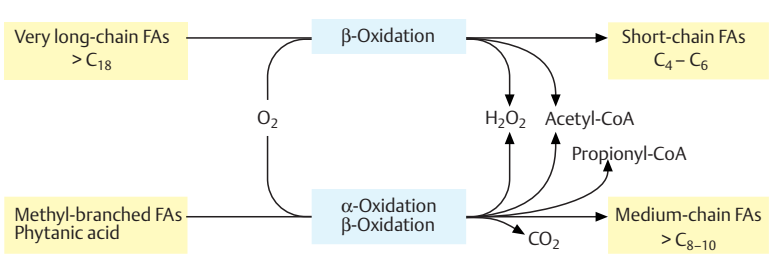
D. Peroxisomal diseases

These are caused either by defective steps in peroxisome biogenesis or by mutations of enzymes involved in peroxisomal metabolism.

Zellweger syndrome is a very rare, severe disease caused by defects in peroxisome development. The liver and brain in particular are damaged by the absence of peroxisomes.

Adrenoleukodystrophy is due to defects of certain *ABC transporters* (p. 328) in the membrane of peroxisomes. An increase in very long-chain fatty acids in the plasma is characteristic of the disease.

Refsum syndrome is caused by a deficiency of *phytanoyl-CoA hydroxylase*, which catalyzes the first step in the α -oxidation of phytanic acid, which can then no longer be degraded (p. 148). The accumulation of *phytanic acid* results in visual and cardiac disorders and also neuropathies.

A. Oxidative metabolism

B. Functions

C. Degradation of very long and methyl-branched fatty acids

D. Peroxisomal diseases

Disease	Cause	Defect
Zellweger syndrome	Disturbed peroxisome biosynthesis due to enzyme transport defect	Organ damage especially in liver and brain, conspicuous facial changes
Adreno-leukodystrophy	Deficient ABC transporter for very long-chain fatty acids	Neurological disorders, weakness, dizziness, adrenal medullary atrophy, demyelination in the brain
Refsum syndrome	Defect of phytanic acid degradation	Neuropathies, vision, hearing and cardiac disorders

Molecular Genetics

Overview

Molecular genetics (“molecular biology”) deals with the biochemistry of the processes involved in the storage, transmission, and expression of genetic information.

A. “Central dogma” of molecular biology

It was only in the 1950s and 1960s, long after classical genetics had explained the laws governing the inheritance of traits, that the role played by nucleic acids and proteins was recognized. It became clear that *genes* (p. 232) are DNA segments that contain information for protein or RNA synthesis in coded form. The “central dogma” of molecular biology, formulated in 1958, describes the information flow in the *expression* of genetic information (DNA → RNA → protein).

B. Expression of genetic information

The decisive factor involved in most molecular biological processes is the ability of nucleic acids to enter into specific base-pairings with each other (p. 68).

Storage. The genetic information of all cells is stored in the base sequence of their DNA (RNA only occurs as a genetic material in viruses; p. 456). Most genes code for proteins—i.e., they contain the information for the sequence of amino acid residues of a protein (its **sequence**). Every amino acid residue is represented in DNA by a code word (a **codon**) consisting of a sequence of three base pairs (a **triplet**). At the level of DNA, codons are defined as sequences of the sense strand read in the 5′ → 3′ direction (p. 68). A DNA codon for the amino acid *phenylalanine*, for example, is thus *TTC* (2; p. 246).

Replication. During cell division, all of the genetic information has to be passed on to the daughter cells. To achieve this, the whole of the DNA is copied during the S phase of the cell cycle (p. 444). In this process, each strand serves as a matrix for the synthesis of a complementary second strand (1; p. 238).

Transcription. For expression of the genetic information, a DNA sequence has to be converted into a protein sequence. As DNA itself does not take part in protein synthesis, the information has to be transferred from the nucleus to the site of synthesis in the cytoplasm. To achieve this, the *template strand* in the relevant part of the gene is transcribed into a **hnRNA** (heterogeneous nuclear RNA). The sequence of this

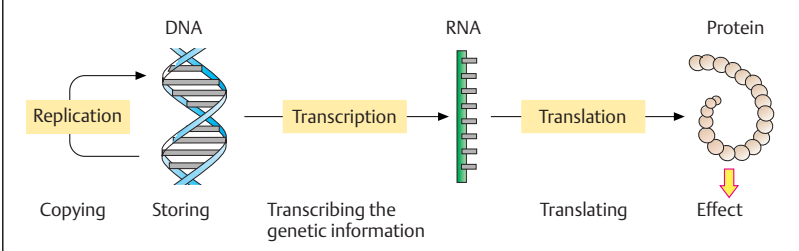
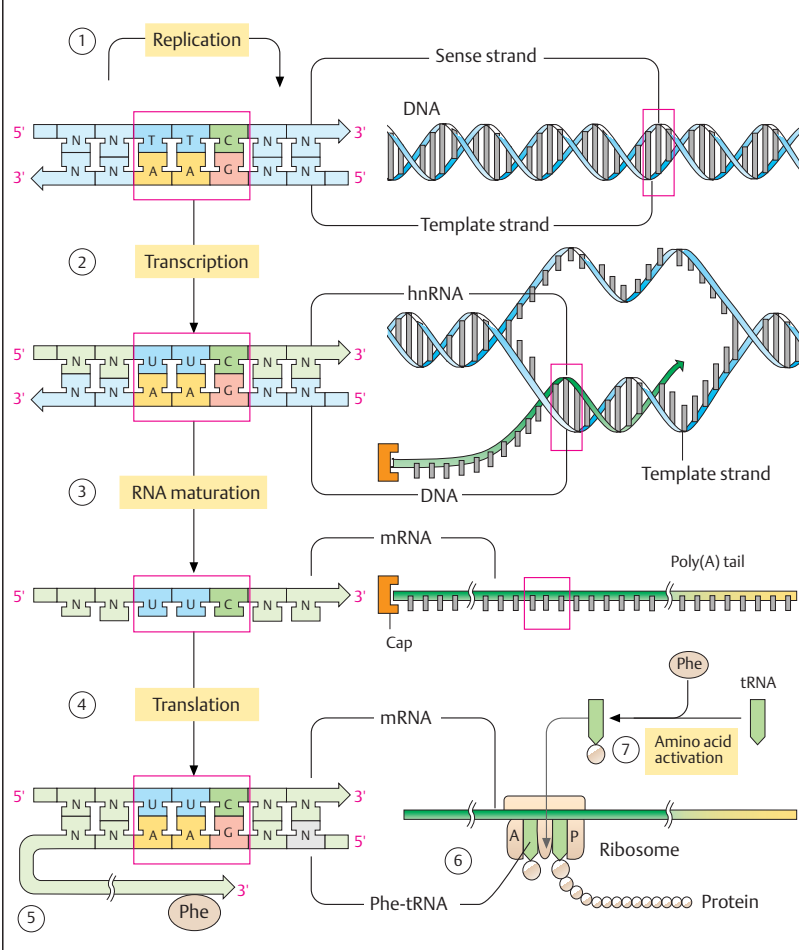
RNA is thus complementary to that of the *template strand* (3), but—with the exception of the exchange of thymine for uracil—it is identical to that of the sense strand. In this way, the DNA triplet *TTC* gives rise in hnRNA to the RNA codon *UUC*.

RNA maturation. In eukaryotes, the hnRNA formed is initially modified several times before it can leave the nucleus as **messenger RNA** (mRNA, 4). During RNA maturation, superfluous sequences (introns) are removed from the molecule, and both ends of the transcript are modified by the addition of further nucleotides (p. 244).

Translation. Mature mRNA enters the cytoplasm, where it binds to **ribosomes**, which convert the RNA information into a peptide sequence. Ribosomes (pp. 66, 248) consist of more than 100 proteins and several ribosomal RNA molecules (rRNAs). The rRNA plays a role as a structural element and is also involved as a ribozyme in the binding of mRNA to the ribosome and the formation of the peptide bond.

The actual information transfer is based on the interaction between the mRNA codons and another type of RNA, **transfer RNA** (tRNA, p. 66). The tRNAs always provide the correct amino acid to the ribosome according to the sequence information in the mRNA, which they carry at the 3′ end. Approximately in the center, they present a triplet that is complementary to the corresponding mRNA codon, known as the *anticodon* (*GAA* in the example shown). If the codon *UUC* appears on the mRNA, a molecule of Phe-tRNA^{Phe} binds to the mRNA by means of its anticodon (5) and thus brings the phenylalanine residue at the other end of the molecule into a position in which it can take over the growing polypeptide chain from the neighboring tRNA (6).

Amino acid activation. Before binding to the ribosomes, tRNAs are loaded with the correct amino acids by specific ligases (7; p. 246). It is the *amino acid tRNA ligases* that carry out the transfer (translation) of the genetic information from the nucleic acid level to the protein level.

A. "Central dogma" of molecular biology**B. Expression of genetic information**

Genes and Genomes

Genes are DNA segments that carry information for the synthesis of an RNA. In addition to the coding regions, genes usually also contain segments for regulation (e.g., the *promoter* region; see below). Eukaryotic genes also include “intervening” sequences (*introns*) without any direct information content (**B**). The entire DNA of an organism is called the **genome** (**C**).

A. Gene structure in prokaryotes

Microorganisms contain a relatively large number of genes although their genome is small in size (see **C**). Bacterial genes do not contain any introns and are often regulated in groups. An **operon** is a DNA segment in which several functionally related genes are arranged behind a shared promoter. When the operon is transcribed, several mRNAs are produced at the same time. On page 242, we describe the *lac* operon of the bacterium *Escherichia coli*, together with its regulation.

B. Gene structure in eukaryotes

The gene for the gluconeogenesis enzyme *phosphoenolpyruvate carboxykinase* (PEP-CK; p. 134) is typical of a eukaryotic gene. The PEP-CK gene in rats has a length of almost 7000 bp (base pairs). However, only 1863 bp carry the information for the protein's 621 amino acids, distributed over 10 coding segments (**exons**, dark blue). The remainder comprises the **promoter** (pink) and **introns** (light blue). The promoter region of the gene (roughly 1000 bp) serves for regulation (p. 242). Transcription (p. 240) starts at the 3' end of the promoter (“transcription start”) and continues until the so-called polyadenylation sequence has been passed.

The length of the primary transcript of the PEP-CK gene (**hnRNA**) is roughly 6200 bp. It still has sequences at both ends which are not translated (untranslated regions, UTRs). In RNA maturation (p. 244), the UTRs and the noncoding segments corresponding to the introns are removed, and both ends are modified. The translatable mature **mRNA** is only half as long as the hnRNA. In many eukaryotic genes, the proportion of introns is even higher. For instance, the gene for *dihydrofolate reductase* (p. 454) has a length of over 30 000 bp. The information is distributed over 6 exons, which together are only about 6000 bp long.

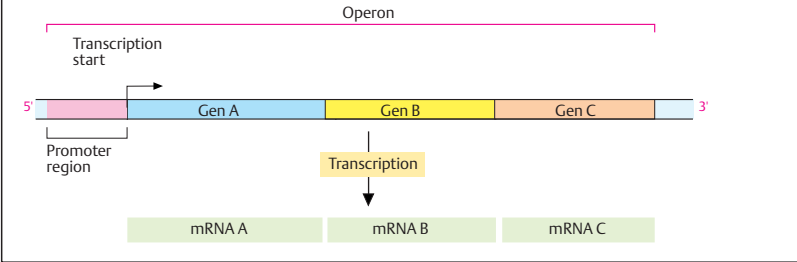
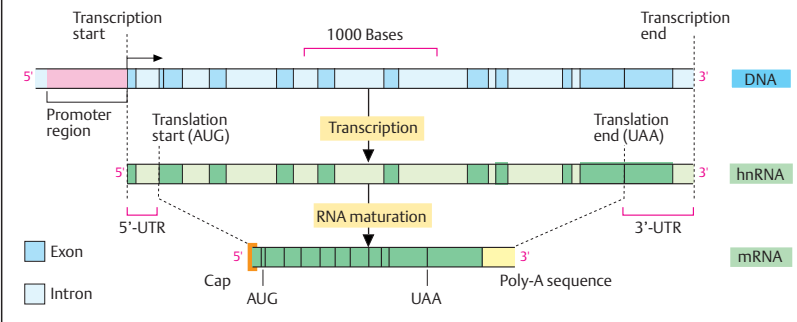
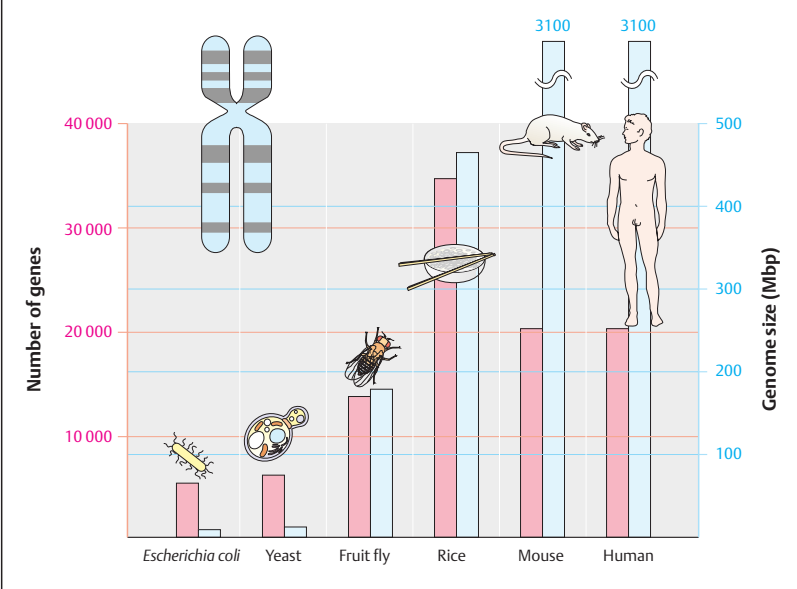
C. Genomes

Advances in DNA sequencing (p. 258) have made it possible to determine the nucleotide sequence of entire genomes. By now, hundreds of genomes have been sequenced in full, including the human genome. Comparative analysis of these data yielded unexpected results. For example, the **total number of genes** (left axis, red) is a little more than 20 000 in *humans* or *mice*, which is only about three times greater than that of *yeast*, and even bacterial genomes can contain up to 6000 genes. Plants like *rice* usually have a more complex metabolism than animals and therefore require a correspondingly larger number of genes.

As regards **genome size** (right axis, blue), the human genome is unremarkable compared to that of other mammals. The human haploid genome (single, complete set of genetic material) contains just under 3.1×10^9 bp (i.e., 3100 mega base pairs, Mbp), distributed over 23 **chromosomes** (22 **autosomes** and 1 **sex chromosome**). The body's cells contain two sets of these chromosomes: they are **diploid**. Each chromosome consists of a single DNA molecule of 50 to 250 Mbp and associated proteins (p. 235).

Only about a quarter of the human genome is represented by genuine *genes*, *pseudogenes*, and *genelike sequences*, while the majority is occupied by *repetitive sequences* (e.g., STR, p. 260), *mobile elements*, and other noncoding sequences. The DNA segments that code for proteins (exons, **B**) account for only 1%–2% of the genome. The function(s) of the noncoding DNA segments is still largely unclear.

The total number of proteins that humans can synthesize (the *proteome*) is probably far higher than the number of genes in the genome, as a gene can code for several different proteins by *alternative splicing* (p. 244). In this respect, the “central dogma” of molecular biology (p. 230) is not strictly valid.

A. Gene structure in prokaryotes**B. Gene structure in eukaryotes****C. Genomes**

Chromatin

A. Chromatin

In the nuclei of eukaryotes (p. 204), DNA is present in the form of **chromatin**. This consists of nucleoprotein complexes, with a DNA proportion of approximately one-third. It is only during mitosis (p. 444) that chromatin condenses into **chromosomes** that are visible under light microscopy, while it is largely loosened during the interphase of the cell cycle. A morphological distinction can be made between tightly packed **heterochromatin** and the less dense **euchromatin**. Euchromatin is the site of active transcription (B).

The proteins contained in chromatin are classified as either histone or nonhistone proteins. **Histones** are small, strongly basic proteins that are directly associated with DNA. They contribute to the structural organization of chromatin, and their basic amino acids also neutralize the negatively charged phosphate groups of DNA. This results in extremely dense packing of DNA in the nucleus, which makes it possible for the 46 DNA molecules of the diploid genome, with a total length of about 2 m, to be accommodated in a nucleus with a diameter of only 10 μm . The amino acid sequence of the histones has hardly altered at all in the course of evolution. The H4 histones in humans and wheat, for instance, differ only in a single amino acid residue.

Two histone molecules each of types **H2A** (blue), **H2B** (green), **H3** (yellow), and **H4** (pink) form an octameric complex, around which 146 bp of DNA are wound in 1.8 turns. These particles, with a diameter of 7 nm, are referred to as **nucleosomes**. Another histone (**H1**) binds to DNA segments that are not directly in contact with the histone octamers ("linker" DNA). It covers about 20 bp and supports the formation of spirally wound superstructures with diameters of 30 nm, known as **solenoids**. When chromatin condenses into chromosomes, the solenoids form **loops** about 200 nm long, which already contain about 80 000 bp. The loops are bound to a protein framework (the **nuclear scaffolding**), which in turn organizes some 20 loops to form **minibands**.

The **nonhistone proteins** are very heterogeneous. This group includes *structural proteins* of the nucleus, as well as many *enzymes* and *transcription factors* (p. 240), which selectively bind to specific segments of DNA and regulate gene expression and other processes.

B. Chromatin remodeling

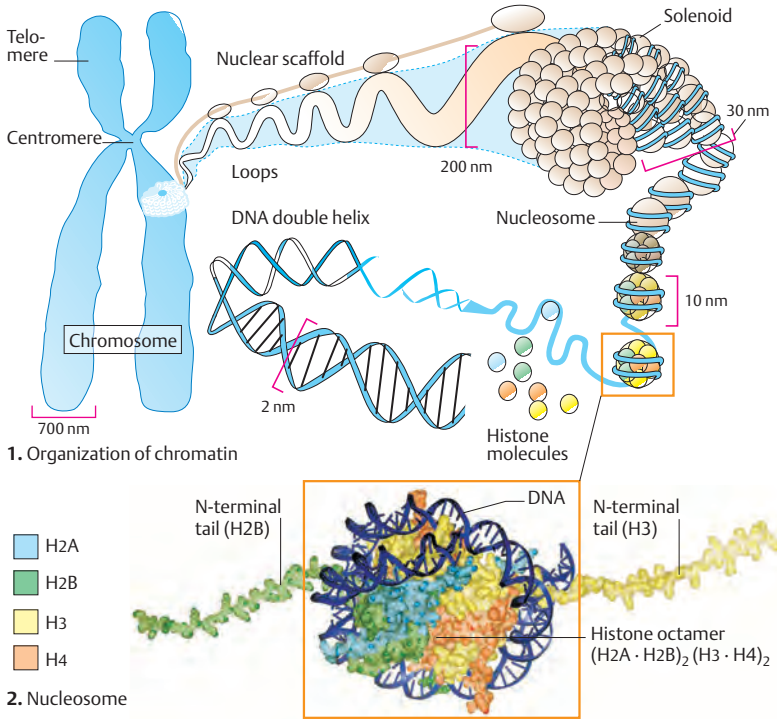
The histones in chromatin organize and protect the genome. In heterochromatin however they also shield the DNA from the enzymes that catalyze replication, transcription, or repair processes. The chromatin structure has therefore to be loosened locally before such processes can take place.

Chromatin remodeling is based mainly on covalent modifications of nucleosomes. The histones in the octamer carry N-terminal mobile "tails" consisting of some 20 amino acid residues that project out of the nucleosomes (see A, only 2 of the 8 tails are shown in full length). They are particularly rich in basic, positively charged amino acid residues (lysine and arginine), which bind the phosphate groups of the DNA, thus stabilizing the nucleosomes. In the condensed chromatin (top), all the genes are therefore inaccessible ("gene off").

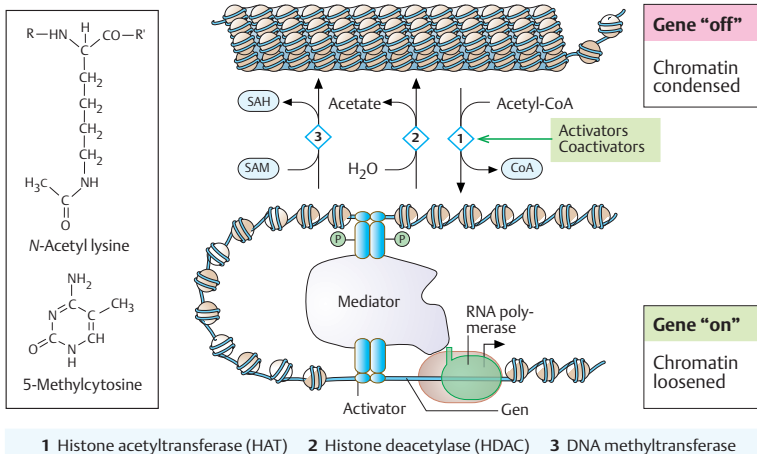
Prior to transcription, *histone acetyltransferases* (HAT, [1]) become active. They acetylate lysine residues in the tails, which thereby lose their charge and separate from the DNA. In the loosened chromatin (bottom), other proteins bind to the DNA and start off transcription ("gene on"). Once it is complete, *histone deacetylases* (HDACs, [2]) remove the acetyl residues again, thus enabling the chromatin to become condensed again. Further condensation into chromosomes is initiated by additional phosphorylation of the histone tails ("gene off").

Although the cells of an organism are the same genetically, they differentiate during embryonic development into many different types of cells and tissues. This is due to the fact that in every cell type certain genes are turned off permanently. This is accomplished by *DNA methyltransferases* [3], which covalently methylate some DNA bases. *RNA interference*, another mechanism for inactivating genes, is discussed on page 262. **Epigenetics**, a new branch of research, deals with processes of this type.

A. Chromatin



B. Chromatin remodeling



Nucleic Acid-Modifying Enzymes

Enzymes that synthesize or modify nucleic acids are of interest not only in connection with the storage and expression of genetic information. Enzymes from this group are also indispensable tools for sequencing and sequence-specific manipulation of DNA in genetic engineering. Practical applications of nucleic-acid modifying enzymes are presented on page 356f.

A. Enzymes in nucleic acid metabolism

Like all biological macromolecules, nucleic acids too are degraded by *hydrolysis* (p. 10). Enzymes that catalyze this reaction are called **nucleases**. As in the degradation of peptides or polysaccharides, there are *exonucleases* and *endonucleases*. Nonspecific endonucleases are present in pancreatic secretion (DNases, RNases; p. 268). *Restriction endonucleases* are discussed in more detail in B. Enzymes that cleave nucleotides from the end of the chain are subdivided into 5'→3' and 3'→5' exonucleases, depending on the direction of degradation.

Nucleic acid polymerases generate complementary copies of RNA or DNA strands. Different groups are distinguished according to the template ("matrix") and product. The *reverse transcriptases*, which transcribe the RNA matrices to DNA, are unusual. Apart from their role in the multiplication of RNA viruses (p. 456), they are also important in genetic engineering (p. 262). **Ligases** and **polynucleotide kinases** are used in the laboratory as well. **Topoisomerases** is the name given to enzymes that modify the conformation of the DNA during replication and transcription. Bacterial topoisomerases are among the sites where antibiotics attack (p. 252).

B. Restriction endonucleases

In many genetic engineering procedures, defined DNA fragments have to be isolated and recombined with other DNA segments (*cloning*; p. 256). For these steps the same enzymes are used which cut DNA inside cells and link them again. The *restriction endonucleases*, a large group of enzymes that occur only in bacteria and cleave DNA double strands at specific sequences, are particularly important. Restriction enzymes are named by abbreviations that indicate the organism of origin. Thus, the enzyme *EcoRI* was isolated from the bacterium *Escherichia coli* (hence *Eco*). Like

most restriction enzymes, *EcoRI* cleaves DNA double strands at *palindromes*, i.e., DNA segments in which the strand and counterstrand (read in the 5'→3' direction) have the same sequence, in this case 5'-GAATTC-3'. *EcoRI* cleaves the phosphate diester bonds of both strands between G and A. This results in complementary *overhanging ends* (AATT), which are held together by base pairing. However, these can be separated easily, e.g., by heating. If the fragments are cooled, the overhanging ends hybridize again in the correct order. The cleavage sites can be closed again by a *DNA ligase*.

C. Mode of action of DNA polymerases

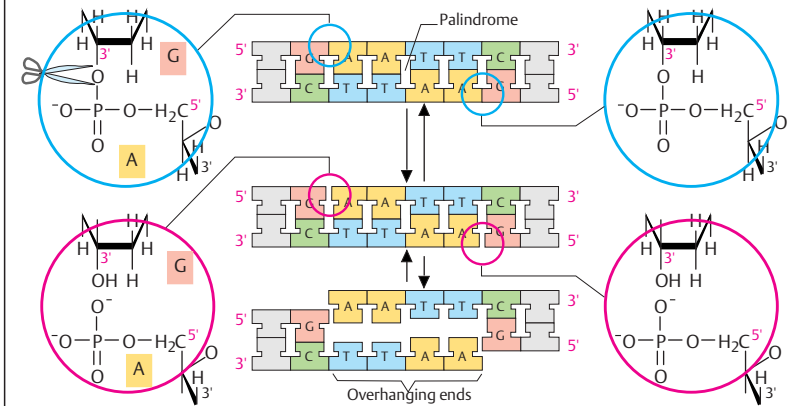
DNA-dependent DNA polymerases require a single-stranded DNA as a template. Based on its sequence, they synthesize a complementary second strand, starting from a short RNA or DNA oligonucleotide (the **primer**) already bound to the template. The four deoxyribonucleoside triphosphates **dATP**, **dGTP**, **dCTP**, and **dTTP** are the substrates of the DNA polymerases.

At each step, the nucleotide that is complementary to the current base in the template strand is initially bound by base pairing. The 3'-OH group of the last component incorporated in the new strand then attacks an α -phosphate residue of the bound nucleoside triphosphate, as a nucleophile. This results in formation of a new phosphate diester bond with the release of diphosphate. The described mechanism means that the template can be read only in the 3'→5' direction, i.e., the newly synthesized strand always grows in the 5'→3' **direction**. *DNA-dependent RNA polymerases* use a similar mechanism for transcription (p. 240). DNA and RNA polymerases are large proteins consisting of several subunits, the role of which is still partly unclear.

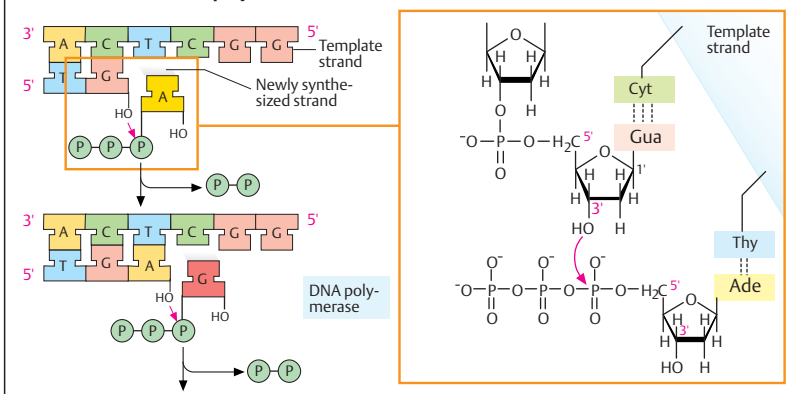
A. Enzymes in nucleic acid metabolism

Enzyme	Acts on	Effect	Page
Nucleases			
Nucleases	RNA, DNA	Hydrolysis of phosphodiester bonds	268
Endonucleases	DNA	Sequence-specific cleavage of the two strands	236
Exonucleases	RNA, DNA	Nucleotide cleavage from the end	–
Polymerases			
DNA-directed DNA polymerases	DNA	Synthesis of a complementary DNA segment	236
DNA-directed RNA polymerases	DNA	Synthesis of a complementary RNA segment	240
RNA-directed DNA polymerases	DNA	Synthesis of a complementary DNA segment	456
Polynucleotide kinases	DNA, RNA	Phosphate transfer to the 5' OH group	–
Ligases	DNA	Linking of two polynucleotide strands	238
Topoisomerases	DNA	Despiralization and unwinding of the double helix	238
Methyltransferases	DNA	Transfer of methyl groups to C or A	234

B. Restriction endonucleases



C. Mechanism of DNA polymerases



Replication

For genetic information to be passed on to both daughter cells during cell division, a complete copy of the genome (p. 232) has to be produced before each mitosis. This process, which takes place in the S phase of the cell cycle (p. 444), is known as **replication** (short for “identical reduplication”).

A. Replication forks

Replication starts at specific sites in the DNA, the **replication origins** (“ori”) and continues in both directions. This means that two **replication forks** are produced, running in opposite directions, in which both strands are replicated at the same time (B). To allow replication within a manageable time, large animal genomes contain numerous replication origins at intervals of 10 kbp to several hundred kilobase pairs, at which many forks can form at the same time. Nevertheless, replication of animal cells takes some hours.

Several proteins are involved in DNA replication. Only the most important are shown here. In human cells, these are *topoisomerase I*, which unwinds twisted DNA, *helicase* to separate the two DNA strands, *single strand-binding proteins*, a *primase complex* (Pol α /primase) and a “*sliding clamp*.” This is a ring-shaped protein complex (red), which holds the replicating *polymerase* (Pol δ) on the DNA during replication. There are many different DNA polymerases (Pol) in animals (p. 236). In humans, the nuclear genome is replicated by Pol δ (and partly by Pol ϵ). Pol γ replicates mitochondrial DNA (p. 126), while Pol β is involved in repair processes (p. 254).

B. Process of replication

Since DNA is always read only from 3' to 5' (p. 236), only one of the two strands (known as the **leading strand**; violet) can undergo *continuous* replication. For the **lagging strand** (light blue), the reading direction is the *opposite* of the direction of movement of the replication fork. If continuous replication were to take place on this strand also, the fork would break up. To prevent this, the new strand is first synthesized in individual pieces on the lagging strand; these are known as **Okazaki fragments** after their discoverer.

Each of these fragments starts at its 5' end with a primer consisting of roughly 10 RNA nucleotides (green), which are produced by the primase activity of the *Pol α /primase complex* and act as the starting sequence for DNA polymerase. The primer is first extended by Pol α and then by Pol β (2, orange). After a few thousand nucleotides, synthesis is interrupted and a new fragment is begun.

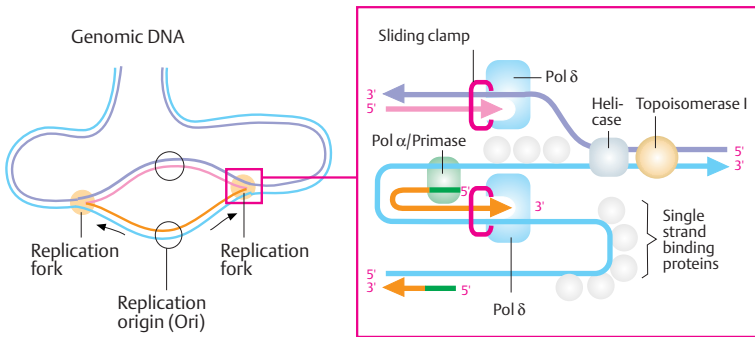
The newly formed Okazaki fragments (OFs) are initially not bound to one another and still have RNA at the 5' end (3). At some distance from the fork, *RNase H1* and *flap endonuclease I* (FEN I) therefore remove the RNA primer. The gap is filled with DNA components by Pol δ before it is finally closed by a *DNA ligase*. In the new DNA double helices, only *one* of the strands has been newly synthesized—i.e., replication is *semiconservative*.

While 1000 or more nucleotides (nt) are replicated per second in bacteria, replication takes place much more slowly in eukaryotes (~ 50 nt $\cdot$ s $^{-1}$), because the new nucleotides are checked for correct base-pairing shortly after they are incorporated. This “proof-reading” is performed by subunits of Pol δ with 3'→5' exonuclease activity (p. 236), which remove incorrect elements. For this reason, an error occurs on average only once in every 100 000 nucleotides.

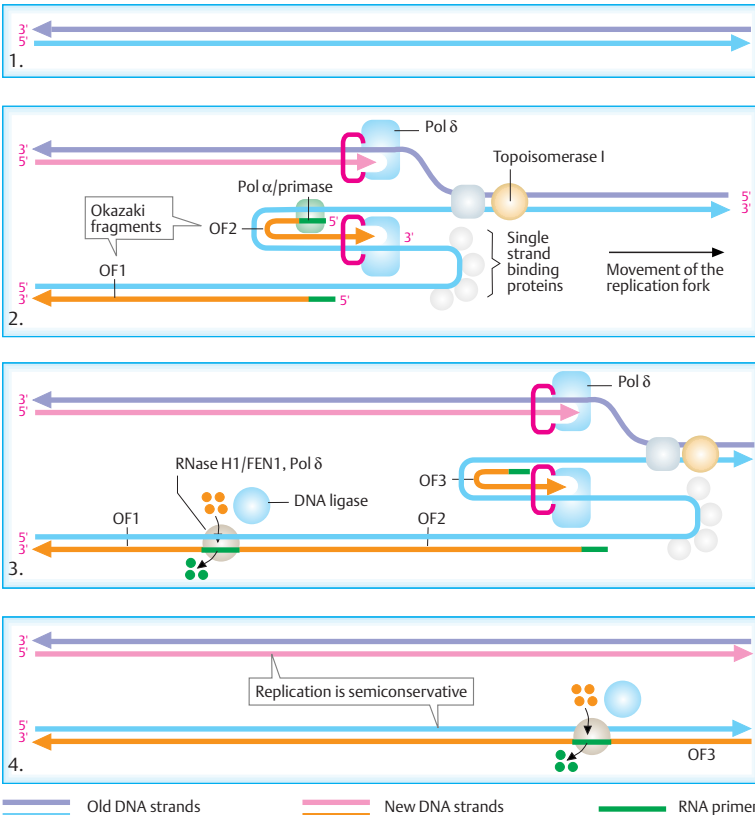
■ Further information

In most of the body's cells, the ends of the chromosomes (the **telomeres**) cannot be completely duplicated during replication. They therefore become shorter with each cell division until finally no further replication is possible. Animal unicellular organisms, embryonic cells, stem cells, and also tumor cells (p. 452) possess an enzyme (**telomerase**), which adds nucleotides to the telomers. Because of this, these cells are potentially immortal.

A. Replication forks



B. Process of replication



Transcription

For the genetic information coded in DNA to become biologically effective, it has to be re-written (transcribed) initially into RNA (p. 230). DNA only serves as a template and is not altered in any way by the **transcription** process. Transcribable segments of DNA that carry information for a defined product are called **genes** (p. 232).

A. Transcription and maturation of RNA: overview

Transcription is catalyzed by *DNA-dependent RNA polymerases* (p. 236). These act in a similar way to DNA polymerases (p. 238), except that they incorporate *ribonucleotides* (ATP, GTP, CTP, and UTP) instead of deoxyribonucleotides into the newly synthesized strand. Unlike DNA polymerases, they do not require a primer.

Eukaryotic cells contain at least three different types of RNA polymerase. *RNA polymerase I* synthesizes RNA molecules with a sedimentation coefficient of 45 S (45S-RNA), which serve as precursor for three ribosomal RNAs. The products of *RNA polymerase II* are hnRNAs, from which translatable mRNAs later develop, as well as precursors for snRNAs. Finally, *RNA polymerase III* transcribes genes that code for tRNAs, 5S rRNA, and certain snRNAs. These precursors give rise to functional RNA molecules by a process called **RNA maturation** (p. 244). Polymerases II and III are inhibited by *α -amanitin*, a toxin in the *Amanita phalloides* mushroom.

B. Basal transcription complex

Before RNA polymerase II can start transcription, it has to combine with numerous other proteins to form a **basal transcription complex**. The complex is formed by associating *basal transcription factors* at the 3' end of the *promoter region* (p. 232) in a defined order: first with the DNA, then with one another, and then with the polymerase. The process often starts at a short DNA segment known as a "**TATA box**" because of its typical sequence. The TATA box is about 25 bp "upstream" (i.e., on the 5' side) from the transcription starting point (initiator; symbol: $\rightarrow$). Other transcription factors (TFs) such as *CTF* and *SP1* support formation of the basal complex, after they have bound to DNA elements some distance away ("**CAAT box**" and "**GC box**"). In addition to these elements, which are present in most promoters, there are other,

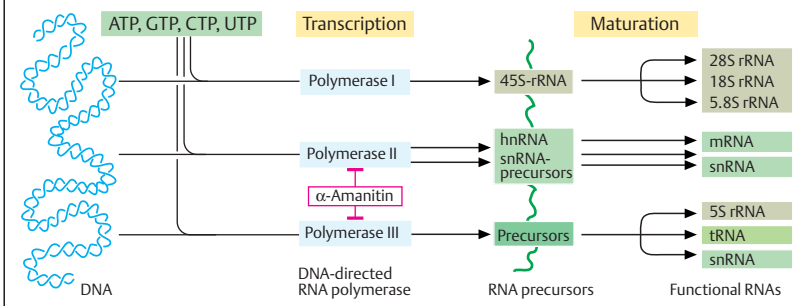
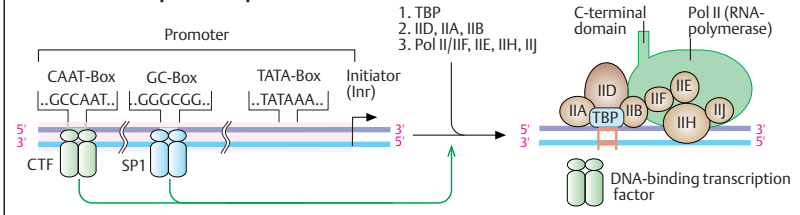
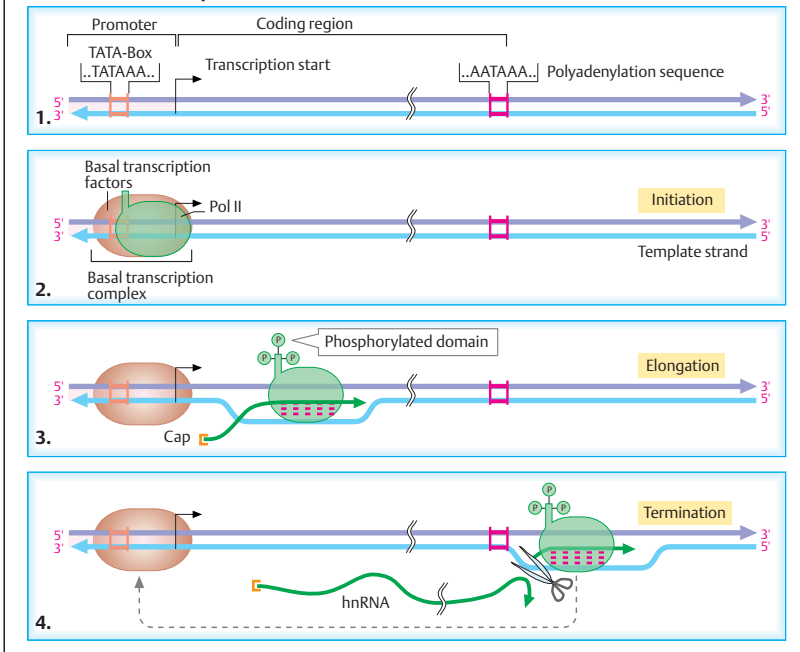
gene-specific control elements, which are involved in the fine regulation of transcription (p. 242).

Because these "boxes" are located on the genes that are to be transcribed, they are called *cis-active elements*. By contrast, the transcription factors are proteins coded by completely different genes and they are therefore known as *trans-active factors*.

To generate the basal complex, factor TFIID first binds to the promoter. TFIID, a combination of numerous proteins, contains a *TATA box binding protein* (TBP) and *TBP-associated factors* or TAFs. RNA polymerase is fixed to this complex with the aid of TFIIB. Before starting transcription, other TFs are bound, including factor TFIIF, which has *helicase* activity and separates the DNA strands during elongation (C3). Roughly 35 different proteins are involved in the basal complex.

C. Process of transcription

At the end of **initiation** (2), the polymerase is repeatedly phosphorylated at a domain in the C-terminal region, frees itself from the basal complex, and starts moving along the DNA in the 5'→3' direction. The helicase activity of the complex separates a short stretch of the DNA double helix into two single strands. Then complementary nucleoside triphosphates are bound by base-pairing to the *template strand* and are linked step by step to the transcript as it grows in the 5'→3' direction (elongation, 3). Shortly after the beginning of **elongation**, the 5' end of the transcript is protected by a "cap" (p. 244). Once the polyadenylation sequence has been reached (typical sequence: AATAA), the completed hnRNA ("heterogeneous nuclear RNA") is released (termination, 4). Shortly after this, the RNA polymerase stops transcribing and dissociates from the DNA.

A. Transcription and maturation of RNA: overview**B. Basal transcription complex****C. Process of transcription**

Transcriptional Control

Although all nucleated body cells contain the complete genome, they only use a fraction of the information coded in it. The genes known as "housekeeping genes," which code for structural molecules and enzymes of intermediate metabolism, are the only ones that undergo constant transcription. Most genes are only active in certain cell types, in specific metabolic conditions, or during differentiation.

Which genes are transcribed and which are not is regulated by **transcriptional control**. In order to be transcribed at all, genes have first to be exposed to the proteins involved by **chromatin remodeling** (p. 234). Fine control of transcription is then brought about by interactions between DNA elements (*control elements*, *cis*-active elements) in the gene's promoter region and regulatory proteins (transcription factors, *trans*-active factors), which bind to these elements and thereby activate or inhibit transcription (p. 240). Control elements with a positive action are called *enhancers*, and those with an inhibitory influence are called *silencers*.

A. Lac operon

The *lactose (lac) operon* of the bacterium *Escherichia coli*, which is subject to both negative and positive control, can serve as an example of transcriptional control in prokaryotes. The operon (p. 232) contains the *structural genes* for three proteins that are required for the utilization of lactose (one transporter and two enzymes), as well as *control elements* that serve to regulate the operon.

Since lactose is first converted to glucose in the cell, there is no point in expressing the genes if glucose is already available. Indeed, they are only transcribed when *glucose is absent* and *lactose is present* (3). This is achieved by interaction between two regulatory proteins. In the absence of lactose, the **lac repressor** blocks the promoter region by binding to the *operator* (2). When lactose is available, it is converted into the isomer *allolactose*, which binds to the repressor and thereby detaches it from the operator (3). However, this is still not sufficient for the transcription of the structural genes. For binding of the RNA polymerase to take place, an **inducer—catabolite activator protein (CAP)**—is required, which only binds to the DNA when it is present as a complex with 3',5'-cyclic-AMP (cAMP; p. 406). cAMP is

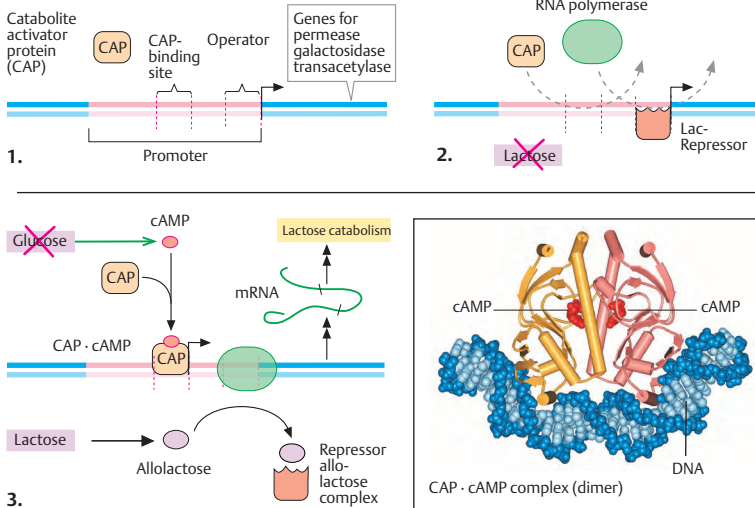
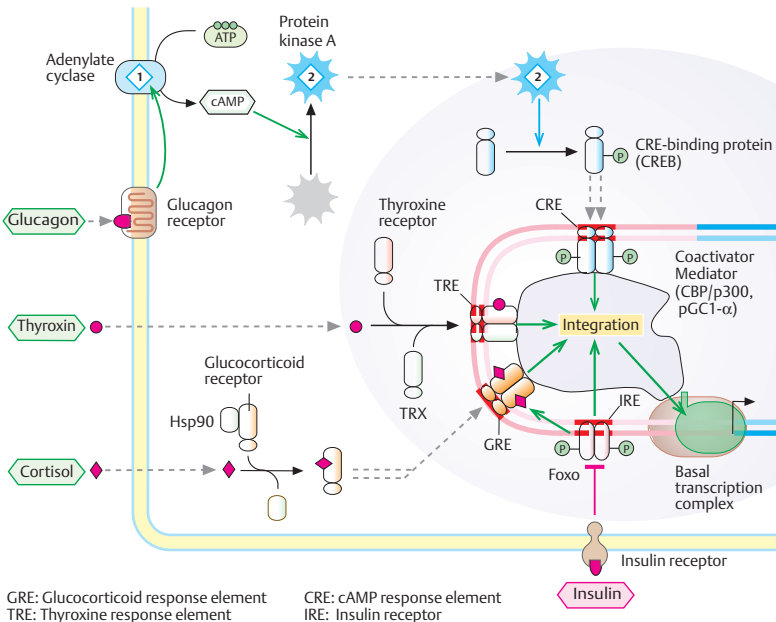
formed by *E. coli* in the *absence* of glucose and is therefore a *signal for nutrient deficiency*.

B. Regulation of PEP-CK transcription in the liver

Phosphoenolpyruvate carboxykinase (PEP-CK), a key enzyme in gluconeogenesis (p. 134), is regulated by several hormones. Cortisol, glucagon, and thyroxine promote transcription of the PEP-CK gene, while insulin inhibits it.

More than ten **control elements**, distributed over about 1000 bp, have so far been identified in the promoter of the PEP-CK gene (p. 232). These include hormone response elements (p. 418) for glucocorticoids (GRE) and thyroxine (TRE). While the glucocorticoid receptor, which is activated by **cortisol**, binds to GRE as a homodimer, the receptor for **thyroxine** has to dimerize with the receptorlike protein, TRX, before it can bind to TRE. The transcription factor, Foxo, stimulates the transcription of several enzymes involved in gluconeogenesis, including PEP-CK, by binding to insulin response elements (IRE). **Insulin** inhibits gluconeogenesis in part by inhibiting the production of active Foxo via protein kinase B (p. 428). Another control element, CRE (cAMP-responsive element) binds the transcription factor CREB (cAMP-responsive element binding protein). CREB is activated by cAMP-dependent protein kinase A through phosphorylation. This in turn is stimulated by **glucagon**, which raises the cAMP level via its G-protein-coupled receptor (p. 410f.).

All of these transcription factors are in contact with a **coactivator/mediator complex** known as **CBP/p300**, which integrates the individual inputs like a computer and transmits the result in the form of more or less strongly activating signals to the basal transcription complex (p. 240). In addition to p300, there is a range of other coactivators with a similar action, e.g., PGC-1 and SRC3.

A. Lac-operon**B. Regulation of PEP-CK transcription in the liver**

RNA Maturation

Before the hnRNA produced by RNA polymerase II (p. 240) can leave the nucleus to serve as a template for protein synthesis in the cytoplasm, it has to undergo several modifications first. Even during transcription, the two ends of the transcript have additional nucleotides added (**A**). The sections that correspond to the intervening gene sequences in the DNA (introns) are then cut out (splicing; **B**). Other transcripts—e.g., the 45S precursor of rRNA formed by polymerase I—are broken down into smaller fragments by nucleases before export into the cytoplasm.

A. 5' and 3' modification of mRNA

Shortly after transcription begins in eukaryotes, the end of the growing RNA is blocked in several reaction steps by a structure known as a “**cap**.” In hnRNAs, this consists of a GTP residue that is methylated at N-7 of the guanine ring. The γ -phosphate residue of the cap is linked to the free 5'-OH group of the terminal ribose via an ester bond. After the “polyadenylation signal” has been reached (typical sequence: ...AAUAAA...; p. 240), a **polyadenylate “tail”** consisting of up to 200 AMP nucleotides is also added at the free 3' end of the transcript. This reaction is catalyzed by a special **polyadenylate polymerase**. It is only after these steps that the mRNA leaves the nucleus as a complex with RNA-binding proteins.

Both the cap and the poly-A tail play a vital part in initiating eukaryotic translation (p. 248) by helping to position the small ribosome subunit correctly on the mRNA. The protection which the additional nucleotides provide against premature enzymatic degradation appears to be of lesser importance.

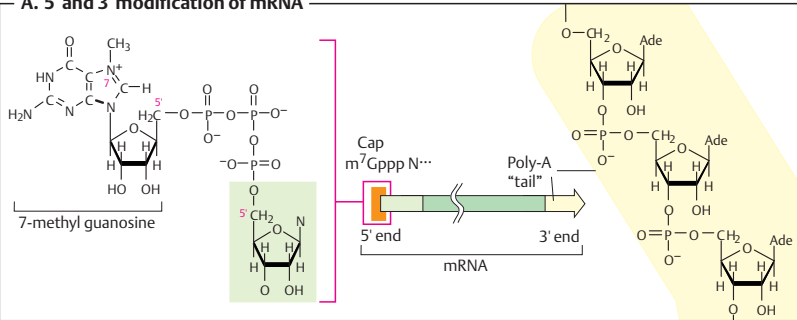
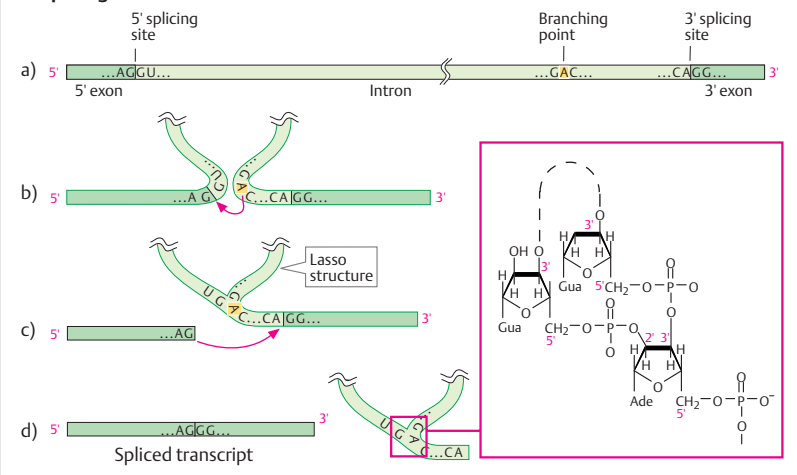
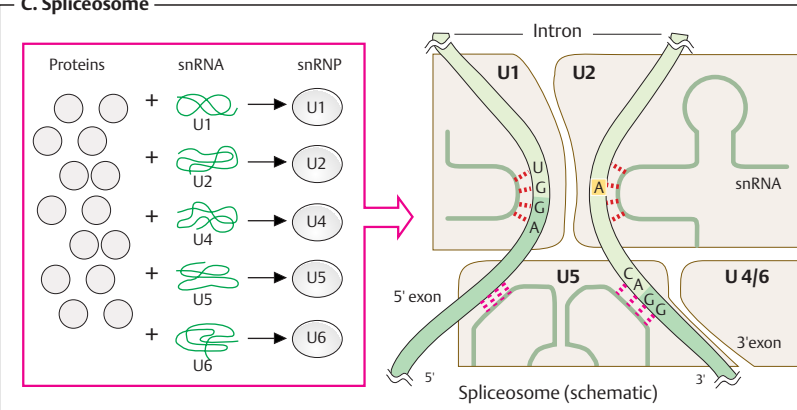
B. Splicing of hnRNA: mechanism

Immediately after transcription, the hnRNA introns are removed and the exons are linked to form a continuous coding sequence. This process, known as **splicing**, is supported by complicated RNA-protein complexes in the nucleus, the so-called **spliceosomes**. The components of these macromolecular machines are called **snRNPs** (*small nuclear ribonucleoprotein particles*, pronounced “snurps”). SnRNPs occur in five different forms (U1, U2, U4, U5, and U6). They consist of numerous **proteins** and one molecule of **snRNA** each (see p. 66).

To ensure that the RNA message is not destroyed, splicing has to take place in a very precise fashion. The start and end of the hnRNA introns are recognized by characteristic sequences (...AGGT... at the 5' end or ...[C,U]AGG... at the 3' end). Another important structure is the so-called **branching point** inside the intron. Its sequence is less conserved than the terminal splicing sites, but it always contains one adenosine residue (**A**). During splicing, the 2'-OH group of this residue—supported by the spliceosome (see **C**)—attacks the phosphate diester bond at the 5' end of the intron and cleaves it (**b**). Simultaneously, an unusual 2'→5' bond is formed inside the intron, which thereby takes on a **lasso shape** (**c**; see formula on right). In the second step of the splicing process, the free 3'-OH group at the end of the 5' terminal exon attacks the A-G bond at the 3' end of the intron. As a result, the two exons are linked and the intron is released, still in a lasso shape (**d**).

C. Spliceosome

As described above, it is residues of the hnRNA that carry out bond cleavage and bond formation during the splicing process. It is therefore not a protein enzyme that acts as a catalyst here, but rather RNA itself. Catalytic RNAs of this type are called **ribozymes** (see also p. 72). The task of the spliceosomes is to fix and orientate the reacting groups by establishing base pairings between snRNAs and segments of the hnRNA. The probable situation before the adenosine attack at the branching point on the 5' splicing site (see [**b**] in **B**) is shown schematically on the right side of the illustration. In this phase, the U1 snRNA fixes the 5' splicing site, U2 fixes the branching site, and U5 fixes the ends of the two exons.

A. 5' and 3' modification of mRNA**B. Splicing of hnRNA: mechanism****C. Spliceosome**

Genetic Code

A. The genetic code

Most of the relevant genetic information stored in the genome codes for amino acid sequences of proteins. For these proteins to be expressed, a text in “nucleic acid language” therefore has to be translated into “protein language.” This is the origin of the use of the term **translation** to describe protein biosynthesis. The dictionary used for the translation is the **genetic code**.

As there are 20 proteinogenic amino acids (p. 50; 21 if selenocysteine is counted, p. 52), the nucleic acid language has to contain at least as many words (**codons**) if all of them are to be named. However, there are only four letters in the nucleic acid alphabet (A, G, C, and U or T). To obtain 20 different words from these, each word has to be at least three letters long (with two letters, there would only be $4^2 = 16$ possibilities). And in fact the codons do consist of three sequential bases (**base triplets**).

The illustration shows the standard code in “RNA language” as a sequence of triplets in the RNA, read in the 5′→3′ direction, represented as a *circular diagram* (2). An example of how the code is read is shown on the left (1). The circular diagram is read from the inside to the outside. For example, the triplet CAU (or CAT) in DNA codes for the amino acid histidine. With the exception of the exchange of U for T, the DNA codons on the *sense strand* (p. 68) are identical to those of mRNA.

As the genetic code provides $4^3 = 64$ codons for the 20 amino acids, there are several synonymous codons for most amino acids—the code is **degenerate**—and accordingly, there are several tRNAs for each amino acid. The different triplets for a given amino acid differ only in the 3rd position. Therefore, point mutations of these “wobble” nucleotides do not inevitably lead to an exchange of the coded amino acid.

There is only one codon for two of the amino acids, methionine and tryptophan, and the methionine codon **AUG** at the same time marks the start of translation (**start codon**). This is why all proteins synthesized on the ribosome have a methionine residue at the N-terminal initially, though this is usually removed later. Three other triplets (**UAA**, **UAG**, and **UGA**) do not code for amino acids, but instead signal the end of translation (**stop codons**). In certain conditions, UGA can also lead to incorporation of selenocysteine (Sec; p. 52).

The code shown here is almost universally applicable in nature; only the genomes of mito-

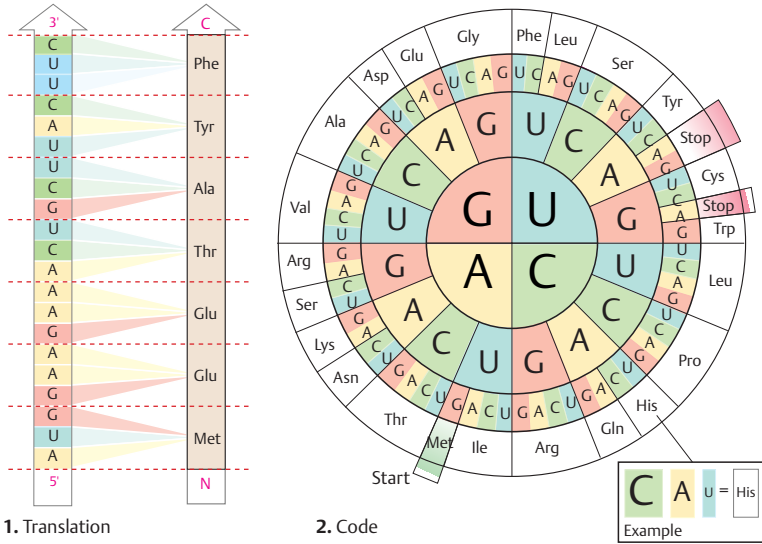
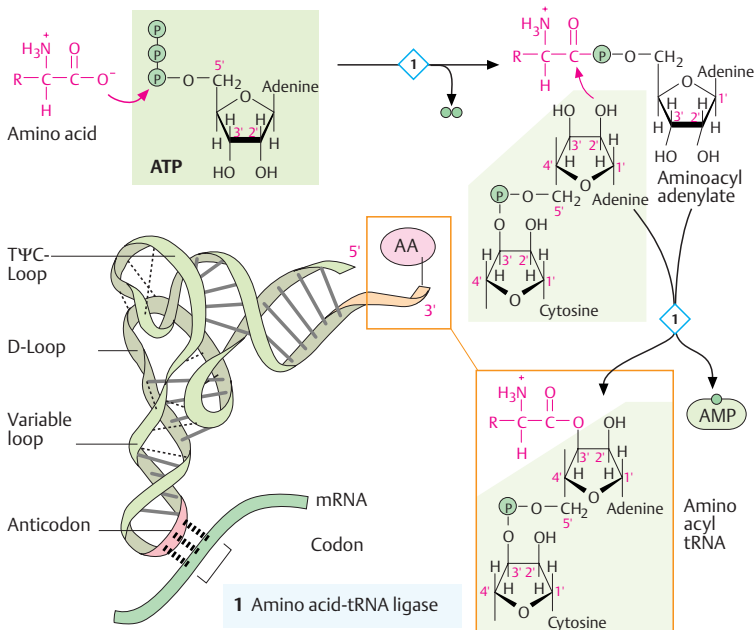
chondria (p. 126) and those of a few microorganisms deviate from it slightly. Thus, it is entirely possible for human proteins to be expressed in bacteria (p. 260).

B. Amino acid activation

For each of the 20 different amino acids, there is one type of *amino acid tRNA ligase* [1], which loads the various transfer RNAs (p. 66) with the corresponding amino acid. This reaction, known as **amino acid activation**, is an endergonic process and is therefore coupled to ATP cleavage in two steps.

First, the amino acid is bound by the enzyme and reacts there with ATP to form diphosphate and an “energy-rich” mixed acid anhydride (**aminoacyl adenylate**). In the second step, which is catalyzed by the same enzyme, the 3′-OH group of the terminal ribose residue of the tRNA (in other ligases it is the 2′-OH group) attacks the mixed anhydride bond of the aminoacyl adenylate. This nucleophilic substitution yields the loaded aminoacyl tRNAs and AMP. In **aminoacyl tRNAs**, the carboxyl group of the amino acid is esterified with the ribose residue of the terminal adenosine of the sequence ...CCA-3′.

The accuracy of translation primarily depends on the substrate specificity of the amino acid tRNA ligases, as incorrectly incorporated amino acid residues are not recognized by the ribosome later. A “*proofreading mechanism*” in the active center of the ligase therefore ensures that incorrectly incorporated amino acid residues are immediately removed again. On average, an error only occurs once every 1300 amino acid residues. This is a surprisingly low rate considering how similar some amino acids are—e.g., leucine and isoleucine.

A. The genetic code**B. Amino acid activation**

Translation I: Initiation

Like amino acid activation (p. 246), protein biosynthesis (**translation**) takes place in the cytoplasm. It is catalyzed by complex nucleoprotein particles, the **ribosomes**, and mainly requires GTP to cover its energy requirements.

A. Structure of eukaryotic ribosomes

Ribosomes consist of two subunits of different size, made up in turn of **ribosomal RNA (rRNA)**; p. 66) and a large number of relatively small ribosomal **proteins** (the numbers in the illustration apply to rat liver ribosomes). It is customary to give the **sedimentation coefficients** of ribosomes and their components instead of their masses. These can be calculated from their behavior during centrifugation. For example, a complete eukaryotic ribosome has a sedimentation coefficient of about 80 Svedberg units (80S), while those of its subunits are 40S and 60S (S values are not additive).

Prokaryotic ribosomes have a similar structure but are somewhat smaller than those of eukaryotes (70S for a complete ribosome, 30S and 50S for the subunits, p. 66). The ribosomes of mitochondria (p. 126) and plant chloroplasts are similar to those of the prokaryotes.

The small **40S subunit** in eukaryotes consists of one molecule of 18S rRNA and 33 proteins. The larger **60S subunit** contains three types of rRNA with sedimentation coefficients of 5S, 5.8S, and 28S, and 49 proteins. In the presence of mRNA and other factors (see **B**), the subunits assemble to form the complete ribosome, with a mass of roughly 4200 kDa, about 650 times larger than that of a hemoglobin molecule.

The arrangement of the individual components of a ribosome has now been determined for some bacterial ribosomes. It is known that filamentous mRNA passes through a cleft between the subunits near the characteristic "horn" on the small subunit. tRNAs also bind near this site. The illustration also shows the size of a tRNA molecule for comparison (green part in the center of the ribosome).

In cells that are carrying out intensive protein synthesis, ribosomes are often found in a linear arrangement like a string of pearls; these are known as **polysomes**. This arrangement arises because several ribosomes are translating a single mRNA molecule simultaneously. The ribosomes first bind at the 5' end and

move during translation in the direction of the 3' end until they reach a **stop codon** and are released again from the mRNA.

B. Initiation of translation

Translation starts with an **initiation phase**. In eukaryotes, this leads in several steps to the **80S initiation complex** (bottom right) in which the starter tRNA loaded with methionine (Met-tRNA^{Met}) is bound to the start codon of the mRNA.

Initiation is supported by numerous initiation factors, of which *initiation factors* eIF-1 to eIF-4 are particularly important. **Initiation factors** eIF-1 and eIF-3 first bind to the small 40S subunit. Another factor (eIF-2) binds as a complex with GTP to the Met-tRNA, which then combines with the 40S subunit to form a **start complex** (center). At the same time, the mRNA exported from the nucleus (p. 244), which has until this time been protected from degradation by bound proteins (not shown), is activated by factor eIF-4; this binds to the "cap" at the 5' end of the mRNA (p. 244). The activated mRNA then associates with the start complex, but in such a way that the initiator tRNA cannot yet make contact with the start codon. In the next step, the entire complex, consisting of 40S subunit, Met-tRNA^{Met}, and the bound factors, moves along the mRNA in the 3' direction until the tRNA reaches the start codon AUG. This ATP-dependent process is called "**scanning**."

Finally, the 60S subunit binds to the complex. The initiation factors are released again, and the GTP bound to eIF-2 is hydrolyzed to GDP and P_i. In the **80S initiation complex**, the methionine tRNA^{Met} is located at a binding site known as the **peptidyl site (P)**. A second binding site, the **acceptor site (A)**, is not yet occupied during this phase of translation (see p. 250).

Translation II

After translation has been initiated (p. 248), the peptide chain is extended by the addition of further amino acid residues (**elongation**) until the ribosome reaches a stop codon on the mRNA and the process is interrupted (**termination**).

A. Elongation and termination of protein biosynthesis

Elongation can be divided into four phases:

- [1] **Binding of aminoacyl tRNA.** At the start of the elongation cycle, the *peptidyl site* (P) of the ribosome is occupied by a tRNA that carries at its 3' end the complete peptide chain formed up to this point (top left). Another tRNA, loaded with the next amino acid (Val-tRNA^{val} in the example shown), then binds via its complementary anticodon (pp. 66, 246) to the mRNA codon exposed at the *acceptor site* (in this case GUG). The tRNA binds as a complex with a GTP-containing protein, the *elongation factor eEF-1 α* . It is only after GTP bound to the factor has been hydrolyzed to GDP and phosphate that eEF-1 α dissociates again. As the binding of the tRNA to the mRNA is still loose before this, GTP hydrolysis acts as a delaying factor, making it possible to check whether the correct tRNA has been bound. A further protein, the *elongation factor eEF-1 β* , later catalyzes the exchange of GDP for GTP and in this way regenerates the eEF-1 α -GTP complex. eEF-1 α is related to the G-proteins involved in signal transduction (p. 404).
- [2] **Synthesis of the peptide bond** takes place in the next step. Ribosomal *peptidyltransferase* catalyzes (without consumption of ATP or GTP) the transfer of the peptide chain from the tRNA at the P-site to the terminal NH₂ group of the amino acid residue of the tRNA at the A-site. The ribosome's peptidyltransferase activity is not located in one of the ribosomal proteins, but in the 28S rRNA. Catalytically active RNAs of this type are known as *ribozymes* (cf. p. 72). It is thought that the few surviving ribozymes are remnants of the "RNA world"—an early phase of evolution in which proteins were not as important as they are today.

- [3] After the transfer of the growing peptide to the A-site, the free tRNA at the P-site dissociates and another GTP-containing elongation factor (eEF-2·GTP) binds to the ribosome. Hydrolysis of the GTP in this factor provides the energy for **translocation**. During this process, the entire ribosome moves three bases along the mRNA in the direction of the 3' end. The tRNA carrying the peptide chain is stationary relative to the mRNA and reaches the ribosome's P-site during translocation, while the next mRNA codon (in this case GUG) appears at the A-site.

The now unloaded Val-tRNA then dissociates from the E-site. The ribosome is now ready for the next elongation cycle. When one of the three stop codons (UAA, UAG, or UGA) appears at the A site instead of an amino acid codon, **termination** starts.

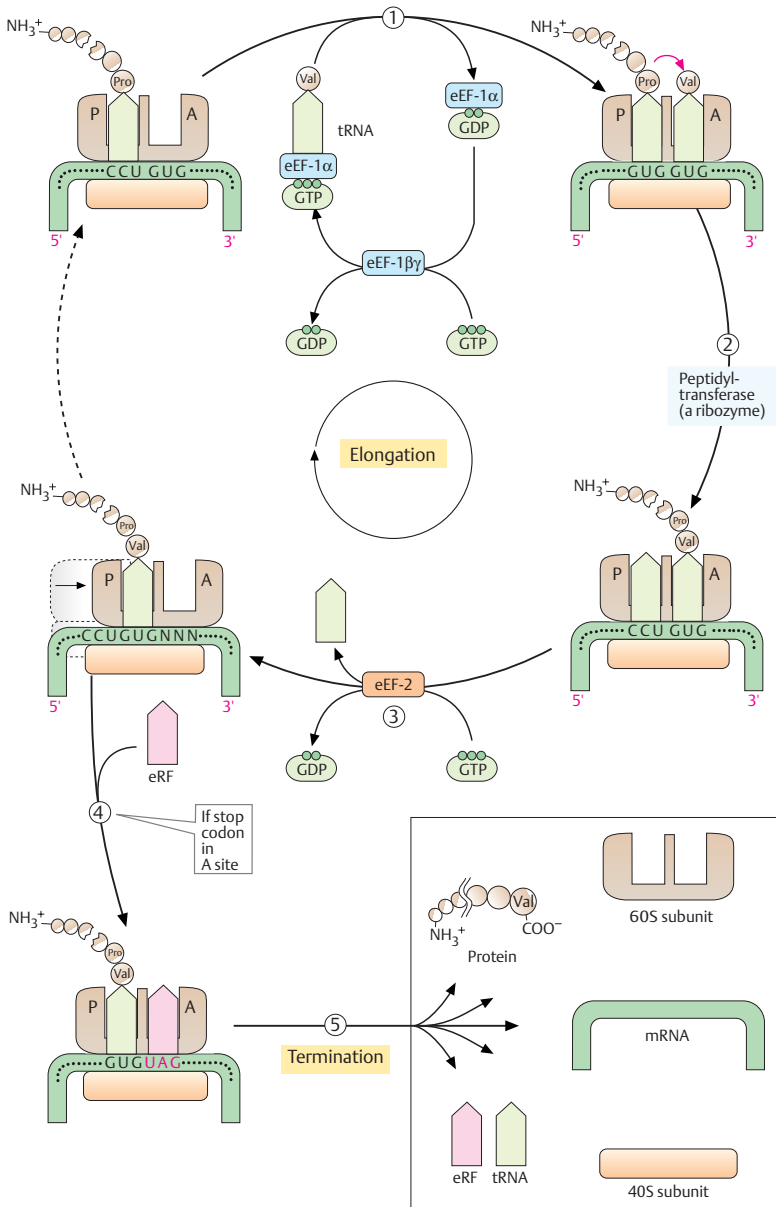
- [4] There are no complementary tRNAs for the stop codons. Instead, a *releasing factor* (eRF) binds to the ribosome. It catalyzes hydrolytic cleavage of the ester bond between the tRNA and the C-terminus of the peptide chain, thereby releasing the protein.
- [5] Hydrolysis of GTP by factor eRF supplies the energy for the dissociation of the whole complex into its component parts, which can enter initiation again.

Energy requirements in protein synthesis are high. Four energy-rich phosphoric acid anhydride bonds are hydrolyzed for each amino acid residue. Amino acid activation uses up two energy-rich bonds per amino acid (ATP → AMP + PP_i; p. 246), and two GTPs are consumed per elongation cycle. In addition, initiation and termination each require one GTP per chain.

■ Further information

In prokaryotic cells, the elongation and termination of translation are similar to in eukaryotes, while initiation has a somewhat different pathway. In prokaryotes, the ribosome binds to a particular binding site on the mRNA. Moreover, the starter tRNA contains *N*-formyl-methionine (fMet) and not methionine. Scanning (p. 248) does not take place. Several steps of bacterial translation are the sites of action of important antibiotics (p. 252).

A. Elongation and termination of protein biosynthesis



Antibiotics

Antibiotics are substances which, even at low concentrations, inhibit the growth or reproduction of bacteria and fungi. The treatment of infectious diseases would be inconceivable today without antibiotics. Substances that only restrict the reproduction of bacteria are described as **bacteriostatic** (or **fungistatic** for fungi). If the target cells are not only inhibited from growing but are killed, then the term **bactericidal** (or **fungicidal**) is used.

A. Antibiotics: overview

Almost all natural antibiotics are produced by microorganisms—mainly bacteria of the genus *Streptomyces* and certain fungi. However, there are also synthetic antibacterial substances, such as sulfonamides or gyrase inhibitors. The illustration shows therapeutically important antibiotics and their sites of action in bacterial metabolism.

Substances known as **intercalators**, such as *daunomycin* and *actinomycin D* (center), fit in between two base pairs of the DNA double helix and thereby interfere with both replication and transcription. As DNA is the same in all cells, intercalating antibiotics are also extremely toxic for eukaryotes, however. They are therefore used exclusively as cytostatic agents (p. 454). Synthetic inhibitors of bacterial DNA topoisomerase II (p. 238), known as **gyrase inhibitors** (center), restrict replication of bacteria and thus their reproduction.

A large group of antibiotics attack the bacterial ribosome. The **inhibitors of translation** (left) include the **tetracyclines**—broad-spectrum antibiotics that are effective against a large number of different pathogens. The **aminoglycosides**, of which *streptomycin* is the best-known, affect all phases of translation. *Erythromycin* impairs the normal function of the large ribosomal subunit. *Chloramphenicol*, one of the few natural nitro compounds, inhibits ribosomal peptidyltransferase. Finally, *puromycin* mimics an aminoacyl tRNA and therefore leads to premature interruption of elongation.

The **β -lactam antibiotics** (lower right) are also frequently used. The members of this group, the **penicillins** and **cephalosporins**, are synthesized by fungi and have a reactive β -lactam ring. They are mainly active against Gram-positive pathogens, in which they inhibit cell wall synthesis (see B).

The first purely synthetic antibiotics were the **sulfonamides** (upper right). As analogues of *p*-aminobenzoic acid, these affect the synthesis of *folic acid*, an essential precursor of the coenzyme tetrahydrofolic acid (THF; p. 90). **Transport antibiotics** such as *gramicidin* or *valinomycin* (upper center) have the properties of ion channels (p. 402). When they are deposited in the plasma membrane of bacteria, it leads to a loss of ions that damages the cells.

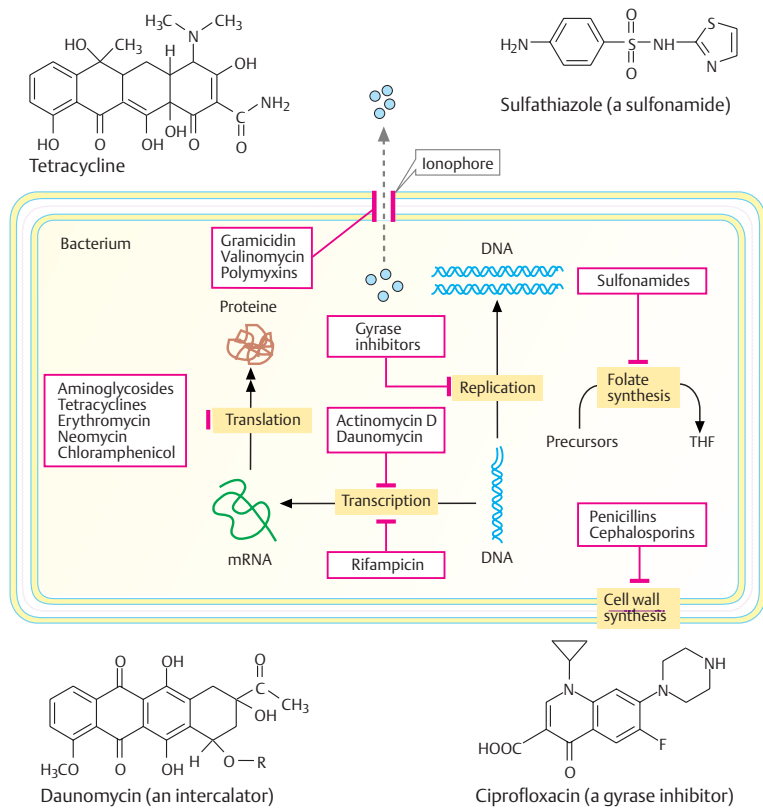
B. Penicillin as a “suicide substrate”

The site of action of the β -lactam antibiotics is an enzyme that is essential for the formation of bacterial cell walls. In Gram-positive bacteria, the cell wall consists of long chains of the polysaccharide murein, which are cross-linked by short peptides. One of the steps in the cross-linking process is catalyzed by *muramoylpentapeptide carboxypeptidase*. Penicillin and the other β -lactam antibiotics resemble the substrate of this peptidase, which is a peptide with the C-terminal sequence D-Ala–D-Ala, and are therefore reversibly bound in the active center of the enzyme. This brings the β -lactam ring into proximity to an essential serine residue of the enzyme. Nucleophilic substitution then results in the formation of a stable covalent bond between the enzyme and the inhibitor, blocking the active center. Inhibitors of this type are called “**suicide substrates**” as they are bound by the enzyme-like substrates before they inactivate it irreversibly. In dividing bacteria, the loss of carboxypeptidase activity leads to the formation of unstable cell walls so that the bacteria break up.

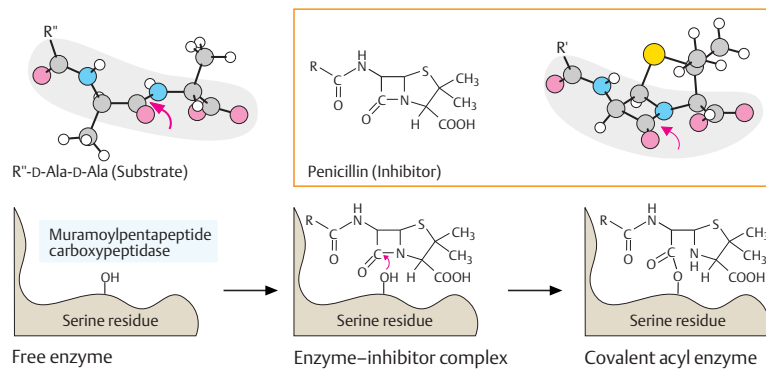
■ Further information

A constantly increasing problem in antimicrobial treatment is the development of resistant pathogens that no longer respond to classical antibiotics. The major causes of this development are the massive use of antibiotics in animal husbandry and therapeutic errors that favor the selection of resistant strains.

A. Antibiotics: overview



B. Penicillin as a “suicide substrate”



Mutation and Repair

Since genetic information is set down in the base sequence of DNA, changes in DNA bases or their sequence usually have **mutagenic** effects. Many mutagens also damage growth regulation in cells, and they are then also **carcinogenic** (p. 452).

Gene alterations (**mutations**) are among the decisive positive factors in biological evolution. On the other hand, an excessive mutation frequency would threaten the survival of individual organisms or entire species. For this reason, every cell has **repair mechanisms** that eliminate most of the DNA changes arising from mutations (C).

A. Mutagenic agents

Mutations can arise as a result of physical or chemical effects, or they can be due to accidental errors in DNA replication and recombination.

The principal physical mutagen is **ionizing radiation** (γ radiation, X-rays, 1). In cells, it produces **free radicals** (molecules with unpaired electrons), which are extremely reactive and can damage DNA. Short-wavelength **ultraviolet light** (UV light) also has mutagenic effects, mainly in skin cells (sunburn). The most common chemical change due to UV exposure is the formation of **thymine dimers**, in which two neighboring thymine bases become covalently linked to one another (2). This results in errors when the DNA is read during replication and transcription.

Only a few examples of the group of **chemical mutagens** are shown here. *Nitrous acid* (HNO_2 ; salt: nitrite) and *hydroxylamine* (NH_2OH) both deaminate bases and thereby convert cytosine to uracil and adenine to inosine.

Alkylating compounds carry reactive groups that can form covalent bonds with DNA bases (see also p. 454). *Methylnitrosamines* (3) release the reactive methyl cation (CH_3^+), which methylates OH and NH_2 groups in DNA. The dangerous carcinogen *benzo(a)pyrene* is an aromatic hydrocarbon that is only converted into the active form in the organism (4). Multiple hydroxylation of one of the rings produces a reactive epoxide that can react with NH_2 groups in guanine residues, for example. Free radicals of benzo(a)pyrene also contribute to its toxicity.

B. Effects of mutagens

Nitrous acid causes **point mutations** (1). For example, base C is converted to U, which in the next replication pairs with A instead of G. The alteration thus becomes permanent. Mutations in which several nucleotides not divisible by three are inserted or removed lead to reading errors in whole segments of DNA, as they move the reading frame (**frameshift mutations**). This is shown in the figure (2) using a simple example. From the inserted C onwards, the resulting mRNA is interpreted differently during translation, producing a completely new protein sequence.

C. Repair mechanisms

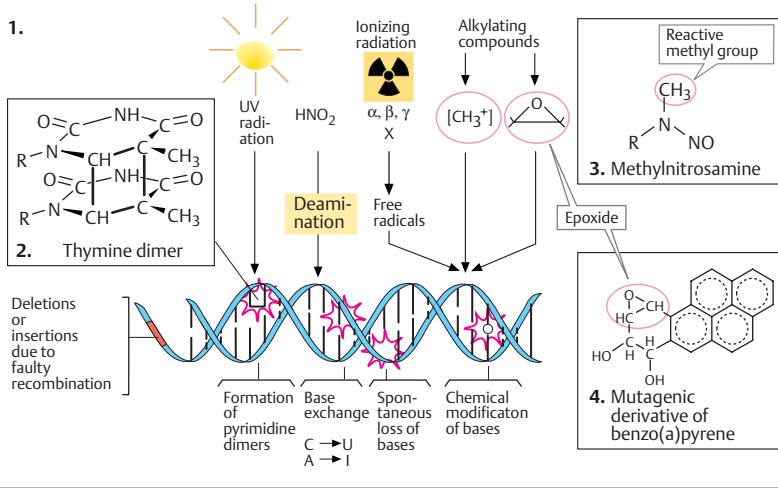
An important mechanism for the removal of DNA damage is **excision repair** (1). In this process, a specific *excision endonuclease* removes a complete segment of DNA on both sides of the error site. Using the sequence of the opposite strand, the missing segment is then replaced by a *DNA polymerase*. Finally, a *DNA ligase* closes the gaps again.

Thymine dimers in the skin can be removed by **photoreactivation** (2). A specific *photolyase* binds at the defect and, when illuminated, cleaves the dimer to yield two single bases again.

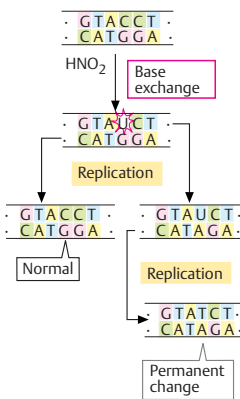
A third mechanism is **recombination repair** (3, shown in simplified form). In this process, the defect is skipped during replication. The gap is closed by shifting the corresponding sequence from the correctly replicated second strand. The new gap that results is then filled by polymerases and ligases. Finally, the original defect is corrected by excision repair as explained above (1) (not shown).

Absence of repair enzymes can cause severe disease. In the rare condition *xeroderma pigmentosum*, defects in excision repair lead to radiation damage of the skin and a greatly increased cancer risk.

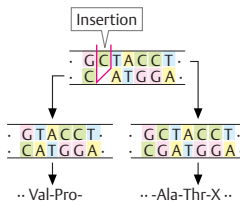
A. Mutagenic agents



B. Effects

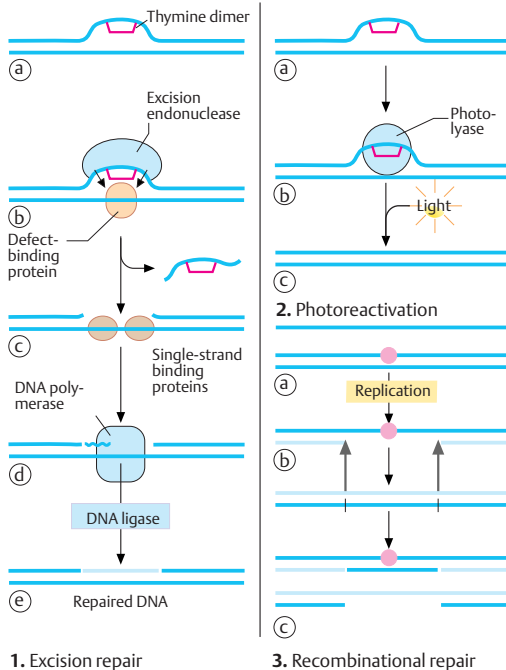


1. Point mutation



2. Frameshift mutation

C. Repair mechanisms



DNA Cloning

The growth of molecular genetics since 1970 has mainly been based on the development and refinement of methods of analyzing and manipulating DNA. **Genetic engineering** has practical applications in many fields. It has provided new methods of diagnosing and treating diseases (B, see also p. 262), and it is now also possible to create targeted changes in specific characteristics of organisms. For example, the susceptibility of crop plants to pests can be reduced by insertion of additional genes. Since biological risks cannot be completely ruled out with genome interventions, it is particularly important to act responsibly when dealing with genetic engineering. A short overview of important methods involved in genetic engineering is provided here and on the following pages.

A. DNA cloning

Most DNA segments—e.g., genes—occur in very small quantities in the cell. To be able to work with them experimentally, a large number of identical copies (“**clones**”) first have to be produced. The classic procedure for cloning DNA takes advantage of the ability of bacteria to take up and replicate short, circular DNA fragments known as **plasmids**.

The segment to be cloned is first cut out of the original DNA using suitable **restriction endonucleases** (p. 236). For the sake of simplicity, cleavage using one enzyme alone (the endonuclease *EcoRI*) is shown here, but in practice two different enzymes are usually used. As a vehicle (“**vector**”), a plasmid is needed that has only *one EcoRI* cleavage site. The plasmid rings are first opened by cleavage with *EcoRI* and then mixed with the isolated DNA fragments. Since the fragment and the vector have the same overhanging ends (p. 236), some of the molecules are hybridized in such a way that the fragment being cloned is incorporated into the vector DNA. When the cleavage sites are now closed again using **DNA ligase**, a newly combined (“**recombinant**”) plasmid arises.

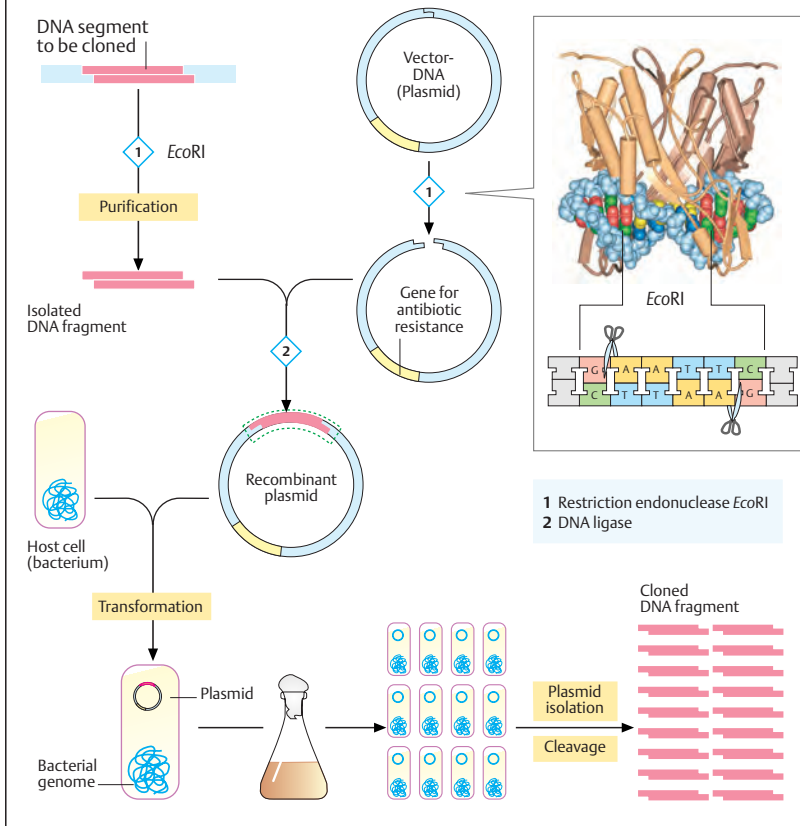
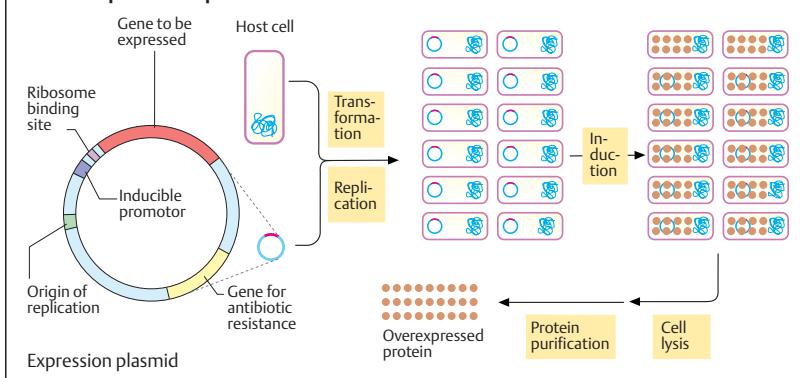
By pretreating a large number of host cells, one can cause some of them to take up the plasmid (a process known as **transformation**) and replicate it along with their own genome when reproducing. To ensure that only bacteria that contain the plasmid replicate, plasmids are used that give the host cells **resistance** to a particular antibiotic. When the bacteria are incubated in the presence of this antibiotic, only

the cells containing the plasmid will then replicate. The plasmid is then isolated from these cells after multiplication, cleaved with *EcoRI* again, and the fragments are separated using agarose gel electrophoresis (p. 260). The desired fragment can be identified by its size and then extracted from the gel and used for further experiments.

B. Overexpression of proteins

To treat some diseases, proteins are needed that occur in such small quantities in the body that isolating them on a large scale would be uneconomical or impossible. Proteins of this type can be obtained by **overexpression** in bacteria or eukaryotic cells. The corresponding mRNA is isolated and copied to DNA using a **reverse transcriptase** (p. 236); the resulting **cDNA** (copy DNA) is then cloned into an **expression plasmid** as described above (A).

Apart from the gene to be expressed, such plasmids must also contain a **replication origin** (p. 238), which enables their replication by the host cell. An inducible promoter must also be present for targeted gene transcription, e.g., the *lac* promoter (p. 242). Following **transformation** and **multiplication** of suitable host cells, efficient **transcription** of the gene is triggered by **induction**. **Translation** of the mRNA in the host cell then produces the desired protein in large quantities. Examples of proteins produced this way include human insulin (p. 428), plasminogen activators to dissolve blood clots (p. 296), and the growth hormone somatotropin (p. 432).

A. DNA cloning**B. Overexpression of proteins**

DNA Sequencing

A. Gene libraries

It is often necessary in genetic engineering to isolate a DNA segment when its details are not fully known—e.g., to determine its nucleotide sequence. In this case, one can use what are known as **DNA libraries**. A DNA library consists of a large number of *vector DNA molecules* containing different fragments of *foreign DNA*. For example, it is possible to take all of the mRNA molecules present in a cell and transcribe them into DNA. These DNA fragments (known as copy DNA or **cDNA**) are then randomly introduced into vector molecules.

A library of genomic DNA can be established by cleaving the total DNA from a cell into small fragments using restriction endonucleases (p. 236), and then incorporating these into vector DNA. Suitable vectors for gene libraries include **bacteriophages**, for example (“phages” for short). Phages are viruses that only infect bacteria and are replicated by them (p. 456). Gene libraries have the advantage that they can be searched for specific DNA segments, using hybridization with oligonucleotides.

The first step is to strongly dilute a small part of the library (10^5 – 10^6 phages in a small volume), mix it with host bacteria, and plate out the mixture onto nutrient medium. The bacteria grow and form a continuous cloudy layer of cells. Bacteria infected by phages grow more slowly. In their surroundings, the bacterial “lawn” is less dense, and a clearer circular zone known as a **plaque** forms. The bacteria in this type of plaque exclusively contain the offspring of a single phage from the library.

The next step is to make an impression of the plate on a plastic sheet, which is then heated. This causes the phage DNA to adhere to the plastic. When the sheet is incubated with a DNA fragment that hybridizes to the DNA segment of interest (a **gene probe**), the probe binds to the sites on the imprint at which the desired DNA is attached. Binding of the gene probe can be detected by prior radioactive or other labeling of the probe. Phages from the positive plaques in the original plate are then isolated and replicated. Restriction cleavage finally provides large amounts of the desired DNA.

B. DNA sequencing

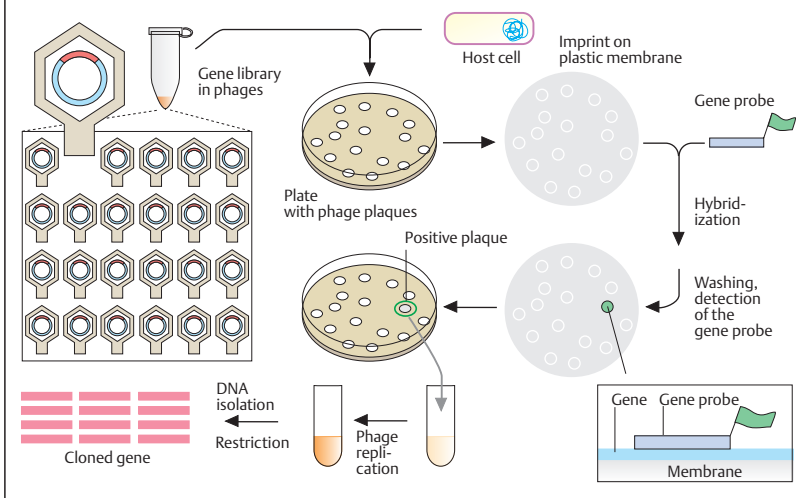
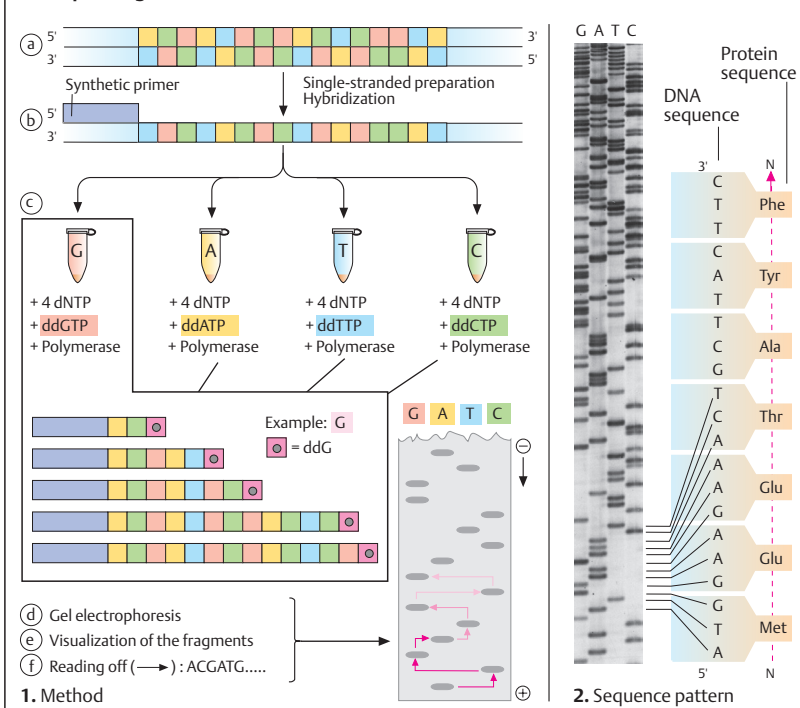
The nucleotide sequence of DNA is often determined using the so-called **chain termination method** (1). In single-strand sequencing, the

DNA fragment to be sequenced (a) is cloned in a suitable vector, e.g., in *bacteriophage M13* (p. 456), from which the coded single strand can be easily isolated. This is **hybridized** with a **primer**—a short, synthetically produced DNA fragment that binds to the 3' end of the introduced DNA segment (b).

Based on this hybrid, the missing second strand can now be generated in the test tube by adding the four *deoxyribonucleoside triphosphates* (**dNTP**) and a suitable *DNA polymerase* (c). The trick lies in also adding small amounts of *dideoxynucleoside triphosphates* (**ddNTPs**). Incorporating a ddNTP leads to the **termination of second-strand synthesis**. This can occur whenever the corresponding dNTP ought to be incorporated. The illustration shows this in detail using the example of ddGTP. In this case, fragments are obtained that each include the primer plus 3, 6, 8, 13, or 14 additional nucleotides. *Four separate reactions*, each with a different ddNTP, are performed (c), and the products are placed side by side on a supporting material. The fragments are then separated by *gel electrophoresis* (d; see also p. 260), in which they move according to their lengths.

Following **visualization** (e), the sequence of the fragments in the individual lanes is simply read from bottom to top (f) to directly obtain the nucleotide sequence. Details from such a sequencing gel and the corresponding protein sequence are shown in the figure (2).

In the automated dideoxy sequencing used nowadays, the four ddNTPs are covalently marked with fluorescent dyes, which produce a different color for each ddNTP on laser illumination. This allows the sequence in which the individual fragments appear at the lower end of the gel to be continuously recorded. More recently, entirely different approaches were developed that greatly accelerate DNA sequencing.

A. Gene libraries**B. Sequencing of DNA**

Polymerase Chain Reaction (PCR)

A. DNA electrophoresis

Gel electrophoresis is a technically simple but very efficient process for separating DNA fragments. The mobility of molecules in an electrical field of a given strength depends on the size and shape of the molecules as well as their charge. In contrast to proteins, in which all three factors vary, the ratio of mass to charge is constant in nucleic acids, as all of the nucleotide components have similar masses and carry one negative charge. When electrophoresis is performed in a wide-meshed matrix that does not separate according to size and shape, the mobility of the molecules depends on their mass alone. The supporting material generally used in genetic engineering is a gel of the polysaccharide **agarose** (see p. 32). Agarose gels are not very stable and are therefore poured horizontally into a plastic chamber in which they are used for separation.

To make the separated fragments visible after electrophoresis the gels are placed in solutions of **ethidium bromide**. This is an intercalator (p. 252) that shows pink fluorescence in UV light after binding to DNA, while it barely fluoresces in aqueous solution. The right side of the illustration shows the result of electrophoresis of two DNA fragments of different length (lanes 1 and 2). Comparing their distances with those of polynucleotides of known length (lane 3, bp = base pairs) yields lengths of about 800 bp for fragment 1 and 1800 bp for fragment 2. After staining, the bands can be cut out of the gel and the DNA can be extracted from them and used for other experiments.

B. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is an important procedure in genetic engineering that allows any DNA segment to be replicated (**amplified**) without the need for restriction enzymes, vectors, or host cells (p. 256). However, the nucleotide sequence of the segment has to be known. Two oligonucleotides (**primers**) are needed, which each hybridize with one of the strands at each end of the DNA segment to be amplified; also needed are sufficient quantities of the four deoxyribonucleoside triphosphates and a special heat-tolerant **DNA polymerase**. The primers are produced by chemical synthesis, and the polymerase is obtained from thermostable bacteria.

First, the sample is heated to around 90°C to separate the DNA double helix into single

strands (a). The mixture is then cooled to allow hybridization of the primers (b). Starting from the primers, complementary DNA strands are now synthesized in both directions by the polymerase (c). This **cycle** (cycle 1) is **repeated 20–30 times** with the same reaction mixture (cycle 2 and subsequent cycles). The cyclic heating and cooling are performed by **computer-controlled thermostats**.

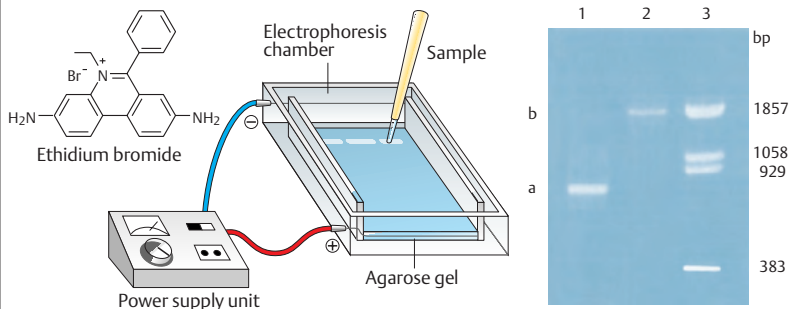
After only the third cycle, double strands start to form with a length equal to the distance between the two primers. The proportion of these approximately doubles during each cycle, until almost all of the newly synthesized segments have the correct length.

C. DNA typing

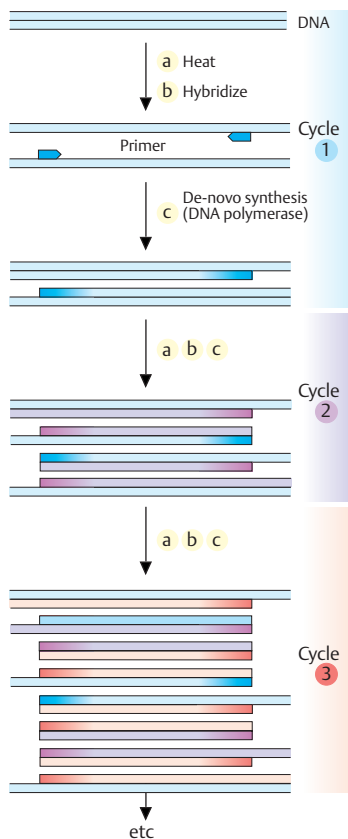
The possibility of amplifying tiny amounts of DNA by PCR forms the basis of DNA typing. This process is used to link small amounts of biological material, e.g., traces from the site of a crime, to a specific person.

The method employed today is based on the fact that the human genome contains noncoding repetitive DNA sequences (p. 232), the length of which varies slightly from individual to individual. These include **short tandem repeats (STRs)**, in which dinucleotides (e.g., -T-X-) are frequently repeated. Each STR occurs in 5–15 different lengths (alleles), of which each individual possesses only one or two. When the various allele combinations for several STRs are determined after PCR amplification of the DNA being investigated, a “**genetic fingerprint**” of the individual from whom the DNA originates is obtained. Using comparative material, e.g., saliva samples, definite identification is then possible.

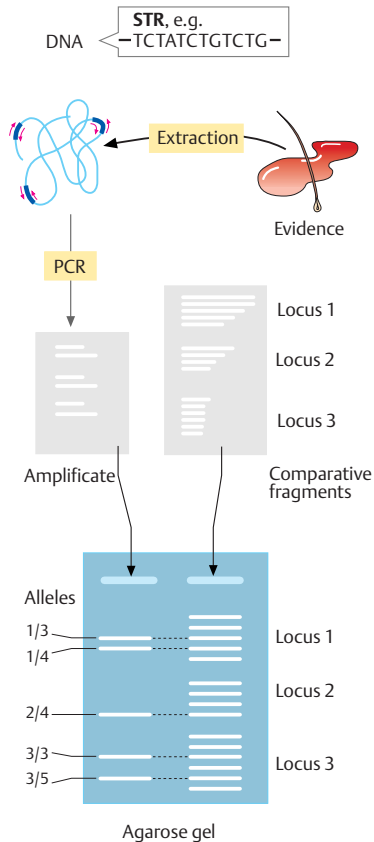
A. DNA electrophoresis



B. Polymerase chain reaction (PCR)



C. DNA typing



Genetic Engineering in Medicine

Genetic engineering procedures are becoming more and more important in medicine for diagnostic purposes (**A**, **B**). Genetic approaches to the treatment of severe diseases are still in the developmental stage ("gene therapy," **C**). *RNA interference* is a newly discovered approach, with which genes can also be "switched off" experimentally; this is particularly promising for research and therapy (**D**).

A. Diagnosis of sickle-cell anemia using RFLP

Point mutations in DNA can destroy cleavage sites for restriction endonucleases (p. 236). Since in this case the restriction digest of normal and mutated DNA yields fragments of different length, the process is called **RFLP** (restriction fragment length polymorphism). The example illustrates use of the method for diagnosing a point mutation in the β -globin gene that leads to *sickle-cell anemia* (p. 300). The mutation in the first exon destroys a cleavage site for endonuclease *Mst* II. When the DNA of healthy and diseased individuals is cleaved with *Mst* II in the region of the gene, DNA fragments of different length (1.2 kbp + 0.2 kbp and 1.4 kbp) are produced, which can be separated by electrophoresis (p. 260) and then detected using specific probes (p. 258). In addition, heterozygous carriers of the sickle-cell gene (trace 2) can be distinguished from homozygous ones (trace 3).

B. Identification of viral RNA using RT-PCR

In viral infections, it is often difficult to determine the species and strain of the pathogen precisely. **RT-PCR** can be used to identify RNA viruses. In this procedure, *reverse transcriptase* (RT, see p. 236) is first used to transcribe the viral RNA into double-stranded DNA, and then PCR (p. 260) is employed to amplify a small segment of this DNA with virus-specific primers. In this way, an amplificate with a characteristic length can be obtained for each pathogen and identified using gel electrophoresis as described on p. 260.

C. Gene therapy

Many diseases, such as hereditary metabolic defects and tumors, can still not be adequately treated. Ways are therefore being sought to

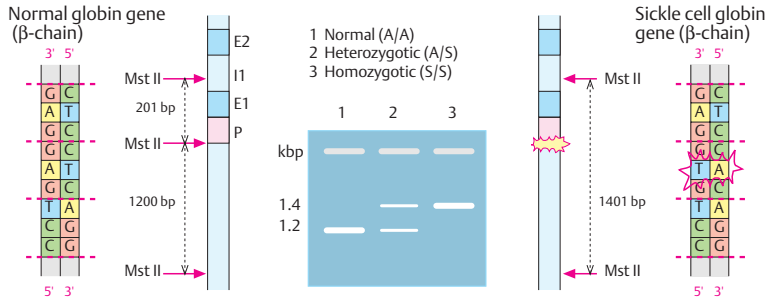
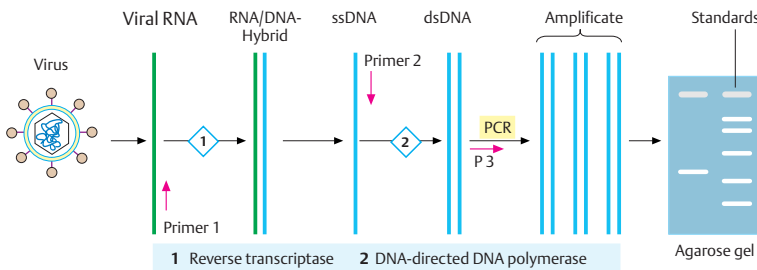
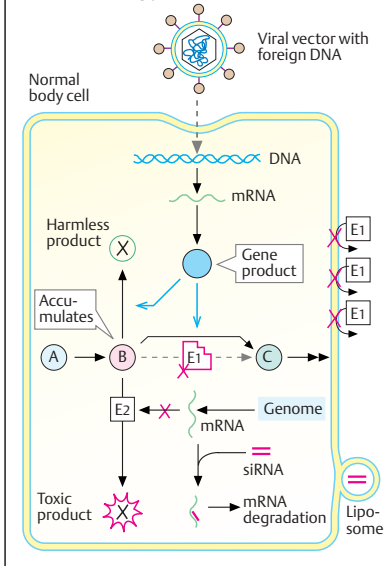
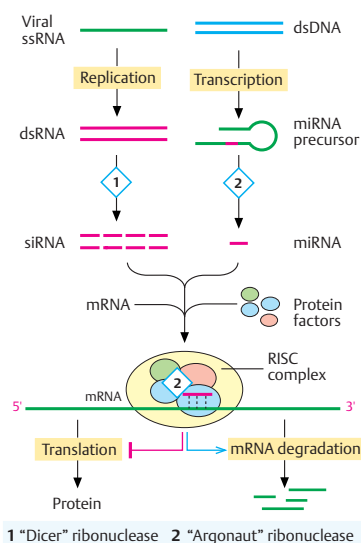
treat diseases of this type by transferring genes into the affected cells (*gene therapy*). However, none of these methods has yet become established in clinical practice.

Of the many conceivable applications of gene therapy, only one will be discussed here. If mutation leads to failure of an *enzyme E1* (center), its substrate B will no longer be converted into C and will accumulate intracellularly. This can lead to cell damage by B itself or by a toxic product, X, formed from it. An attempt at treatment with intact enzyme E1 would fail, as proteins are not capable of passing through the cell membrane. By contrast, it is in principle possible to use a virus (p. 456) as vector to introduce a *foreign gene* into the cell (adenoviruses or retroviruses are mainly used). The gene product could replace the defective E1 or convert B into a harmless product Y. Another approach uses the so-called *siRNA* (see **D**). In the case shown here, the synthesis of E2 and thus the production of the toxic product X could be blocked, for example.

D. RNA interference

It was discovered recently that gene expression is not regulated only at the transcription level (p. 242) but also by small RNA molecules. So-called small interfering RNAs (**siRNAs**) are produced, for example, from viral DNA by replication and cleavage with the aid of a ribonuclease by the name of *dicer* [1]. The single-stranded RNA fragments produced consist of 21–23 nucleotides and combine with proteins into a *RISC complex* (RNA-induced silencing complex); these then bind specifically to specific mRNAs. This inhibits translation of the mRNA in question and also leads to its degradation with the aid of the catalytic component of the complex ("*argonaut*," [2]). The result is that the gene can no longer become active (hence "silencing").

The precursors of **miRNAs** (micro RNAs) are coded by the genome itself. The transcripts are altered while still in the nucleus and after export to the cytoplasm they also undergo hydrolytic cleavage by "dicers". The action of mature miRNAs is similar to that of the siRNAs and is also mediated by the RISC complex.

A. Diagnosis of sickle-cell anemia using RFLP**B. Identification of viral DNA using RT-PCR****C. Gene therapy****D. RNA interference**

Tissues and Organs

Overview

Most components of food (p. 382ff.) cannot be absorbed directly by the organism. It is only after they have been broken down into smaller molecules that the organism can take up the essential nutrients. *Digestion* refers to the mechanical and enzymatic breakdown of food and the absorption of the resulting products.

A. Hydrolysis and absorption of food components

Digestion begins in the mouth with mechanical fragmentation of food during chewing. Salivary enzymes probably serve more for oral hygiene than for digestive purposes. The process of enzymatic degradation of food components then starts in the stomach. For this purpose, the chyme is mixed with *digestive enzymes* that occur in the various digestive secretions or in membrane-bound form on the surface of the intestinal epithelium (p. 272ff.). Almost all digestive enzymes are *hydrolases* (class 3 enzymes; p. 72); they catalyze the cleavage of composite bonds with the uptake of water.

Proteins are first denatured by the stomach's *hydrochloric acid* (p. 270), making them more susceptible to attack by the *endopeptidases* (proteinases) present in gastric and pancreatic juice. The peptides released by endopeptidases are further degraded into amino acids by *exopeptidases*. Finally, the amino acids are absorbed by the intestinal mucosa in cotransport with Na⁺ ions (p. 210). There are separate transport systems for each of the various groups of amino acids (p. 272).

Carbohydrates mainly occur in food in the form of polymers (starches and glycogen). They are cleaved by *pancreatic amylase* into oligosaccharides and are then hydrolyzed by *glycosidases*, which are located on the surface of the intestinal epithelium, to yield monosaccharides. Glucose and galactose are taken up into the enterocytes by secondary active cotransport with Na⁺ ions (p. 272). In addition, monosaccharides also use passive transport systems in the intestine.

Nucleic acids are broken down into their components by *nucleases* from the pancreas and small intestine (*ribonucleases* and *deoxyribonucleases*). Further breakdown yields the nucleobases (purine and pyrimidine derivatives), pentoses (ribose and deoxyribose), phosphate, and nucleosides (nucleobase pentose). These

cleavage products are absorbed by the intestinal wall in the region of the jejunum.

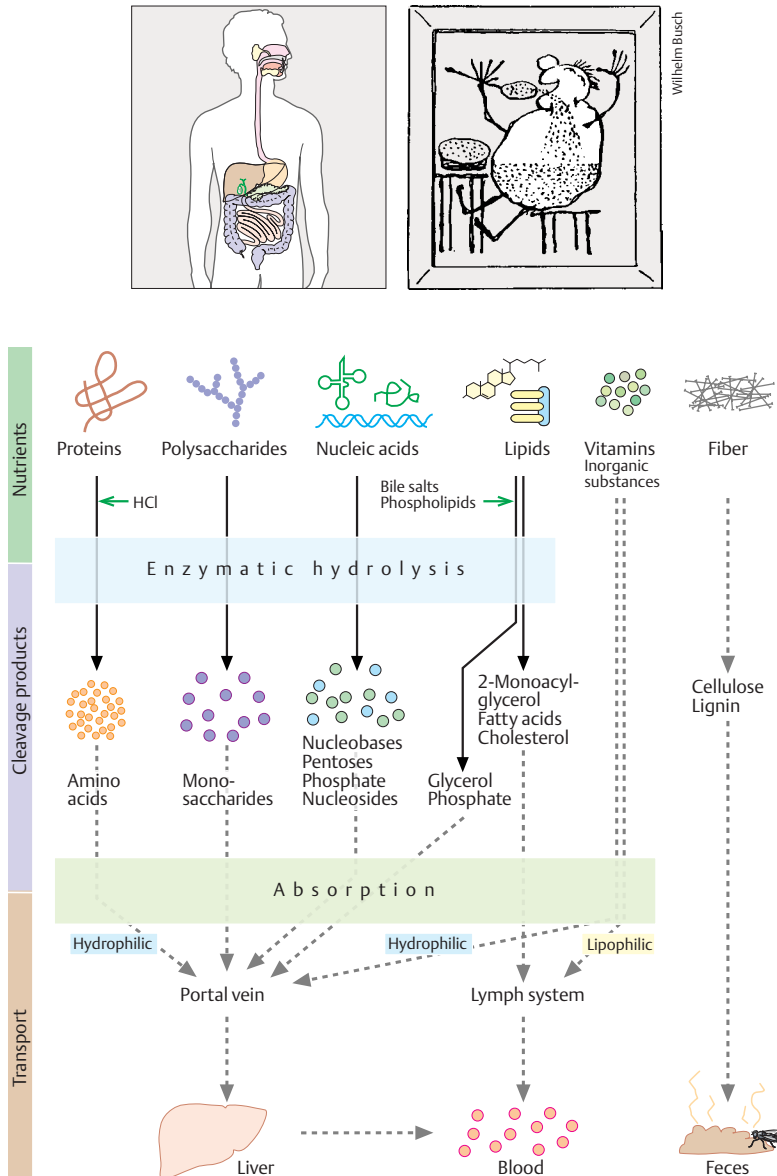
Lipids are a special problem for digestion, as they are not soluble in water. Before enzymatic breakdown, they have to be emulsified by *bile salts* and *phospholipids* from the bile (p. 320). At the water–lipid interface, *pancreatic lipase* then attacks triacylglycerols with the help of *colipase* (p. 274). The cleavage products include *fatty acids*, *2-monoacylglycerols*, *glycerol*, and *phosphate* from phospholipid breakdown. After absorption into the epithelial cells, fats are resynthesized from fatty acids, glycerol, and 2-monoacylglycerols and passed into the lymphatic system (p. 274). The lipids in milk are more easily digested, as they are already present in emulsified form, and provide short-chain fatty acids. Cleavage is started by salivary and *gastric lipases*.

Inorganic components such as water, electrolytes, and *vitamins* are directly absorbed by the intestine. Complicated transport systems may be involved.

High-molecular-weight indigestible components, such as the fibrous components of plant cell walls, which mainly consist of cellulose and lignin, pass through the bowel unchanged and form the main component of feces, in addition to cells shed from the intestinal mucosa. Dietary fiber makes a positive contribution to digestion as a *ballast material* by binding water and promoting intestinal peristalsis.

The food components absorbed by the epithelial cells of the intestinal wall in the region of the jejunum and ileum are transported directly to the liver via the *portal vein*. Fats, cholesterol, and lipid-soluble vitamins are exceptions. These are first released by the enterocytes in the form of *chylomicrons* (p. 274) into the *lymph system*, and only reach the blood via the thoracic duct.

A. Hydrolysis and absorption of food components



Digestive Secretions

A. Digestive juices

Saliva. The salivary glands produce a slightly alkaline secretion which—in addition to water and salts—contains **glycoproteins** (mucins) as lubricants, **antibodies**, and **enzymes**. **α -Amylase** acts on polysaccharides, while a lipase starts the hydrolysis of neutral fats with short- and medium-chain fatty acids. **α -Amylase** and **lysozyme**, a murein-cleaving enzyme (p. 32), probably serve to regulate the oral bacterial flora rather than digestion (p. 348).

Gastric juice. In the stomach, the chyme is mixed with gastric juice. Due to its hydrochloric acid content, this secretion of the gastric mucosa is strongly acidic (pH 1–3; p. 14). It also contains **mucus** (mainly glycoproteins known as mucins), which protects the mucosa from the hydrochloric acid, **salts**, and **pepsinogen**—the proenzyme (“zymogen”) of the aspartate proteinase **pepsin** (pp. 162, 270). In addition, the gastric mucosa secretes **haptocorrin** and what is known as “**intrinsic factor**.” Both factors can bind vitamin B₁₂ and are necessary for its transport in the bowel and its absorption in the ileum.

In the stomach, pepsin and related enzymes initiate the enzymatic digestion of proteins, which takes 1–3 hours. The acidic gastric contents are then released into the duodenum in batches, where they are neutralized by alkaline pancreatic secretions and mixed with cystic bile.

Pancreatic secretions. In the acinar cells, the pancreas forms a secretion that is alkaline due to its **hydrogen carbonate** (HCO₃⁻) content, the buffer capacity of which is sufficient to neutralize the stomach's hydrochloric acid. The pancreatic secretion also contains many **enzymes** that catalyze the hydrolysis of high-molecular-weight food components. All of these enzymes are hydrolases with pH optima in the neutral or weakly alkaline range. Many of them are formed and secreted as proenzymes (“**zymogens**”) and are only activated in the bowel lumen (p. 272).

Trypsin, **chymotrypsin**, and **elastase** are endopeptidases that belong to the group of serine proteinases (p. 162). Trypsin hydrolyzes specific peptide bonds on the C side of the basic amino acids Arg and Lys, while chymotrypsin prefers peptide bonds of the nonpolar amino acids Tyr, Trp, Phe, and Leu. Elastase mainly cleaves on the C side of the aliphatic amino acids Gly, Ala, Val, and Ile. Smaller peptides are attacked by **carboxypeptidases**, which as exo-

peptidases cleave individual amino acids from the C-terminal end of the peptides (p. 162).

α -Amylase, the most important endoglycosidase in the pancreas, catalyzes the hydrolysis of α 1→4 bonds in the polymeric carbohydrates starch and glycogen. This releases maltose, maltotriose, and some other oligosaccharides. Various pancreatic enzymes hydrolyze lipids, including **lipase** with its auxiliary protein **colipase** (p. 274), **phospholipase A₂**, and **sterol esterase**. Bile salts activate the lipid-cleaving enzymes through micelle formation (see below). Several hydrolases—particularly **ribonuclease** (RNase) and **deoxyribonuclease** (DNase)—break down the nucleic acids contained in food.

Bile. The liver forms a thin secretion (bile) that is stored in the gallbladder after water and salts have been extracted from it. From the gallbladder, it is released into the duodenum. The most important constituents of bile are **water** and inorganic **salts**, **bile acids** and **bile salts** (p. 320), **phospholipids**, **bile pigments** (p. 190), and **cholesterol**. Bile salts, together with phospholipids, emulsify insoluble food lipids and activate the lipases. Without bile, fats would be inadequately cleaved, if at all, resulting in “fatty stool” (steatorrhea). Absorption of fat-soluble vitamins would also be impaired.

Small-intestinal secretions. The glands of the small intestine (the Lieberkühn and Brunner glands) secrete additional **digestive enzymes** into the bowel. Together with enzymes on the microvilli of the intestinal epithelium (**peptidases**, **glycosidases**, etc.), these enzymes ensure almost complete hydrolysis of the food components previously broken down by the endoenzymes.

Absorption of the food components can then begin.

A. Digestive juices

Saliva

Daily secretion 1.0–1.5 L

pH 7

Water ————— Moistens food

Salts —————

Mucus ————— Lubricant

Antibodies ————— Bind to bacteria

α -amylases ————— Starch ↓

Lysozyme ————— Attacks bacterial cell walls ↓

Lipase ————— Cleaves fats

Bile

Daily secretion 0.6 L

pH 6.9–7.7

Water —————

HCO_3^- ————— Neutralizes gastric juice

Bile salts ————— Facilitate lipid digestion

Phospholipids ————— Facilitate lipid digestion

Bile pigments ————— Waste products

Cholesterol ————— Waste product

Pancreatic secretions

Daily secretion 0.7–2.5 L

pH 7.7 (7.5–8.8)

Water —————

HCO_3^- ————— Neutralizes gastric juice

Trypsin ————— Proteins ↓

Chymotrypsin ————— Proteins ↓

Elastase ————— Proteins ↓

Carboxypeptidases ————— Peptides ←

α -amylase ————— Starch and glycogen ↓

Triacylglycerol lipase ————— Fats

Co-lipase ————— Cofactor for lipase

Phospholipase A_2 ————— Phospholipids

Cholesterol esterase ————— Cholesterol esters

Ribonuclease ————— RNA ↓

Desoxyribonuclease ————— DNA ↓

Secretions of the small intestine

Daily secretion unknown

pH 6.5–7.8

Aminopeptidases ————— Peptides ←

Dipeptidases ————— Dipeptides

α -Glucosidase ————— Oligosaccharides ↓

Oligo-1,6-glucosidase ————— Oligosaccharides ↓

β -Galactosidase ————— Lactose

Saccharase ————— Saccharose

α, α' -Trehalase ————— Trehalose

Alkaline phosphatase ————— Phosphoric acid esters

Polynucleotidases ————— Nucleic acids, nucleotides ↓

Nucleosidases ————— Nucleosides ←

Phospholipases ————— Phospholipids

↓ Endoenzyme
← Exoenzyme

Component

Function or substrate

Gastric juice

Daily secretion 2–3 L

pH 1

Water —————

Salts —————

HCl ————— Denatures proteins, kills bacteria

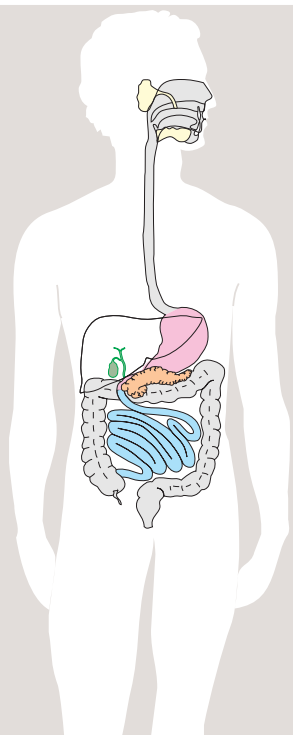
Mucus ————— Protects stomach lining

Pepsins ————— Proteins ↓

Chymosin ————— Precipitates casein ↓

Triacylglycerol lipase ————— Cleaves fats

Intrinsic factor, haptocorrin ————— Protects vitamin B_{12}



Digestive Processes

After food has been ground mechanically by chewing, the first intensive chemical digestive process takes place in the stomach. **Gastric juice**, which is the product of several cell types, is secreted. The **parietal cells** produce hydrochloric acid, **chief cells** release pepsinogen, and **accessory cells** protect the surface of the stomach with mucus containing **mucins**, which are very large glycoproteins.

A. Formation of hydrochloric acid

The secretion of **hydrochloric acid** (H^+ and Cl^-) by the parietal cells is an active process that consumes ATP and takes place against a concentration gradient. In the gastric lumen, with a pH of 1, the H^+ concentration is some 10^6 times higher than in the parietal cells, which have a pH of 7.

The precursors of the exported H^+ ions are carbon dioxide (CO_2) and water (H_2O). CO_2 diffuses from the blood into the parietal cells, and in a reaction catalyzed by **carbonate dehydratase** (carbonic anhydrase [1]), it reacts with H_2O to form H^+ and hydrogen carbonate (HCO_3^-). The H^+ ions are transported into the gastric lumen in exchange for K^+ by a membrane-bound H^+/K^+ -exchanging ATPase [2] (a transport ATPase of the P type; p. 212). The remaining hydrogen carbonate is released into the interstitium in electroneutral antiport in exchange for chloride ions (Cl^-), and from there into the blood. The Cl^- ions follow the secreted protons through a channel into the gastric lumen.

The hydrochloric acid in gastric juice is important for digestion. It activates the zymogen **pepsinogen** to **pepsin**, the active protease (p. 272). Hydrochloric acid also creates an optimal pH level for pepsin to take effect. In addition it denatures food proteins so that they are more easily attacked by proteinases, and it kills microorganisms, thus sterilizing gastric contents. The key enzyme of HCl secretion, H^+/K^+ -ATPase, can be inhibited very effectively by **prazoles**, e.g., omeprazole (p. 276).

B. Regulation of hydrochloric acid, mucin, and pepsinogen secretion

The secretory activity of the **parietal cells** (secretion: HCl), **accessory cells** (mucins) and **chief cells** (pepsinogen) is regulated by neurotransmitters, hormones, and mediators. It is now clear that the regulation of the stomach's secretory activities takes place through a

network of signal pathways, including both external stimuli (peptides and amino acids in the gastric lumen) and internal stimuli (vagus nerve) and a feedback loop (H^+ in the gastric lumen).

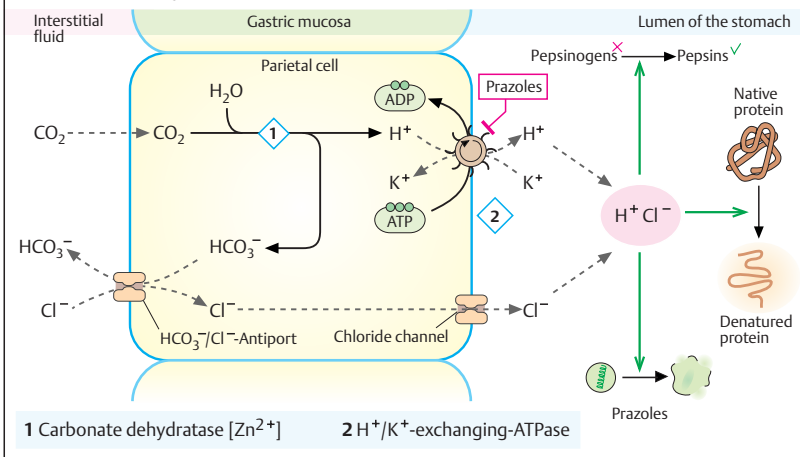
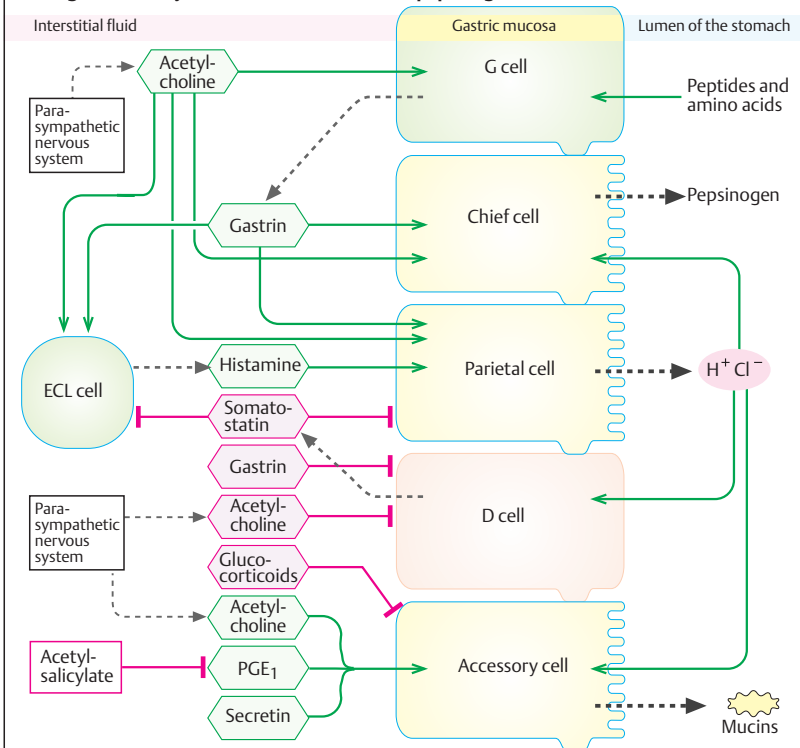
HCl secretion is stimulated by the tissue peptide hormone **gastrin**, derived from the **G cells**, the mediator **histamine** (p. 436), secreted by the **ECL** (enterochromaffin-like) cells, and—via the neurotransmitter **acetylcholine**—by the autonomic nervous system. The peptide **somatostatin** from the **D cells** and certain **prostaglandins** (p. 438) have inhibitory effects. Gastrin belongs to the group of **gastrointestinal hormones** (p. 436).

Pepsinogen production and secretion in the **chief cells** are also stimulated by gastrin and acetylcholine.

Mucin secretion from the **accessory cells** is stimulated by acetylcholine, **secretin**, and **prostaglandin** E_1 (PGE_1) and inhibited by **glucocorticoids**.

■ Further information

Protection against self-digestion. The stomach and pancreas release most proteolytic enzymes into the duodenum in an inactive form as **proenzymes** (zymogens) to prevent self-digestion. In the acidic milieu of the **stomach**, pepsinogen is converted to pepsins by cleavage of peptides. Since their pH optimum is in the acid range, pepsins are active only at the low pH of gastric juice. The mucins protect the gastric wall against self-digestion by forming a protective film. In the **pancreas**, proteinase inhibitors provide additional protection from the effects of unwanted activation of pancreatic proteinases. Zymogens prematurely activated in the pancreas are immediately inactivated by forming complexes with these inhibitors (p. 272). **Trypsinogen** plays a key role in pancreatic secretions. It is proteolytically converted only in the bowel into active trypsin by **enteropeptidase**, a membrane enzyme on the surface of the enterocytes. Trypsin then autocatalytically activates additional trypsinogen molecules and the other proenzymes.

A. Formation of hydrochloric acid

B. Regulation of hydrochloric acid, mucin, and pepsinogen secretion


Absorption I

Absorption of enzymatic cleavage products takes place primarily in the small intestine. Only ethanol and short-chain fatty acids are absorbed to some extent in the stomach.

The absorption process is facilitated by the large inner surface of the intestine. Lipophilic molecules penetrate the plasma membrane of the mucosal cells by simple diffusion, whereas polar molecules require *transporters* (facilitated diffusion; p. 210). In many cases, carrier-mediated cotransport with Na^+ or H^+ ions is observed. In this case, the difference in the concentration of the Na^+ ions, which is high in the intestinal lumen and low in the mucosal cells, drives the import of nutrients against a concentration gradient (*secondary active transport*; p. 218).

A. Digestion of proteins and polysaccharides

The digestion of proteins starts in the stomach and continues in the small intestine. After denaturing by hydrochloric acid (p. 270), gastric *pepsin* [1] and the pancreatic enzymes *trypsin* [2], *chymotrypsin* [3], and *elastase* [4] cleave the polypeptides to oligopeptides. These are further hydrolyzed by *carboxypeptidases A* and *B* [5]. In protein digestion, proteinases with different substrate specificities work together. Finally, many oligopeptides are degraded into amino acids by *aminopeptidases* [6] in the brush border.

The most important digestible **carbohydrates** in the diet are starch, lactose, and sucrose. They are degraded completely to monosaccharides by enzymatic hydrolysis in the small intestine. The **polysaccharides** are hydrolyzed by α -*amylase* [7], an endoglycosidase in saliva and pancreatic secretions, which acts on α -1,4-glycosidic bonds. The resulting **oligosaccharides** (limit dextrins) are then broken down further by exoglycosidases. These are the oligosaccharidases and disaccharidases: *glucoamylase* [8], *saccharase isomaltase* [9], *trehalase* [10] and the β -*glycosidase* complex (with lactase activity [11]). These enzymes are firmly bound to the plasma membrane of the brush border, as integral membrane proteins or via a GPI anchor.

Cellulose, hemicellulose, lignins, pectins and similar *plant carbohydrates* cannot be degraded by human enzymes.

B. Zymogen activation

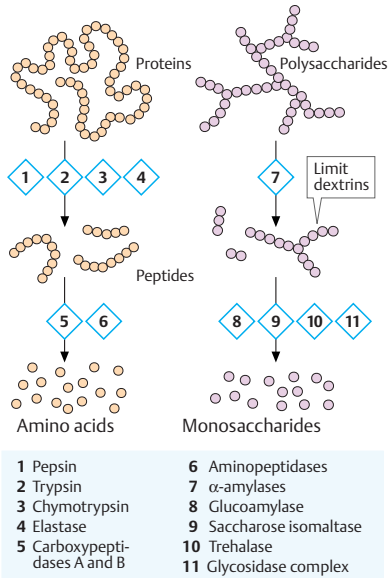
Many digestive enzymes are secreted into the digestive tract as proenzymes and are only activated there by *limited proteolysis*. *Pepsinogen* is converted by H^+ in the stomach to *pepsin* (p. 270). *Trypsinogen* in pancreatic secretion is cleaved to *trypsin* in the small intestine by *enteropeptidase* ("enterokinase"). Trypsin then activates the following zymogens in the pancreatic secretion: *chymotrypsinogen*, *proelastase*, *procarboxypeptidases*, and *prophospholipase A₂*. In addition, trypsin activates itself by autocatalysis (p. 162). Premature activation of trypsinogen in the pancreas is prevented by the presence of **trypsin inhibitors**.

C. Absorption of sugars and amino acids

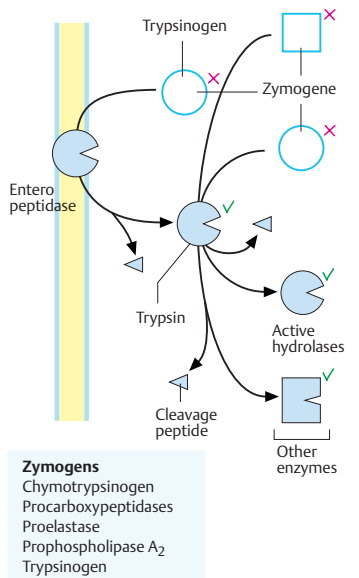
The **monosaccharides** released by glycosidases pass with the help of various *sugar-specific transporters* into the cells of intestinal epithelium. *Secondary active transport* on the luminal side serves for the uptake of **glucose** and **galactose**, which are cotransported with Na^+ by a **Na^+ -glucose transporter** (SGLT). The Na^+ gradient is maintained on the basal side of the cells by Na^+/K^+ -ATPase. A passive glucose transporter, Glut-2 (p. 212), then releases glucose and galactose into the blood. **Fructose** and other monosaccharides are taken up into the enterocytes by facilitated diffusion with the aid of Glut-5 and released into the blood by means of Glut-2 (p. 212).

Individual groups of **amino acids** have *group-specific transport systems*. Anionic (i.e., acidic), and neutral amino acids are absorbed by *secondary active transport* (cotransport with Na^+), while the cationic (basic) ones are taken up via an antiport with neutral amino acids. **Di-** and **tripeptides** are absorbed by cotransport with H^+ and then cleaved intracellularly by peptidases (not shown). Finally, the amino acids are released into the blood by *facilitated diffusion*.

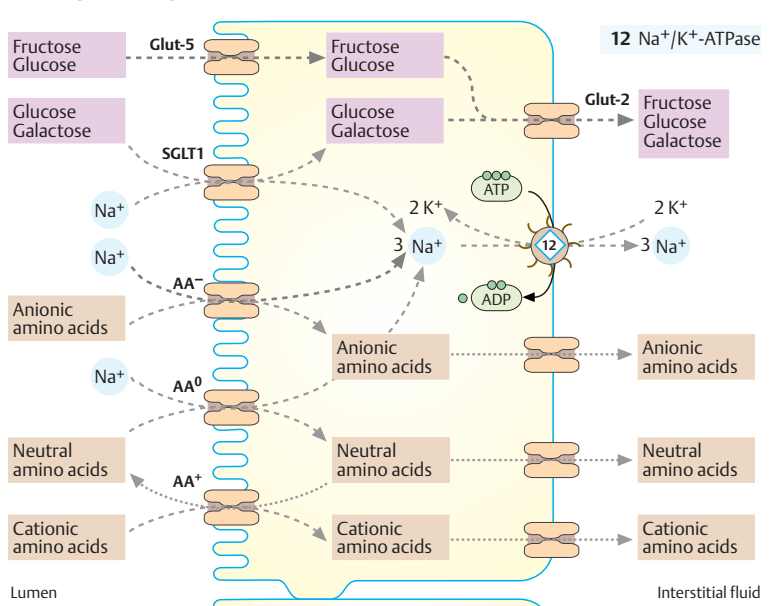
A. Digestion of proteins and polysaccharides



B. Zymogen activation



C. Absorption of sugars and amino acids



Absorption II

Fats, the most important group of lipids in diet, are poorly soluble in water. The greater their surface area—i.e., the better the fats are emulsified to small droplets—the more easily they can be hydrolyzed by enzymes.

A. Fat hydrolysis

Cleavage of fats in the gastrointestinal tract is catalyzed by lipases, the more important of which is *pancreatic lipase* [1]. Triacylglycerols, their substrates, are poorly accessible in the aqueous environment of the bowel lumen because they aggregate into larger fat drops because of the “hydrophobic effect” (p. 24). Amphipathic substances in bile (**bile acids**, **bile salts**, **phospholipids**) help digestion by attaching to the dietary lipids to create an **emulsion** of finely distributed droplets with a large surface area. Assisted by the auxiliary protein, *colipase*, the bile salts mediate binding of *triacylglycerol lipase* to the emulsified lipids and also its activation. This is triggered by a conformational change in the C-terminal domain of the enzyme, which uncovers the active center.

Activated lipase hydrolyzes the ester bonds of fats, converting the emulsified droplets into small **micelles** (p. 24), the components of which are ultimately absorbed by the enterocytes. The bile acids are also re-absorbed (in the ileum; p. 320).

Lipid digestion requires contraction of the gallbladder and secretion of pancreatic enzymes. Both processes are triggered by the intestinal hormone **cholecystokinin** (p. 436) when gastric contents pass into the small bowel.

Lipases occur in saliva, gastric juice, and pancreatic secretions. The lipases have a certain degree of substrate specificity with regard to fatty acid chain length, optimal pH, and the position of the cleavage. While *triacylglycerol lipase* specializes in fats, *phospholipase A₂* (PLA₂) preferentially cleaves phospholipids in position C-2 of the glycerol. PLA₂ is produced in the pancreas as a proenzyme (p. 272).

B. Lipid absorption and resynthesis

Pancreatic lipase [1] hydrolyzes the ester bonds of fats (triacylglycerols) especially in position 1 and 3 of the glycerol. This releases **fatty acids** and **2-monoacylglycerols**, which remain in the micelles together with other dietary lipids (lysolecithins, cholesterol, lipid-soluble vitamins). These lipids are then ab-

sorbed through contacts between the micelles and the microvilli of the intestinal epithelial cells, probably followed by diffusion.

The more water-soluble short- and medium-chain fatty acids (length: C₄–C₁₀) can be absorbed without the aid of the micelles.

Complete hydrolysis of triacylglycerols also releases some glycerol.

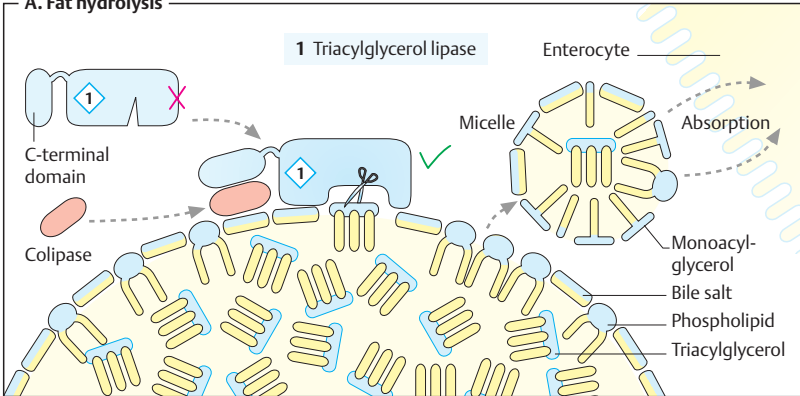
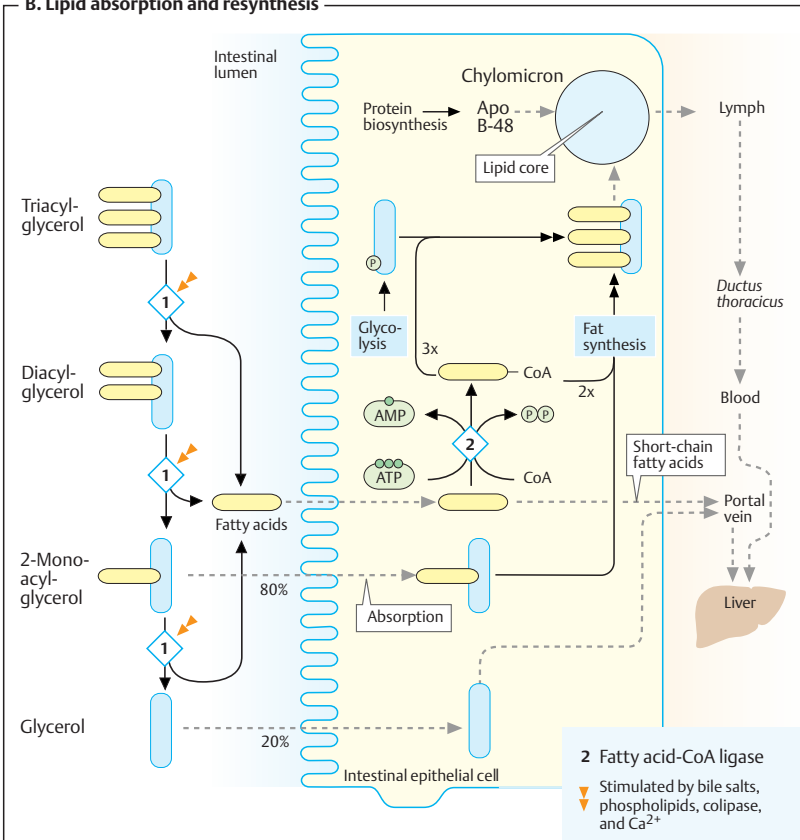
In the mucosal cells, the *long-chain fatty acids* (C₁₀–C₁₈) are activated to acyl-CoA by an *ATP-dependent ligase* [2] and resynthesized to triacylglycerols (fat) from 2-monoacylglycerols. This fat synthesis pathway differs from fat synthesis in the liver and adipose tissue, which proceeds via phosphatidic acids (p. 154).

The resynthesized fats are packaged into **chylomicrons** by the intestinal cells. These are **lipo-protein complexes** (p. 282f.), which the intestinal epithelial cells produce to convert the absorbed lipids into a water-soluble form for transport.

Production of chylomicrons in the enterocytes starts with synthesis of **apoprotein B-48** (Apo B-48). This protein is coded by the same gene as Apo B-100 of the liver, but Apo-B-48 is much shorter because a stop codon is generated in its primary transcript by *RNA editing* (not shown).

After the chylomicrons are packed with lipids, they are released into the lymph by exocytosis, and bypass the liver to reach the bloodstream via the *thoracic duct*. They transport not only fats but also **phospholipids**, **cholesterol**, and **fat-soluble vitamins**.

Short-chain fatty acids (< C₈), e.g., from the lipids in milk, are not incorporated into fats but passed directly into the blood by the enterocytes. That way, they reach the liver directly through the portal vein. Absorbed glycerol can also travel by this route.

A. Fat hydrolysis**B. Lipid absorption and resynthesis**

Pathobiochemistry

A. Gastritis and ulcer

In the stomach there is equilibrium between the production of **hydrochloric acid** (HCl) and **mucins** (p. 268). Disturbances of this equilibrium can result in the stomach starting to digest itself. **Gastritis** (inflammation of the mucosa of the stomach) and an **ulcer** in the longer term can have various causes. Mucin production can be reduced, for example, by **glucocorticoids** (endogenous cause: stress; exogenous cause: medications). **Inhibitors of prostaglandin synthesis** (nonsteroidal anti-inflammatory drugs, NSAIDs; e.g., *acetylsalicylate*, p. 438) also have this effect. On the other hand, hypersecretion of HCl can be triggered by increased vagal stimulation (by *nicotine* and *alcohol*) or increased concentrations of *histamine* or *gastrin* (p. 270). The acid-adapted bacterium *Helicobacter pylori* is frequently also involved in the development of gastritis and ulcer.

Administering acid-neutralizing medications (**antacids**) is a first step to reduce the HCl concentration. Acid production can be blocked directly by **H⁺/K⁺-ATPase inhibitors**, e.g., *omeprazole*. These inhibitors are prodrugs which only become reactive in an acid environment and then block the proton pump irreversibly by forming a covalent bond. **Antihistamines** (H₂ blockers) like *ranitidine* interfere with the control of HCl production (p. 270). *Helicobacter* infections can be treated with **antibiotics**.

B. Disorders of carbohydrate digestion

Undigested carbohydrates serve as a source of nutrients for bacteria in the large bowel. Bacterial metabolism in the colon produces **gases** (mainly H₂, CO₂, and CH₄), **short-chain fatty acids**, and **lactate**. The gases lead to flatulence, the fatty acids are absorbed by the mucosal cells of the colon and lactate causes retention of water, which can produce diarrhea.

Peas, beans, and lentils (pulses) contain oligosaccharides consisting of 1,6-linked galactose, as well as starch, which are incompletely cleaved by human disaccharidases.

Lactase deficiency in adults (= **lactose intolerance**) is particularly widespread. Symptoms can be relieved by avoiding milk products that contain lactose or by giving gastroresistant lactase preparations.

Other disorders of digestion involve genetic deficiencies of disaccharidases in the brush border and of transporters for individual monosaccharides. The result is malabsorption

of the sugars in question. Examples are **sucrose intolerance** (disorder of *saccharase-isomaltase*) and a certain form of **fructose intolerance** (defects of Glut-5; p. 212).

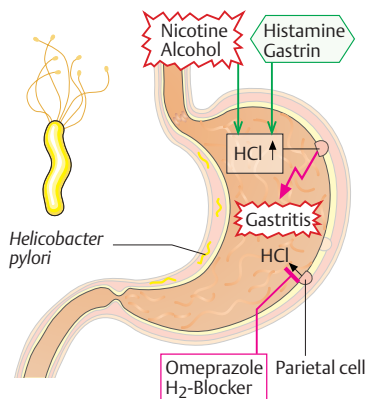
C. Fat blockers and fat substitutes

There are various approaches for limiting nutrient absorption to treat severe obesity. One way is to block fat utilization with drugs. Inhibitors of gastric and pancreatic lipase, known as **fat blockers**, are used for this purpose, e.g., *orlistat* (Xenical), a derivative of bacterial lipstatin. A large part of dietary fats is then excreted undigested. Another approach is to replace fats by substances that have the flavor and consistency of fats but are indigestible. One of these **fat substitutes** is *Olestra* (not licensed in Europe), a sucrose derivative completely esterified with fatty acids (*octa-acylsucrose*). As it cannot be cleaved by the lipases of the digestive tract it is excreted unchanged.

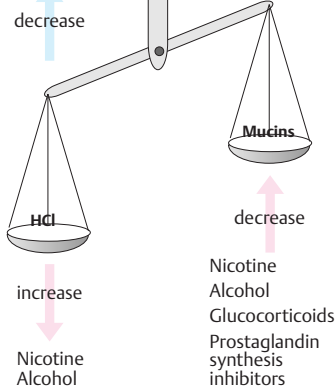
Disorders of amino acid absorption (not shown)

Various transport systems for amino acids can be defective (p. 272). In **cystinuria**, absorption of basic amino acids (Arg, Lys, Orn) and *cystine* from the intestinal lumen and from primary urine in the proximal renal tubule is disturbed. In the rare **Hartnup disease**, the absorption of neutral amino acids is disturbed. A *tryptophan deficiency* follows, in particular.

A. Gastritis and ulcer



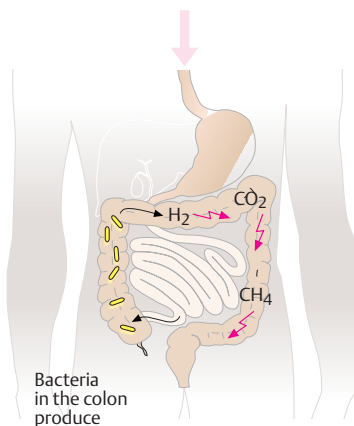
Antacids
Proton pump
inhibitors
Antihistamines



B. Disorders of carbohydrate digestion

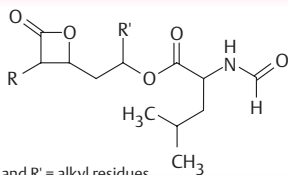
Causes:

Lactose in lactase deficiency
Fructose in Glut-5 deficiency
Sucrose in saccharase-isomaltase disorder
Oligosaccharides from pulses

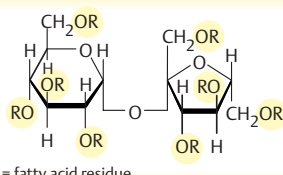


Gases	Short-chain fatty acids	Lactate
H ₂ CO ₂ CH ₄	Acetic acid Propionic acid Butyric acid	Water retention
Flatulence	Absorption	Diarrhea

C. Fat blockers and fat substitutes



Orlistat



Olestra®

Blood: Composition and Functions

Human blood constitutes about 8% of the body's weight. It consists of **cells** and cell fragments in an aqueous medium, the **blood plasma**. The proportion of cellular elements, known as *hematocrit*, in the total volume is approximately 45%.

A. Functions of the blood

The blood is the most important transport medium in the body. It serves to keep the "internal milieu" constant (homeostasis) and it plays a decisive role in defending the body against pathogens.

Transport. The *gases* oxygen and carbon dioxide are transported in the blood. The blood mediates the *exchange of substances between organs* and takes up *metabolic end products* from tissues to transport them to the lungs, liver, and kidney for excretion. The blood also distributes *hormones* throughout the organism (p. 414).

Homeostasis. The blood ensures that a balanced distribution of water is maintained between the vascular system, the cells (intracellular space), and the extracellular space. The *acid-base balance* is also regulated by the blood in cooperation with the lungs, liver, and kidneys (p. 292). The regulation of *body temperature* also depends on the controlled transport of heat by the blood.

Defense. The body uses both nonspecific and specific mechanisms to defend itself against pathogens that have penetrated into the body. The defense system includes the *cells of the immune system* and certain *plasma proteins* (p. 302ff.).

Self-protection. To prevent blood loss when a vessel is injured, the blood has systems for stanching blood flow and coagulating the blood (hemostasis; p. 294). The dissolution of blood clots (fibrinolysis) is also managed by the blood itself (p. 296).

B. Cellular elements

The solid elements in the blood are the erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets).

The **erythrocytes** provide for gas transport in the blood. They are discussed in greater detail on pages 286–290.

The **leukocytes** include various types of granulocyte, monocyte, and lymphocyte. All of these have immune defense functions (p. 302). The

neutrophil granulocytes, *monocytes*, and the *macrophages* derived from monocytes are phagocytes. They can ingest and degrade invading pathogens. The *lymphocytes* are divided into two groups, B lymphocytes and T lymphocytes. B lymphocytes produce *antibodies*, while T lymphocytes regulate the immune response and destroy virus-infected cells and tumor cells. *Eosinophilic* and *basophilic granulocytes* have special tasks for defense against animal parasites.

Platelets are cell fragments that arise in the bone marrow from large precursor cells, the megakaryocytes. Their task is to promote hemostasis (p. 294).

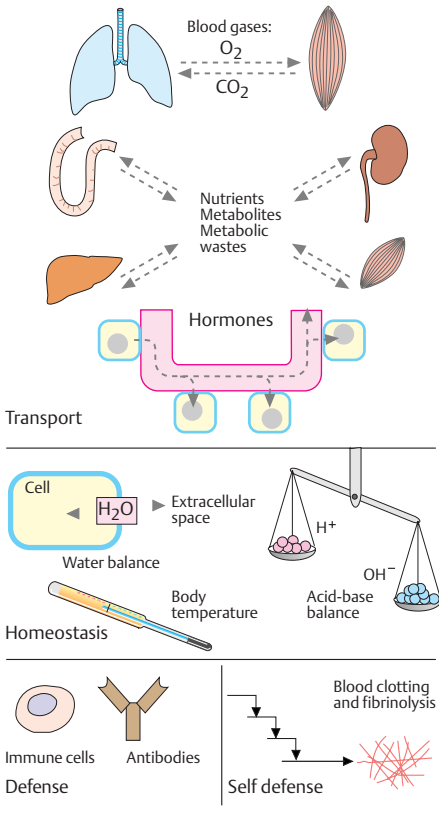
C. Blood plasma: composition

The **blood plasma** is an aqueous solution of electrolytes, nutrients, metabolites, proteins, vitamins, trace elements, and signaling substances. The fluid phase of coagulated blood is known as **blood serum**. It differs from the plasma in that it lacks fibrin and other coagulation proteins (p. 294).

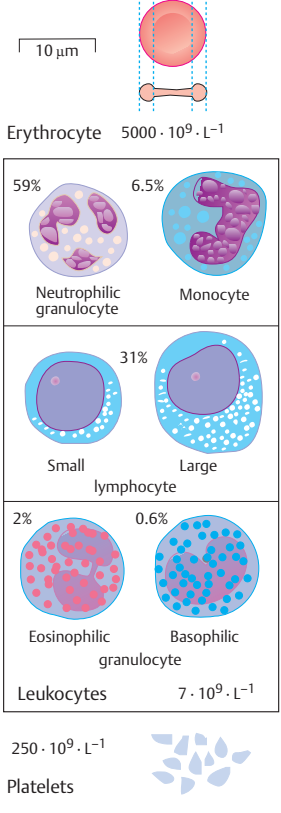
Laboratory assessment of the composition of the blood plasma is often performed in clinical chemistry. Among the **electrolytes**, there is a relatively high concentration of Na^+ , Ca^{2+} , and Cl^- ions in the blood in comparison with the cytoplasm. By contrast, the concentrations of K^+ , Mg^{2+} , and phosphate ions are higher in the cells. Proteins also have a higher intracellular concentration. The electrolyte composition of blood plasma is similar to that of seawater, due to the evolution of early forms of life in the sea. The solution known as "**isotonic saline**" ("normal saline" NaCl at a concentration of 0.9 weight%, $0.15 \text{ mol} \cdot \text{L}^{-1}$) is almost isotonic with blood plasma.

A list of particularly important **metabolites** and their concentrations in the blood plasma is given on the right.

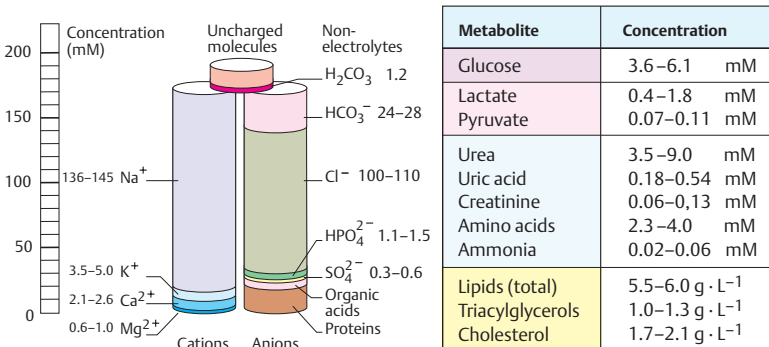
A. Functions of the blood



B. Cellular elements



C. Blood plasma: composition



Plasma Proteins

Quantitatively, proteins are the most important part of the soluble components of the blood plasma. With concentrations of between 60 and 80 g · L⁻¹, they constitute approximately 4% of the body's total protein. Their tasks include transport, regulation of the water balance, hemostasis, and defense against pathogens.

A. Plasma proteins

Some 100 different proteins occur in human blood plasma. Based on their behavior during electrophoresis (see below), they are broadly divided into *five fractions*: **albumins** and α_1 -, α_2 -, β - and γ -**globulins**. Historically, the distinction between the albumins and globulins was based on differences in the proteins' solubility—albumins are soluble in pure water, whereas globulins only dissolve in the presence of salts.

The most abundant protein in the plasma, at around 45 g · L⁻¹, is **albumin**. Due to its high concentration, it plays a crucial role in maintaining the blood's **colloid osmotic pressure** and represents an important amino acid reserve for the body. Albumin has binding sites for nonpolar substances and therefore functions as a **transport protein** for long-chain fatty acids, bilirubin, drugs, and some steroid hormones and vitamins. In addition, serum albumin binds Ca²⁺ and Mg²⁺ ions, and also Zn²⁺ and Cu⁺. It is the only important plasma protein that is not glycosylated. The albumin fraction also includes *transthyretin* (prealbumin), which together with other proteins transports the hormone thyroxine and its metabolites (p. 426).

The table also lists important **globulins** in blood plasma, with their mass and function. The α - and β -globulins are involved in the transport of lipids (lipoproteins; p. 282f.), hormones, vitamins, and metal ions. In addition, they provide coagulation factors, protease inhibitors, and the proteins of the complement system (p. 308). Soluble antibodies (immunoglobulins; p. 310) make up the γ -globulin fraction.

Synthesis and degradation. Most plasma proteins are synthesized by the liver. Exceptions to this include the immunoglobulins, which are secreted by B lymphocytes known as plasma cells (p. 304) and peptide hormones, which derive from endocrine gland cells.

With the exception of albumin, almost all plasma proteins are **glycoproteins**. They carry oligosaccharides in *N*- and *O*-glycosidic bonds (p. 34). *N*-acetylneuraminic acid (sialic acid; p. 30) often occurs as a terminal carbohydrate among sugar residues. *Neuraminidases* (sialidases) on the surface of the vascular endothelia gradually cleave the sialic acid residues and thereby release galactose units on the surfaces of the proteins. These *asialoglycoproteins* ("asialo-" = without sialic acid) are recognized and bound by galactose receptors on hepatocytes. In this way, the liver takes up aged plasma proteins by endocytosis and breaks them down. The oligosaccharides on the protein surfaces thus determine the half-life of plasma proteins, which is a period of days to weeks.

Not all important plasma proteins are listed in the table. For example, some factors of the clotting system and fibrinolysis (p. 294f.), complement factors (p. 308), the protease inhibitors α_1 -antiprotease (α_1 -antitrypsin) and α_2 -macroglobulin, and various lectins (carbohydrate-binding proteins) are absent.

In healthy individuals, the concentration of plasma proteins is constant. Diseases in organs that are involved in protein synthesis and breakdown can shift the protein pattern. For example, via cytokines (p. 440), severe injuries trigger increased synthesis of **acute-phase proteins**, which include *C-reactive protein*, *haptoglobin*, *fibrinogen*, *complement factor C-3*, and others. The concentrations of individual plasma proteins are altered in some diseases (known as *dysproteinemias*). This is often apparent on electrophoresis.

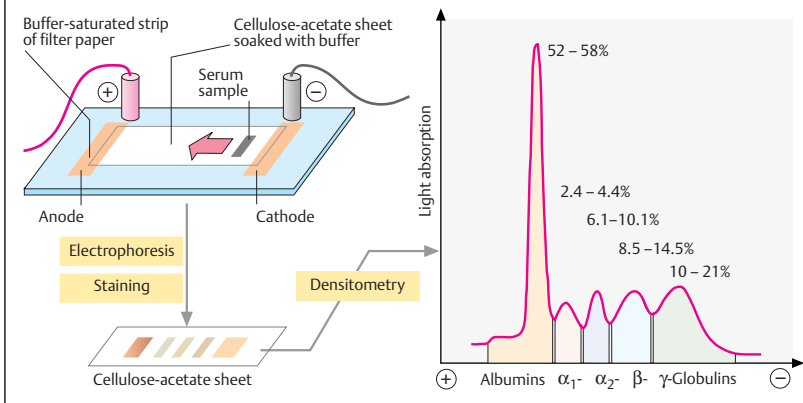
B. Carrier electrophoresis

Proteins and other electrically charged macromolecules can be separated using electrophoresis (p. 260). Among the various procedures used, **carrier electrophoresis** on cellulose acetate foil is particularly simple. Using this method, serum proteins—which at slightly alkaline pH values all move toward the anode, owing to their excess of negative charges—can be separated into the five fractions mentioned. After the proteins have been fixed and stained with dyes, the resulting bands can be quantitatively assessed using densitometry.

A. Plasma proteins

Group	Protein	Mass (kDa)	Function
Albumins	Transthyretin Albumin: 45 g · L ⁻¹	50–66 67	Transport of thyroxine and triiodothyronine Maintenance of osmotic pressure; transport of fatty acids, bilirubin, bile acids, steroid hormones, pharmaceuticals, and inorganic ions.
α ₁ -Globulins	Antitrypsin	51	Inhibition of trypsin and other proteases
	Antichymotrypsin	58–68	Inhibition of chymotrypsin
	High density lipoprotein (HDL)	200–400	Transport of lipids
	Prothrombin	72	Coagulation factor II, thrombin precursor
	Transcortin	51	Transport of cortisol, corticosterone, and progesterone
	Acid glycoprotein	44	Transport of progesterone
	Thyroxine-binding globulin	54	Transport of thyroxine and triiodothyronine
α ₂ -Globulins	Transcuprein	135	Transport of copper ions
	Antithrombin III	58	Inhibition of blood clotting
	Haptoglobin *	100	Binding of hemoglobin
	Cholinesterase	ca. 350	Cleavage of choline esters
	Plasminogen	90	Precursor of plasmin, breakdown of blood clots
	Macroglobulin	725	Binding of proteases, transport of zinc ions
	Retinol-binding protein	21	Transport of vitamin A
	Vitamin D-binding protein	52	Transport of calcium
β-Globulins	Low-density lipoprotein (LDL)	2000–4500	Transport of lipids
	Transferrin	80	Transport of iron ions
	Fibrinogen *	340	Coagulation factor I
	Sex hormone-binding globulin	65	Transport of testosterone and estradiol
	Transcobalamin	38	Transport of vitamin B ₁₂
	C-reactive protein *	110	Complement activation
γ-Globulins	IgG	150	Late antibodies
	IgA	360	Mucosa-protecting antibodies
	IgM	935	Early antibodies
	IgD	172	B-lymphocyte receptors
	IgE	196	Reagents
			* Acute phase protein

B. Carrier electrophoresis



Lipoproteins I

Most lipids are barely soluble in water, while some have *amphipathic properties*; i.e., they are both water-soluble and fat-soluble. In the blood, the hydrophobic triacylglycerols in free form would coalesce into drops that could cause fat embolisms. By contrast, amphipathic lipids would be deposited in the blood cells' membranes and would dissolve them. Special precautions are therefore needed for lipid transport in the blood. Long-chain **fatty acids** (C_{12} – C_{20}) are transported in the blood bound to albumin. By contrast, medium-chain (C_8 – C_{10}) and short-chain (C_4 – C_6) ones are dissolved freely in the blood because of their greater hydrophilicity (p. 274). **Fats, phospholipids, cholesterol** and its esters, and also **fat-soluble vitamins** are transported in the form of water-soluble **lipoprotein complexes**, of which there are several types in the blood plasma, with different sizes and composition.

A. Composition of lipoprotein complexes

Lipoproteins are spherical or discoid aggregates of **lipids** with proteins that are referred to as **apoproteins**. Lipoproteins consist of a nucleus of nonpolar lipids (mainly triacylglycerols and cholesterol esters) surrounded by a single-layered shell about 2 nm thick of amphipathic lipids (phospholipids and cholesterol; see next page for LDL as an example). The shell, in which the apoproteins are also deposited, gives the surfaces of the particles polar properties and thereby prevents them from aggregating into large particles. The larger the lipid nucleus of a lipoprotein is—i.e., the larger the number of nonpolar lipids it contains—the lower its density is. Lipoproteins are usually classified into five groups. In order of decreasing size and increasing density, these are: *chylomicrons and chylomicron remnants*, *VLDLs* (very-low-density lipoproteins), *IDLs* (intermediate-density lipoproteins), *LDLs* (low-density lipoproteins), and *HDLs* (high-density lipoproteins). The proportion of apoproteins ranges from 1% in chylomicrons to over 50% in HDLs. Apoproteins, the most important of which are listed in the table, serve not only for solubility purposes, but also function as recognition molecules for the membrane receptors on target cells (see next page) and as activators for enzymes or proteins that are involved in lipid exchange.

B. Transport functions

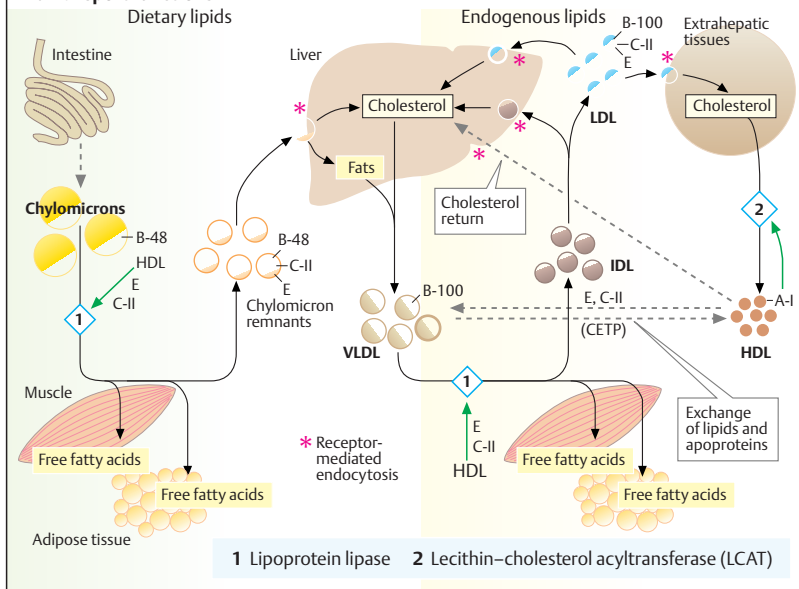
The classes of lipoproteins differ not only in their composition, but also in the ways in which they originate and function.

The **chylomicrons** are produced in the intestinal mucosa (p. 274) and bypass the liver to reach the blood via the lymphatic system. They take care of the transport of dietary lipids from the intestine to the tissues. Their characteristic apoprotein is **Apo B-48**. In the blood, HDLs stimulate the degradation of chylomicrons by transfer of Apo E and Apo C-II, which occurs particularly in muscle and adipose tissue. Apo C-II in the chylomicrons activates *lipoprotein lipase* [1], an enzyme located on the surface of the vascular endothelia. It hydrolyzes most of the triacylglycerols. The released fatty acids are taken up locally by the cells, while glycerol is transported to the liver. Due to the depletion of the lipids they contain, the chylomicrons gradually shrink to **chylomicron remnants**, which are ultimately removed from the blood by the liver with the aid of Apo-E receptors.

VLDLs, **IDLs**, and **LDLs** are closely related to one another. VLDLs are formed in the liver. They are used to transport endogenously produced lipids such as triacylglycerols, cholesterol, and phospholipids to extrahepatic tissues. **Apo-B-100** is the characteristic apoprotein of VLDLs. Like chylomicrons, VLDLs shrink gradually under the influence of *lipoprotein lipase* [1] to become IDL and finally LDL. This process is also stimulated by transfer of various proteins from HDL. Compared with VLDL and IDL, **LDLs** contain a much larger proportion of cholesterol and cholesterol esters. Cells that have a demand for cholesterol bind LDL, and take up the complete particles through **receptor-mediated endocytosis** (see next page), while the rest is collected by the liver.

A. Composition of lipoprotein complexes

Type	Density [g · cm ⁻³]	Diameter [nm]	Apoproteins	Lipid proportion [%]			Function
				Triacyl- glycerol	Chole- sterol	Phospho- lipid	
Chylo- microns	0.930	75–1200	B-48, C-II, E	80–95	2–7	3–9	Transport of dietary lipids from the intestine
Chylo- micron remnants	0.930– 1.006	30–80	B-48, C-II, E				Transport of dietary lipids to the liver
VLDL	0.930– 1.006	30–80	B-100, C-II, E	55–80	5–15	10–20	Transport of endo- genous lipids from the liver
IDL	1.006– 1.019	25–35	B-100 C-II, E	20–50	20–40	15–25	Transport of endo- genous lipids from the liver
LDL	1.019– 1.063	18–25	B-100	5–15	40–50	20–25	Provision of cholesterol to cells
HDL	1.063– 1.210	5–12	C-II, E	5–10	15–25	20–30	Return of cholesterol to the liver

B. Transport functions

Lipoproteins II

A. Function and fate of HDL

The **HDLs** (high density lipoproteins; see table on previous page) are the lipoprotein group in the blood plasma with the highest density and smallest size. They are produced by various mechanisms, mainly by biosynthesis in the liver and intestine.

In their outer layer, HDLs contain phospholipids, cholesterol, and a range of **apoproteins** such as A-I, A-II, C-I, C-II, D, and E. Fats and cholesterol esters are located inside the particles. The HDL grows by taking up phospholipids and cholesterol from the walls of blood vessels and thereby change from a disc-like structure to a spherical shape.

HDLs can exchange lipids and apoproteins with tissues and other lipoproteins (see illustration on previous page).

1. By reversible **transfer of Apo E and Apo C-II** to chylomicrons and VLDLs their maturation and discharge are promoted.
2. HDLs collect the excess cholesterol produced in the tissues and return it to the liver for disposal (known as **reverse cholesterol transport**).
3. This process is assisted by the fact that the cholesterol taken up by HDL is esterified with fatty acids. Because of their greater lipophilicity, these **cholesterol esters** move into the core of the HDL. This acylation of cholesterol is catalyzed by *lecithin-cholesterol acyltransferase* (LCAT), which the HDL takes up from the blood. The donor of the acyl groups is the phospholipid lecithin, which is converted to lysolecithin by transfer of the fatty acid residue from C-2 of the glycerol to cholesterol.
4. By means of a **cholesterol ester transfer protein** (CETP), HDL with VLDL can exchange cholesterol esters for triacylglycerols and phospholipids. VLDL passes the cholesterol esters on to the liver via IDL and LDL.
5. Mature HDLs are bound to **Apo E** receptors on hepatocytes, taken up by endocytosis, and finally broken down. In addition, a few cells in the body, especially the macrophages, have **"scavenger" receptors**, to which HDLs and other lipoproteins can be bound reversibly in order to discharge their contents. Scavenger receptors usually have high binding capacity and low specificity for their ligands.

HDLs contribute decisively to reducing the blood cholesterol level by collecting cholesterol from peripheral tissues and providing it to the liver directly or via VLDL. In the liver, cholesterol is converted into bile acids or excreted directly into the bile (p. 318). HDLs are therefore regarded as **"good cholesterol,"** in contrast to LDL.

B. LDL binding to the LDL receptor

Certain target cells of lipoproteins bear membrane receptors for different apoproteins. The **LDL receptor**, a very large membrane protein that binds the proteins **Apo B-100** and **Apo E** specifically and with high affinity, has been particularly well studied. By means of the LDL receptors, **LDL** is bound by cells in the periphery of the body and endocytosed. This is a good example of **receptor-mediated endocytosis** (p. 214).

The LDL receptor is an integral membrane protein with a modular structure. It consists of five domains. The outermost, cysteine-rich binding domain binds Apo B-100 and Apo E and thus serves for direct interaction with LDL. In an acidic environment (after endocytosis), the conformation of this protein domain changes and leads to dissociation of the bound LDL. The second domain is homologous to epidermal growth factor (EGF). The third domain contains N- and O-glycosidically linked oligosaccharides, which apparently ensure that the receptor projects far out from the membrane. With the fourth domain the receptor is anchored in the plasma membrane. The fifth domain inside the cell is represented by the C-terminal end, which is responsible for endocytosis.

Through endocytosis of LDLs, their cholesterol-rich lipid content is removed from the blood and absorbed by the cells. The density of LDL receptors is regulated by the intracellular cholesterol level. Disturbances in this process therefore can lead to **hypercholesterolaemia** (p. 300).

Hemoglobin and Gas Transport

The most important task of the erythrocytes is to **transport** molecular oxygen (O_2) from the lungs into the tissues, and carbon dioxide (CO_2) from the tissues into the lungs. To achieve this, the higher organisms require hemoglobin (Hb), since O_2 is *poorly soluble in water*. Hb is also responsible for the majority of the blood's buffer capacity (p. 292).

A. Hemoglobin

In adults, hemoglobin (**HbA**; **1**) is a *heterotetrameric protein* consisting of four globin molecules (two α -chains and two β -chains, each of 16 kDa) which have different sequences but are similarly folded (p. 56). Some 80% of the amino acid residues form α -helices.

Each subunit carries a **heme group** (p. 86), with a central Fe^{2+} ion. When O_2 binds to the heme iron (**oxygenation**), the oxidation stage of the iron does *not* change. *Methemoglobin* (Met-Hb, $Hb \cdot Fe^{3+}$), which is unable to bind O_2 , is formed only rarely. It usually amounts to only 1%–2% (p. 290).

Four of the six coordination sites of the iron are occupied by the nitrogen atoms of the pyrrole rings, and another is occupied by a histidine residue (the *proximal histidine*). The sixth is coordinated with oxygen in oxyhemoglobin and with H_2O in the deoxy form.

Because of *cooperative interactions* between the subunits (see **B**), the O_2 affinity of Hb increases with the O_2 partial pressure (pO_2), leading to an **S-shaped** (sigmoidal) **saturation curve** (**2**, see also p. 80). The shape of the curve is adapted to the O_2 partial pressures in the body: at the pO_2 in the arteries (80–100 mmHg), Hb is almost completely saturated with O_2 , while even slight pO_2 shifts in peripheral tissues (to 30–50 mmHg) greatly alter the degree of saturation (**2**, a–c). This effect is further intensified by *allosteric effectors* of Hb (**B**). Doubling of the normal pCO_2 of 40 mmHg shifts the curve to the right and thereby leads to doubled O_2 release (**3**, cf. distances a and b). This phenomenon has long been familiar as the *Bohr effect*.

B. Allosteric regulation

Like ACTase (p. 80), Hb can exist in two different states (*conformations*), the T form and the R form. The **T form** (for *tense*; top) has a much *lower* O_2 affinity than the R form (for *relaxed*; bottom).

Binding of O_2 to the T form leads to a conformational change that weakens the association between the chains. Increasing pO_2 thus means that more and more molecules convert to the higher-affinity R form.

Various **allosteric effectors** influence the equilibrium between the T and R forms and thereby regulate the binding behavior of hemoglobin. The most important effectors are CO_2 , H^+ , and 2,3-bisphosphoglycerate (BPG; p. 290).

C. Gas transport in the blood

Hemoglobin is also decisively involved in the transport of carbon dioxide (CO_2) between the tissues and lungs.

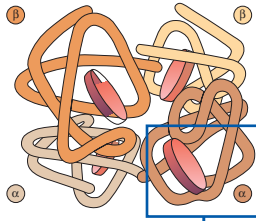
Some 5% of the CO_2 is covalently bound to the N-terminus of Hb as **carbamino-Hb** (not shown). Over 90% is first converted in peripheral tissues into *hydrogen carbonate* (HCO_3^-), which is more soluble (bottom). In the lungs (top), CO_2 is regenerated again from HCO_3^- and can then be exhaled.

These two processes are coupled to the oxygenation and deoxygenation of Hb. *Deoxy-Hb* is a stronger base than *oxy-Hb*. It binds additional protons ($\sim 0.7 H^+$ per tetramer), which promotes the conversion of CO_2 to HCO_3^- in the tissues. The resulting HCO_3^- is released into the plasma via an antiporter in exchange for Cl^- , and is transported to the lungs. There, the reactions described above proceed in reverse order: deoxy-Hb is oxygenated and releases protons. The protons shift the HCO_3^-/CO_2 equilibrium and thereby promote CO_2 release.

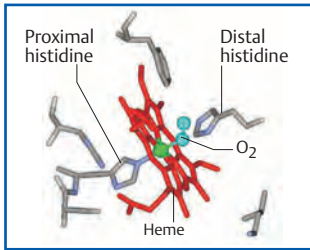
O_2 binding to Hb is regulated by H^+ ions (i.e., by the pH) via the same mechanism. High concentrations of CO_2 in tissues with intensive metabolism locally increase the local H^+ concentration and thereby reduce the O_2 affinity of Hb (**Bohr effect**; see above). This leads to increased O_2 release and thus to an improved oxygen supply.

The adjustment of the equilibrium between CO_2 and HCO_3^- is relatively slow in the uncatalyzed state. It is therefore accelerated in blood and other organs by the zinc-containing enzyme *carbonic anhydrase* [**1**], which occurs in high concentrations in the erythrocytes.

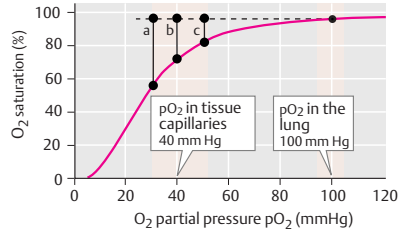
A. Hemoglobin



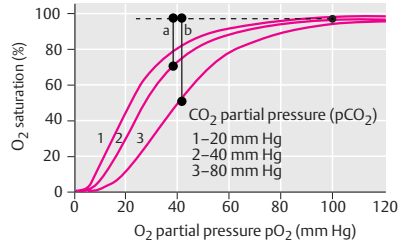
Hemoglobin A ($\alpha_2\beta_2$)



1. Structure



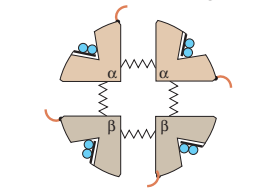
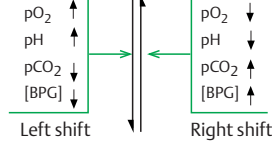
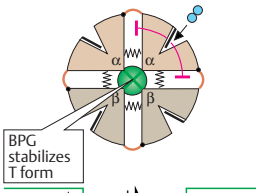
2. Saturation curve



3. Bohr effect

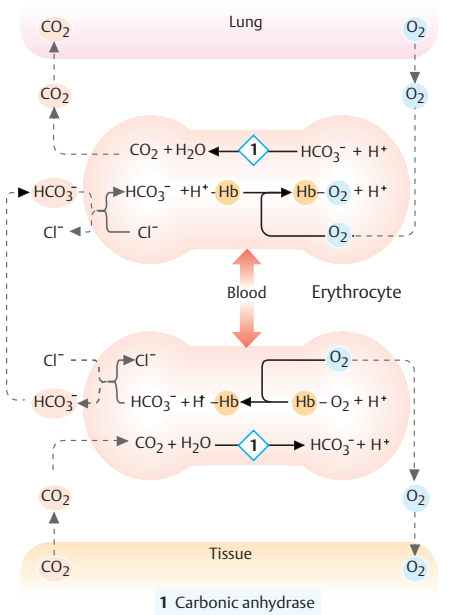
B. Allosteric regulation

Deoxyhemoglobin (T form)
low O_2 affinity



Oxyhemoglobin (R form)
high O_2 affinity

C. Gas transport in the blood



Reactive Oxygen Species

Cells living in aerobic conditions are dependent on molecular oxygen for energy production (p. 110). On the other hand, O_2 constantly gives rise to small quantities of toxic substances known as **reactive oxygen species** (ROS). These substances are powerful *oxidation agents* or extremely reactive *free radicals* (p. 12), which damage cells and functional molecules. Due to their role in O_2 transport, the erythrocytes are constantly exposed to high concentrations of O_2 and are therefore particularly at risk from ROS.

A. Reactive oxygen species

The oxygen molecule (O_2) contains two unpaired electrons—i.e., it is a *diradical*. Despite this, O_2 is relatively stable due to its special electron arrangement. However, if the molecule takes up an extra electron (a), the highly reactive **superoxide radical** ($\cdot O_2^-$) is produced. Another reduction step (b) leads to the **peroxide anion** (O_2^{2-}), which binds protons and thus becomes **hydrogen peroxide** (H_2O_2). Addition of a third electron (c) leads to cleavage of the molecule into the ions O^{2-} and O^- . While O^{2-} can form **water** by taking up two protons, protonation of O^- provides the extremely dangerous **hydroxy radical** ($\cdot OH$). A fourth electron transfer (d) and subsequent protonation also convert O^- into water.

The synthesis of ROS can be catalyzed by Fe^{2+} ions, for example. Reaction of O_2 with FMN or FAD (p. 12) also constantly produces ROS. In addition to antioxidants (**B**), **enzymes** also provide protection against ROS: *superoxide dismutase* [1] breaks down (“disproportionates”) two superoxide radicals into O_2 and the less damaging H_2O_2 . The latter is in turn disproportionated into O_2 and H_2O by heme-containing *catalase* ([2]; p. 226).

B. Biological antioxidants

To protect them against ROS and other radicals, all cells contain **antioxidants**. These are *reducing agents* that react readily with oxidizing agents and thus protect more important molecules from oxidation. Biological antioxidants include **vitamins C and E** (p. 392f.), **ubiquinol** (coenzyme Q; p. 86), and several **carotenoids** (p. 394). **Bilirubin** (p. 190) and **uric acid** (p. 180), also serves for protection against oxidation. The tripeptide **glutathione** is particularly important.

C. Glutathione

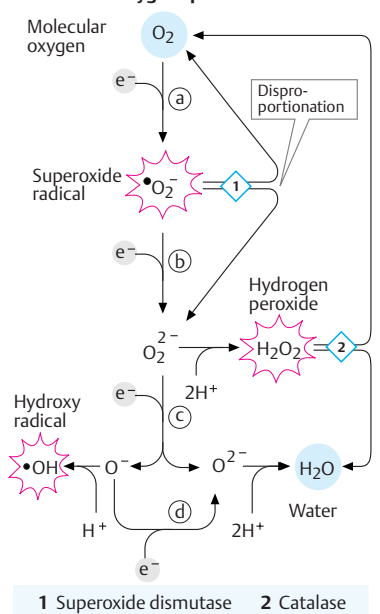
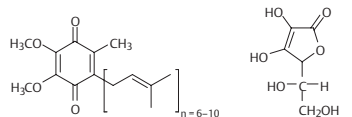
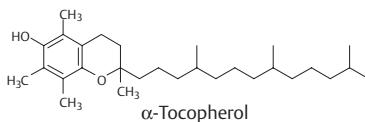
Glutathione (sequence: γ -Glu-Cys-Gly; p. 175) contains an atypical γ -peptide bond between Glu and Cys. The *thiol group* of the cysteine residue is redox-active. Two molecules of the reduced form (**GSH**, top) are joined to the disulfide (**GSSG**, bottom) during oxidation, and this can be split back into two GSH molecules by reduction (p. 12). Glutathione is present in millimolar concentrations in all cells, including erythrocytes.

D. Membrane protection

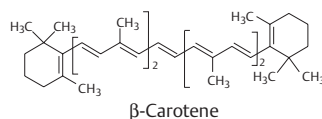
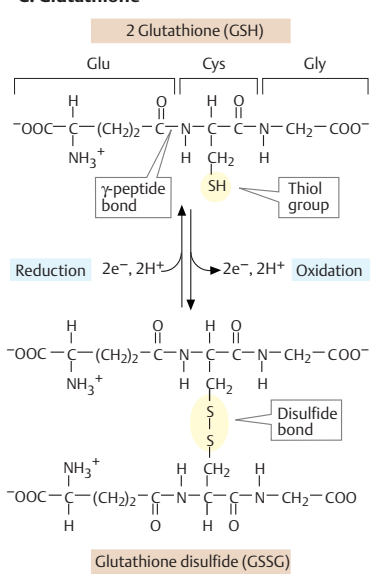
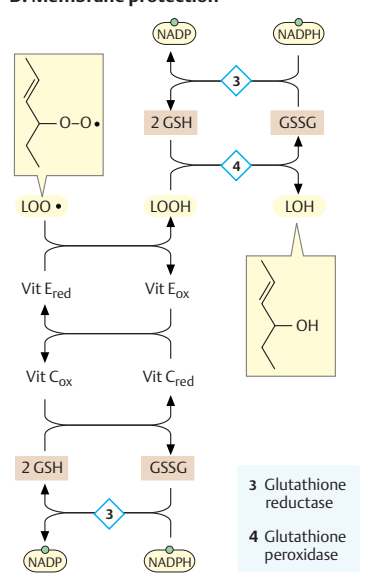
The acyl residues of lipids in the erythrocyte membrane are particularly susceptible to ROS. They can form *alkyl radicals*, $L\cdot$ (not shown), which are further converted to *lipid peroxide radicals* ($LOO\cdot$) by reaction with O_2 . This **lipid peroxidation** triggers further reactions, which over time damage the membrane and hemoglobin irreversibly.

The illustration shows how lipid peroxide radicals can be converted to harmless products by the interplay of chemical antioxidants and enzymatic reactions. The primary antioxidant is **vitamin E** (α -tocopherol, p. 392), which “floats” in the membrane because of its hydrophobic isoprenoid side chain. It reduces the peroxide radical to the more harmless *lipid peroxide* $LOOH$. This is reduced further to harmless lipid alcohols (LOH) by the selenium-containing enzyme *glutathione peroxidase* ([4]; p. 52). **Glutathione** (**GSH**, **C**), which is oxidized to the disulfide **GSSG**, is the reaction's coenzyme. In the last step of the redox chain, *glutathione reductase* [3] regenerates functional GSH with the aid of NADPH. Vitamin E oxidized by $LOO\cdot$ is also regenerated by GSH, with **vitamin C** interposed as a further redox system.

While erythrocytes can survive for more than four months under optimal conditions, they perish within a few days if their oxidation protection systems fail.

A. Reactive oxygen species**B. Biological antioxidants**

Quinols and enols	α -Tocopherol (vitamin E) Ubiquinol (coenzyme Q) Ascorbic acid (vitamin C)
Carotinoids	β -Carotene Lycopene
Others	Glutathione Bilirubin

**C. Glutathione****D. Membrane protection**

Erythrocyte Metabolism

Mammalian erythrocytes are non-nucleated cells with a greatly reduced **maintenance metabolism** which is essentially limited to **anaerobic glycolysis** (p. 130) and the **pentose phosphate pathway** (PPP, p. 132). Erythrocytes are therefore viable only when they are constantly supplied with glucose. Regardless of the metabolic situation, the 3×10^{13} erythrocytes in human blood consume about 36 g glucose per day and release about the same amount of *lactate*, which can be converted to glucose again by gluconeogenesis in the liver (p. 378).

A. Erythrocyte metabolism

The **ATP** formed during glycolysis serves mainly as a coenzyme of *Na⁺/K⁺-ATPase* (p. 108), which maintains the erythrocytes' membrane potential. A second byproduct of glycolysis is the allosteric effector of hemoglobin, **2,3-bisphosphoglycerate (BPG)**. A small amount of the NADH produced in glycolysis supplies reducing equivalents for the reduction of methemoglobin (see below).

The main function of the pentose phosphate pathway is to provide **NADPH**, which is needed to regenerate **glutathione (GSH)** from glutathione disulfide (GSSG) with the help of *glutathione reductase* [3]. Glutathione, the most important antioxidant in the erythrocytes, is as a coenzyme for *glutathione peroxidase* [4]. This enzyme detoxifies H₂O₂ and lipid hydroperoxides (LOOH), which arise during the reaction of ROS with unsaturated fatty acids in the erythrocyte membrane (p. 288). Glutathione reductase is one of the few enzymes with a **selenocysteine residue** in its active center (p. 52).

Normally, O₂ is only bound to Hb by coordination to the heme iron, without reacting with it. However, occasionally a redox reaction occurs that produces **methemoglobin** (Hb · Fe³⁺) and a **superoxide radical** (p. 288). *Met-Hb reductase* [2] reduces Hb · Fe³⁺ back to functional Hb · Fe²⁺, with reduced cytochrome acting as electron donor. This in turn is regenerated by *cytochrome b₅ reductase* [1] with the aid of NADH produced in glycolysis. The superoxide anion is detoxified by *superoxide dismutase* (p. 288).

B. 2,3-Bisphosphoglycerate

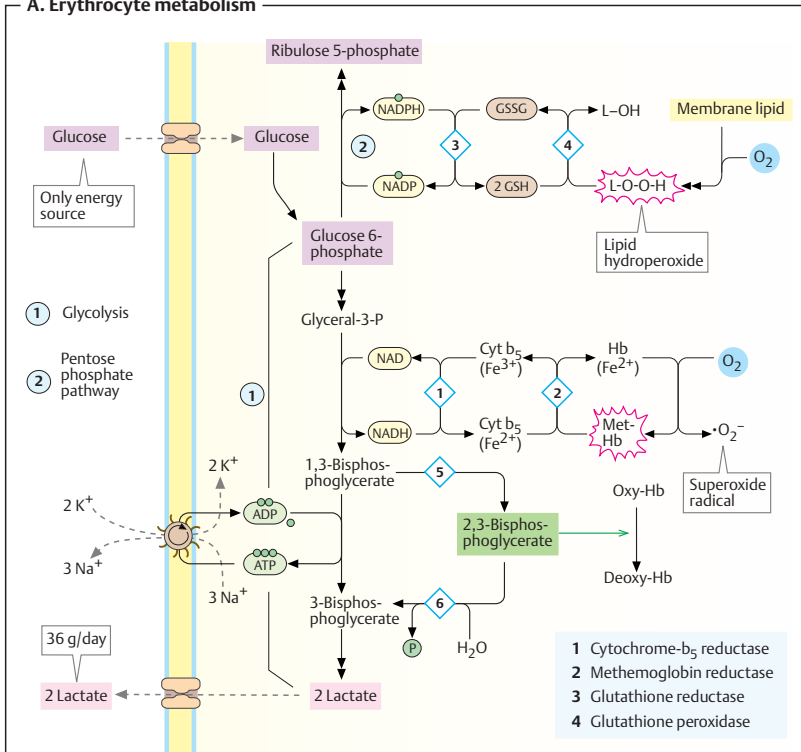
Besides H⁺ ions and CO₂ (p. 286), **2,3-bisphosphoglycerate (BPG)** is the most important regulator of O₂ binding to hemoglobin. BPG is formed in erythrocytes by *bisphosphoglycerate*

mutase [5] from the glycolysis intermediate *1,3-bisphosphoglycerate* (p. 130) and can reenter glycolysis as 2-phosphoglycerate via *bisphosphoglycerate phosphatase* [6]. Because the phosphoglycerate kinase reaction is bypassed, the "detour" via BPG is associated with loss of an ATP, as compared to glycolysis (p. 130). BPG binds selectively to *deoxy-Hb* as an **allosteric effector** (p. 80), thereby increasing its proportion in the equilibrium. The result is **increased O₂ release** at constant pO₂. In the diagram, this corresponds to a **right shift** of the saturation curve (1→2). CO₂ and H⁺ ions act similarly (p. 286). The effects of CO₂ (red curve, 1→2) and BPG on the O₂ saturation curve are **additive**. If both effectors are added to a solution of pure Hb, curve 1 (blue) changes to curve 3 (green), which corresponds to that of whole blood (p. 286).

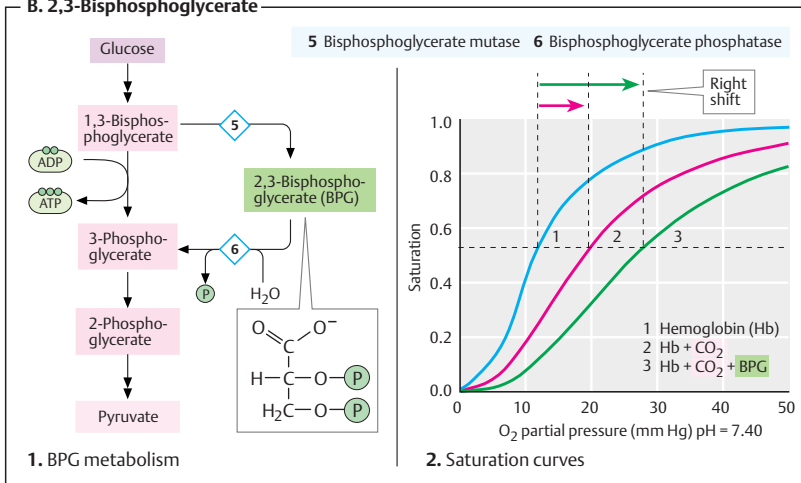
■ Further information

Donor erythrocytes that will not be used for transfusion purposes immediately can be preserved by appropriate measures for up to 6 weeks. They are first separated from plasma and leukocytes and the resulting erythrocyte concentrate is then stabilized with additives, e.g., in the form of *CPDA solution*. The acronym "CPDA" stands for the components of the solution, namely, *citrate*, which inhibits clotting (p. 296), *phosphate*, which is needed for ATP production, "*dextrose*," i.e., glucose as energy substrate for the erythrocytes, and *adenine*, which helps to maintain the ATP level.

A. Erythrocyte metabolism



B. 2,3-Bisphosphoglycerate



Acid–Base Balance

A. Hydrogen ion concentration in the blood plasma

The H^+ concentration in the blood and extracellular space is approximately 40 nM ($4 \times 10^{-8} \text{ mol} \times \text{L}^{-1}$). This corresponds to a **pH of 7.40**. The body tries to maintain this value, as large shifts in pH are incompatible with life.

The pH value is kept constant by **buffer systems (C)** that cushion minor disturbances in the *acid–base balance*. In the longer term, the decisive aspect is maintaining a balanced equilibrium between H^+ production plus uptake and H^+ release. If the blood's buffering capacity is not sufficient, or if the acid–base balance is not in equilibrium—e.g., in kidney disease or during *hypoventilation* or *hyperventilation*—shifts in the plasma pH value can occur. A reduction by more than 0.03 units is known as **acidosis**, and an increase is called **alkalosis**.

B. Acid–base balance

Protons are mainly derived from two sources—free acids in the diet and sulfur-containing amino acids. *Acids* taken up with food—e.g., **citric acid**, **ascorbic acid**, and **phosphoric acid**—release protons in the alkaline pH of the intestinal tract. More important for proton balance, however, are the amino acids **methionine** and **cysteine**, which arise from protein degradation. Their sulfur atoms are oxidized in the liver to form sulfuric acid, which releases protons by dissociation into sulfate.

During anaerobic glycolysis in the muscles and erythrocytes, glucose is converted into **lactate**, also releasing protons in the process (p. 130). The synthesis of the **ketone bodies** acetoacetic acid and 3-hydroxybutyric acid in the liver (p. 318) also yields protons. If acids are formed in excessive amounts, however (e.g., during *starvation* or in *diabetes mellitus*; p. 430), they strain the buffer systems and can lead to a reduction in pH (**metabolic acidosis**; *lactacidosis* or *ketoacidosis*). Normally, however, the amounts of these acids are small and of little influence on the proton balance.

Only the kidneys (but not by the lungs) can excrete protons directly or in exchange for Na^+ ions. In the urine, the excreted H^+ ions are buffered by NH_3 and phosphate (p. 336).

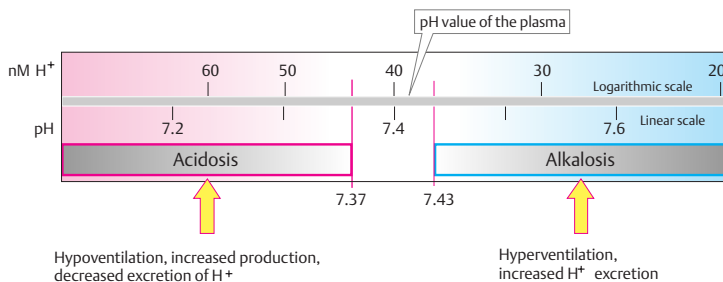
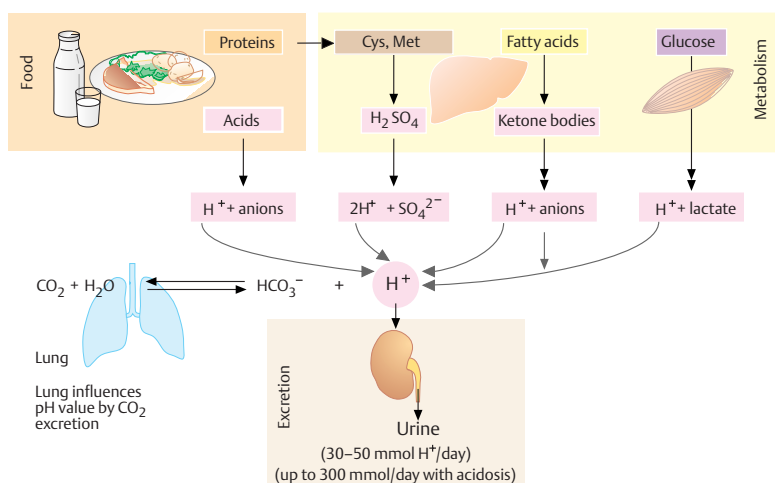
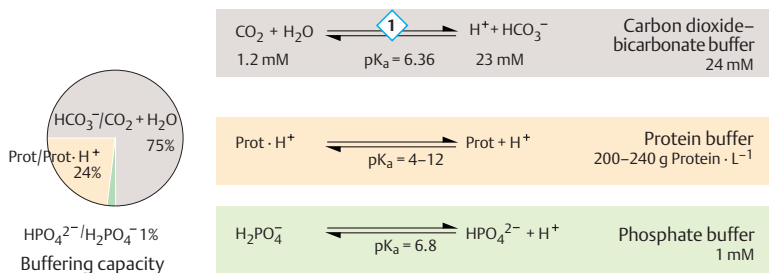
C. Buffer systems in the plasma

The **buffering capacity** of a buffer system depends on its concentration and its pK_a value. The strongest buffering effect is achieved if the pH value corresponds to the buffer system's pK_a value (see p. 30). For this reason, weak acids with pK_a values of around 7 are best suited for buffering purposes in the blood.

The most important buffer in the blood is the **CO_2 /bicarbonate buffer**. This consists of water, **carbon dioxide** (CO_2 , the anhydride of carbonic acid H_2CO_3), and **hydrogen carbonate** (HCO_3^- , bicarbonate). The adjustment of the balance between CO_2 and HCO_3^- is accelerated by the zinc-containing enzyme *carbonate dehydratase* (carbonic anhydrase [1]; p. 286). At the pH value of the plasma, HCO_3^- and CO_2 are present in a ratio of about 20 : 1. However, the CO_2 in solution in the blood is in equilibrium with the gaseous CO_2 in the pulmonary alveoli. The CO_2/HCO_3^- system is therefore a powerful *open buffer system*, despite having a not entirely optimal pK_a value of 6.36 (acid constant of CO_2). Faster or slower respiration increases or reduces CO_2 release in the lungs. This shifts the CO_2/HCO_3^- ratio and thus the plasma pH value (**respiratory acidosis** or **alkalosis**). In this way, respiration can compensate to a certain extent for changes in plasma pH values. However, as stated above it does *not* lead to the excretion of protons.

Due to their high concentration, **plasma proteins**—and **hemoglobin** in the erythrocytes in particular—provide about one-quarter of the blood's buffering capacity. The buffering effect of proteins involves contributions from all of the ionizable side chains. At the pH value of blood, the acidic amino acids (Asp, Glu) and histidine are particularly effective.

The second dissociation step of **phosphate** ($H_2PO_4^- / HPO_4^{2-}$) also contributes to the buffering capacity of the blood plasma. Although the pK_a value of this system is nearly optimal, its contribution remains small due to the low total concentration of phosphate in the blood (around 1 mM).

A. Hydrogen ion concentration in the blood plasma**B. Acid–base balance****C. Buffer systems in the plasma**

1 Carbonate dehydratase (carbonic anhydrase)

Blood Clotting

Following injury to blood vessels, **hemostasis** ensures that blood loss is minimized. Initially, platelet activation leads to contraction of the injured vessel and the formation of a loose clot consisting of platelets (**hemostasis**). Slightly later, the action of the enzyme **thrombin** leads to the formation and deposition in the thrombus of polymeric fibrin (**coagulation, blood clotting**). Only the coagulation process is discussed here.

A. Blood clotting

The most important reaction in blood clotting is the enzymatic conversion of the soluble plasma protein **fibrinogen** (factor I) into polymeric **fibrin**, which is deposited as a fibrous network in the primary thrombus. This reaction is catalyzed by **thrombin** (factor IIa), a serine proteinase (p. 162) that cleaves small peptides from fibrinogen. This exposes binding sites that spontaneously allow the fibrin molecules to aggregate into polymers. Subsequent covalent cross-linking of fibrin by a **transglutaminase** (factor XIII) further stabilizes the thrombus.

Normally, thrombin is present in the blood as an inactive proenzyme. Prothrombin is activated in two different ways, both of which represent cascades of enzymatic reactions in which inactive proenzymes (zymogens, symbol: circle) are proteolytically converted into active **proteinases** (symbol: sector of a circle). The proteinases activate the next proenzyme in turn, and so on. Several steps in the cascade require additional **protein factors** (factors III, Va, and VIIIa) as well as anionic **phospholipids** (PL; see below) and Ca^{2+} ions. Both pathways are activated by injuries to the vessel wall.

In the **extravascular pathway** (right), *tissue thromboplastin* (factor III), a membrane protein in the deeper layers of the vascular wall, activates coagulation factor VII. The activated form of this (VIIa) autocatalytically promotes its own formation and also generates the active factors IXa and Xa from their precursors. With the aid of factor VIIIa, PL, and Ca^{2+} , factor IXa then produces additional Xa, which finally—with the support of Va, PL, and Ca^{2+} —releases active thrombin.

The **intravascular pathway** (left) is probably also triggered by vascular injuries. It leads in five steps via factors XIIa, XIa, IXa, and Xa to the activation of prothrombin. The significance of this pathway *in vivo* has been controversial

since it was found that a genetic deficiency in factor XII does not lead to coagulation disturbances.

B. Protein complexes

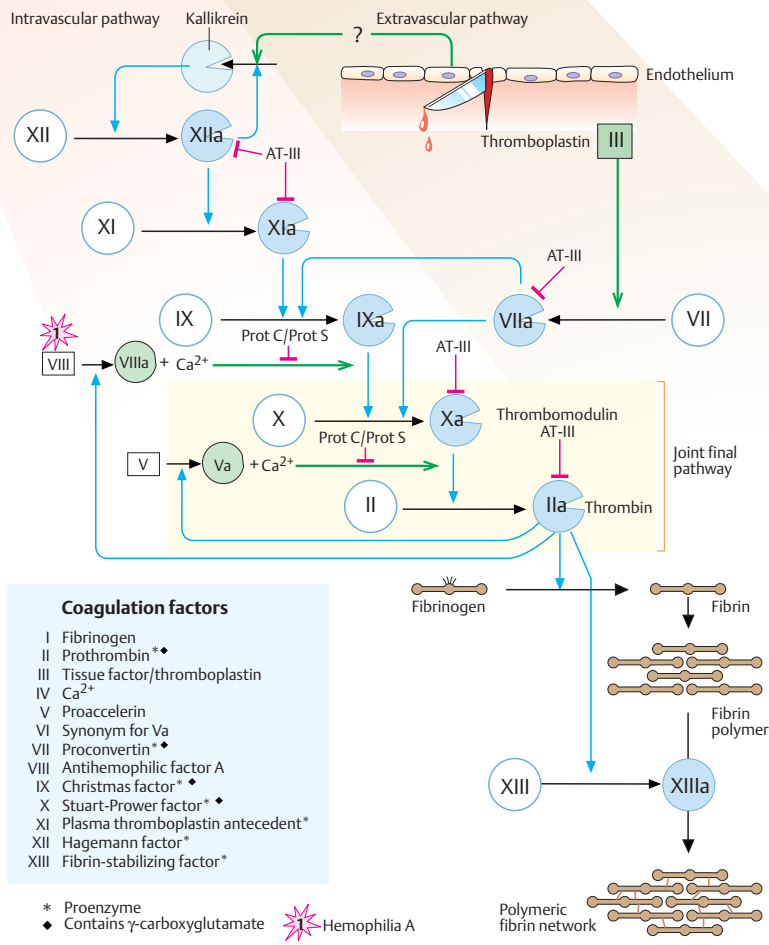
Ca^{2+} and phospholipids (PL) in the platelet membrane are decisively involved in the clotting cascade. They lead to the formation of local complexes involving proteins that contain amino acid residues of the Glu type (γ -carboxyglutamate, p. 52).

Glu residues are found in groups in special domains of clotting factors II, VII, IX, and X where they mediate contacts to phospholipids of the thrombocyte membrane via bound Ca^{2+} ions. The **tenase complex** (1, left) is formed from factors IXa and VIIIa, thereby activating factor X to Xa. This then binds with Va into the **prothrombinase complex** (2, right), which catalyzes the crucial activation of prothrombin (II) to thrombin (IIa) on the platelet membrane.

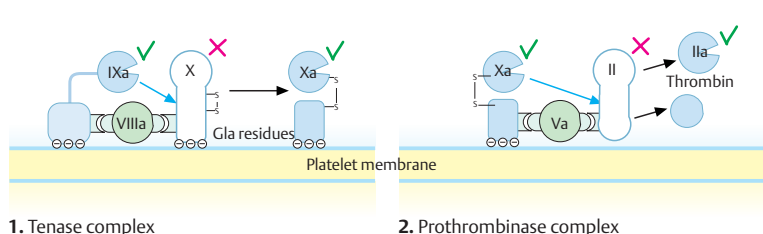
Active **thrombin** not only converts fibrinogen into fibrin, but also indirectly promotes its own synthesis by stimulating the activation of factors V and VIII. In addition, it catalyzes the activation of factor XIII and thereby triggers the cross-linking of the fibrin. This greatly reinforces the effect when clotting has been triggered. Fibrinolysis is also triggered by thrombin (p. 296), though with a delayed onset.

Disorders of blood clotting. There are various known defects that slow blood clotting. The most common genetic disease is a deficiency of **von Willebrand factor** in the subendothelium of blood vessels, a factor that promotes platelet adhesion. **Hemophilia A** is caused by a deficiency of functional factor VIII, while **hemophilia B** is due to mutations in the gene for factor IX. Since both forms of hemophilia have *X-linked recessive inheritance*, men are affected almost exclusively.

A. Blood clotting



B. Protein complexes



Inhibition of Blood Clotting, Fibrinolysis

Hemostasis, the formation of *thrombi* from fibrin and platelets (p. 294), is in a delicate equilibrium with the dissolution of these thrombi by **fibrinolysis**. Disturbances of this equilibrium can lead, on the one hand, to *hemorrhages* due to excessively slow hemostasis, and on the other hand to *thrombosis* and *embolism* due to an increased clotting tendency. When there is a risk of thrombosis, anticoagulants are employed to inhibit clotting, while fibrinolytic enzymes are used in myocardial infarction to dissolve blood clots.

A. Natural anticoagulation

Active **thrombin** can lose its clotting activity by binding to **thrombomodulin**, a membrane protein of endothelial cells. In its bound form, thrombin has anticoagulant properties; it activates **protein C**. This binds to its cofactor **protein S**, resulting in an **APC complex** (activated protein C), which blocks the clotting cascade by destroying clotting factors Va and VIIIa.

In blood plasma various serine protease inhibitors are present, which are known as **serpins** (**Serine Protease Inhibitors**). Serpins by acting as *pseudosubstrates* can block enzymes of both the clotting cascade and fibrinolysis (see below). For example, thrombin is regulated by the serpin **antithrombin III** (ATIII). Inactivation of thrombin by the formation of a thrombin-ATIII complex is greatly activated by **heparin** and similar glycosaminoglycans (p. 352). Heparin stimulates ATIII and thereby indirectly inactivates not only thrombin but also other clotting factors.

B. Artificial anticoagulation

Blood clotting can be artificially inhibited to avoid *thromboses* and *emboli*. This is achieved by giving **anticoagulant drugs**.

Heparin (p. 352), a *glycosaminoglycan* that occurs naturally in mast cells and connective tissue, activates the serpin ATIII (see above). Since heparin would be digested in the bowel, this fast-acting medication has to be injected. By contrast, the **vitamin K antagonists**, which can be taken orally, act much more slowly. They inhibit the regeneration of active vitamin K in the liver, thus indirectly blocking the **γ -carboxylation** of glutamate residues (p. 62) involved in the biosynthesis of clotting factors II, VII, IX, and X and proteins C and S. These

proteins can then no longer bind Ca^{2+} ions and are unable to function. The **coumarins** are especially important vitamin K antagonists. *Phenprocoumon* (shown here) and the similar compound *warfarin*, have an obvious structural similarity to vitamin K (p. 393).

The anticoagulants also include **inhibitors of platelet aggregation**, which are used as “*blood thinners*.” **Acetylsalicylate** (ASA, Aspirin) is one of the drugs in this group. It inhibits platelet aggregation by blocking prostaglandin synthesis (p. 438).

Blood clotting *in vitro* can be prevented by heparin or **Ca^{2+} chelators** like **citrate** or **EDTA** (ethylenediamine tetraacetate).

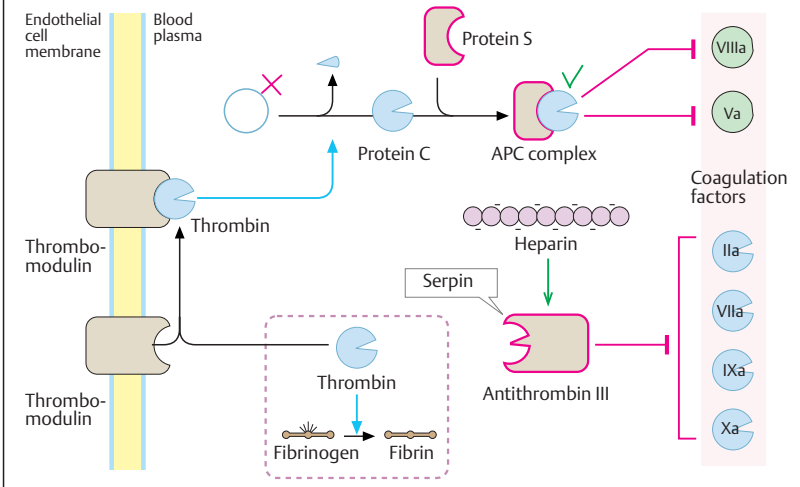
The effect of the anticoagulants is assessed by determining the so-called **INR** (*international normalized ratio*), or formerly the **prothrombin time**. The INR states the factor by which the clotting time is prolonged.

C. Fibrinolysis

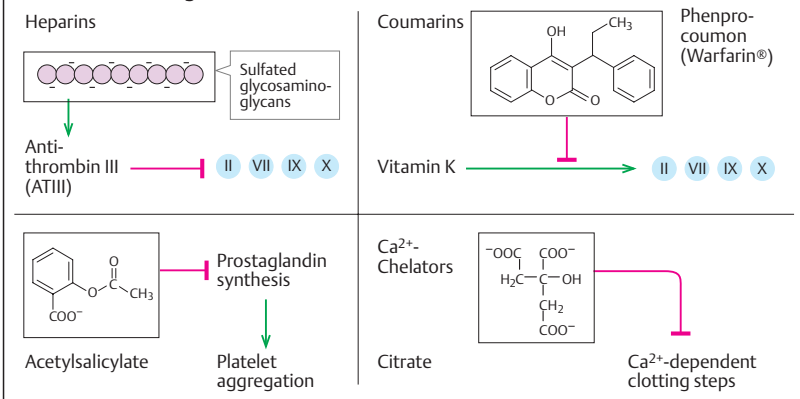
In fibrinolysis, the fibrin thrombus resulting from blood clotting (p. 294) is dissolved again by **plasmin**. Plasmin, also a serine proteinase, is produced from the zymogen *plasminogen*, which is already incorporated in the thrombus. The activating enzymes include a *plasminogen activator* from kidney (*urokinase*, *uPA*) and *tissue plasminogen activator* (*tPA*) from vascular endothelial cells (p. 354). By contrast, the plasma protein α_2 -antiplasmin, which binds to active plasmin and thereby inactivates it, inhibits fibrinolysis.

Urokinase, t-PA, and streptokinase, a bacterial activator of human plasminogen, are used clinically to dissolve thrombi following *heart attacks*.

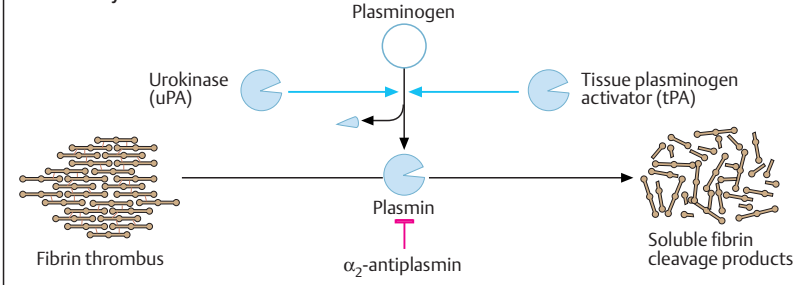
A. Natural anticoagulation



B. Artificial anticoagulation



C. Fibrinolysis



Blood Groups

During blood transfusions, immune reactions can occur that destroy the erythrocytes transfused from the donor. These reactions result from the presence of soluble antibodies (p. 304) directed to certain surface structures on the erythrocytes. These surface structures, known as **blood group antigens**, are present on the surface of erythrocytes and many other cells. They are *proteins* or *glycolipids* that can differ from individual to individual. More than 20 different blood group systems are now known. The ABO system and the Rh system are of particular clinical importance.

A. Blood groups: the ABO system

In the **ABO system**, the terminal carbohydrate parts of glycoproteins or glycolipids act as antigens. In this relatively simple system, there are four **blood groups**: A, B, AB, and O. In individuals with blood groups A and B, the antigens consist of tetrasaccharides that only differ in the terminal sugar residue (galactose or N-acetylgalactosamine). Carriers of the AB blood group have both antigens (A and B). Blood group O arises from an oligosaccharide (the **H antigen**) that lacks the terminal residue of antigens A and B. The molecular causes for the differences between blood groups are mutations in the *glycosyl transferases* that transfer the terminal sugar to the core oligosaccharide. What is distinctive about the ABO blood group system is that every individual forms antibodies against those surface antigens not present in his or her own blood. This occurs in the first year of life, probably as a reaction to similar glycostructures in the membranes of intestinal bacteria. For example, antibodies against antigen B ("**anti-B**") are found in carriers of blood group A, while antibodies against antigen A ("**anti-A**") are found in carriers of group B. Individuals with blood group O form anti-A and anti-B, and those with blood group AB do not form any of these antibodies.

B. Blood group compatibility

If an **erythrocyte concentrate** from a blood group A donor were to be transfused into the circulation of an individual with blood group B, then the anti-A present there would bind to the A antigens of the erythrocytes. The donor erythrocytes marked in this way would then be recognized and destroyed by the complement system of the recipient (p. 308). In the

test tube, **agglutination** of the erythrocytes is observed when donor and recipient blood are incompatible. The recipient's serum should therefore not contain any antibodies against the donor erythrocytes.

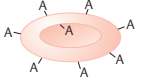
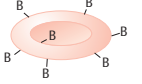
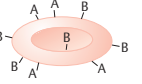
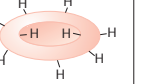
When **plasma** or **whole blood** is transfused, the donor serum should not contain any antibodies against the recipient's erythrocytes. For this reason, erythrocyte concentrate is usually employed for transfusion, and not whole blood. Transfusion of an erythrocyte concentrate with blood group O is unproblematic, as its erythrocytes do not possess any ABO antigens and therefore do not react with anti-A or anti-B in the recipient's blood. Conversely, erythrocytes from the AB group can only be administered to recipients with the AB group, as these are the only ones without antibodies to the A or B antigen.

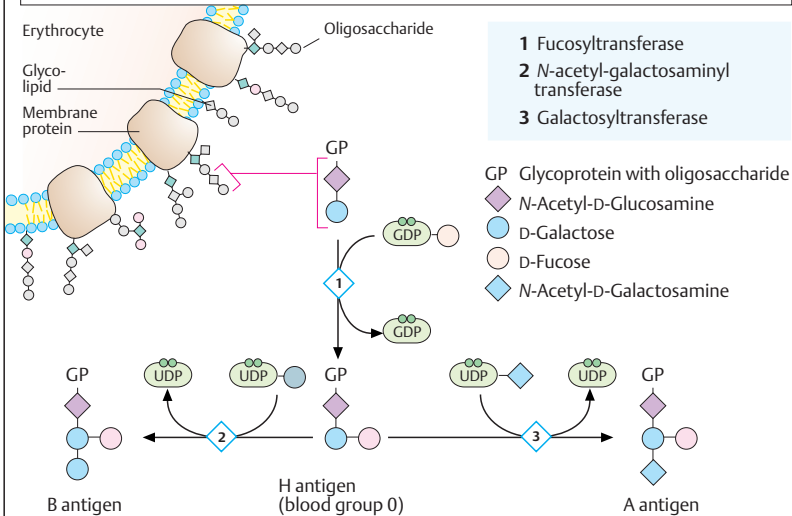
Prior to transfusion, **cross-matching** of donor erythrocytes and the recipient's blood is performed to check that no agglutination occurs.

In the **Rh system**, *proteins* on the surface of the erythrocytes act as antigens (not shown). These are known as "*rhesus factors*," as the system was first discovered in rhesus monkeys.

The **rhesus D** antigen is particularly widespread, occurring in 84% of all white individuals, who are therefore "*Rh-positive*." If an Rh-positive child is born to an Rh-negative mother, fetal erythrocytes can enter the mother's circulation during birth and lead to the formation of antibodies (IgG) against the D antigen. This initially has no acute effects on the mother or child. Complications only arise when there is a second pregnancy with an Rh-positive child, as maternal anti-D antibodies cross the placenta to the fetus even before birth and can trigger destruction of the child's Rh-positive erythrocytes (*fetal erythroblastosis*). However, erythroblastosis of the second child can be prevented by giving the mother anti-D prophylaxis.

A. Blood groups: the ABO system

Blood group	A	B	AB	O
Genotypes	AA and AO	BB and BO	AB	OO
Antigens				
Antibodies in blood	anti-B	anti-A	—	anti-A anti-B
Frequency in central Europe	42.5%	14%	6.5%	37%



B. Blood group compatibility

	A anti-B	B anti-A	AB —	O anti-A anti-B
Donor erythrocytes				
Recipient's blood				

Pathobiochemistry

Of the many diseases that affect the blood system, only anemia, atherosclerosis, and defects of lipoprotein lipase are discussed below.

A. Anemia

The concentration of **hemoglobin** (p. 286) in the blood has characteristic values for newborn infants, men, and women (1). Values below this concentration are called **anemia**. It can have a great variety of causes (2) and is therefore classified into different categories, e.g., according to the size and shape of the erythrocytes and according to the hemoglobin concentration.

Nutritional anemias can be due to a *deficiency of iron, folic acid, or vitamin B₁₂*, which are required for the biosynthesis of hemoglobin (p. 188).

Other forms of anemia are caused by *poisoning*, e.g., with lead. Another group of anemias is caused by *genetic defects*, e.g., mutations of the hemoglobin gene (*hemoglobinopathies*) like sickle cell anemia and the thalassemias.

Sickle cell anemia. Sickle cell anemia (3) is medically the most important of the many hundreds of known mutations of the globin gene. This *missense point mutation* is caused by exchange of a single base: GAG → GTG (p. 262). This leads to the exchange of a hydrophilic glutamate residue in position 6 of the β -chain for a hydrophobic valine residue. The sickle cell hemoglobin HbS is less soluble than the HbA of healthy individuals. In the absence of oxygen, it tends to form fibers by aggregation, which alter the structure of erythrocytes from rounded to *sickle-shaped* cells. Sickle cells clump readily, are more unstable and are degraded faster in the spleen. Sickle cell anemia provides *heterozygous carriers* resistance against malaria and is therefore particularly widespread in malarial regions.

Thalassemia. In normal hemoglobin, the α - and β -chains are present in a 1:1 ratio (p. 286). In the thalassemias, this ratio is disturbed by mutations of the globin genes: in **α -thalassemia** too little α -globin is formed, and in the much more frequent **β -thalassemia** too little β -globin is produced. Homozygous carriers suffer from severe anemia because they do not produce enough functional hemoglobin. Fewer and defective erythrocytes are then formed. Degradation of the erythrocytes is also faster. On the other hand, the thalassemias also confer resistance against **malaria** and therefore

occur more frequently in the Mediterranean region.

B. Marker enzymes in blood serum

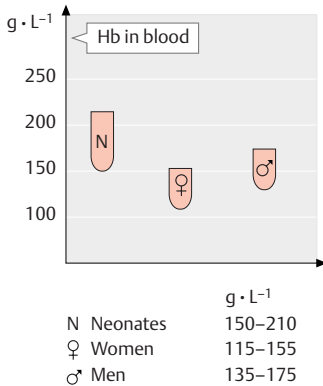
The presence of elevated levels of intracellular enzymes in the blood indicates that tissue damage has occurred somewhere in the body leading to enhanced plasma membrane permeability or cell necrosis. **Enzyme analysis** of serum samples can therefore provide evidence of organ diseases (p. 94). The table gives examples of such marker enzymes. Sometimes it is necessary to distinguish between **isoenzymes**. The quotient of the activities of different enzyme forms often provides a more specific indication of the affected tissue (not shown).

■ Further information

Atherosclerosis is a pathological change of the inner surface of blood vessels that underlies **arteriosclerosis** (*hardening of the arteries*). It starts with damage of the endothelial cells due to various noxious agents, such as high blood pressure, raised concentrations of glucose and lipoproteins, or smoking. Macrophages then accumulate, which take up LDL with the aid of *scavenger receptors* (p. 282). These lipid-laden macrophages (known as foam cells) produce fatty streaks on the intima. Further cell damage results in the development of fibrous **plaques** with cholesterol crystals. Subsequent deposition of calcium leads to *calcification*. This altered inner surface of the blood vessels can trigger the formation of thrombi, which can give rise to **emboli** and myocardial **infarctions**.

Defects of lipoprotein lipase (LPL) lead to disorders of chylomicron and VLDL degradation. As a result, the *triglyceride level* of the blood increases greatly. In diabetics, too, LPL activity is reduced because of the absence of insulin. Their blood lipid levels are therefore often raised (**hyperlipidemia**). Measurement of the activity of LPL in the serum is facilitated by prior injection of heparin (p. 354), which detaches the LPL from the capillary walls

A. Anemia



Reduced erythrocyte production

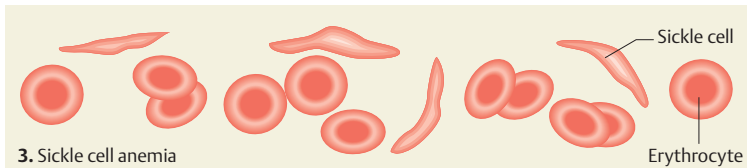
Poor diet:
lack of iron, folic acid or
vitamin B₁₂
Genetic defects:
Sickle cell anemia, thalassemia

Increased consumption and decay of erythrocytes

Hemorrhage
Genetic defects:
Glucose-6-phosphate
dehydrogenase, pyruvate kinase,
spherocytosis
Poisoning:
lead, drugs
Infectious diseases:
malaria

1. Hemoglobin concentration in healthy individuals

2. Causes of anemia



3. Sickle cell anemia

B. Marker enzymes in plasma and serum

Enzyme (acronym)	Tissue(s) of origin
Aspartate transaminase (AST, GOT)	Liver (cirrhosis, fatty liver) Heart (infarction)
Alkaline phosphatase (AP)	Liver, bile duct Bone (fractures, osteomalacia)
Amylase	Pancreas (pancreatitis)
Alanine aminotransferase (ALT, GPT)	Liver (cirrhosis, fatty liver)
Cholinesterase (CE)	Liver (cirrhosis, fatty liver)
Creatine kinase (CK, isoenzyme MB)	Heart (infarction)
Glutamate dehydrogenase (GLDH)	Liver (necrosis)
γ-Glutamyl transpeptidase (GGT)	Liver (toxins, hepatitis) Bile duct (cholestasis)
Lactate dehydrogenase (LDH)	Liver (shock), muscle, heart (infarction)
Pancreatic lipase (PLP)	Pancreas (pancreatitis)

Immune System

Immunity means the organism's ability to recognize and eliminate foreign bodies such as invading microorganisms, foreign cells or tissues, or altered endogenous tissue. *Congenital immunity* is distinguished from *acquired immunity*.

A. Immune response

The **innate** (nonspecific) **immune system** (left) is responsible for the early immune response to disease pathogens (reaction within hours). It recognizes structures typical of **microorganisms**, and distinguishes exogenous from endogenous structures. It can rapidly eliminate invading pathogens.

The first barrier for invading microorganisms is the **epithelium**. **Macrophages** are located there, which phagocytose microorganisms and release interleukins (p. 440). **Granulocytes** occur in the blood, which are capable of killing bacteria (see **B**), and also **natural killer cells (NK cells)**, which kill virus-infected cells.

The humoral elements of the innate immune system found in the blood are factors of the **complement system** (p. 308), which lyses bacteria following activation, and the **interferons** (p. 440), which inhibit intracellular viral replication.

The **acquired** (adaptive) **immune system** (right) reacts more slowly to invading foreign bodies (reaction in days; see next page). It is directed against **antigens**, which it recognizes and binds with a high degree of specificity (*primary response*). The system has a memory. On second contact with the same antigen, it reacts considerably faster and more intensely (*secondary response*). Acquired immunity is mediated by lymphocytes. **B-lymphocytes** (B-cells) produce **antibodies** (p. 310) directed against antigens. By means of **T-cell receptors** (p. 306), **T lymphocytes** (T cells) can specifically recognize endogenous cells infected by pathogens such as viruses, bacteria and protozoa, and destroy these cells.

Antibodies recognize a broad range of different antigens ($>10^{10}$). Antibodies of a certain specificity are produced by a B-lymphocyte **clone**, the specificity of which is genetically fixed (see below). **Antibody variety** arises during embryonic development through a *combination of multiple genes*, *somatic recombination* (selection and linking of gene segments) and by *somatic mutation* (point mutation during B-cell maturation).

Before antigen contact, B lymphocytes produce IgM and IgD antibodies, which are expressed on the cell surface. After antigen exposure, the B cells differentiate into **plasma cells** and secrete antibodies of either the IgM, IgG, IgE, or IgA class (p. 310). Regardless of this change of **antibody class**, binding specificity is preserved.

B. Killing of bacteria by granulocytes

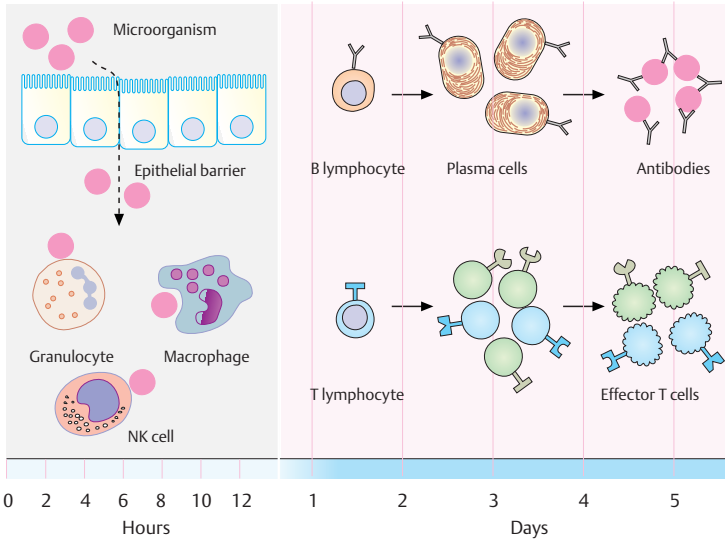
Granulocytes belong to the phagocytic blood cells. They contain enzymes that attack bacterial membranes and produce toxic substances. The bacteria to be killed are taken up into **phagosomes**. Vesicles with the enzymes fuse with the phagosomes and attack the bacteria. **Lysozyme** and different **proteases** produce holes in the bacterial membrane. An **NADPH oxidase** generates **superoxide anions** (O_2^-). From this, hydrogen peroxide and **hydroxyl radicals** ($OH^\cdot$) are produced by **superoxide dismutase** (p. 288). In addition, a **myeloperoxidase** catalyzes the formation of **hypochlorite** (OCI^-). All of these toxic reaction products kill the phagocytosed bacteria but also cause the granulocytes to perish later.

C. Organs of the immune system

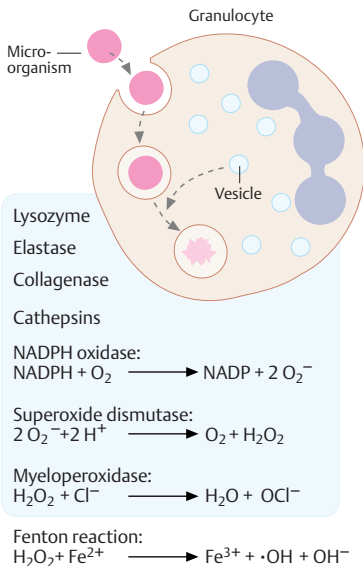
Development of the immune system takes place in the **primary organs** independent of antigens. In the **thymus**, mature **T cells** are formed from precursor cells. On the other hand, T cells directed against endogenous antigens are removed there. By contrast, development of **B cells** in humans takes place in the **fetal liver** and in the **bone marrow**.

The **secondary organs** of the immune system include the **lymph nodes**, into which **dendritic cells** migrate and induce an immune response, and the **spleen**, in which antigens from the blood stream are trapped to trigger an immune response.

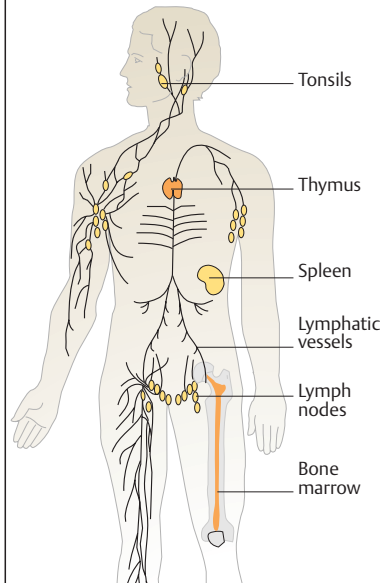
A. Immune response



B. Killing of bacteria by granulocytes



C. Organs of the immune system



Specific Immune Response

Viruses, bacteria, fungi, and parasites that enter the body of vertebrates are recognized and attacked by the **immune system**. Endogenous cells that have undergone alterations—e.g., tumor cells—are also usually recognized as foreign and destroyed. The immune system is supported by physiological changes in infected tissue, known as **inflammation**. This reaction which involves the release of cytokines makes it easier for the immune cells to reach the site of infection (p. 440).

As described on the previous page, two systems are involved in an immune response, the *innate* and the *acquired* immune systems.

The **acquired** (adaptive, specific) **immune system** is based on the ability of *lymphocytes* to express highly specific antigen receptors “on suspicion,” without ever having met the corresponding antigen. In humans, there are several billion different lymphocytes, each of which carries a different antigen receptor. If this type of receptor recognizes “its” cognate antigen, the lymphocyte carrying it is activated and then plays its special role in the immune response.

In addition, a distinction is made between cellular and humoral immune responses. The *T lymphocytes* (T cells) are responsible for **cellular immunity**. They are named after the thymus, in which the decisive steps in their differentiation take place (p. 302). Depending on their function, another distinction is made between *cytotoxic T cells* (green in A) and *helper T cells* (blue). **Humoral immunity** is based on the activity of the *B lymphocytes* (B cells, light brown), which mature in the fetal liver and bone marrow. After activation by T cells, B cells are able to release soluble forms of their specific antigen receptors, the *antibodies* (p. 310), into the blood plasma. The immune system’s “memory” is represented by memory cells. These are particularly long-lived cells that can arise from any of the lymphocyte types described.

A. Simplified diagram of the immune response

Pathogens that have invaded the body—e.g., viruses (top)—are taken up by **antigen-presenting cells** (APCs) and proteolytically degraded (1). The viral fragments produced in this way are then presented on the surfaces of these cells with the help of special membrane proteins (MHC proteins; p. 306) (2). The APCs in-

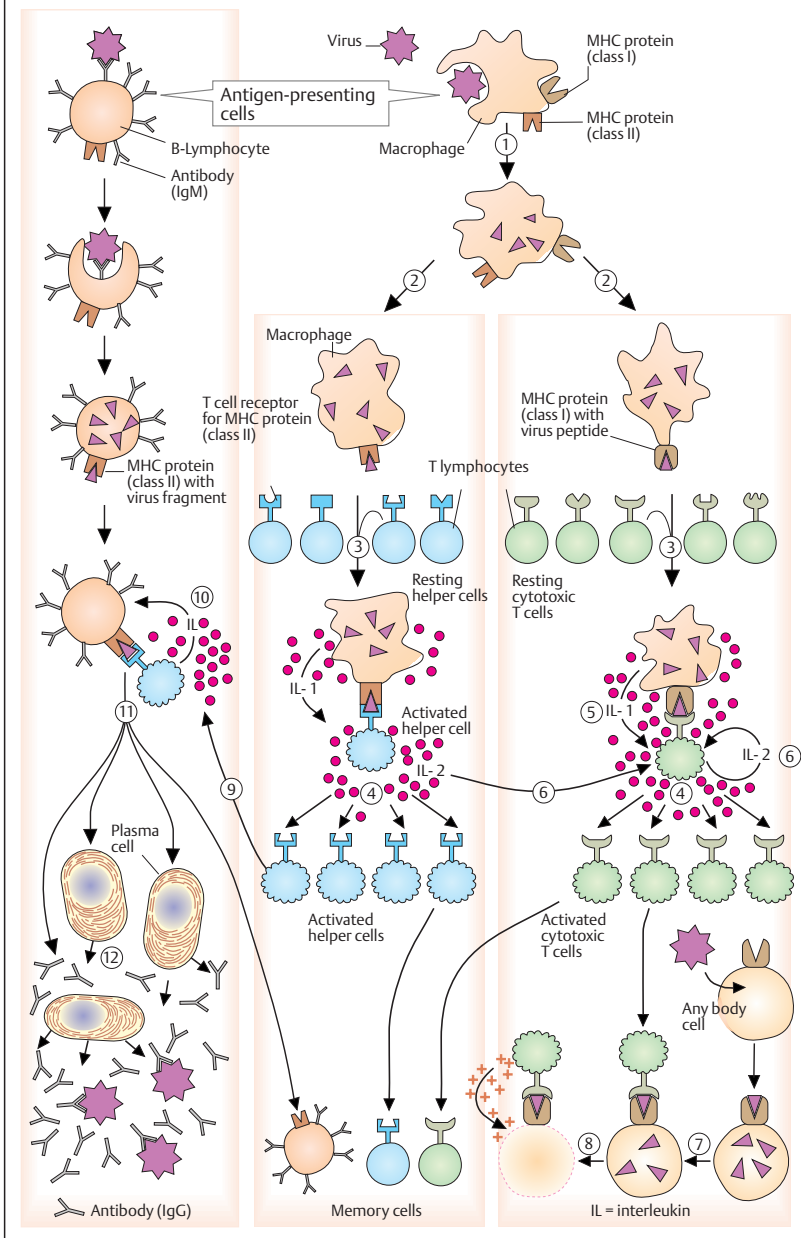
clude *B lymphocytes*, *macrophages*, and *dendritic cells* such as the skin’s Langerhans cells.

The complexes of MHC proteins and viral fragments displayed on the APCs are recognized by T cells in lymph nodes that carry a receptor which matches the antigen (“T-cell receptors”; p. 306) (3). Binding leads to activation of the T cell concerned and its selective replication (4, “clonal selection”). The proliferation of immune cells is stimulated by *interleukins* (ILs). These are a group of more than 20 signaling substances belonging to the cytokine family (p. 440), through which immune cells communicate with each other. For example, activated macrophages release IL-1 (5), while T cells stimulate their own replication and that of other immune cells by releasing IL-2 (6).

Depending on their type, activated T cells have different functions. **Cytotoxic T cells** (green) are able to recognize and bind virus-infected body cells or tumor cells (7). They then drive the infected cells into apoptosis (p. 448) or kill them directly. They achieve this by secreting *perforin* and *granzyme B*. Perforin perforates the target cell’s plasma membrane and lets in the protease *granzyme B*, which triggers apoptosis (8). Alternatively, Fas ligands on the cell surface of the cytotoxic T-cells activate Fas receptors on the target cells, thus initiating the caspase cascade that leads to apoptosis (details on p. 448).

B lymphocytes, which as APCs present viral fragments on their surfaces, are recognized by **helper T cells** (blue) through their T-cell receptors (9). Stimulated by interleukins, selective clonal replication of those B cells that carry antigen receptors matching antigens of the pathogen then takes place (10). These mature into **plasma cells** (11) and finally secrete large amounts of soluble **antibodies** (12).

A. Simplified scheme of the immune response



T-cell Activation

For the selectivity of the immune response, the cells involved must be able to recognize foreign antigens and proteins on other immune cells safely and reliably. To do this, they have **antigen receptors** on their cell surfaces and co-receptors that support recognition.

A. Antigen receptors

Many antigen receptors belong to the **immunoglobulin superfamily**. The common characteristic of these proteins is that they are made up from “immunoglobulin domains.” These are characteristically folded substructures consisting of 70–110 amino acids, which are also found in soluble immunoglobulins (Ig; p. 310). The illustration shows schematically a few of the important proteins in the Ig superfamily. They consist of constant regions (brown or green) and variable regions (orange). Homologous domains are shown in the same colors in each case. All of the receptors have transmembrane helices at the C-terminus, which anchor them in cell membranes. Intramolecular and intermolecular disulfide bonds are also usually found in proteins belonging to the Ig superfamily.

Immunoglobulin M (IgM), a membrane protein on the surface of B lymphocytes, serves to bind free antigens to the B cells. By contrast, **T-cell receptors** only bind antigens when they are presented by another cell as a complex with an MHC protein (see below). Interaction between MHC-bound antigens and T-cell receptors is supported by **co-receptors**. This group includes **CD8**, a membrane protein that is typical in cytotoxic T cells and that binds to class I MHC proteins. T helper cells use **CD4** as a co-receptor instead (not shown) and bind to class II MHC proteins. The abbreviation “CD” stands for “cluster of differentiation.” It is the term for a large group of proteins that are all located on the cell surface and can therefore be identified by antibodies. In addition to CD4 and CD8, there are many other co-receptors on immune cells (not shown).

The **MHC proteins** are membrane proteins of body cells that present antigenic peptides to T lymphocytes. They are named after the “*major histocompatibility complex*”—the DNA segment that codes for them. Human MHC proteins are also known as HLA antigens (“human leukocyte-associated” antigens). Their polymorphism is so large that it is unlikely that any

two individuals carry the same set of MHC proteins—except for monozygotic twins.

Class I MHC proteins occur in almost all nucleated cells. They mainly interact with cytotoxic T cells and are the reason for the rejection of transplanted organs. Class I MHC proteins are heterodimers ($\alpha\beta$). The β subunit is also known as β_2 -microglobulin.

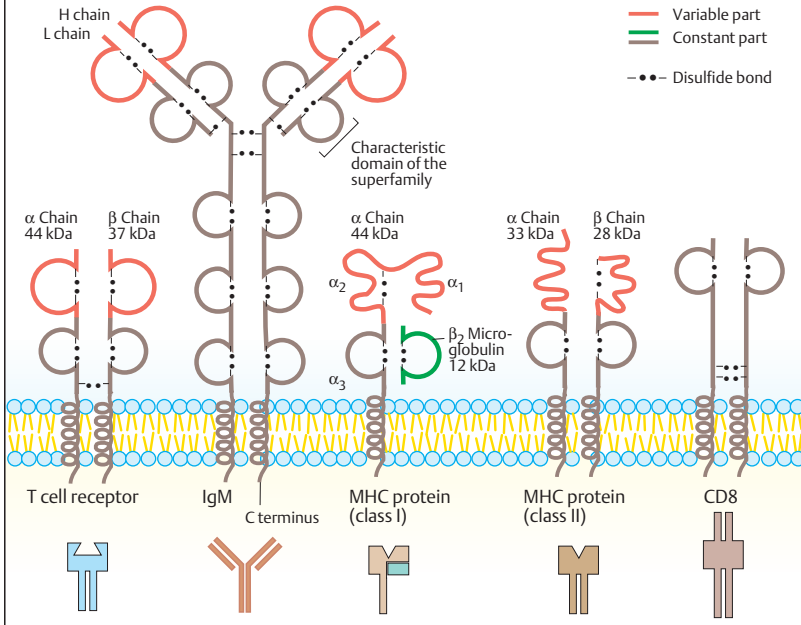
Class II MHC proteins also consist of two peptide chains, which are related to each other. MHC II molecules are found on all antigen-presenting cells in the immune system. They serve for interaction between these cells and CD4-carrying T helper cells.

B. T-cell activation

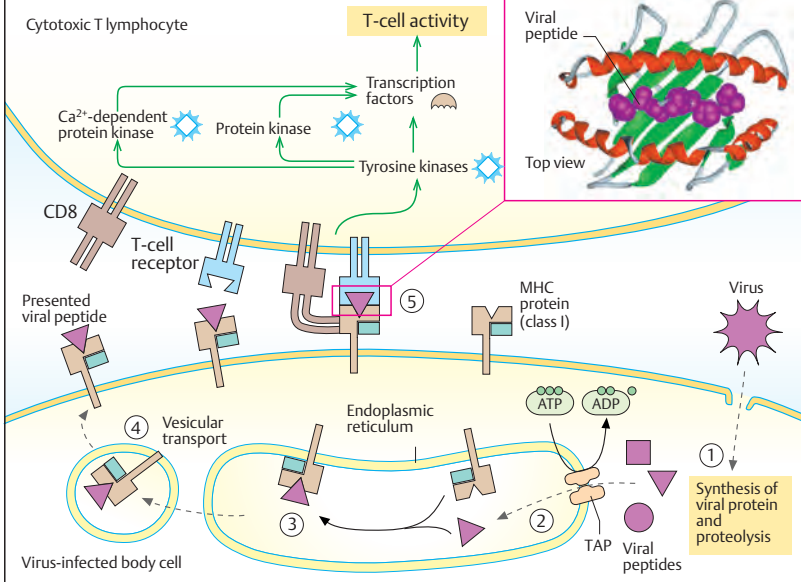
The illustration shows the interaction between a virus-infected body cell (bottom) and a CD8-carrying cytotoxic T lymphocyte (top). The infected cell synthesizes viral proteins in its cytoplasm, cleaves them to peptides (1) and transports the peptide fragments into the endoplasmic reticulum with the help of a special transporter (*TAP*) (2). Newly synthesized class I MHC proteins on the endoplasmic reticulum are loaded with the viral peptides (3) and then transferred to the cell surface by vesicular transport (4). The viral peptides are bound on the surface of the α_1 domain of the MHC protein in a depression (5) formed by a β -sheet as a “floor” and two helices as “walls” (see enlarged inset).

A T cell with a matching T-cell receptor binds to the MHC–peptide complex. This is supported by several co-receptors, including CD8, which binds to the invariant part of MHC. Binding of the MHC complex to the T-cell receptor activates protein kinases in the interior of the T cell, which trigger a chain of additional reactions (*signal transduction*). Finally, destruction of the virus-infected cell by the cytotoxic T lymphocytes takes place (*T-cell activity*).

A. Antigen receptors



B. T-cell activation



Complement System

The complement system is part of the innate immune system (p. 302). It supports *nonspecific defense* against microorganisms. The system consists of some 30 different proteins produced in the liver, the “*complement factors*.” Normally they are found in the blood where they represent about 4% of all proteins. When inflammatory reactions occur, the complement factors also enter the infected tissue to take effect there.

The complement system is a self-reinforcing enzyme cascade that triggers three different processes when activated:

Opsonization. Certain complement factors (“opsonins”) bind to the invading microbes and mark them as targets for phagocytosing cells (e.g., macrophages).

Membrane attack. Other complement factors are deposited in the bacterial membrane, where they create pores that lyse the cells (**B**).

Immune response. Complement factors stimulate the acquired immune system response, e.g., by binding to receptors on B cells.

Chemotaxis. Fragments of complement factors act as diffusible substances to attract immune cells that can attack and phagocytose the microorganisms.

A. Complement activation

The reactions that take place in the complement system can be initiated in three ways (*early phase*), which all take place on the surface of the invading microorganism. They take the form of a cascade-like activation of serine proteases and culminate in cleavage of complement factor C3 into C3a and C3b by a C3 convertase [1].

Alternative pathway. At the start of an infection, bacterial lipopolysaccharides (endotoxins) on the surface of the pathogens trigger the *alternative pathway* (center). Factors B and D form the reactive components.

Classic pathway. If antibodies against the pathogens are already available, the antigen-antibody complexes formed activate the *classic pathway* (left). Factors C1, C2, and C4 are involved. The classic pathway is triggered by binding of factor C1 to IgG or IgM on the surface of microorganisms.

Lectin pathway. Acute-phase proteins (p. 280) are also able to start the complement cascade by binding to typical bacterial carbohydrates, like mannose. The elements of this lectin pathway (right) are complement factors MBL (man-

nose-binding lectin), MASP (MBL-associated serine protease), C2, and C4. Endogenous cells are protected by various proteins and surface modifications on their cell surface from activating the complement system—in most cases by neuraminic acid, a frequent component of glycoproteins and glycolipids on the cell surface.

As in blood coagulation, the *components* of the early phase of the complement system are pro-enzymes of *serine proteinases*, which activate each other by limited proteolysis. They create a self-reinforcing **enzyme cascade**. Factor **C3** is central to the complement system. Its cleavage products **C3a** and **C3b** are involved in several functions and initiate the late phase of complement activation.

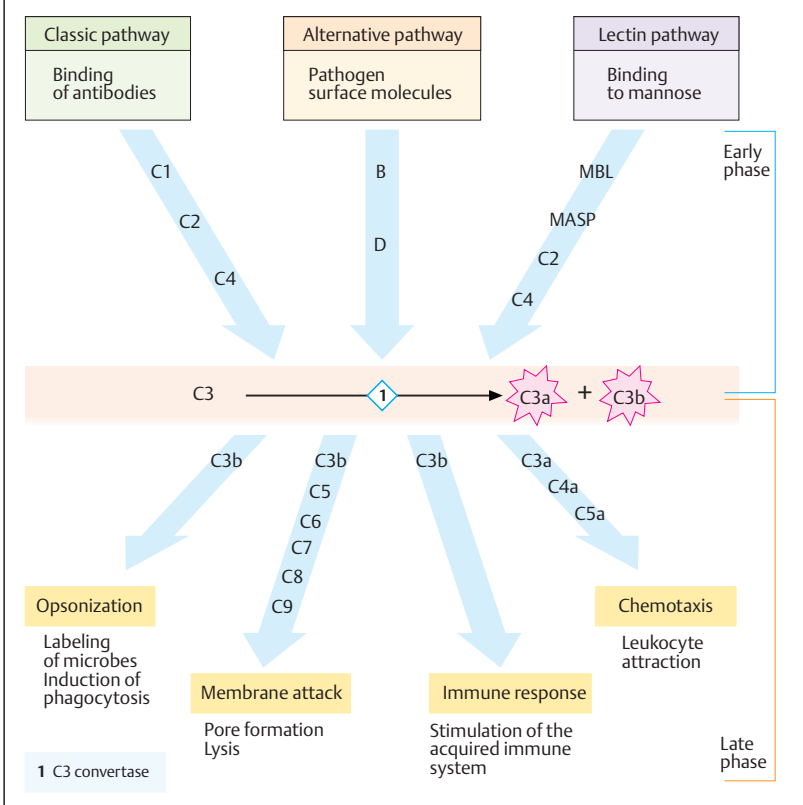
The reaction exposes a highly *reactive thioester group* in C3b, which reacts with hydroxyl or amino groups in the immediate environment. This allows C3b to bind covalently to molecules on the bacterial surface (*opsonization*, left). In addition, C3b initiates a chain of reactions leading to the formation of the *membrane attack complex* (bottom). Together with other complement factors, the smaller product, C3a, promotes the inflammatory reaction and has chemotactic effects (right).

The complement system is regulated by the short lifetime of its components and by inhibitors in the blood serum (not shown).

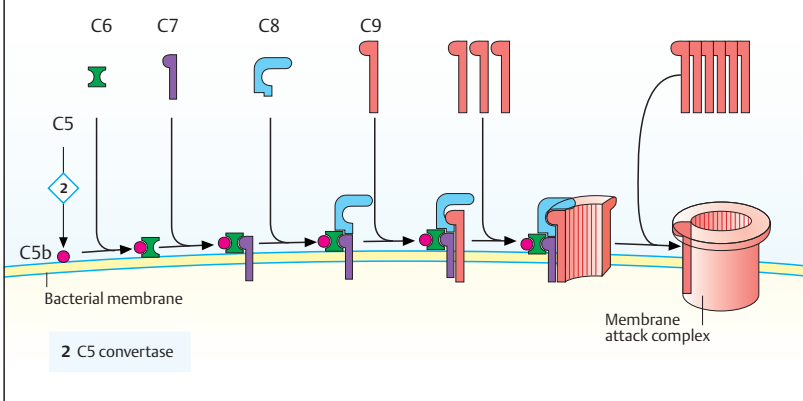
B. Membrane attack

Factors C5 to C9 of the “late phase” are responsible for the development of the **membrane attack complex**. They create an ion-permeable pore in the bacterial membrane, which leads to lysis of the pathogen. This reaction is triggered by C5 convertase [2]. Depending on the type of complement activation, this enzyme has the structure *C4b2a3b* or *C3bBb3b*, and it cleaves C5 into C5a and C5b. The complex of C5b and C6 allows deposition of C7 in the bacterial membrane. C8 and numerous C9 molecules—which form the pore—then bind to this core.

A. Complement activation



B. Membrane attack



Antibodies

Soluble antigen receptors, which are formed by activated B cells (plasma cells; p. 304) and released into the blood, are known as **antibodies**. They are members of the immunoglobulin family (Ig; pp. 280, 306). Antibodies are an important part of the humoral immune defense system. They have no antimicrobial properties themselves, but support the cellular immune system in various ways:

1. They bind to antigens on the surface of pathogens and thereby prevent them from interacting with body cells (toxin and virus *neutralization*).
2. They link single-celled pathogens into aggregates (immune complexes), which are more easily taken up by phagocytes (*agglutination*).
3. They activate the complement system by the classic pathway (p. 308) and thereby promote the innate immune defense system (*opsonization*).

In addition, antibodies have become indispensable aids in medical and biological diagnosis (e.g., in immunofluorescence or immunoassays).

A. Immunoglobulin classes

Human immunoglobulins (Ig) are divided into five classes: **IgA** (with two subgroups), **IgD**, **IgE**, **IgG** (with four subgroups), and **IgM**. The classes are defined by different heavy (H) chains (see **B**), which are designated by the Greek letters α , δ , ϵ , γ , and μ . By contrast, there are only two types of light (L) chain (κ and λ). IgD and IgE (like IgG) are tetramers with the structure H_2L_2 . By contrast, soluble IgA and IgM are multimers that are held together by disulfide bonds and additional J peptides (joining peptides).

The immunoglobulins have different tasks and locations:

IgAs mainly occur in the intestinal tract and in body secretions.

IgDs are receptors on B lymphocytes. Their plasma concentration is also very low.

IgEs are found in low concentrations in the blood. As they can trigger degranulation of mast cells (p. 436), they play an important role in allergic reactions (mediation of immediate allergy).

IgGs account for the majority of serum antibodies (ca. 75%). They are the mediators of the secondary response (p. 302). IgGs occur in the blood and interstitial fluid. As they can pass

the placenta with the help of receptors, they are transferred from mother to fetus.

IgMs are the first Ig formed after contact with a foreign antigen (primary response; p. 302). Their early forms are located on the surface of B cells (p. 304), while the later forms are secreted by plasma cells as pentamers. Their action targets microorganisms in particular.

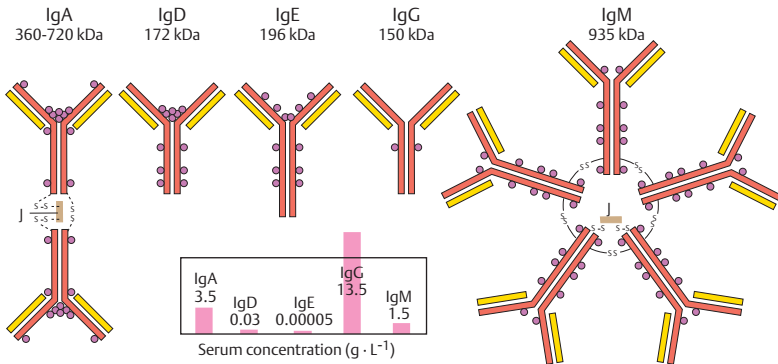
B. Structure of immunoglobulin G

Type G immunoglobulins (**IgGs**) are quantitatively the most important antibodies in the blood, where they form the fraction of γ -globulins (p. 280). IgGs (mass 150 kDa) are Y-shaped molecules that can bind antigens selectively at the ends of both arms. As tetramers they consist of two **heavy chains** (H chains; red or orange) and two **light chains** (L chains; yellow). Both H chains are glycosylated (violet; structure p. 34).

IgGs are cleaved into two F_{ab} fragments and one F_c fragment by the proteinase *papain*. Each F_{ab} ("antigen-binding") fragment, which consists of one L chain and the N-terminal part of an H chain, is able to bind antigens. The F_c ("crystallizable") fragment is made up of the C-terminal halves of the two H chains. This segment serves to bind the IgG to cell surfaces, for interaction with the complement system and antibody transport.

Immunoglobulins are constructed in a modular fashion from several **immunoglobulin domains** (shown in the diagram on the right as Ω shapes). The H chains of IgG contain four of these domains (V_H , C_{H1} , C_{H2} , and C_{H3}) and the L chains contain two (C_L and V_L). The letters C and V designate constant or variable regions.

Disulfide bonds link the two heavy chains to each other and also link the heavy chains to the light chains. Inside the domains, there are also disulfide bonds that stabilize the tertiary structure. The domains are about 110 amino acids long and are homologous with each other. The antibody structure evidently developed as a result of gene duplication. In its central "hinge" region, the antibodies are highly mobile.

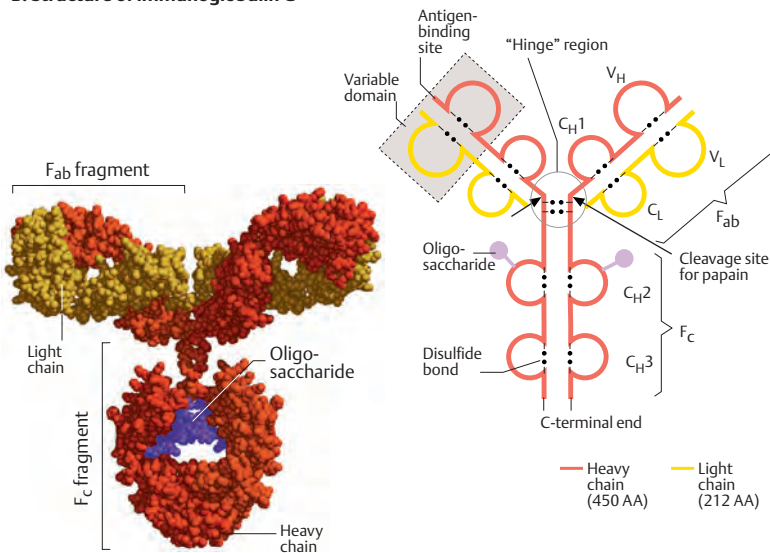
A. Immunoglobulin classes

Chain:

H	α	δ	ϵ	γ	μ
L	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ

Structure:

$(\alpha_2 \kappa_2)_n$	$\delta_2 \kappa_2$	$\epsilon_2 \kappa_2$	$\gamma_2 \kappa_2$	$(\mu_2 \kappa_2)_5$
$(\alpha_2 \lambda_2)_n$	$\delta_2 \lambda_2$	$\epsilon_2 \lambda_2$	$\gamma_2 \lambda_2$	$(\mu_2 \lambda_2)_5$
n = 1, 2, or 3				

B. Structure of immunoglobulin G

Pathobiochemistry

A. Allergies

Allergies are excessive responses of the immune system to **allergens**—i.e., antigens that are normally harmless but can trigger immunological hypersensitivity in sensitive persons. On repeated contact with allergens, their immune system reacts with inappropriate vigor by initiating inflammatory-type processes. Mucous membranes, respiratory tract, skin, and the gastrointestinal tract are affected particularly often.

In the common **type I (immediate) allergy**, allergens (e.g., pollen) that have entered the body activate antigen-presenting cells (APCs). By releasing cytokines (p. 440; especially IL-10), the APCs convert type TH0 T helper cells to TH2 cells, which secrete further cytokines, thereby bringing B cells to produce **IgE** antibodies. The IgEs bind to **Fc receptors** on **mast cells**, inducing their degranulation (p. 436). Mediators such as histamine, cytokines, platelet activating factor and others are released in the process. These can cause bronchospasm and intestinal spasm, a drop in blood pressure, edema and many other disorders, even anaphylactic shock.

The described *acute allergic inflammation* may be followed by a *chronic inflammatory response*, in which the chemokines of the TH2 cells attract eosinophilic granulocytes and activate them to release further signaling substances. In the long term, this can lead, for example, to asthma.

B. Autoimmune diseases

The ability to distinguish “self” from “foreign” is an important property of the immune system. However, even in healthy individuals, there are a few antibodies and immune cells directed against endogenous molecular structures. However, they lack co-stimulatory signals for autostimulatory destruction of endogenous cells and tissues. This tolerance is disrupted in **autoimmune diseases**. When *immune tolerance is lost*, the immune system mistakenly attacks endogenous structures and triggers inflammation and cell lysis. This leads to local cell destruction or systemic inflammatory diseases. The illustration lists a number of organ-specific and non-organ-specific autoimmune diseases.

The causes of these diseases are not yet fully understood. Involvement of the genetic constitution is likely, as the incidence of many auto-

immune diseases correlates with certain genes of the **HLA system** (genes for MHC proteins, p. 306). In addition, there are environmental influences that lead to the onset of the autoimmune disease. This kind of induction of an autoimmune reaction can be caused, for example, by infection with *viruses* or by pathogens with a great similarity to body structures (*molecular mimicry*).

Autoantibodies produced against the invaders can be directed against endogenous remnants of cell membranes, DNA fragments or proteins. They attach to these target structures and mark them for degradation and destruction by macrophages or CD8-positive T cells.

C. Leukemia

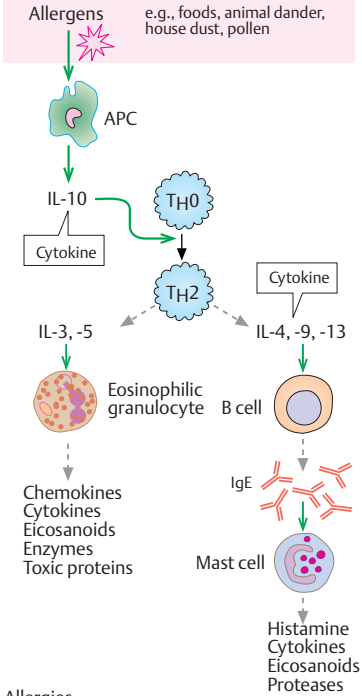
The leukemias (“white blood”) are *malignant diseases of the blood-forming system*, in which transformed precursors of white blood cells (leukocytes) are present in the blood in large numbers.

The leukemias are classified into *chronic* and *acute* forms depending on the clinical course and into *myeloid* and *lymphatic leukemias* depending on the origin of the malignant cell clone.

The presence of immature, nonfunctional blood cells in the bone marrow interferes with the normal formation of blood cells and life-threatening alterations of the blood count occur. The illustration shows stained blood cells of a healthy individual on the left (leukocytes stained purple) and on the right that of a patient with chronic myeloid leukemia. The number of leukocytes is greatly increased and they demonstrate unusual morphology.

The main causes of the leukemias are considered to be genetic predisposition, cytogenetic anomalies, certain viruses, and exposure to physical or chemical carcinogens (p. 452), which lead to transformation of a hematopoietic precursor cell. Chemotherapy (p. 454) and radiotherapy are used to treat them.

A. Allergies



Allergies

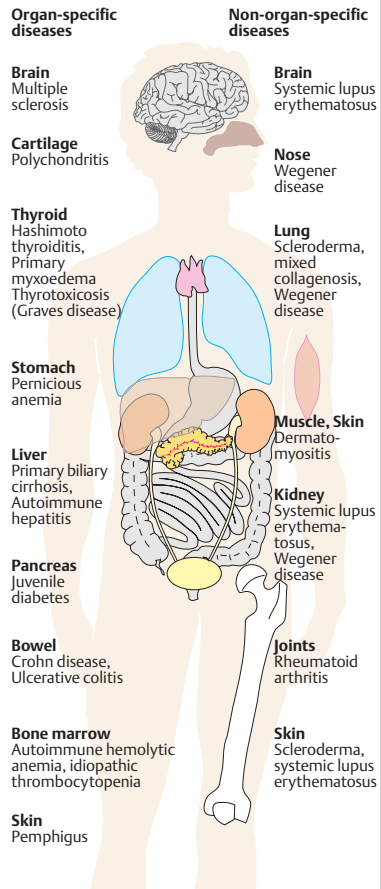
Type I
Bronchial asthma
Hay fever
House dust allergy
Food allergy

Type II
Drug allergies

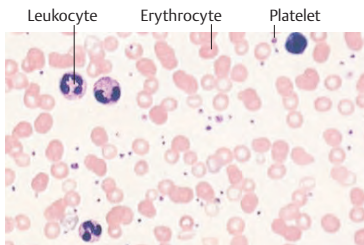
Type III
Allergies to foreign proteins, e.g. in antisera

Type IV
Contact dermatitis to nickel, zinc, or household chemicals

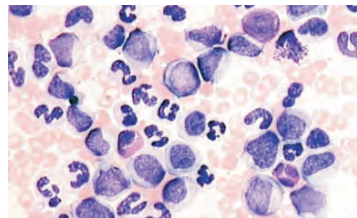
B. Autoimmune diseases



C. Leukemia



Blood film of a healthy individual



Blood film of a patient with chronic myeloid leukemia

Functions

Weighing 1.5 kg, the liver is one of the largest organs in the human body. Although it only represents 2%–3% of the body's mass, it accounts for 25%–30% of oxygen consumption.

A. Functions of the liver

The most important functions of the liver are:

1. **Uptake** of nutrients supplied by the intestines via the portal vein.
 2. **Metabolism**, i.e., biosynthesis of endogenous compounds as well as their storage, conversion, and degradation into excretable molecules. In particular, the liver is responsible for the biosynthesis and degradation of almost all plasma proteins.
 3. **Supply** of the body with metabolites and nutrients. The liver often regulates the plasma concentration of these substances.
 4. **Detoxification** of toxic compounds by biotransformation.
 5. **Excretion** of substances with the bile.
- In addition, there are several special functions, such as:
6. Involvement in the body's **acid-base regulation**.
 7. Activation and inactivation of **hormones**.
 8. Involvement in **nonspecific defense processes** against foreign substances and organisms with the aid of Kupffer cells.

B. Hepatic metabolism

The liver is involved in the metabolism of practically all groups of metabolites. Its functions primarily serve to cushion fluctuations in the concentration of these substances in the blood, to ensure a constant supply to the peripheral tissues (*homeostasis*).

Carbohydrate metabolism. The liver takes up glucose and other monosaccharides from the plasma. Glucose is then either stored in the form of the polysaccharide *glycogen* or converted into fatty acids. When there is a drop in the blood glucose level, the liver releases glucose again by breaking down glycogen. If the glycogen store is exhausted, glucose can also be synthesized by *gluconeogenesis* from lactate, glycerol, or the carbon skeleton of amino acids (pp. 134, 316).

Lipid metabolism. The liver synthesizes fatty acids from acetate units. The fatty acids formed are then used to synthesize fats and phospholipids, which are released into the blood in the form of *lipoproteins*. The liver can

convert fatty acids into *ketone bodies* and release these (p. 318). The liver synthesizes cholesterol, which is transported to other tissues as a component of lipoproteins. Excess cholesterol is converted into bile acids or directly excreted with the bile (p. 320).

Amino acid and protein metabolism. The liver controls the plasma levels of most amino acids. Excess amino acids are broken down. With the help of the urea cycle (p. 172), the nitrogen from the amino acids is converted into urea and excreted via the kidneys. The carbon skeleton of the amino acids enters the intermediary metabolism and serves for glucose synthesis or energy production. In addition, most of the plasma proteins are synthesized or degraded in the liver (p. 280).

Biotransformation. Steroid hormones and bilirubin, as well as drugs, ethanol, and other xenobiotics are inactivated by the liver and converted into highly polar metabolites by conversion reactions (p. 322).

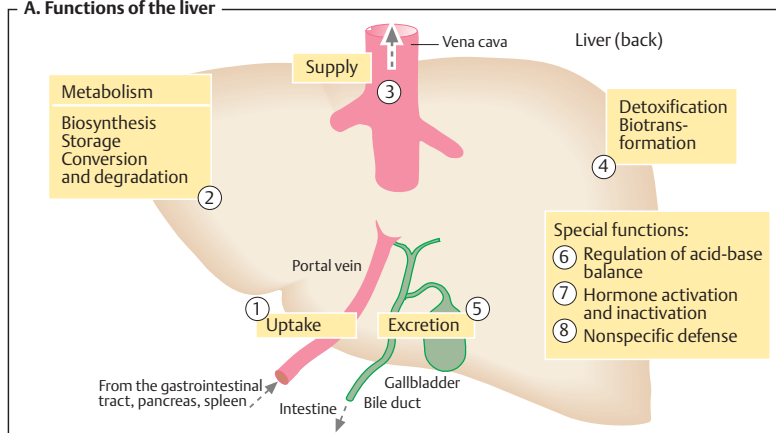
Storage. The liver stores energy reserves (glycogen) and nutrients, mineral substances, trace elements, and vitamins, including iron, retinol, and vitamins A, D, K, folic acid, and B₁₂.

C. Zoning of liver metabolism

The roughly 3×10^{11} cells in the liver—particularly the **hepatocytes**, which make up about 80% of the cell mass—are the central location for the body's *intermediary metabolism*. The other cells in the liver include epithelial cells, **Kupffer cells**, and **stellate (Ito) cells**.

The left half of the illustration shows part of a liver lobule. The hepatocytes are in close contact with the blood, which enters the liver from the portal vein and the hepatic arteries (left), flows through the sinusoids, and is collected again in the central veins of the hepatic lobules (right). Hepatocytes carry out different metabolic functions depending on their location along the intrahepatic capillaries (sinusoids) (**metabolic zoning**). Periportal hepatocytes (left, pink) are distinguished from perivenous hepatocytes (right, blue).

A. Functions of the liver



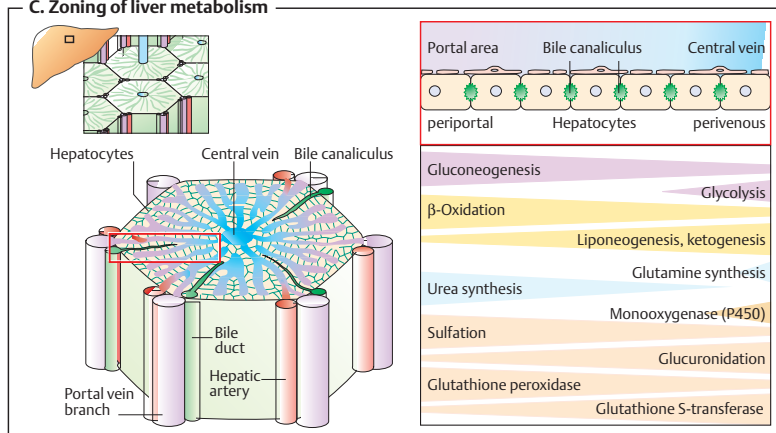
B. Hepatic metabolism

Carbohydrate metabolism	Lipid metabolism	Amino acid metabolism	Biotransformation
Glucose BSC	Fatty acids BC	Amino acids C	Steroid hormones EC
Galactose C	Fats BC	Urea B	Bile pigments EC
Fructose C	Ketone bodies B		Ethanol C
Mannose C	Cholesterol BEC		Drugs EC
Pentoses BC	Bile acids BE		
Lactate C	Vitamins SC		
Glycerol BC			
Glycogen BSC			

Plasma proteins	
Lipoproteins BC	
Albumin BC	
Coagulation factors BC	
Hormones C	
Enzymes BC	

B Biosynthesis	
C Conversion and degradation	
E Excretion	
S Storage	

C. Zoning of liver metabolism



Carbohydrate Metabolism

Besides fatty acids and ketone bodies, glucose is the body's most important energy supplier. The concentration of glucose in the blood (the "blood glucose level") is maintained at 4–6 mM (0.8–1.0 g.L⁻¹) by precise regulation of glucose-supplying and glucose-utilizing processes (p. 372). Glucose suppliers include the intestine (glucose from food), liver, and kidneys. The liver plays the role of a "**glucostat**."

Glucose transporters of the **Glut-2** type (p. 212) in the plasma membrane of hepatocytes enable glucose and other sugars to be transported both into and out of cells independent of insulin. In contrast to muscle, the liver possesses the enzyme *glucose-6-phosphatase*, which can release glucose from glucose-6-phosphate, thus enabling export of glucose. The liver is also capable of forming glucose by converting other sugars—e.g., *fructose* and *galactose*—or by synthesizing from other metabolites, especially amino acids. The conversion of lactate to glucose in the *Cori cycle* (p. 344) and the conversion of alanine to glucose with the help of the *alanine cycle* (p. 374) are particularly important for metabolic interaction with erythrocytes and muscle cells.

Apart from glucose, the liver can also produce other sugars such as fructose, galactose, mannose and ribose, and also amino sugars such as neuraminic acid.

A. UDP-glucose Metabolism

Glucose is activated to glucose-6-phosphate by phosphorylation [1]. After isomerization [2] to glucose-1-phosphate, **UDP-glucose**, which has a key position in sugar metabolism, is produced by transfer of a UMP residue [3]. For example, the glucose moiety can be linked with other sugars through a glycosidic bond. **Glycogen** or the oligosaccharide and polysaccharide side chains of **proteoglycans**, **glycoproteins**, and **glycolipids** are produced in the liver by these reactions, which are catalyzed by *glucosyltransferases*.

UDP-glucose can also be oxidized to **UDP-glucuronate** [4]; a compound also needed for the formation of proteoglycans and glycoproteins and for formation of conjugates (**glucuronides**; phase II of biotransformation; p. 322). UDP-glucose can also be epimerized in the liver to **UDP-galactose** ([5], **B**), from which **lactose** is produced in the mammary gland by reaction [6] with glucose. However, this disaccharide is synthesized only during lactation.

B. Fructose and galactose metabolism

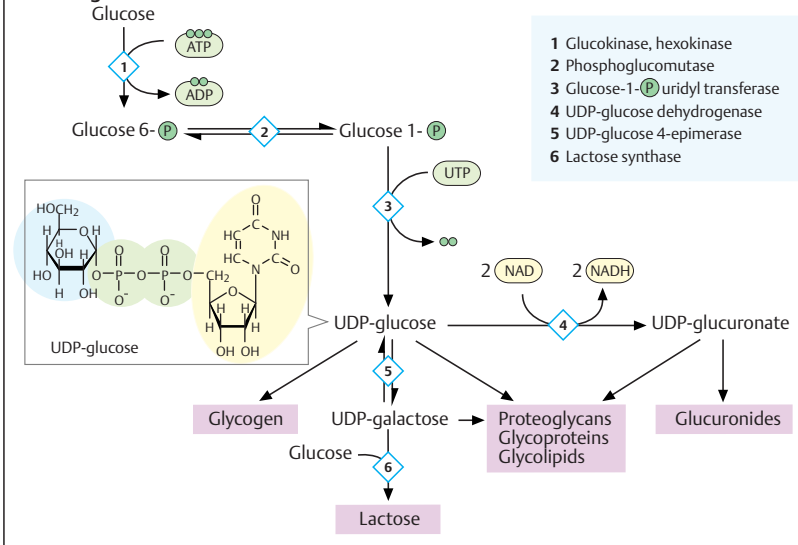
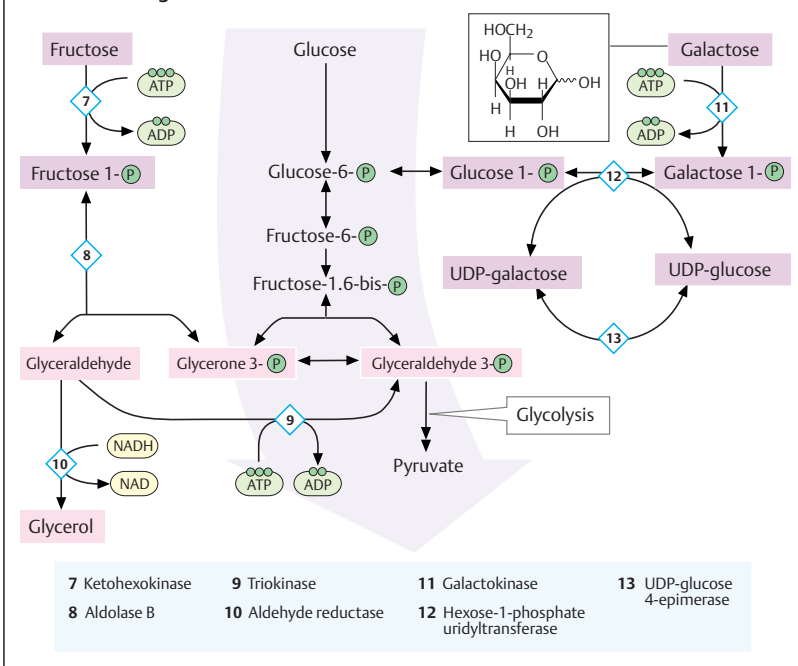
Fructose is mainly metabolized by the liver, which channels it into glycolysis (left side of the illustration).

A special *ketohexokinase* [7] initially phosphorylates fructose into **fructose 1-phosphate**. This is then cleaved by an *aldolase B* [8], which is fructose-specific, to yield **glycerone 3-phosphate** (dihydroxyacetone phosphate) and **glyceraldehyde**. Glycerone 3-phosphate is already an intermediate of glycolysis (center), while glyceraldehyde can be phosphorylated into glyceraldehyde 3-phosphate by a *trikinase* [9].

To a smaller extent, glyceraldehyde is also reduced to glycerol [10] or oxidized to glycerate, which can be channeled into glycolysis following phosphorylation (not shown). The reduction of glyceraldehyde [10] uses up NADH. As the rate of degradation of alcohol in the hepatocytes is limited by the supply of NAD⁺, fructose degradation accelerates alcohol degradation (p. 326).

Outside of the liver, fructose is channeled into the sugar metabolism by reduction at C-2 to yield **sorbitol** and subsequent dehydration at C-1 to yield glucose (the **polyol pathway**; not shown).

Galactose is also synthesized or broken down in the liver (right side of the illustration). As is usual with sugars, the metabolism of galactose starts with a phosphorylation to yield galactose 1-phosphate [11]. The connection to the glucose metabolism is established by C-4 *epimerization* to form **glucose 1-phosphate**. However, this does not take place directly. Instead, a *transferase* [12] transfers a uridine 5'-monophosphate (UMP) residue from uridine diphosphoglucose (UDP-glucose) to galactose 1-phosphate. This releases glucose 1-phosphate, while galactose 1-phosphate is converted into uridine diphosphogalactose (**UDP-galactose**). This then is isomerized into UDP-glucose [7]. The *biosynthesis* of galactose also follows this reaction pathway, which is freely reversible up to reaction [11]. Genetic defects of enzymes [11] or [6] can lead to the clinical condition of *galactosemia* (p. 142).

A. UDP-glucose metabolism**B. Fructose and galactose metabolism**

Lipid Metabolism

The liver is the most important site for the formation of *fatty acids*, *glycerolipids*, *sphingolipids*, *ketone bodies*, and *cholesterol*. Most of these products are released into the blood. In contrast, the triacylglycerols synthesized in adipose tissue are also stored there.

A. Lipid Metabolism

Lipid metabolism in the liver is closely linked to its carbohydrate and amino acid metabolism.

Absorptive phase (p. 376). When there is a good supply of nutrients, the liver converts glucose and other monosaccharides via acetyl-CoA into **fatty acids**. The liver can also take up fatty acids from *chylomicrons*, which are supplied by the intestine, or from fatty-acid-albumin complexes. Fatty acids from both sources are converted into glycerolipids, i.e., **fats** and **phospholipids**, and also sphingolipids. Together with apoproteins, these are packed into very-low-density lipoproteins (**VLDLs**; p. 282f.) and then released into the blood by exocytosis. The VLDLs supply extrahepatic tissue, e.g., adipose tissue and muscle, with lipids.

Post-absorptive phase (p. 378). When there is no supply of nutrients—particularly during fasting and starvation—lipid metabolism is re-adjusted and the body falls back on its own reserves. Adipose tissue then releases fatty acids. They are taken up by the liver and are mainly converted into **ketone bodies** (**B**).

Cholesterol can be derived from two sources—food or endogenous synthesis from acetyl-CoA. A substantial percentage of endogenous cholesterol synthesis takes place in the liver. Some cholesterol is required for the synthesis of **bile acids** (p. 320). In addition, it serves as a building block for cell membranes (p. 208), or can be esterified with fatty acids and stored in lipid droplets. The rest is released together into the blood in the form of lipoprotein complexes (VLDLs) and supplies other tissues. The liver also contributes to the cholesterol metabolism by taking up from the blood and breaking down lipoproteins that contain cholesterol and cholesterol esters (HDLs, IDLs, LDLs; p. 282f.). The liver converts cholesterol to bile acids and excretes these in the bile along with unaltered cholesterol (p. 320).

B. Biosynthesis of ketone bodies

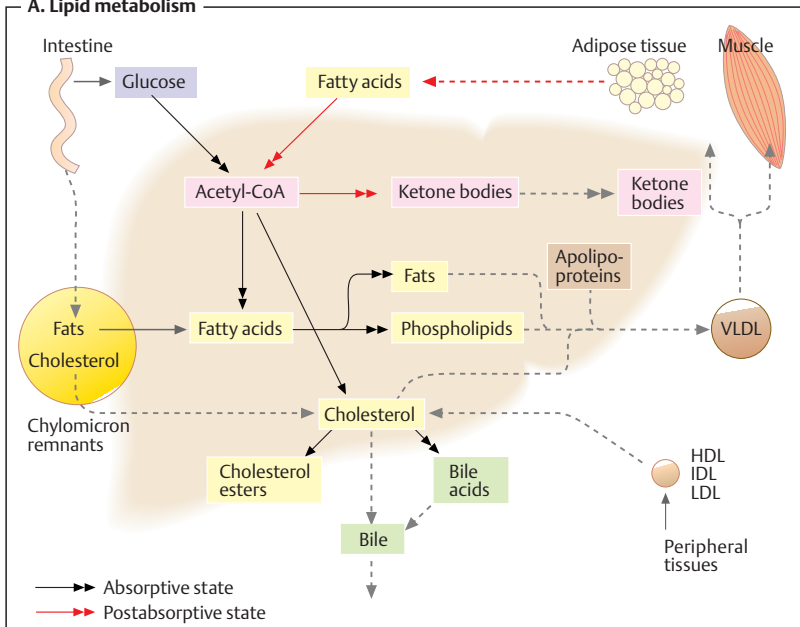
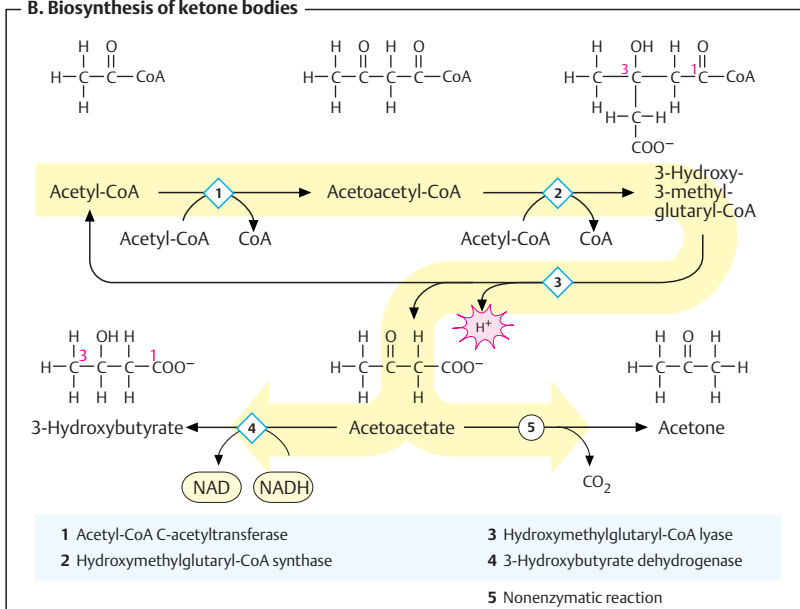
When there is an abundant supply of **fatty acids**, β -oxidation in the liver degrades them to acetyl-CoA, most of which is converted to **ketone bodies**. The liver uses these soluble sources of energy to supply extrahepatic tissues.

Ketone body synthesis takes place exclusively in the mitochondrial matrix of the liver. In the first step, two molecules of acetyl-CoA condense to form **acetoacetyl-CoA** [1]. The transfer of another acetyl group [2] gives rise to 3-hydroxy-3-methylglutaryl-CoA (**HMG-CoA**), which after release of acetyl-CoA [3] yields free **acetoacetate** (*Lynen cycle*). Acetoacetate can be converted to **3-hydroxybutyrate** by reduction [4], or can give rise to **acetone** by non-enzymatic decarboxylation [5]. These three compounds are together referred to as “*ketone bodies*,” although in fact 3-hydroxybutyrate is not actually a ketone. As reaction [3] releases an H^+ ion, *metabolic acidosis* can occur as a result of increased ketone body synthesis (p. 292).

The ketone bodies are released by the liver into the blood, in which they are readily soluble. Blood levels of ketone bodies rise during periods of hunger. Together with free fatty acids, 3-hydroxybutyrate and acetoacetate are then important energy suppliers for many tissues (including brain and heart muscle). Acetone cannot be metabolized and is exhaled via the lungs or excreted with urine.

The channeling of ketone bodies into energy metabolism is discussed on page 374.

If the production of ketone bodies exceeds the demand for them, there is an increase in the concentration of ketone bodies in the plasma (*ketonemia*) and they are also eventually excreted in the urine (*ketonuria*). Both phenomena are observed after prolonged starvation and in inadequately treated *type 1 diabetes mellitus*. Severe ketonuria with ketoacidosis can cause electrolyte shifts and loss of consciousness, and is therefore life-threatening (*ketoacidotic coma*).

A. Lipid metabolism**B. Biosynthesis of ketone bodies**

Bile Acids

Bile is an important product released by the hepatocytes. It promotes the digestion of dietary lipids by *emulsifying* them in the small intestine (p. 274). The emulsifying components of bile, apart from phospholipids, mainly consist of **bile acids** and **bile salts** (see below). The bile also contains free **cholesterol**, which is excreted in this way.

A. Bile acids and bile salts

Bile acids are steroids consisting of 24 C atoms carrying one carboxylate group and several hydroxyl groups. They are formed from cholesterol in the liver. Cytochrome P450 enzymes in the smooth endoplasmic reticulum (sER) of hepatocytes are involved in many of the 14 steps (p. 324).

Initially, cholesterol is converted to 7 α -hydroxycholesterol by a 7 α -hydroxylase. The double bond of the cholesterol is then transferred. Next, another OH group is introduced into the sterane framework at C-12. Finally, the double bond is reduced, the side chain is shortened by three C atoms, and the terminal C atom is oxidized to a carboxylate group.

It is important that the arrangement of the A and B rings is altered from *trans* to *cis* during bile acid synthesis. The result of this is that all of the hydrophilic groups in the bile acids lie on one side of the molecule. While cholesterol, which is weakly amphipathic (top left), has a small polar "head" and an extended apolar "tail," the bile acid molecules (bottom left) are much more strongly amphipathic and resemble disks with polar groups above and apolar groups beneath.

Cholic acid and **chenodeoxycholic acid**, known as the **primary bile acids**, are quantitatively the most important metabolites of cholesterol. After being biosynthesized, they are mostly activated with coenzyme A and then conjugated with *glycine* or the nonproteinogenic amino acid *taurine*, a derivative of cysteine (p. 174). The acid amides formed in this way are known as **conjugated bile acids**. They have pK_a values of around 4 (glycocholic acid) or 2 (taurocholic acid) and are therefore almost fully dissociated as "**bile salts**." Conjugated bile salts are therefore even more amphipathic than the unconjugated compounds.

Deoxycholic acid and **lithocholic acid** are only formed in the intestine by microorganisms by enzymatic cleavage of the OH group at C-7 (see B). They are therefore referred to as **secondary bile acids**.

B. Metabolism of bile salts

Bile salts are exclusively synthesized in the liver. The slowest step in their biosynthesis is hydroxylation at position 7 by a 7 α -hydroxylase. Cholic acid and other bile acids inhibit this reaction (*end-product inhibition*, 1). In this way, the bile acids present in the liver regulate the rate of cholesterol utilization.

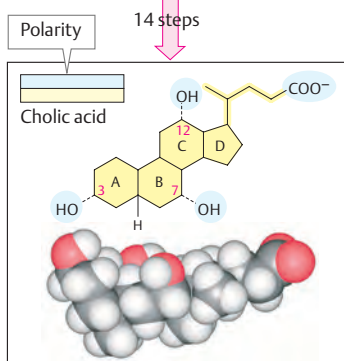
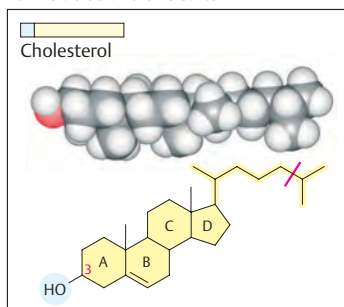
Before leaving the liver, a large proportion of the bile acids is activated with CoA and then conjugated with the amino acids *glycine* or *taurine* (2; cf. A). In this way, cholic acid gives rise to **glycocholic acid** and **taurocholic acid**. The **liver bile** secreted by the liver becomes denser in the gallbladder as a result of the removal of water (*bladder bile*; 3).

Intestinal bacteria produce enzymes that can chemically alter the bile salts (4). The acid amide bond in the bile salts is cleaved, and dehydroxylation at C-7 yields the corresponding secondary bile acids from the primary bile acids (5). Most of the intestinal bile acids (> 95%) are reabsorbed in the ileum (6) and returned to the liver via the portal vein (**enterohepatic circulation**). In the liver, the secondary bile acids give rise to primary bile acids again, from which bile salts are again produced. Of the 15–30 g bile salts that are released with the bile per day, only around 0.5 g therefore appears in the feces. This approximately corresponds to the amount of daily de-novo synthesis of cholesterol.

■ Further information

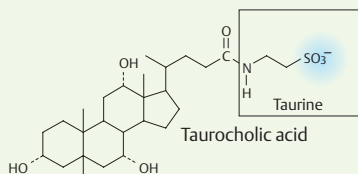
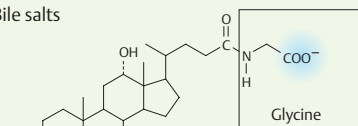
The **cholesterol** excreted with the bile is poorly water-soluble. Together with phospholipids and bile acids, it forms micelles (p. 24), which keep it in solution. If the proportions of phospholipids, bile acids and cholesterol shift, **gallstones** can arise. These mainly consist of precipitated cholesterol (cholesterol stones), but can also contain Ca²⁺ salts of bile acids and bile pigments (pigment stones).

A. Bile acids and bile salts



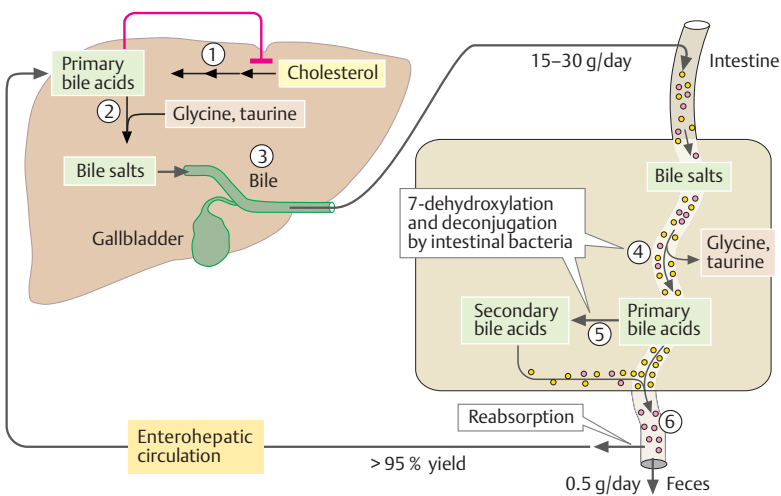
Bile acids	HO- in position		
Primary bile acids			
– Cholic acid	C-3	C-7	C-12
– Chenodeoxycholic acid	C-3	C-7	–
Secondary bile acids			
– Deoxycholic acid	C-3	–	C-12
– Lithocholic acid	C-3	–	–

Bile salts



Bile salts = conjugated bile acids

B. Metabolism of bile salts



Biotransformation

The body is constantly taking up **foreign substances** (= *xenobiotics*) from food or through contact with the environment, via the skin and lungs, which can be natural in origin, or may have been synthetically produced by humans. Some of these substances are toxic, particularly at high concentrations. However, the body has effective mechanisms for inactivating and then excreting foreign substances through **biotransformations**. The mechanisms of biotransformation are similar to those with which **endogenous substances** such as bile pigments and steroid hormones are inactivated and made excretable. Biotransformations mainly take place in the *liver*, but also in the *intestine* and *kidneys*.

A. Biotransformation

Phase I reactions (*interconversion reactions*)

Type I reactions introduce functional groups into inert, apolar molecules or alter functional groups that are already present. In many cases, this is what first makes it possible for foreign substances to conjugate with polar molecules via phase II reactions (see below).

Phase I reactions usually reduce the *biological activity* or *toxicity* of a substance ("**detoxification**"). However, some substances only become biologically active as a result of the interconversion reaction (see, e.g., benzo[a]pyrene, p. 254) or become more toxic after interconversion than the initial substance ("**toxification**").

Important phase I biotransformation reactions include:

- **Hydrolytic cleavages** of ether, ester, and peptide bonds. Example (1) shows hydrolysis of the painkiller *acetylsalicylic acid*.
- **Oxidations**: hydroxylations (2), epoxide formation, sulfoxide formation, dealkylation, deamination. For example, ethanol is oxidized to ethanal (p. 326), benzene to phenol, and toluene (methylbenzene) to benzoic acid.
- **Reductions** of carbonyl, azo-, or nitro-compounds, dehalogenation.
- **Methylations**.
- **Desulfurations**.

The phase I reactions take place in the hepatocytes on the sER. Many of the oxidation reactions are catalyzed by **cytochrome P450 systems** (p. 324). These monooxygenases are induced by their lipophilic substrates and usually show wide specificity.

Phase II reactions (*conjugate formation*)

Type II reactions couple their substrates (bilirubin, steroid hormones, drugs, and products of phase I reactions) via ester or amide bonds to highly polar, negatively charged molecules. The enzymes involved are transferases, and their products are known as **conjugates**.

The most common type of conjugate formation is coupling with **glucuronate** (GlcUA) as an *O*- or *N*-glucuronide. The coenzyme for the reaction is UDP-glucuronate, the "**active glucuronate**" (pp. 92, 316). Bonding with the anionic glucuronate makes an apolar (hydrophobic) molecule more strongly polar, and it becomes sufficiently water-soluble and capable of being excreted. Example (3) shows the glucuronidation of *tetrahydrocortisol*, a metabolite of the glucocorticoid cortisol (p. 420).

The biosynthesis of **sulfate** esters with the help of *phosphoadenosine phosphosulfate* (PAPS), the "**active sulfate**," (p. 92), linking with **glutathione**, and amide formation with **glycine** and **glutamine** also play a role in conjugation. For example, benzoic acid is conjugated with glycine to form the more soluble and less toxic *hippuric acid* (*N*-benzoylglycine).

The conjugates are eliminated from the liver either by the *biliary* route—i.e., by receptor-mediated excretion into the bile—or by the *renal* route, via the blood and kidneys by filtration.

■ Further information

To detoxify **heavy metals**, the liver contains **metallothioneins**, a group of cysteine-rich proteins with a high affinity for divalent metal ions such as Cd^{2+} , Cu^{2+} , Hg^{2+} , and Zn^{2+} . These metal ions induce the formation of metallothioneins via a special metal-regulating element in the gene's promoter.

Cytochrome P450 Systems

During the first phase of **biotransformation** in the liver and other tissues (p. 322), compounds that are weakly chemically reactive are enzymatically hydroxylated. This makes it possible for them to be conjugated with polar substances. The hydroxylating enzymes are generally **monooxygenases** that contain a **heme b** (protoporphyrin IX) as the redox-active coenzyme (p. 86). In the reduced form, the heme can bind *carbon monoxide* (CO), and it then shows typical light absorption at 450 nm. This property characterizes the **cytochrome P450 systems**.

A. Reaction

Cytochrome P450-dependent monooxygenases (**CYP** [1]) catalyze reductive cleavage of molecular **oxygen**. One of the two oxygen atoms is transferred to a lipophilic substrate molecule, while the other is released as a water molecule. The necessary reducing equivalents are supplied by **NADPH + H⁺**.

CYP have many different lipophilic substrates of endogenous and exogenous origin. They are involved not only in phase I reactions in the biotransformation of **xenobiotics**, **drugs**, and **chemicals**, but also in the metabolism of **sterols**, **steroid hormones** (p. 424), **bile acids** (p. 320) and **eicosanoids** (p. 438), **unsaturated fatty acids** (p. 153), and **retinoic acid** (p. 44). These substrates can be hydroxylated, epoxidized, dealkylated, oxidized, or dehalogenated by CYP-catalyzed reactions. Usually, their polarity is increased and their ability to be metabolized is improved as a result.

B. Structure

CYP are components of electron transport chains involving several membrane proteins bound to the sER and the inner mitochondrial membrane. The substrate oxygen attaches to heme-bound iron in the active center of **cytochrome P450** (Cyt P450) and is activated by accepting electrons. The ER and mitochondrial systems differ in how electrons are transported to the cytochrome. In the ER, a *Cyt-P450 reductase* that contains **FAD** and **FMN** transfers electrons from NADPH to Cyt P450. In the mitochondria, a FAD-containing *adrenodoxin reductase* first transfers them from NADPH to two **adrenodoxins**, before the electrons reach Cyt 450.

C. Properties

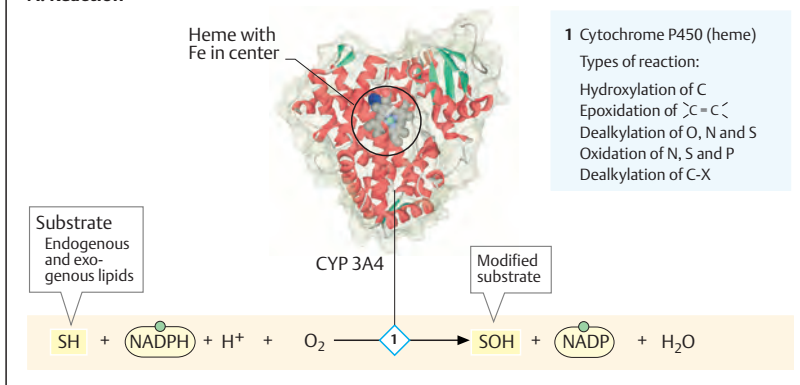
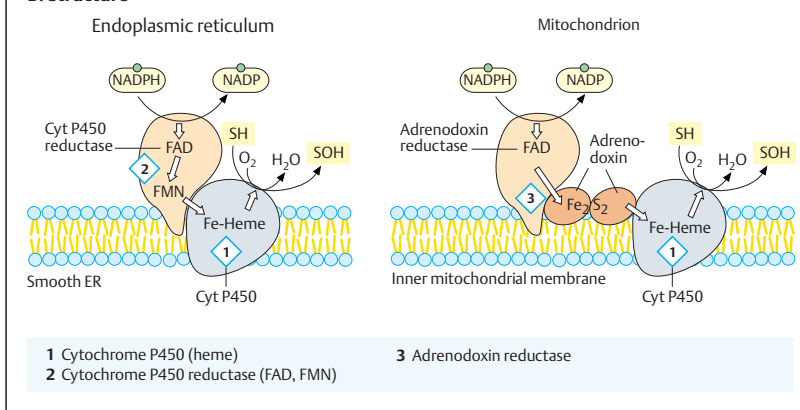
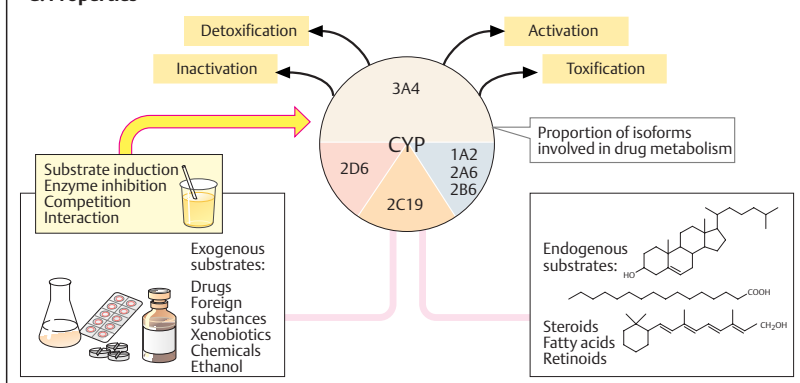
The cytochrome P450-dependent monooxygenases constitute a superfamily, which is classified into 19 families with 41 subfamilies on the basis of homology criteria. In humans, there are roughly 60 different **CYP isoforms** with different properties.

CYP occur in all nucleated cells, especially the liver, steroid hormone-producing glands, the intestine, and the lungs. They are involved in the metabolism of lipophilic **endogenous metabolites**, e.g., the biosynthesis of cholesterol, bile acids, and steroid hormones. With their assistance, alkanes and fatty acids are hydroxylated to initiate their degradation. CYP also catalyze the biosynthesis of eicosanoids from arachidonic acid.

By contrast, the substrate specificity of some CYP isoforms directed at **exogenous substances** such as xenobiotics and drugs is lower, and sometimes even overlapping. Isotype **CYP 3A4** catalyzes the conversion of about half of all known drugs. A further 20% of drugs are metabolized by **CYP 2D6**, 15% by **CYP 2C19**, and the remainder by **CYP 1A2**, **2A6**, and **2B6**.

While the CYP reaction in many cases leads to **inactivation** of drugs and exogenous substances (**detoxification**), in other cases it can produce toxic compounds (**toxification**). For example, *epoxidation* of aromatics yields products that are highly reactive and often toxic.

Many substrates can induce their CYP (**substrate induction**). For example, *barbiturates* induce CYP 3A4 and related CYP. Other compounds can inhibit individual CYP isotypes (**enzyme inhibition**), including natural substances such as those present in grapefruit juice. Substrate **competition** at the active center of the CYP ensures that they interfere with each other's degradation (**substrate interaction**). About 40% of CYP genes occur as multiple alleles (**genetic polymorphism**). This is why major individual differences are observed in patients' ability to metabolize drugs ("*good*" and "*poor metabolizers*").

A. Reaction**B. Structure****C. Properties**

Ethanol Metabolism

A. Blood ethanol level

Ethanol (EtOH, “alcohol”) is produced in small amounts by intestinal bacteria and traces occur in fruit. Alcoholic drinks contain much higher concentrations. Their alcohol content is given as percentage by volume. To estimate alcohol uptake and the blood alcohol level, it is useful to convert the amount to grams of ethanol (density $0.79 \text{ kg} \cdot \text{L}^{-1}$). For example, a bottle of beer (0.5 L at 4% v/v alcohol) contains 20 mL = 16 g of ethanol, while a bottle of wine (0.7 L at 12% v/v alcohol) contains 84 mL = 66 g ethanol.

Ethanol is membrane-permeable and is quickly absorbed. The maximum **blood level** is already reached within 60–90 minutes after drinking. The *absorption rate* depends on various conditions, however. An empty stomach, a warm drink (e.g., mulled wine), and the presence of sugar and carbonic acid (e.g., in champagne) promote ethanol absorption, whereas a heavy meal reduces it. Ethanol is rapidly distributed throughout the body. A large amount is taken up by the muscles and brain, but comparatively little by adipose tissue and bones. Roughly 70% of the body is accessible to alcohol. Complete absorption of the ethanol contained in one bottle of beer (16 g) by a person weighing 70 kg (distribution in $70 \text{ kg} \times 70/100 = 49 \text{ kg}$) leads to a blood alcohol level of 33 mg/dL (7.2 mM). The lethal concentration of alcohol is ~350 mg/dL (76 mM).

B. Ethanol metabolism

The major site of ethanol degradation is the liver, although the stomach is also able to metabolize ethanol. Most **ethanol** is initially oxidized by *alcohol dehydrogenase* [1] to form **ethanal** (acetaldehyde). Subsequent oxidation, catalyzed by *aldehyde dehydrogenase* [2], leads to acetate. Acetate is then activated in an ATP-dependent reaction catalyzed by *acetate-CoA ligase* [3] to yield **acetyl-CoA**, providing a link to the intermediary metabolism. In addition to cytoplasmic alcohol dehydrogenase, *catalase* and inducible *microsomal alcohol oxidase* (“**MEOS**”, especially CYP 2E1; p. 324) also contribute to a lesser extent to ethanol degradation. The enzymes of ethanol metabolism are present as isoenzymes with greatly differing properties. There is considerable genetic polymorphism.

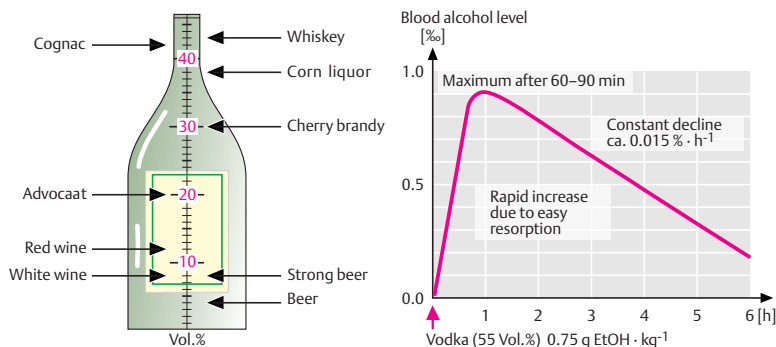
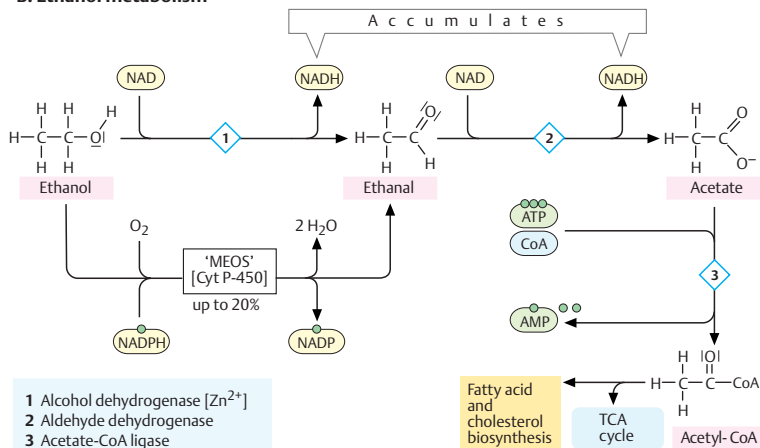
The rate of ethanol degradation in the liver is determined by alcohol dehydrogenase activity. The amount of NAD^+ available is the limiting factor. As the maximum degradation rate (roughly 15 mg/dL per h) is already reached at low concentrations of ethanol, the ethanol level therefore declines at a constant rate (zero-order kinetics). The *calorific value* of ethanol is $29.4 \text{ kJ} \cdot \text{g}^{-1}$. Alcoholic drinks—particularly in alcoholics—can therefore represent a substantial proportion of dietary energy intake.

C. Acute effects of ethanol

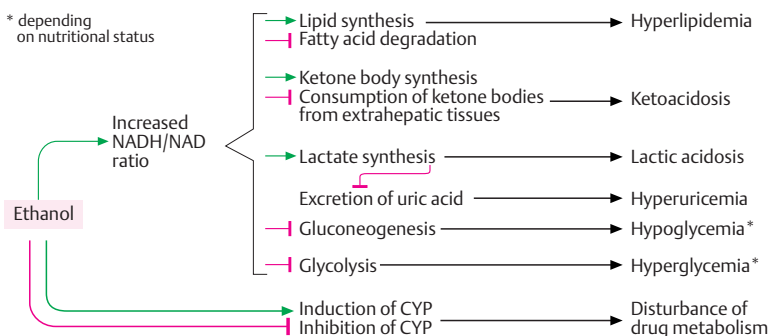
Ethanol degradation leads to an increased **NADH/NAD^+ ratio**, which significantly influences hepatic metabolism. Inhibition of the tricarboxylic acid (TCA) cycle is the central effect. Moreover, pyruvate is reduced to lactate and reaches the blood in this form (**lactic acidosis**). The elevated blood lactate level inhibits the excretion of uric acid in the kidney (**hyperuricemia**). The excess acetyl-CoA produced is used for the synthesis of ketone bodies (**ketoadidosis**) and fatty acids. At the same time, fatty acid degradation is inhibited. Lipid synthesis and lipid release into the blood increase (**hyperlipidemia**). Gluconeogenesis is also diminished because of the inhibition of the TCA cycle. If carbohydrate supply is insufficient, **hypoglycemia** occurs. By contrast, when carbohydrates are available, they cannot be degraded normally because of inhibition of glycolysis (**hyperglycemia**).

At higher concentrations EtOH is also broken down by cytochrome P450 systems, especially CYP 2E1. As a substrate, EtOH induces various CYP and at the same time inhibits others (**disturbance of drug metabolism**).

In the brain, ethanol is deposited in membranes owing to its amphipathic properties, where it affects receptors for neurotransmitters (p. 362). The effect of the inhibitory transmitter γ -aminobutyric acid (GABA) is enhanced, while that of the excitatory transmitter glutamate is reduced.

A. Blood ethanol level**B. Ethanol metabolism****C. Acute effects of ethanol**

* depending on nutritional status



Pathobiochemistry

Increased **ethanol consumption** over many years leads to damage to the liver and other organs. The recommended daily limit is about 60 g EtOH for men and about 30 g for women. However, these values are highly dependent on age, body weight, health status, and other factors.

A. Long-term effects of alcohol

Besides the impact of alcohol on intermediary metabolism (p. 326), other toxic effects are due to accumulation of **ethanal** (acetaldehyde). As an aldehyde ethanal reacts nonspecifically with amino groups and thiol groups of amino acids and proteins. Protein synthesis is impaired as a result, and *export of proteins and VLDL* from the liver is reduced. By a direct reaction with *glutathione*, ethanal compromises protection against H_2O_2 and other ROS (p. 288). *Lipid peroxidation* increases, mitochondria are damaged, and the respiratory chain is decoupled. These injuries promote alcohol-induced **hepatitis**. The biochemical signs of hepatitis include a rise in hepatic γ -*glutamyltranspeptidase* (= γ -glutamyltransferase, γ -GT), and the *aminotransferases* **ALT** and **AST** (pp. 168, 300) in the blood, increased **bilirubin** blood level because of reduced glucuronidation in the liver (p. 190) and delayed **blood clotting** because of impaired synthesis of coagulation proteins (p. 294).

B. Alcohol-induced fatty liver and cirrhosis

Since lipid synthesis increases with constant ethanol ingestion (see previous page), while the export of lipids with VLDL is diminished, lipids are increasingly stored in the liver (**fatty liver**). The rise in the liver's fat content (from less than 5% to more than 50% of the dry weight) is initially reversible. In chronic alcoholism (about > 160 g EtOH daily), however, the hepatocytes are increasingly replaced by connective tissue (**fibrosis**). **Liver cirrhosis** caused by ethanol and other liver-toxic substances is the final irreversible stage, which is characterized by a progressive loss of liver functions. On the other hand, the moderate (< 20 g EtOH/day) consumption of certain alcoholic beverages, especially red wine, may have a cardioprotective effect. This is ascribed less to ethanol than to *polyphenols*, which act as antioxidants. *Resveratrol* and *procyanidine*,

which occur in certain red grape varieties, may be mentioned.

C. Transport systems for bile components

In the apical (canalicular) membrane that separates the hepatocytes from bile canaliculi, there are various ATP-driven transport systems that assist bile formation. They belong to the large family of **ABC transporters** (i.e., ATP-binding cassette transporters), which transport lipids, bile salts, drugs, toxic compounds, and short peptides against a concentration gradient with hydrolysis of ATP. They are made up of two domains that transverse the membrane, which are responsible for translocation of their substrate, and two hydrophilic domains that bind and hydrolyze ATP.

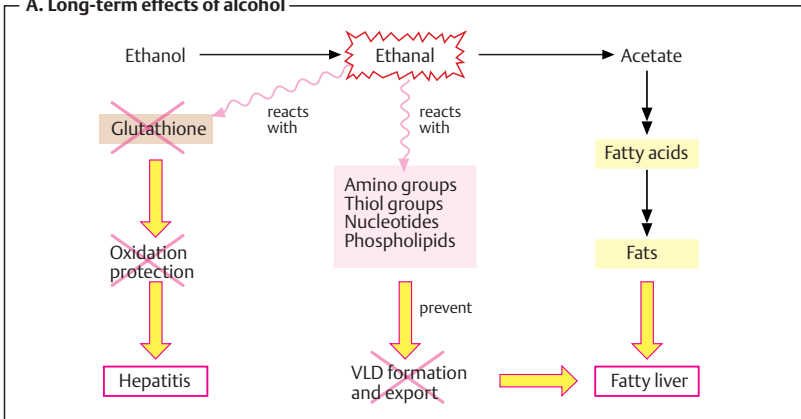
Important transport systems in the hepatocytes are **MRP2** (*multidrug resistance-related protein*; 1, transport of conjugates of bilirubin and xenobiotics), **MDR1** (*multidrug resistance glycoprotein*; 2, cations, drugs and xenobiotics), **MDR3** (3, phospholipids), **ABC G5/G8** (4, cholesterol), **BSEP** (*bile salt export protein*; 5, bile salts), **NTCP** (sodium-taurocholate co-transporting polypeptide; 6, bile acids/ Na^+), **OATP** (*organic anion transport polypeptide*; 7, bilirubin, anions/glutathione), **OCT** (*organic cation transporter*; 8, cationic xenobiotics, drugs), **MRP3** and **MRP4** (9, bile acids, bilirubin conjugates).

■ Further information

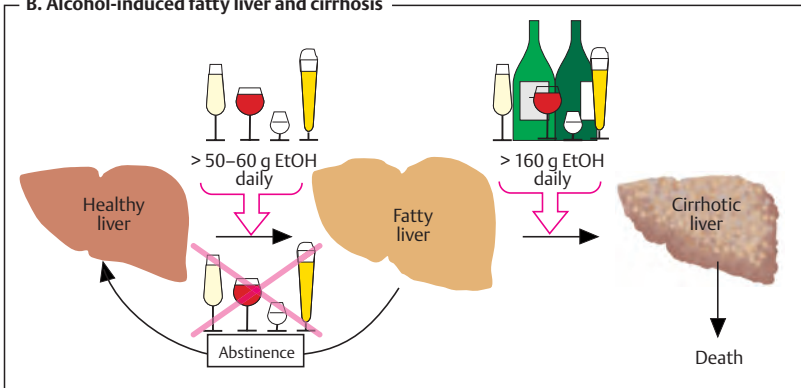
Cholestasis develops due to a disturbance of bile drainage from the liver. Bile acids, bilirubin, and other constituents of bile are then retained and damage the hepatocytes. The concentrations of the bile acids and bilirubin in the blood rise. Skin itching due to bile acid deposits and the development of jaundice are characteristic (p. 190). Enzymes in the blood typical of cholestasis are alkaline phosphatase (AP) and γ -GT (pp. 94, 300).

Gallstones or tumors can be *extrahepatic causes* of cholestasis. *Intrahepatic causes* include infections of the liver, and disorders or congenital defects of the **transport systems**.

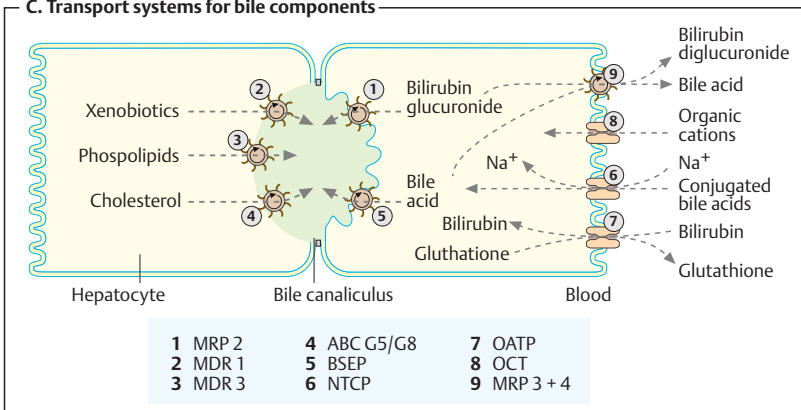
A. Long-term effects of alcohol



B. Alcohol-induced fatty liver and cirrhosis



C. Transport systems for bile components



Functions

Adipose tissue is a specialized connective tissue. It occurs primarily as **white adipose tissue**, the cells of which, the *adipocytes*, are characterized by a large vacuole in which fats are deposited. The cytoplasm and nucleus are located at the edge of the cell close to the membrane. White fat is the body's *most important energy store* (p. 332), and it is also a good *heat insulator* and *mechanical protective padding*. It acts as a *store* for provitamin A (β -carotene) and vitamin E (α -tocopherol).

The second, rarer type, **brown adipose tissue**, is important primarily in babies for *thermogenesis* (p. 124).

A. Lipogenesis and lipolysis in adipocytes

When nutrients are plentiful, adipocytes synthesize fats (**lipogenesis**). To do so, they take up *glucose* from the blood with the aid of the glucose transporter Glut-4 (1) and metabolize it via glycolysis (2) and the pentose phosphate pathway (3).

In the course of **glycolysis**, *glycerone 3-phosphate* (dihydroxyacetone phosphate) is produced, which is reduced by means of *glycerol 3-phosphate dehydrogenase* to *glycerol 3-phosphate* [4]. This metabolite can also be synthesized from other metabolites such as alanine, aspartate or malate: **glyceroneogenesis** [10]. In contrast to hepatocytes, adipocytes do not contain *glycerol kinase*. Free glycerol can therefore not be used to produce glycerol 3-phosphate. Acetyl-CoA is derived in the mitochondria from the pyruvate provided by glycolysis. After conversion to citrate and transfer into the cytosol, it is used for **fatty acid synthesis** (6). The **pentose phosphate pathway** (3) (p. 132) serves above all to produce *NADPH* for fatty acid synthesis (6).

Adipocytes can also take up *fatty acids* from the blood. These originate from lipoproteins (chylomicrons and VLDL), which are hydrolyzed by *lipoprotein lipase* (LPL [7]) on the surface of vascular endothelial cells. LPL is synthesized within the adipocytes and then released into the capillaries of the adipose tissue.

After the fatty acids have been activated to acyl-CoA, they are linked with glycerol 3-phosphate to form fats (8), which ultimately are stored in the vacuoles of the adipocytes.

When the body requires energy, **lipolysis** takes place. By means of this process, the fats in the adipocyte vacuoles are mobilized and hydrolyzed to fatty acids and glycerol with the aid of several *lipases*. The rate-limiting step is catalyzed by *hormone-sensitive triacylglycerol lipase* [9], a lipase that hydrolyzes mainly tri- and diacylglycerols. Fatty acids and glycerol, the products of lipolysis, are then released into the blood.

Lipogenesis and lipolysis are regulated by hormones. **Insulin** promotes lipogenesis and inhibits lipolysis. It stimulates glucose uptake into adipose tissue by inducing transfer of Glut-4 (1) into the plasma membrane (p. 428), and it promotes glycolysis (2) and in particular the pyruvate dehydrogenase reaction [5]. In addition, insulin induces *lipoprotein lipase* (LPL) [7], thereby reducing the blood's lipid content and increasing fatty acid uptake into adipocytes. At the same time, insulin inhibits *hormone-sensitive triacylglycerol lipase* [9].

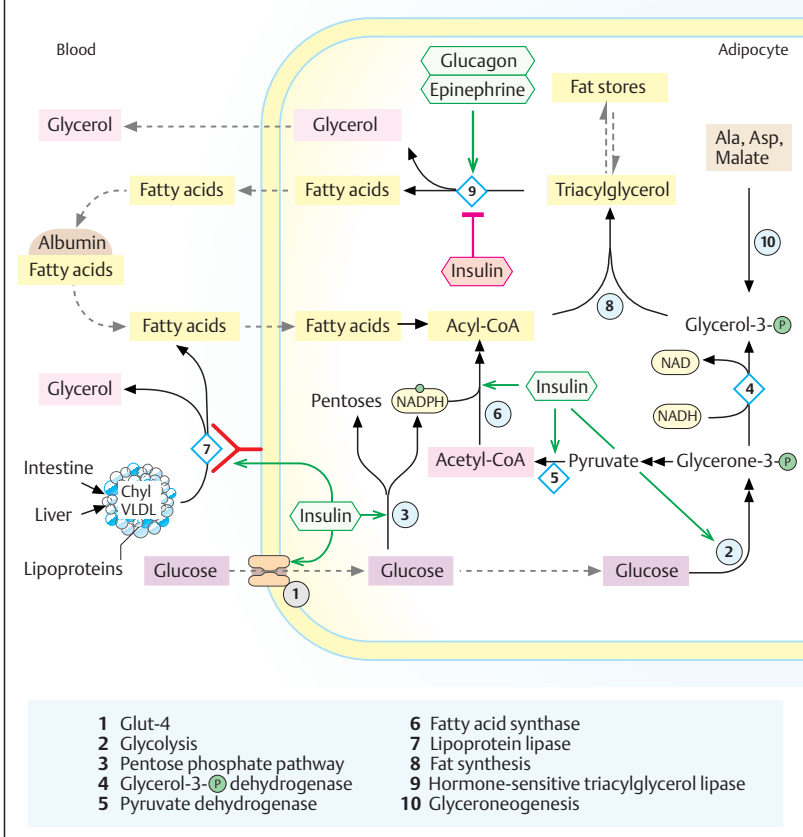
The **catecholamines** are insulin antagonists and are the most important hormones with a lipolytic action. They stimulate *hormone-sensitive lipase* via β_1 and β_2 receptors [9]. **Growth hormone** (GH) increases the sensitivity of adipocytes for catecholamines and reduces their sensitivity for insulin. In this way, it increases the blood level of fatty acids.

B. Hormones of adipose tissue

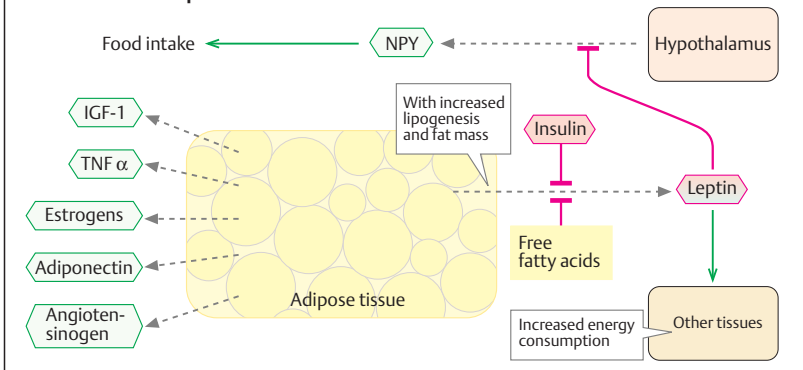
Adipose tissue is also active as an endocrine organ. It synthesizes hormones and mediators, with which it intervenes in *energy balance* and *glucose level homeostasis*.

During active lipogenesis with an increase in fats, the peptide hormone **leptin** is secreted into the blood. This signals to the brain that the fat stores have been filled. In the hypothalamus, leptin inhibits the production of **neuropeptide Y** (NPY) and other neuropeptides that stimulate the appetite and food intake. At the same time, it promotes energy consumption and thermogenesis in other tissues. Like leptin, **adiponectin** also reduces the glucose level. Moreover **estrogens** and other signaling substances are released from adipose tissue.

A. Lipogenesis and lipolysis in adipocytes



B. Hormones of adipose tissue



Pathobiochemistry

A. Fuel reserves

White adipose tissue serves primarily as an energy store. By releasing fatty acids and glycerol during fasting, it is the body's most important fuel supplier.

In an adult of normal weight, white adipose tissue contains roughly 8 kg of fat. This is the body's largest *fuel reserve* (A). Assuming a daily requirement of 9200 and 12 600 kJ, respectively, for women and men (p. 382), this represents an *energy reserve* for about 30 days. The diagram compares the energy content that can be mobilized from *carbohydrates* (glucose or glycogen), *triglycerides* and *proteins* in blood, liver, brain, muscle, and adipose tissue.

B. Obesity

An excessive quantity of adipose tissue is known as obesity. The **BMI** (*body mass index*) is used as a measure of obesity; it is obtained by dividing body weight (in kg) by the square of the height (in m). A BMI of

20–25 is regarded as *normal weight*,

25–30 as *overweight*,

30–40 as *obesity*, and

> 40 as *massive obesity*.

About 40% of the German population has a BMI over 25 and is therefore overweight according to this criterion. About 12% are obese. However, an elevated BMI cannot distinguish between unusually large muscle mass and fat mass. Different indicators have therefore been suggested for the proportion of body fat and its distribution, e.g., *waist circumference* and the *waist-hip ratio*, but these have not yet been finally accepted.

An endocrine (hormonal) *cause* of obesity is rare. Obesity is usually caused by three factors: genetic predisposition, lack of physical activity, and unfavourable dietary habits. In particular, the unchecked consumption of foods with a high fat and carbohydrate content is responsible for obesity. The possible role of industrial chemical in foods as an additional cause of obesity is currently being researched.

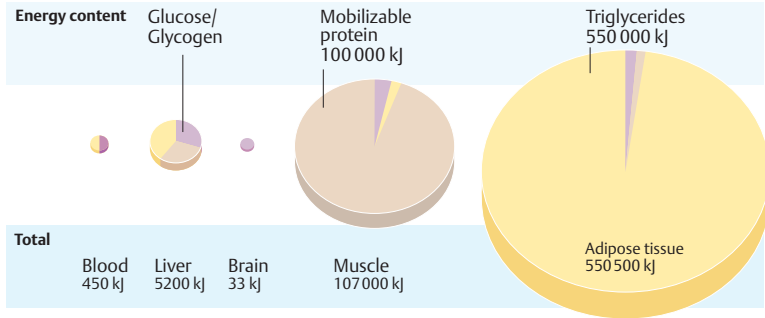
Successful *treatment* of obesity requires a lasting reduction of calorie intake and an increase in energy consumption by more exercise. Since these are behavior changes that are usually not achieved permanently, attempts are also made to reduce obesity with drugs ("appetite suppressants") and by surgical measures. Fat digestion in the intestine can be blocked by pharmacological inhibition of *pancreatic lipase*

(p. 276). Other drugs suppress the appetite or increase thermogenesis. Surgical reduction of the stomach and removal ("liposuction") of adipose tissue are intended to fight obesity. Obesity is a significant factor in the development of **insulin resistance** (p. 430). This is caused by a persistently raised blood level of free fatty acids derived from the increased adipose tissue. Since fatty acids are the preferred substrate for energy production in many tissues, they reduce glucose utilization, especially in muscle. They achieve this by inhibiting glucose uptake via Glut-4, as well as phosphofructokinase and the pyruvate dehydrogenase complex. This results in a raised blood glucose level and a secondary rise in insulin. In about 40% of patients with obesity, this leads to *diabetes mellitus type II* after 5–10 years (p. 430). Obesity is one of the symptoms of the **metabolic syndrome**, a dangerous combination of disease symptoms. The syndrome is characterized by the simultaneous presence of *obesity*, *high blood pressure*, *high triglyceride level*, low lipoprotein level and *insulin resistance*. It represents a high risk for arteriosclerotic complications, especially myocardial infarction. Whether the relatives of the "*Willendorf Venus*" shown in the center suffered from the metabolic syndrome ca. 25 000 years ago is not known.

The World Health Organization (WHO) lists the following *criteria* for the metabolic syndrome:

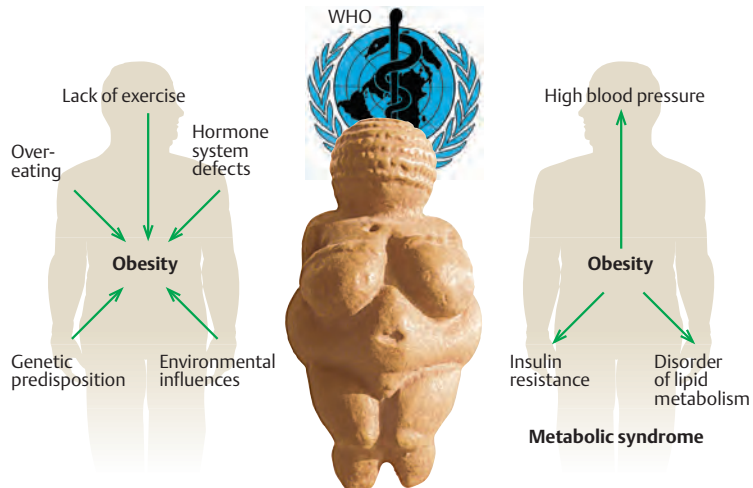
1. Obesity: waist measurement: > 88 cm in women, > 102 cm in men
2. HDL cholesterol < 50 mg/100 mL in women, < 40 mg/100 mL in men
3. Triglycerides > 150 mg/100 mL
4. Blood pressure > 130/85 mmHg
5. Fasting blood sugar > 110 mg/100 mL

A. Fuel reserves



	Blood	Liver	Brain	Muscle	Adipose tissue
Glucose/Glycogen	55%	30%	100%	5%	0.6%
Lipids	45%	40%	0%	2%	99%
Proteins	0%	30%	0%	93%	0.4%

B. Obesity



Functions

A. Functions of the kidneys

The kidneys' main function is **excretion** of water and water-soluble substances. This is closely associated with their role in regulating the body's electrolyte and acid-base balance (**homeostasis**). Finally, they also have functions in intermediary metabolism, especially in gluconeogenesis and amino acid metabolism (p. 338). Excretion, homeostasis, and metabolism are subject to hormonal control. The kidneys are also involved in synthesizing several **hormones**.

The kidneys are perfused with about 1500 L of blood every day. Approximately 180 L of primary urine is filtered out of this. Removal of water leads to concentration of the primary urine (to approximately one-hundredth of the initial volume).

The functional unit of the kidney is the **nephron**. It is made up of the Malpighian bodies or renal corpuscles (consisting of the Bowman capsule and glomerulus), the proximal tubule, Henle loop, and the distal tubule, which passes into a collecting duct. The human kidney contains around one million nephrons. The nephrons form urine in the following three phases.

Filtration. Ultrafiltration of the blood plasma in the glomerulus gives rise to **primary urine**, which is **isotonic** with plasma. The pores in the glomerular basal membrane allow substances with a molecular mass below 15 kDa to pass through unhindered. Molecules with masses greater than 65 kDa, e.g., almost all plasma proteins, are unable to enter the urine.

Reabsorption. Most low-molecular-weight plasma components are transported back into the blood by reabsorption from the ultrafiltrate, to prevent metabolite and electrolyte losses. In the proximal tubule, organic metabolites (especially glucose, amino acids, lactate, and ketone bodies) are recovered by secondary active transport (p. 210). The transporters involved are very similar to those responsible for the absorption of nutrients in the small intestine. The inorganic ions HCO_3^- , Na^+ , K^+ , phosphate, and sulfate are also reabsorbed by ATP-dependent (active) mechanisms in the proximal tubule. The later sections of the nephron mainly serve for additional water recovery and regulated reabsorption of Na^+ and Cl^- (p. 336).

Secretion. A few substances are released into the urine by **active transport**. These substances include H^+ and K^+ ions, uric acid, and creatinine, as well as drugs such as penicillin.

Hormones. The kidneys produce the hormones **erythropoietin** and **calcitriol** and are involved in the production of **angiotensin II** through release of the enzyme **renin**.

B. Urine

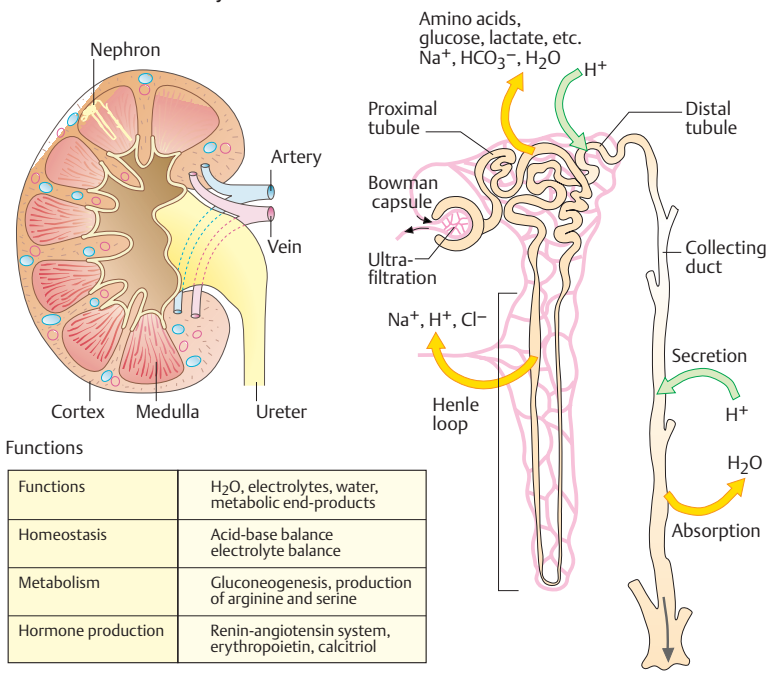
The volume and composition of urine depend on the diet and other factors and demonstrate a marked circadian rhythm. The volume and the amounts of substances excreted are therefore usually given relative to a 24-hour period ("24-hour urine"). An adult produces 0.5–2.0 L urine per day, 95% of which consists of water. Its pH fluctuates between 4.8 and 7.5 depending on metabolic status (the mean is around 5.8).

Electrolytes. The **cations** Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and NH_4^+ , and the **anions** Cl^- , SO_4^{2-} , and HPO_4^{2-} , are found in the urine, along with traces of other ions. Na^+ and Cl^- account for about two-thirds of all electrolytes in the final urine. Ca^{2+} and Mg^{2+} are present in even greater quantities in the feces. In acidosis, the excretion of NH_4^+ can increase greatly (p. 338). The excretion of Na^+ , K^+ , Ca^{2+} and phosphate is controlled by hormones (pp. 336, 386).

Organic components. Nitrogen-containing compounds are among the most important organic components of urine. **Urea**, which is synthesized in the urea cycle in the liver (p. 172), is the form in which amino acid nitrogen is excreted. When the nitrogen balance is constant, the amount of urea excreted reflects protein degradation: 70 g protein in food yields about 30 g urea in the urine.

Uric acid is the end product of purine metabolism. **Creatinine** is derived from the muscle metabolism, where it arises through spontaneous cyclization of creatine and creatine phosphate (p. 344). The amount of **amino acids** excreted is strongly dependent on the diet and on liver function.

A. Functions of the kidneys



B. Urine

Composition of 24 h urine

Elektrolytes			Organic substances		
Ion	g/day	mmol/day	Substance	g/day	mmol/day (as N)
Na ⁺	4-6	175-260	Urea	20-35	650-1200
K ⁺	2-3	50-75	Uric acid	0.4-0.8	10-20
Ca ²⁺	0.1-0.4	2.5-10	Creatinine	0.6-1.8	10-30
Mg ²⁺	0.2	8	Amino acids	< 1	< 10
Cl ⁻	5-10	140-280	NH ₄ ⁺	0-1	0-50
SO ₄ ²⁻	0.8-1.2	8-12			
HPO ₄ ²⁻	0.7-1.5	7-15	Total-N	-	700-1300

Volume: 0.5-2 L/day
 pH: 5.8 (4.8-7.5)
 Density: 1.015-1.022 kg/L
 Osmolarity: 50-1300 mosmol/kg
 Solids: 50-72 g/day

Also present in traces:
 sugar, organic acids,
 pyrrole derivatives, steroids,
 mucins, and enzymes

Electrolyte and Water Recycling

Together with low-molecular-weight organic components, water and inorganic ions (electrolytes) enter the *primary urine* by ultrafiltration. From there, they are recovered for the most part by passive or active reabsorption. The extent of the reabsorption determines the amount of the substances that ultimately is excreted in the urine.

A. Electrolyte and water recycling

Water. Reabsorption of water in the tubules is a mainly *passive process* in which water follows osmotically active particles, particularly Na^+ and Cl^- , through the gaps between endothelial cells (paracellular transport). In this way, most of the filtered water is recovered.

Fine regulation of water excretion (**diuresis**) takes place in the collecting ducts, where the peptide hormone *vasopressin* (adiuretin, antidiuretic hormone, ADH) operates. This is produced in the hypothalamus and secreted by the posterior lobe of the pituitary. It promotes recovery of H_2O by stimulating the transfer of *aquaporin 2* (7; p. 212) from the ER into the plasma membrane of the endothelial cells via G-protein-coupled V2 receptors (translocation, p. 100). A lack of ADH leads to the condition of *diabetes insipidus*, in which up to 30 L of final urine is produced per day by increased diuresis.

H^+ , HCO_3^- . The kidney is the only organ that can control absorption or excretion of H^+ and HCO_3^- ions and therefore plays a central part in *acid-base balance* (p. 292).

The cells of the renal tubules are able to secrete protons (H^+) from the blood into the urine against a concentration gradient, although the H^+ concentration there is up to 1000 times higher than in plasma. To achieve this, CO_2 is taken up from the lumen and reacted with H_2O with the aid of *carbonic anhydrase* [1] to yield a proton and hydrogen carbonate ("bicarbonate," HCO_3^-). HCO_3^- returns to the plasma in co-transport with Na^+ (1), where it contributes to the blood's base reserve, while the proton is released into the urine in an antiport against Na^+ (2). Both transport processes are driven by the Na^+ gradient, which is built up by *Na^+/K^+ -ATPase* ([2]; p. 108)—they are therefore *secondary active* transport processes. In addition to this transport mechanism, there is a V-type H^+ -transporting ATPase in the distal tubule and collecting duct ([3]; p. 212).

Na^+ , K^+ and Cl^- . Controlled reabsorption of Na^+ , K^+ and Cl^- from the primary urine is highly effective, with more than 99% of these ions being reabsorbed.

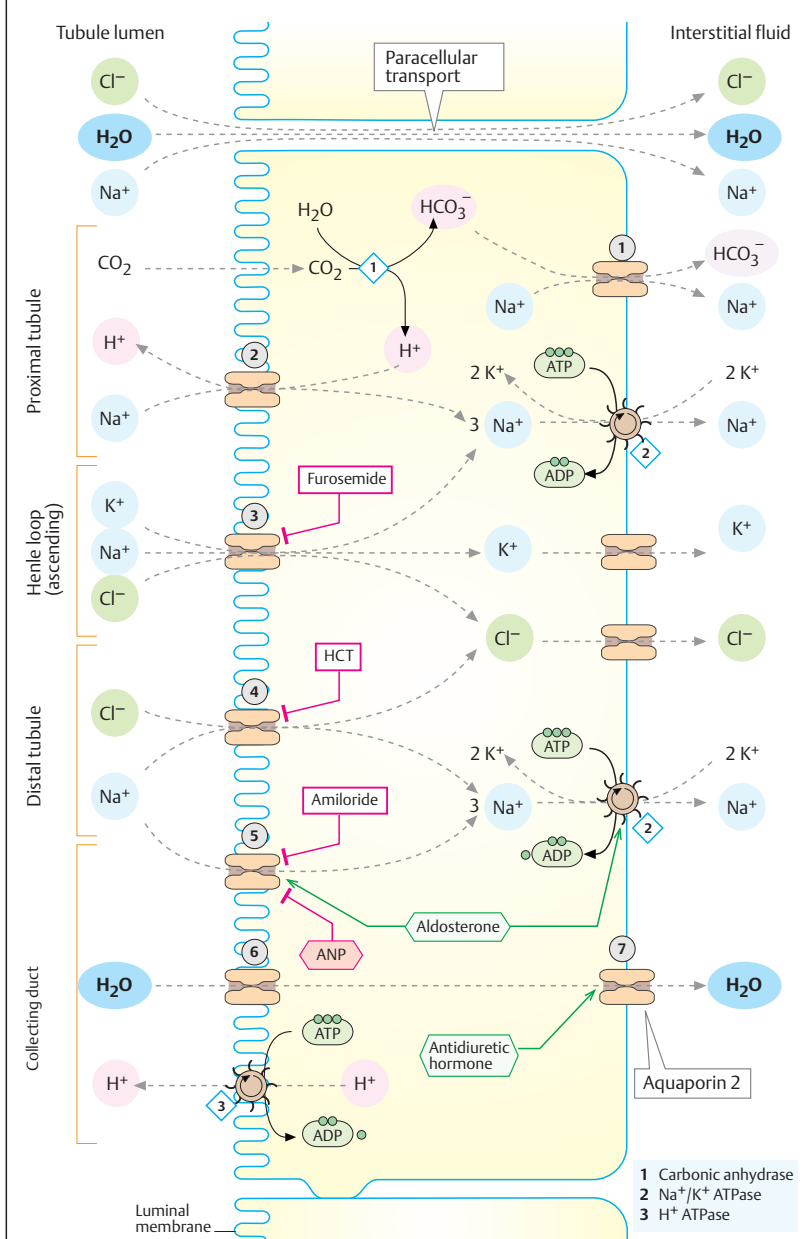
Several mechanisms are involved: some of the Na^+ and Cl^- ions are taken up in the proximal tubule through the junctions between the cells (paracellularly). In addition, there is a secondary active co-transport of Na^+ together with glucose and amino acids (not shown; p. 272). These two pathways are responsible for 60%–70% of total Na^+ absorption.

In the ascending part of the Henle loop, there is another transporter (3), which functions electroneutrally and takes up one Na^+ ion and one K^+ ion together with two Cl^- ions. This symport is also secondarily active. It is inhibited by loop diuretics such as *furosemide*.

This is followed in the distal tubule by a Na^+/Cl^- cotransporter which is also driven by the Na^+ gradient (4). It is the site of action of the diuretic *hydrochlorothiazide* (HCT).

In the distal tubule and in the collecting duct, the steroid hormone aldosterone (p. 420) increases the reuptake of Na^+ . Like all steroids, aldosterone works mainly by stimulating the transcription of certain target genes (transcription control; p. 100). In this way, it increases the expression of Na^+/K^+ -ATPase and the synthesis of a sodium channel (5) that can be inhibited by amiloride. The increase of Na^+ reabsorption is associated with water retention and thus with an increase in blood pressure. Atrial natriuretic peptide (ANP; p. 432), produced in the atrium of the heart, reduces blood pressure by acting as an aldosterone antagonist. It diminishes Na^+ reabsorption in the kidney and at the same time causes vasodilatation via cGMP (p. 408).

A. Electrolyte and water recycling



Metabolism

A. Renin–angiotensin system

The peptide hormone angiotensin II is not synthesized in an endocrine gland, but in the blood. The kidneys take part by releasing the aspartate proteinase (p. 162) *renin* [1]. Renin is produced in the juxtaglomerular apparatus as a precursor (prorenin), proteolytically activated and released into the blood. There it acts on **angiotensinogen**, a plasma protein in the α_2 -globulin group, which like most plasma proteins is synthesized in the liver. **Angiotensin I**, the decapeptide cleaved off, is broken down further in the blood vessels of the lung and other tissues to the octapeptide **angiotensin II** by *angiotensin-converting enzyme* (ACE, [2]). The lifespan of active angiotensin II in the plasma is only minutes, as it is rapidly degraded by peptidases. The plasma level of angiotensin II is determined by renin release, which in turn is triggered by a fall in blood pressure or in the Na^+ concentration in the blood.

Effects. Angiotensin II has effects on the kidneys, brain stem, pituitary gland, adrenal cortex, blood vessel walls, and heart via G-protein-coupled receptors. It increases blood pressure by *vasoconstriction* (narrowing of the blood vessels). In the kidneys, it promotes the *retention of Na^+* and water by stimulating aldosterone synthesis. In the brain stem, it triggers the *sensation of thirst*. In the pituitary gland, angiotensin II stimulates *vasopressin release* (adiuretin). All of these effects lead directly or indirectly to *increased blood pressure*. Components of the renin–angiotensin system are therefore important targets for antihypertensive medications.

B. Metabolism

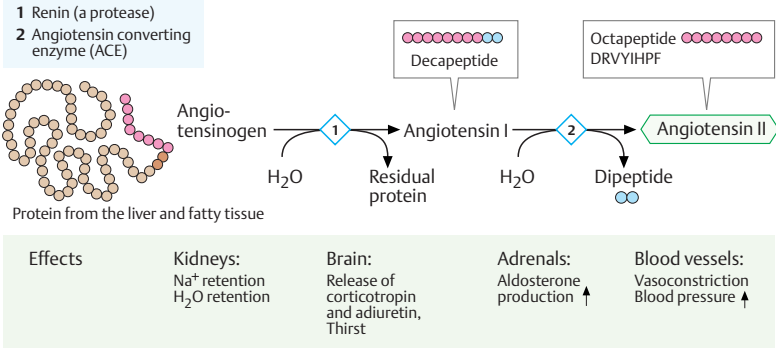
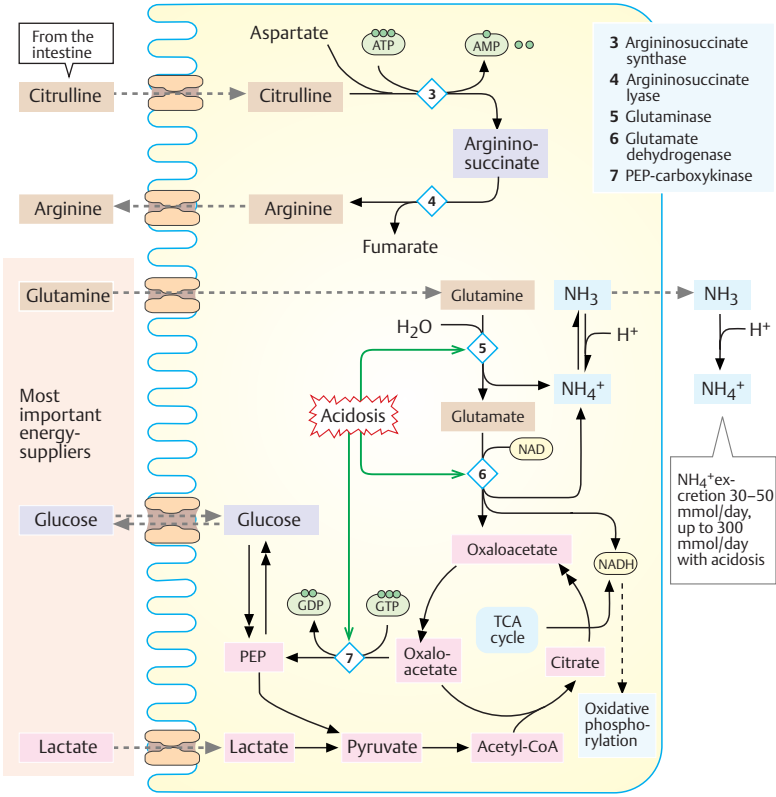
The kidneys have a high ATP requirement and therefore engage in very active metabolism. The most important energy substrates are *glucose*, *lactate*, and *glutamine*. The latter two metabolites and glycerol at the same time are precursors for **gluconeogenesis**, which is highly active in the proximal tubule. The contribution of the kidneys to gluconeogenesis in the body overall is still unclear—current estimates are between 10% and 50%. The kidneys also have central functions in amino acid metabolism, e.g., in the synthesis and release of arginine (see below) and serine.

Glutamine is the most abundant amino acid in the plasma, with a concentration of 0.5–0.7 mM, and is the preferred transport form for amino nitrogen in the blood (pp. 172, 374). Glutamine degradation in the kidney by *glutaminase* [5] and *glutamate dehydrogenase* [6] provides not only the gluconeogenesis precursor 2-oxoglutarate, but also two molecules of NH_4^+ . These play an important part in buffering acids in the urine.

The kidneys excrete 30–50 mmol H^+ ions on average daily in the urine (p. 336). Normally, buffer systems in the urine (especially the $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ system) trap a large proportion of the secreted protons so that the urine is only weakly acid ($> \text{pH } 4.8$). In *metabolic acidosis* (pp. 140, 430) and acidosis of dietary origin, H^+ excretion can increase to 10 times the normal level. In this case, glutamine degradation is intensified to release additional **ammonium ions** (NH_4^+) in the tubule cells. These are in equilibrium with **ammonia** (NH_3), which can diffuse through the tubule membrane into the urine (p. 164), where it neutralizes H^+ ions and is converted back to ammonium ions. The charged NH_4^+ ions can no longer return into the cells and are excreted in the urine.

When acidosis is present, several glutamine degradation enzymes are induced in the kidney. These include not only glutaminase and glutamate dehydrogenase, but also *PEP carboxykinase* [7], a key enzyme in gluconeogenesis. By contrast, the renal excretion of ammonia is limited in *alkalosis*.

Other metabolic functions specific to the kidneys include biosynthesis of **serine** (not shown) and **arginine**. Arginine biosynthesis in the kidney starts from *citrulline*, which is formed especially in the intestine. Two enzymes that also occur in the urea cycle ([3] and [4]) produce arginine from citrulline, which is released into the blood to supply other organs. Arginine synthesized in the urea cycle of the liver cannot be removed without bringing the cycle to a standstill.

A. Renin-angiotensin system**B. Metabolism**

Muscle Contraction

The musculature is what makes movements possible. In addition to the **skeletal muscles**, which can be contracted voluntarily, there are also the autonomically activated **heart muscle** and **smooth muscle**, which is also involuntary. In all types of muscle, contraction is based on the interplay between the proteins actin and myosin.

A. Organization of skeletal muscle

Striated muscle consists of parallel bundles of **muscle fibers**. Each fiber is a single large multinucleate cell. The cytoplasm in these cells contains **myofibrils**, 2–3 μm thick, that can extend over the full length of the muscle fiber.

The *striation* of the muscle fibers is characteristic of skeletal muscle. It results from the regular arrangement of molecules of differing density. The repeating contractile units, the **sarcomeres**, are bounded by Z lines from which thin filaments of **F-actin** (p. 198) extend on each side. In the A bands, there are also thick parallel filaments of **myosin**. The H bands in the middle of the A bands only contain myosin, while only actin is found on each side of the Z lines.

Myosin is quantitatively the most important protein in the myofibrils, representing 65% of the total. It is shaped like a golf club (bottom right; p. 54). The molecule is a hexamer consisting of two identical **heavy chains** (2×223 kDa) and four **light chains** (each about 20 kDa). Each of the two heavy chains has a globular “head” at its N-terminus, which extends into a “tail” about 150 nm long in which the two chains are intertwined to form a superhelix. The small subunits are attached in the head area. Myosin is present as a bundle of several hundred stacked molecules in the form of a “*thick myosin filament*.” The head portion of the molecule acts as an **ATPase**, the activity of which is modulated by the small subunits.

Actin (42 kDa) is the most important component of the “*thin filaments*.” It represents ca. 20%–25% of the muscle proteins. **F-actin** is also an important component of the cytoskeleton (p. 198). This filamentous polymer is in equilibrium with its monomer, **G-actin**. The other protein components of muscle include tropomyosin and troponin. **Tropomyosin** (64 kDa) attaches to F-actin as a rodlike dimer and connects approximately seven actin units to each other. The heterotrimer **troponin** (78 kDa) is bound to one end of tropomyosin.

In addition to the above proteins, several other proteins are also typical of muscle—including **titin** (the largest known protein, at just under 3000 kDa), α - and β -**actinin**, **desmin**, and **vimentin**.

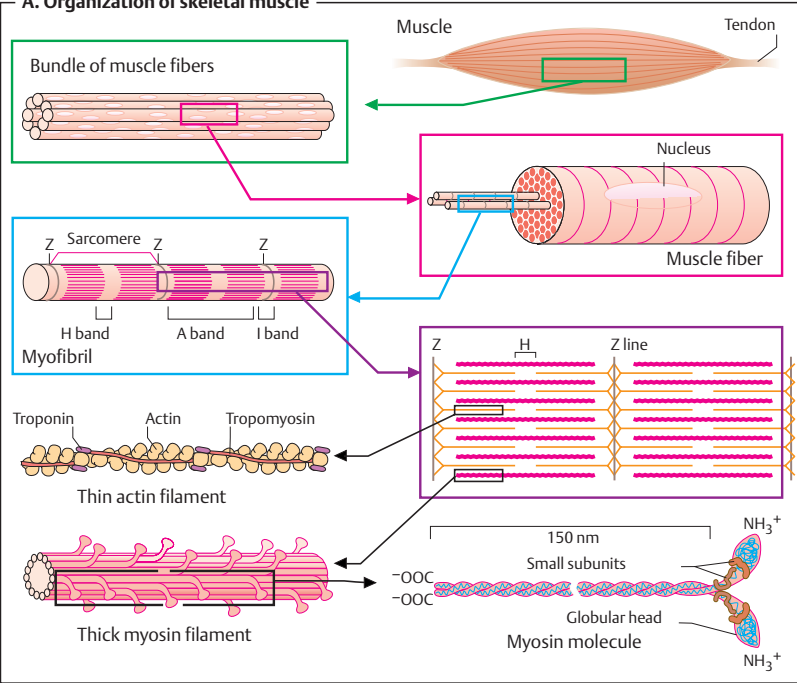
B. Mechanism of muscle contraction

The *sliding filament model* describes the mechanism involved in muscle contraction. In this model, sarcomeres become shorter when the thin and thick filaments slide alongside each other and telescope together, with ATP being consumed. During contraction, the following reaction cycle is repeated several times:

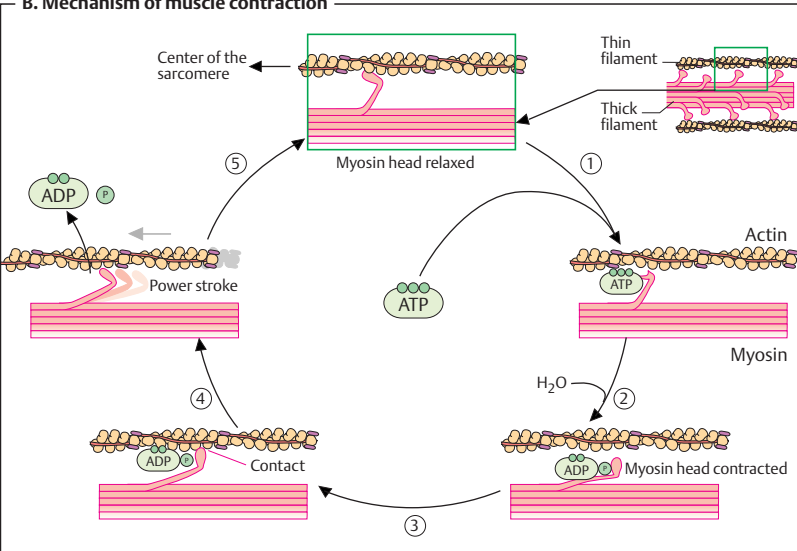
- (1) In the initial state, the myosin heads are attached to actin. When ATP is bound, the heads detach themselves from the actin (the “plasticizing” effect of ATP).
- (2) The myosin head hydrolyzes the bound ATP to ADP and P_i , but initially withholds the two reaction products. ATP cleavage leads to allosteric tension in the myosin head.
- (3) The myosin head now forms a new bond with a neighboring actin molecule.
- (4) The actin causes the release of the P_i and shortly afterward release of the ADP as well. This converts the allosteric tension in the myosin head into a conformational change that acts like a rowing stroke.
- (5) After the movement is performed the cycle can be repeated for as long as ATP is available, so that the thick filaments are constantly moving along the thin filaments in the direction of the Z disk.

Each rowing stroke of the 500 or so myosin heads in a thick filament produces a contraction of about 10 nm. During strong contraction, the process is repeated about five times per second. This leads to the whole complex of thin filaments moving together; the H band becomes shorter and the Z lines slide closer together.

A. Organization of skeletal muscle



B. Mechanism of muscle contraction



Control of Muscle Contraction

A. Neuromuscular junction

Muscle contraction is triggered by *motor neurons* that release the neurotransmitter **acetylcholine** (ACh; p. 362). ACh diffuses through the narrow synaptic cleft and binds to nicotinic **acetylcholine receptors** on the plasma membrane of the muscle cell (the **sarcolemma**), thereby opening the ion channels integrated into the receptors (p. 364). This leads to an inflow of Na^+ , which triggers an **action potential** (p. 360) in the sarcolemma. The action potential propagates from the end plate in all directions and constantly stimulates the muscle fiber. With a delay of a few milliseconds, the contractile mechanism responds to this by contracting the muscle fibers.

B. Sarcoplasmic reticulum

The action potential produced at the neuromuscular junction is transferred in the muscle cell into a transient increase in the Ca^{2+} concentration in the cytoplasm of the muscle fiber (the **sarcoplasm**).

In the resting state, the Ca^{2+} level in the sarcoplasm is very low (less than $10^{-7} \text{ mol} \cdot \text{L}^{-1}$). By contrast, the **sarcoplasmic reticulum** (SR), a special form of the ER, contains Ca^{2+} ions at a concentration of about $10^{-3} \text{ mol} \cdot \text{L}^{-1}$ (p. 408). The SR is a branched organelle that surrounds the myofibrils like a net stocking inside the muscle fibers (illustrated at the top using the example of a heart muscle cell). The high Ca^{2+} level in the SR is maintained by Ca^{2+} -transporting **ATPases** (p. 212). In addition, the SR also contains **calsequestrin**, a 55-kDa protein that is able to bind numerous Ca^{2+} ions via acidic amino acid residues.

The transfer of the action potential to the SR is made possible by **transverse tubules** (T tubules), which are open to the extracellular space and establish a close connection with the SR. The altered membrane potential activates **dihydropyridine receptors** (DHPR), calcium channels on the T tubules, which in turn activate **ryanodine receptors** (p. 408) on the SR. These are also calcium channels, which open by mechanical contact with the DHPR or by binding Ca^{2+} or other second messengers. Calcium ions then leave the SR and enter the sarcoplasm, where they lead to a rapid increase in Ca^{2+} concentration. This in turn causes the myofibrils to contract (C).

Contractions of **cardiac muscle** are triggered in a way similar to that in skeletal muscle, though

the extracellular Ca^{2+} concentration is more important for the rise in the intracellular Ca^{2+} concentration that initiates contraction.

In **smooth muscle**, on the other hand, Ca^{2+} has a different function. The cells lack the troponin present in skeletal muscle cells. Activation of actin/myosin contraction therefore takes place either through **caldesmon**, which binds Ca^{2+} , or by phosphorylation of the **small myosin subunits**.

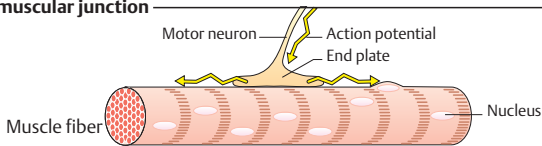
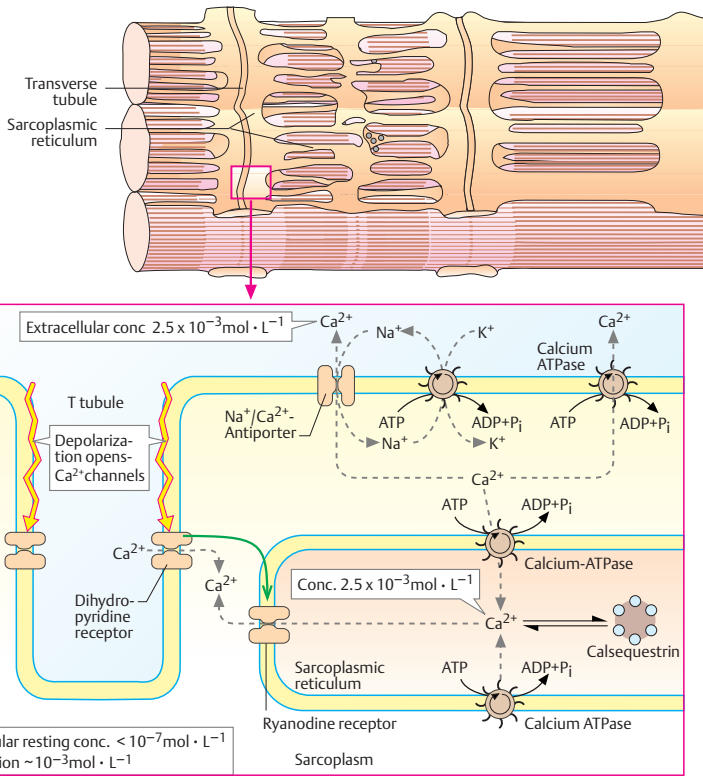
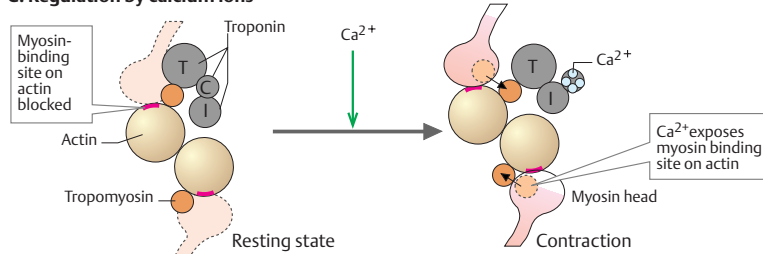
Muscle relaxation is dependent on a fall in the Ca^{2+} concentration to $< 10^{-6} \text{ mol} \cdot \text{L}^{-1}$. This is achieved by transport of Ca^{2+} by **ATP-driven calcium pumps** into the SR (p. 212) and extracellular space. **$\text{Na}^+/\text{Ca}^{2+}$ antiporters** also contribute. These processes consume ATP directly or indirectly (p. 408).

C. Regulation by calcium ions

In relaxed skeletal muscle, the complex consisting of **troponin** and **tropomyosin** blocks the access of the myosin heads to actin (p. 340). Troponin consists of three different subunits (T, C, and I). The rapid increase in cytoplasmic Ca^{2+} concentration caused by opening of the calcium channels in the SR leads to binding of Ca^{2+} to the C subunit of troponin, which closely resembles calmodulin (p. 408). This produces a conformational change in troponin that causes the whole troponin-tropomyosin complex to shift slightly and expose a binding site for myosin (red). This initiates the contraction cycle. When the sarcoplasmic Ca^{2+} concentration is quickly reduced again by active transport back into the SR, troponin loses the bound Ca^{2+} ions and returns to the initial state, in which the binding site for myosin on actin is blocked.

■ Further information

The calcium channels and pumps are important pharmacologic targets for influencing muscle and cardiac work by “**calcium antagonists**.”

A. Neuromuscular junction**B. Sarcoplasmic reticulum****C. Regulation by calcium ions**

Muscle Metabolism

Muscle contraction is associated with a high level of ATP consumption (p. 340). Without constant resynthesis, the ATP available in the resting state would be used up in less than 1 second of contraction.

A. Energy metabolism in the white and red muscle fibers

Skeletal muscles contain two types of fibers. **Red fibers** (slow or type I fibers; right part of illustration) are suitable for prolonged work. Their metabolism is mainly aerobic and therefore depends on an adequate supply of O₂. **White fibers** (fast or type II fibers; left part of illustration) are better suited for rapid, strong contractions. These fibers are able to form sufficient ATP even at low O₂ concentrations.

Red fibers provide for their ATP requirements mainly from **fatty acids**, which are broken down via β -oxidation and the TCA cycle, to synthesize the required ATP in the respiratory chain by **oxidative phosphorylation** (OxPhos). Because of the oxygen requirement of the respiratory chain, red fibers have an oxygen reserve in the form of myoglobin-bound O₂ (see B).

White fibers (left part of illustration) do not have this O₂ reserve. If the oxygen supplied by the blood no longer suffices during major muscular work—e.g., weight-lifting, or very rapid contractions, such as those performed by the eye muscles—they switch from aerobic metabolism to **anaerobic glycolysis**. They then obtain ATP especially by converting glycogen to lactate, drawing on their **glycogen** stores to produce glucose-6-phosphate for glycolysis (p. 136). The NADH formed during glycolysis has to be reoxidized to NAD⁺ to continue glucose degradation and thus ATP formation in the white fibers. When O₂ is lacking, this occurs through reduction of the pyruvate produced to **lactate**, which is released into the blood and synthesized back to glucose in the liver (**Cori cycle**; p. 316).

Another reaction that provides ATP in muscle cells is catalyzed by **adenylate kinase** (*myokinase*, not shown). This reaction disproportionates two molecules of ADP to ATP and AMP. The AMP is further deaminated to IMP in a subsequent step, to shift the equilibrium of the reversible adenylate kinase reaction toward ATP production. The rise in the concentration of AMP is a crucial signal for provision of substrates for energy metabolism (p. 124).

B. Myoglobin

The red color of muscle fibers is due to the heme protein **myoglobin** when it has bound oxygen. Oxy-myoglobin is used as an oxygen store. As the illustration shows, myoglobin has a much higher affinity for O₂ than hemoglobin (p. 286) and therefore only releases the bound oxygen when there is a severe drop in O₂ partial pressure. Myoglobin accepts the oxygen from hemoglobin and stores it for later use by the respiratory chain (OxPhos). As a monomeric protein, myoglobin (left) does not exhibit allosteric binding behavior for O₂ despite its structural similarity to the tetrameric hemoglobin (right).

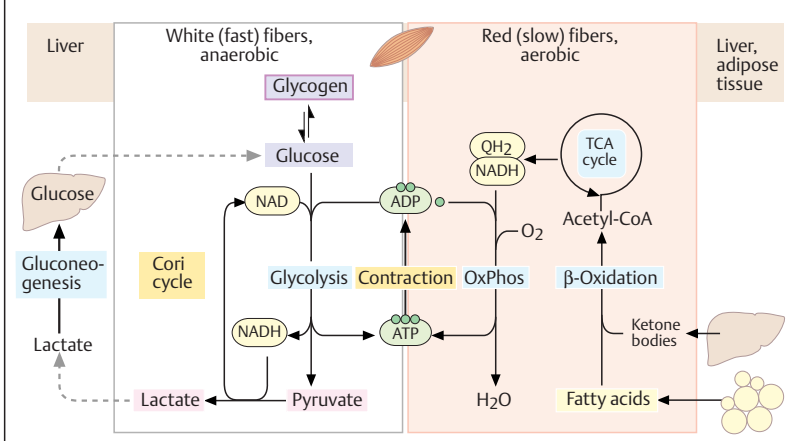
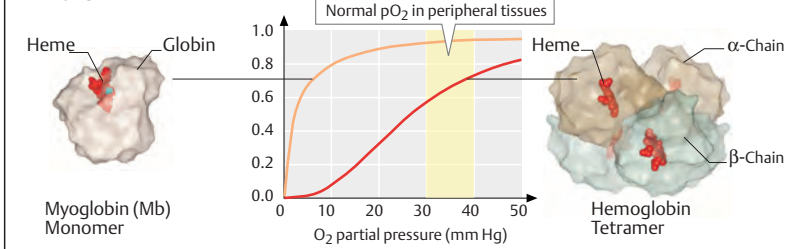
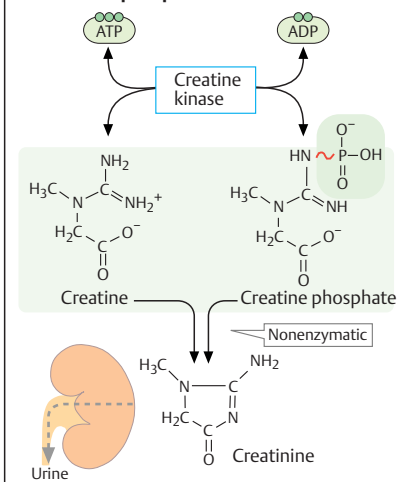
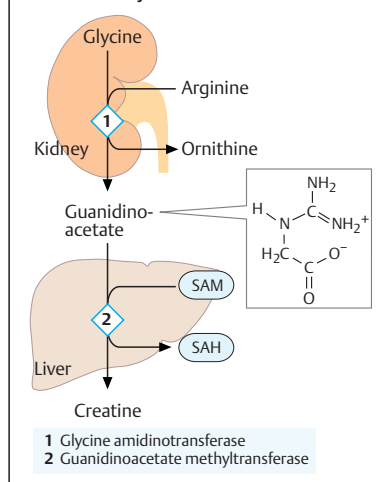
C. Creatine phosphate

Creatine phosphate is the most important short-term buffer for the ATP level in muscle. Creatine phosphate stores are sufficient for about 9 seconds of strong muscular work. When there is an excess of ATP (in resting muscle), creatine phosphate is produced by phosphorylation of **creatine** (*N*-methylguanidoacetic acid), catalyzed by **creatine kinase**. In creatine phosphate, the phosphate residue has a similarly high chemical potential as in ATP and is therefore easily transferred back to ADP when necessary (in working muscle).

Creatine and creatine phosphate are not chemically stable. In a nonenzymatic reaction, they cyclize slowly to form **creatinine**, which can no longer be phosphorylated. It is released into the blood and excreted with the urine at a constant rate, which depends only on muscle mass (~15 mg/kg BW daily). In **urinalysis**, creatinine is used as a reference parameter because of its constant excretion.

D. Creatine synthesis

Creatine does not derive from the muscles themselves, but is synthesized in two steps in the kidneys and liver. Initially, the guanidino group of arginine is transferred to glycine in the kidneys, yielding **guanidinoacetate** [1]. In the liver, *N*-methylation of guanidinoacetate leads to the formation of creatine from this [2]. The coenzyme in this reaction is *S*-adenosyl methionine (**SAM**; p. 88).

A. Energy metabolism in the white and red muscle fibers**B. Myoglobin****C. Creatine phosphate****D. Creatine synthesis**

Pathobiochemistry

A. Muscle diseases

In the *congenital* muscle diseases, genetic defects of structural proteins (e.g., *dystrophies* of the Duchenne and Becker type), ion channels (*myotonias*) or enzymes (e.g., McArdle syndrome) predominate. Diseases of cardiac muscle, especially myocardial infarction, are examples of *acquired* muscle diseases.

Duchenne muscular dystrophy is an example of a congenital muscle disease. It is caused by defects of the muscle protein **dystrophin**, a structural protein that connects actin with proteins of the sarcolemma (the cell membrane around the muscle fibers). The disease leads to progressive muscle atrophy. It is caused by a major deletion in the dystrophin gene. **Becker muscular dystrophy** is a milder form of the disease in which a point mutation in the dystrophin gene leads to changes in the protein.

In **McArdle syndrome**, the *glycogen phosphorylase* in muscle is deficient. Patients with this disease are unable to degrade glycogen (p. 136), so it accumulates in muscle cells. Liver phosphorylase is not affected.

Hereditary myopathies can also be caused by *mutation of mitochondrial genes*, e.g., **MELAS syndrome**, in which the mitochondrial *tRNA* for leucine is altered (p. 108).

Myocardial infarction is caused by **ischemia** of the heart muscle, so that its oxygen supply is inadequate. The cardiomyocytes, which obtain ATP preferentially by oxidative metabolism, then switch to anaerobic glycolysis (p. 108). As a result, the concentration of lactate and protons increases so greatly that regular contraction of the muscle ceases. Finally, the cells die (*necrosis*).

Muscle and brain cells contain considerable amounts of *creatine kinase* (p. 344). When the cells are damaged or die, they release it into the blood. The appearance of the myocardial isoenzyme of creatine kinase (CK-2) in the blood is characteristic of myocardial infarction. It is therefore measured in the serum to diagnose myocardial damage (pp. 94, 300).

Muscle ache, a delayed muscle pain lasting several days, occurs mainly after unusual movements in which the working muscle is strained by external forces. These forces lead to *sarcomere tears* especially in the region of the Z lines. The pain arises secondarily, probably due to autolysis of the destroyed fiber structures and to edema. The production of lactic acid (anion: lactate) does not appear to play

any part. Muscle ache does not leave any lasting damage. It can be relieved by massage, stretching and light dynamic work. There is no known effective drug treatment.

B. Energy sources of muscle cells

The main energy sources of muscle metabolism are **glucose**, **fatty acids**, and **branched-chain amino acids**. Cardiac muscle differs from skeletal and smooth muscle in that respect.

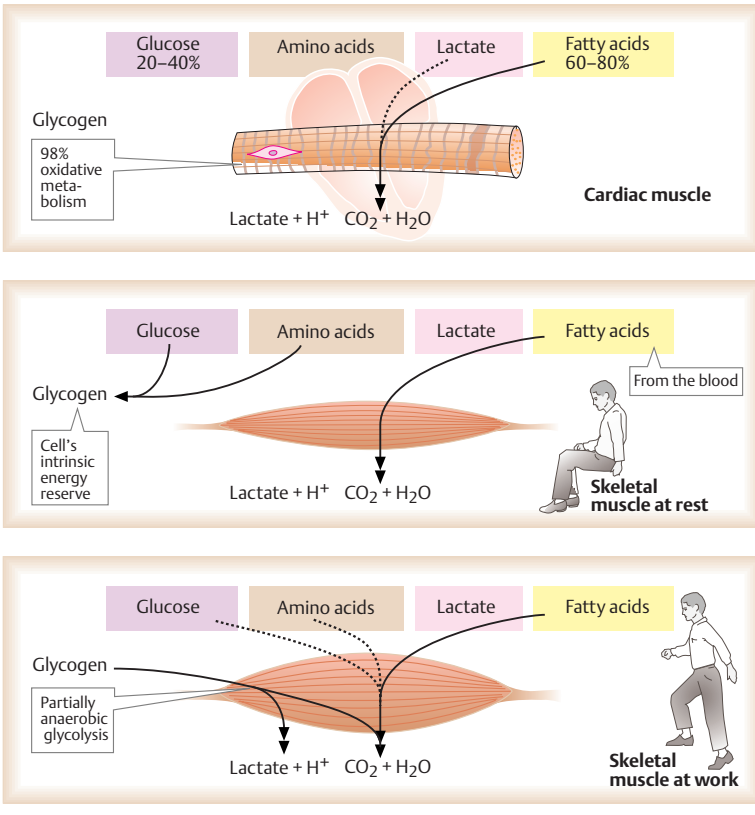
Cardiac muscle is constantly active and obtains its ATP almost exclusively by oxidation of fatty acids from the blood. Glucose is used only when its blood level is high and insulin stimulates glucose transport via Glut-4 into the cardiomyocytes.

In resting **skeletal muscle** the red fibers also use mainly fatty acids for energy production. In these conditions glucose and branched-chain amino acids are preferentially converted to glycogen, which is stored in large quantities (p. 136). During intense work, skeletal muscle quickly falls back on its glycogen stores (already within the first minute) and then obtains ATP mainly by anaerobic glycolysis until the blood supply of O₂ increases again and it can switch to oxidative metabolism. During maximum effort, when skeletal muscle depends on additional ATP synthesis by anaerobic glycolysis, the pH in muscle rapidly decreases which limits work performance and ultimately leads to exhaustion.

Prolonged **muscle training** can shift the proportions of type I and type II fibers. It also enlarges the glycogen stores and increases the number and size of mitochondria.

A. Muscle diseases

Congenital muscle diseases	Cause
Duchenne muscular dystrophy	Absence of dystrophin
Becker muscular dystrophy	Dystrophin deficiency
McArdle syndrome	Glycogen phosphorylase deficiency
MELAS syndrome	Mutation of mt-RNA ^{Leu}
Acquired muscle diseases	Cause
Myocardial infarction	Insufficient of oxygen supply
Muscle ache	Microtears of the sarcomere

B. Energy sources of muscle cells


Bone and Teeth

The family of connective-tissue cells is derived from mesenchymal stem cells. It includes *fibroblasts*, *chondrocytes* (cartilage cells), and *osteoblasts* (bone-forming cells), and also endothelial and mast cells. Connective tissue cells are specialized to secrete extracellular proteins, particularly collagens, which they use to synthesize the **extracellular matrix** (p. 352f.). Bone, cartilage and tendons are formed in this way. Remodeling and degradation of these structures are also performed by connective tissue cells.

A. Bone

Bones are an extremely dense, specialized form of connective tissue. In addition to their supportive function, they serve to store calcium and phosphate ions. In addition, blood cells are formed in the bone marrow. The most important mineral component of bone, accounting for roughly 70%, is **apatite**, a form of crystalline *calcium phosphate*.

Apatites are cationic complexes of three Ca^{2+} and three $(\text{PO}_4)^{3-}$ ligands matched by HPO_4^{2-} , CO_3^{2-} , OH^- , or F^- as anions. Depending on the counter-ion, apatite can occur in the forms *carbonate apatite* $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$, as *hydroxyapatite* $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, or *fluoroapatite* $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$. In addition, alkaline earth carbonates also occur in bone. In adults, more than 1 kg calcium is stored in bone.

Osteoblast and **osteoclast** activity is constantly incorporating Ca^{2+} into bone and removing it again. There are various hormones that regulate these processes: **calcitonin** increases deposition of Ca^{2+} in the bone matrix, while **parathyroid hormone** (PTH) promotes the mobilization of Ca^{2+} , and **calcitriol** improves mineralization (p. 386).

The most important *organic components* of bone are **collagens** (mainly type I; p. 350) and **proteoglycans** (p. 354). These form the extracellular matrix into which the apatite crystals are deposited (*biomineralization*). Various proteins are involved in this process of bone formation, including collagens and phosphatases. *Alkaline phosphatase* is mainly found in osteoblasts and *acid phosphatase* in osteoclasts. Both of these enzymes serve as **marker enzymes** for bone cells (p. 301).

B. Teeth

The illustration shows a longitudinal section through an incisor, one of the 32 permanent teeth in humans. The majority of the tooth consists of **dentine**. The crown of the tooth extends beyond the gums, and it is covered in **enamel**. By contrast, the root of the tooth is coated in dental **cement**.

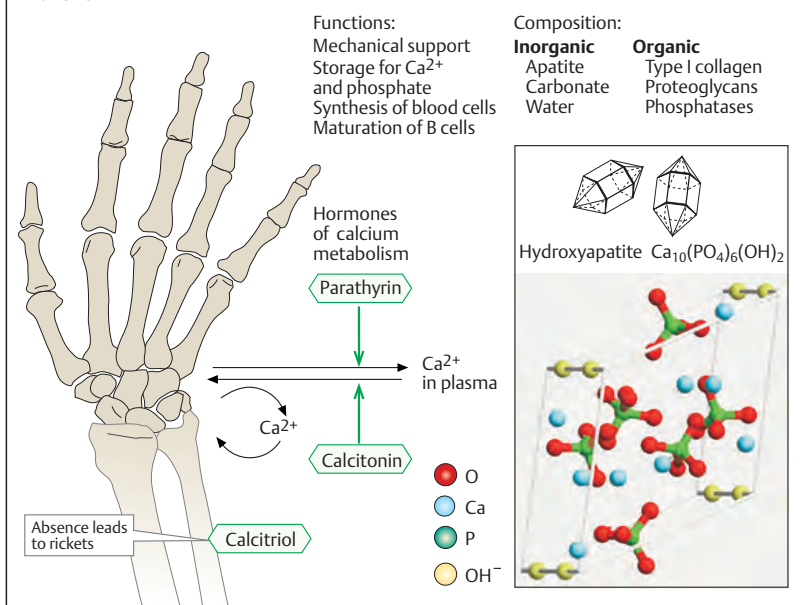
Cement, dentin, and enamel are bonelike substances. The high proportion of inorganic matter they contain (~97% in the dental enamel) gives them their characteristic hardness. The organic components of cement, dentin, and enamel mainly consist of **collagens** and **proteoglycans**; their most important mineral component is **apatite**, as in bone (see above).

A widespread form of dental disease, **caries**, is caused by acids that dissolve the mineral part of the teeth by neutralizing the negatively charged counter-ions in apatite. Acids occur in food, or are produced by microorganisms (e.g., *Streptococcus mutans*) that live on the surfaces of the teeth.

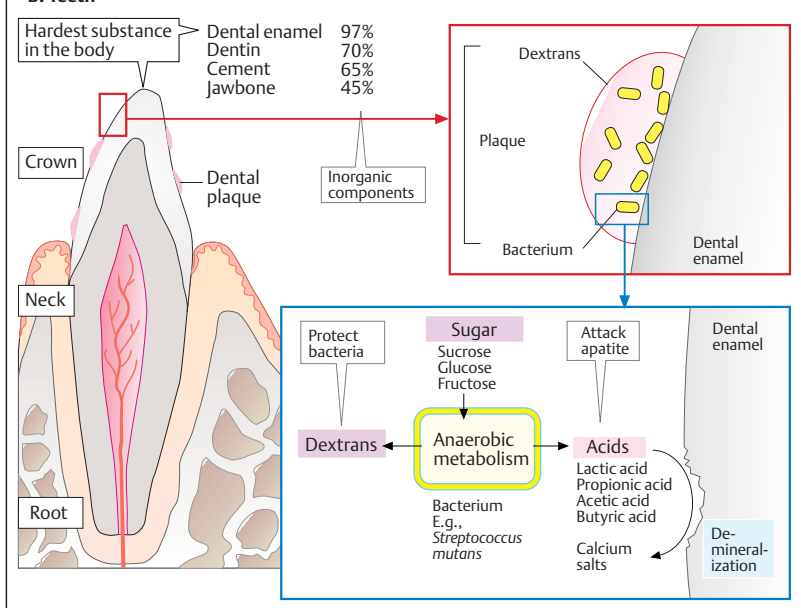
The main product of anaerobic degradation of sugars by these organisms is **lactic acid**. Another product of bacterial carbohydrate metabolism is extracellular **dextrans** (see p. 32)—insoluble polymers of glucose that help bacteria to protect themselves from their environment. Bacteria and dextrans are components of **dental plaque**, which forms on inadequately cleaned teeth. When Ca^{2+} salts and other minerals are deposited in plaque as well, **tartar** is formed.

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

A. Bone



B. Teeth



Collagens

Collagens are quantitatively the most abundant of animal proteins, representing 25% of the total. Their basic structure is described on p. 58. As characteristic elements of the **extracellular matrix** (p. 352f.), the collagens are fibrous, extracellular structural proteins that guarantee the tensile strength of the tissues. Their name (which literally means “glue-producers”) is derived from the *gelatins* that appear as a decomposition product when bone or cartilage are boiled.

A. Collagen structure (type I)

Collagens are synthesized in large amounts particularly by connective tissue cells. There are 28 different types now known, which are distinguished using roman numerals. What they have in common is a right-handed **triple helix** made up of three very long α -chains (p. 58).

The amino acid sequence **Gly-X-Y** is constantly repeated in the sequence of the triple-helical regions. Every third amino acid there is a *glycine* residue. The amino acid *proline* (Pro) is frequently found in positions X or Y; the Y position is sometimes occupied by *4-hydroxyproline* (4Hyp), although *3-hydroxyproline* (3Hyp) and *5-hydroxylysine* (5Hyl) also occur. These amino acids are characteristic components of collagen. They are only produced after protein biosynthesis by **hydroxylation** of the amino acid residues in the peptide chain (p. 62).

Their formation is catalyzed by iron-containing *oxygenases* (“proline and lysine hydroxylase”), whose function is maintained by *ascorbate* (vitamin C) as reducing agent. Many of the symptoms of the vitamin C deficiency disease *scurvy* (p. 394) are explained by disturbed collagen biosynthesis.

The hydroxyproline residues stabilize the triple helix by forming hydrogen bonds between the α -chains, while the hydroxyl groups of hydroxylysine are partly **glycosylated** with a disaccharide (-Glc-Gal).

The various types of collagen have a typical tissue distribution. They consist of different combinations of α -chains ($\alpha 1$ to $\alpha 3$ and other subtypes). Some 90% of all collagens are represented by **type I** (present especially in skin and bone), **type II** (in cartilage), **type III** (in fibers of the reticulum), and **type IV** (in basement membranes).

The collagens can be classified according to their properties:

- Fibrillary: I, II, III, V, and XI
- Reticular: IV, VIII, and X
- Fibril-associated: IX, XII, XIV, and XXII
- String of pearls-like: VI
- Anchoring: VII
- Transmembrane: XIII, XVII, XXIII, and XXV

The **triple helix** of the **type I** collagen shown here consists of 2 $\alpha 1(I)$ and 1 $\alpha 2(I)$ chains.

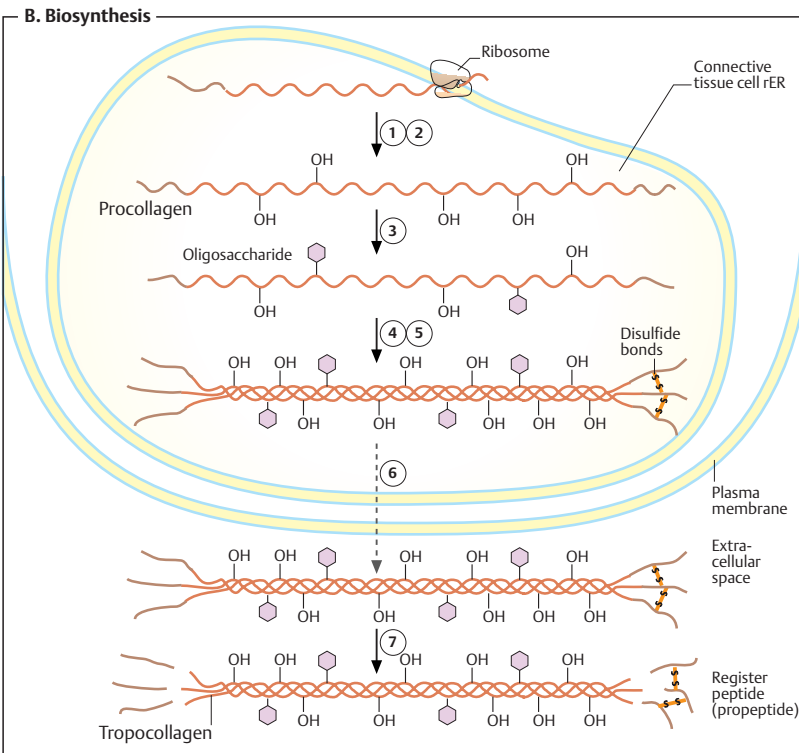
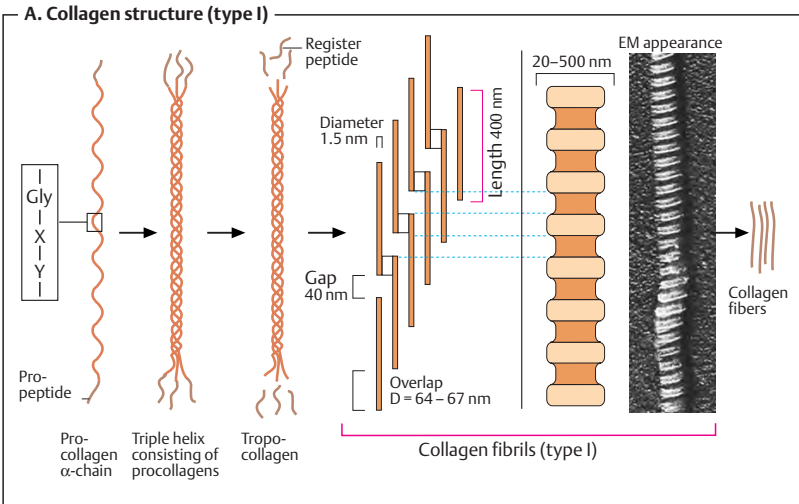
Numerous **tropocollagen** molecules (mass 285 kDa) aggregate extracellularly into a defined arrangement, forming cylindrical **fibrils**. Under the electron microscope (EM), these fibrils are seen to have a characteristic banding pattern.

Tropocollagen molecules are firmly linked together, particularly at their ends, by covalent bonds between altered lysine side chains. The number of these links increases with age. Finally, fibrils come together to form **collagen fibers** and become cross-linked with the cell membrane (p. 354).

B. Biosynthesis

The precursor molecule of collagen (*procollagen*) is formed in the rough endoplasmic reticulum (rER) and subjected there to extensive post-translational modifications (p. 62).

Cleavage of the signal peptide (p. 220) gives rise to **procollagen**, which still carries large extra segments (propeptides) at each end (1). Most proline residues and some lysine residues of procollagen are then hydroxylated (2). The procollagen is then glycosylated at hydroxylysine residues (3). Intramolecular and intermolecular disulfide bonds form between the propeptides (4), allowing correct positioning of the peptide strands to form a triple helix (5). It is only after these steps have been completed that procollagen is transferred to the Golgi apparatus, packed there, and secreted into the extracellular space by exocytosis (6). The N- and C-terminal propeptides are now removed proteolytically (7), producing **tropocollagen**, which aggregates by stacking to form fibrils (see A). Finally, several ϵ -amino groups in lysine residues are oxidatively converted into aldehyde groups. Covalent links between the molecules then form as a result of condensation (not shown). In this way, collagen reaches its final structure, which is characterized by its high *tensile strength* and *proteinase resistance*.



Extracellular Matrix I

The cells of a tissue are held together by direct contacts that are aided by cell adhesion molecules such as *cadherins* and by the **extracellular matrix** (ECM). In many types of tissue—e.g., muscle and liver—the ECM is only a narrow border between the cells, while in others it forms a larger space. The ECM gives many tissues such as skin, muscle or renal glomeruli their characteristic properties. The ECM is particularly strong in connective tissue types such as *bone, cartilage, ligaments, and tendons*.

A. Extracellular matrix

The ECM has a very wide variety of functions: it establishes mechanical connections between cells; it creates structures with special mechanical properties (tensile strength, elasticity, stable shape) as in bone, cartilage, tendons, and joints; it creates filters (e.g., in the basal membrane in the renal corpuscles); it separates cells and tissues from each other (e.g., to allow the joints to move freely); and it provides pathways to guide migratory cells (important for embryonic development). The chemical composition of the ECM is just as diverse as its functions.

The ECM consists essentially of proteins and polysaccharides, which are secreted locally by the involved cells and combined into a complex network that is attached to the surface of the cells. The illustration shows diagrammatically the main components of the extracellular matrix. These are stabilizing *fiber proteins*, cross-linking *adhesion proteins*, space-filling *proteoglycans*, and *ECM receptors* on the cell surface.

The **collagens** are the most important group of **fiber proteins**. They occur in very different varieties, which form fibers, fibrils, networks, and ligaments. Their characteristic properties are *tensile strength and flexibility*. Another fiber protein, **elastin**, ensures the elasticity of the ECM.

Adhesion proteins provide the connections between the various components of the extracellular matrix. Important representatives include **laminin**, **fibrillin**, and **fibronectin** (see C). These multifunctional proteins bind to other different types of matrix components. Cells are attached to the cell surface receptors in the ECM with their help.

ECM receptors are located on the cell surface. The most important representatives are the integrins (see B).

Due to their polarity and negative charge, **glycosaminoglycans** (p. 354) bind water molecules and cations, e.g., the proteins of the ECM. As a homogeneous “cement,” they fill the gaps between the ECM fibers.

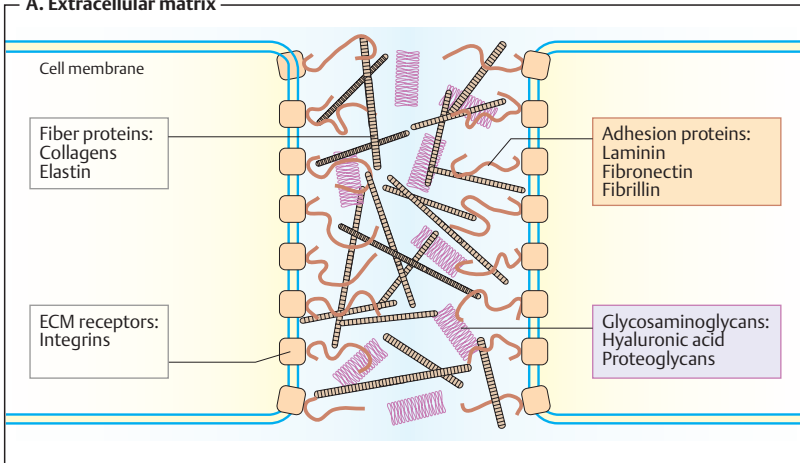
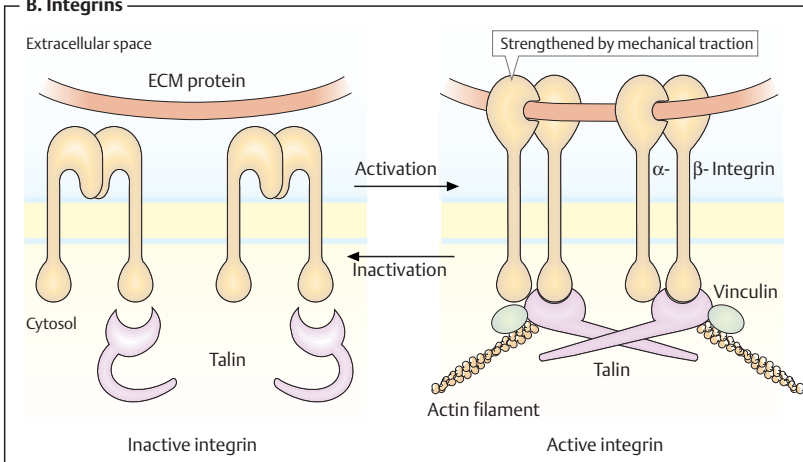
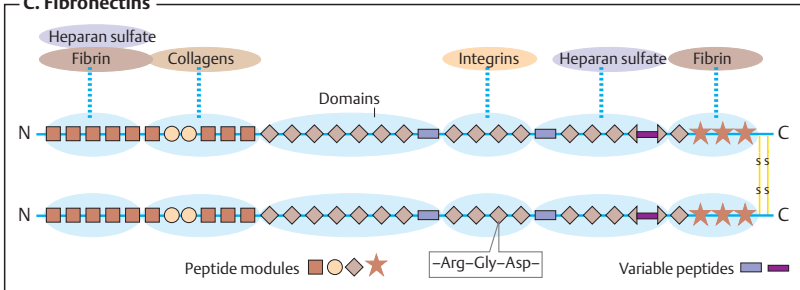
B. Integrins

The integrins are a family of dimeric membrane proteins that connect cells with the ECM or with neighboring cells. Integrins reversibly bind proteins that often share a characteristic sequence of amino acids, viz. -Arg-Gly-Asp- (**RGD sequence**), e.g., in **laminin** and **fibronectin**. On the inside of the cell, integrins in the activated state are linked with **actin filaments**, i.e., with the cytoskeleton, via **talín** and a few other adhesion proteins such as **vinculin**.

Binding of ECM proteins can be signaled into the cell interior (*outside-in signal*). Conversely, conditions inside the cell can also be signaled outward and control binding to ligands (*inside-out signal*). Integrins thus come in an active, *binding* form and in an inactive, *nonbinding* form. Mechanical traction strengthens the binding of ECM proteins.

C. Fibronectins

Fibronectins are typical representatives of adhesion proteins. They are filamentous dimers consisting of two related peptide chains (each with a mass of 250 kDa) linked to each other by disulfide bonds. The fibronectin molecules are divided into different *domains*, which bind to cell-surface integrins, collagens, fibrin, and various proteoglycans. This is what gives fibronectins their “*molecular glue*” characteristics. The domain structure in fibronectins is made up of a few types of *peptide module* that are repeated numerous times. Each of the more than 50 modules is coded for by one exon in the fibronectin gene. *Alternative splicing* (p. 244) of the hnRNA transcript of the fibronectin gene leads to fibronectins with different compositions.

A. Extracellular matrix**B. Integrins****C. Fibronectins**

Extracellular Matrix II

A. Proteoglycans

Proteoglycans are giant molecule complexes consisting of carbohydrates (roughly 95%) and proteins (about 5%), with masses of up to more than 2×10^6 Da. Their bottlebrush-shaped structure is produced by an axis consisting of **hyaluronate**. This threadlike polysaccharide (p. 32) has **proteins** attached to it, from which in turn long polysaccharide chains emerge. These polysaccharides belong to the glycosaminoglycan group (p. 34).

The **glycosaminoglycans** are made up of repeating disaccharide units, each of which consists of one **uronic acid** (glucuronic acid or iduronic acid) and one **amino sugar** (*N*-acetylglucosamine or *N*-acetylgalactosamine) (p. 30). Many of the amino sugars are also esterified with sulfuric acid (sulfated), which further increases their polarity. The five most important glycosaminoglycans are **hyaluronate**, **dermatan sulfate**, **heparin**, **keratan sulfate**, and **chondroitin sulfate**.

Because of their many carboxyl and sulfate groups, proteoglycans have a strongly negative charge. They therefore bind large amounts of ions, which in turn bind large amounts of water. Hydrated proteoglycans fill the gaps between the fibrillar components of the ECM in the form of a hydrogel. Proteoglycans are not only mechanical structural elements of the ECM, but they also bind other secreted proteins, thereby regulating their activity.

B. Basement membrane

The basement membrane is a special form of ECM. It forms a thin layer under all epithelial cells and it also surrounds some other cell types such as muscle and fat cells. The basement membrane separates these cells from the connective tissue and connects them mechanically with their environment. It can have the function of a filter (in the kidney); it controls cell polarity, organizes the proteins of the adjacent plasma membrane, regulates the survival, proliferation and differentiation of cells, and acts as a guide for cell movement.

The main components of the basement membrane are **type IV collagen**, **laminin**, **nidogen** (not shown), and the proteoglycan **perlecan**. In addition, there are other proteins such as collagens and fibronectin in certain tissues.

The ECM protein **laminin** organizes the structure of the basement membrane. It is a long, cruciate molecule consisting of three peptide

chains held together by disulfide bridges. Laminin molecules can aggregate on the surface of cells to form a flat network. This is then linked with other components of the ECM. Nidogen and perlecan function as binding elements, especially between the networks of laminin and collagen.

C. Matrix protein degradation

The metabolism of the components of the ECM is very slow. Synthesis and/or degradation of the ECM is accelerated only in certain situations, e.g., in developmental processes such as growth and cell division, and during cell movement, inflammation, and injury.

The degradation of ECM proteins is catalyzed by specific proteinases. Among these, the **matrix metalloproteinases** (MMPs) play a dominant role. Members of this enzyme family have Zn^{2+} ions in their active center. Individual MMPs specialize in the degradation of specific matrix proteins.

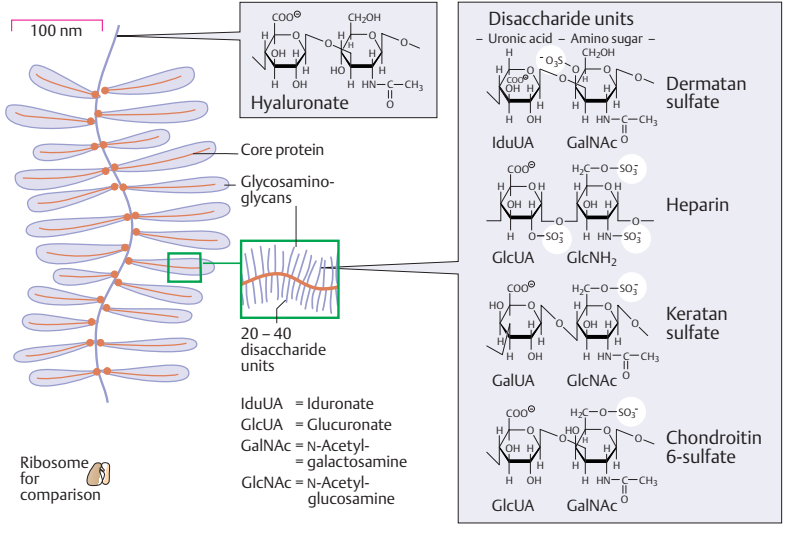
The structure of the ECM is dissolved by the degradation of matrix proteins. This means that bound **tissue growth factors** can be released, and these stimulate restoration of the ECM and tissue growth. Metastatic tumors also use MMPs to break free and migrate.

Besides the MMPs, **serine proteinases** play a part in the degradation of matrix proteins. They have a serine residue in their active center (p. 162). Among these enzymes, **plasmin**, **tPA** (**tissue plasminogen activator**) and **uPA** (urokinase) may be mentioned. They are also involved in the dissolution of blood clots (p. 296).

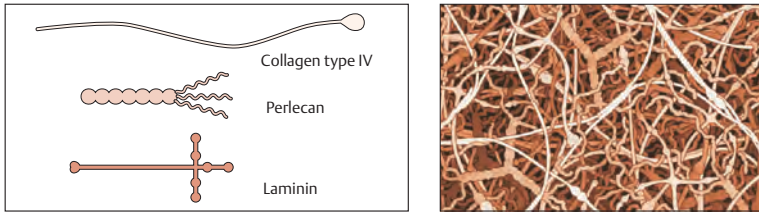
The activity of the matrix proteinases is carefully regulated. This is achieved by:

1. **Secretion of the enzymes in the form of inactive precursors**, which are activated by **limited proteolysis**: In this way pro-MMP becomes MMP and plasminogen becomes plasmin.
2. **Local limiting of their effect**, by binding the proteinases to membrane receptors, e.g., MMP and uPA.
3. **Specific inhibitors**: **TIMP** (**tissue inhibitors of metalloproteinases**) inhibit the MMPs, and **serpins** (p. 296) inhibit the serine proteinases.

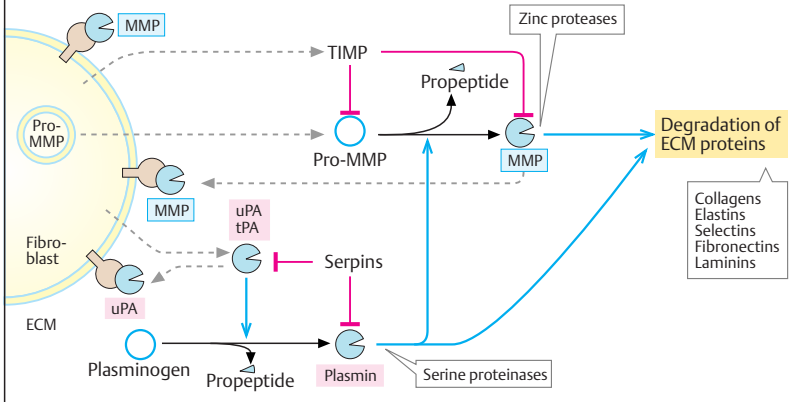
A. Proteoglycans



B. Basement membrane



C. Matrix protein degradation



Pathobiochemistry

A. Diseases of the extracellular matrix

There are many diseases that affect the extracellular matrix (ECM). They are manifested particularly often in the skin, connective tissue, cartilage and bone. The diseases can be divided formally into *congenital* and *acquired disorders*. The table lists examples to show their variety.

In **osteogenesis imperfecta** the biosynthesis of *collagen I* is disturbed. In principle, all organs with high collagen content are affected, but bone deformities are particularly obvious. Formation of the collagen triple helix is usually disturbed by substitution of glycine residues.

In **Ehlers–Danlos syndrome** likewise, the biosynthesis of collagens is affected. Collagen-modifying enzymes, e.g., *lysyl oxidase* (type V), *lysyl hydroxylase* (type VI), or the propeptide of cleavage *peptidases* (type VII) are deficient. In the **mucopolysaccharidoses** the degradation of *glycosaminoglycans* is disturbed so that intermediates accumulate.

In **epidermolysis bullosa**, of which there are various forms, the anchoring complex of keratinocytes with the basement membrane is impaired. The cause can be defects of *keratins*, *collagens*, *laminins*, *plectin*, *integrin*, or of the *desmosomes*. Blistering skin diseases are characteristic of the condition.

In **Marfan syndrome** the gene for *fibrillin-1* is defective. This results in increased stretchability and lower tear strength of connective tissue structures.

Glanzmann disease (Glanzmann thrombasthenia) is due to defects of *integrin*, which functions as a receptor for *fibrinogen* on platelet membranes. Prolonged bleeding occurs as a result.

In **congenital muscle dystrophy** the gene for the $\alpha 2$ chain of *laminin* (p. 354) is deficient. An error of the $\beta 2$ chain leads to blocking of neuromuscular signal transmission. Laminins are heterotrimers consisting of an α , β , and γ chain with isoforms, which can be combined to form different laminin variants. Their expression is development- and organ-specific.

Osteoporosis (see B), **scurvy**, and **rheumatoid arthritis** are examples of acquired defects of the ECM.

B. Osteoporosis

Osteoporosis is a disease of bone in which the equilibrium between bone synthesis and bone degradation (bone homeostasis; p. 386) is disturbed in favor of degradation. The reduction in bone mass due to *deminerallization* leads to bone pain, a reduction in height, and spontaneous fractures.

A moderate reduction in bone mass and deterioration of bone architecture with age are a normal process. **Primary osteoporosis** typically occurs in women after the menopause when the levels of estrogens are declining. Primary osteoporosis can also occur in aging men because of the reduction in *androgens*. Androgens and estrogens prevent excessive osteoclast activity by inhibiting the formation of certain *cytokines* which control osteoblast formation and activity (p. 386, B). Reduced steroid synthesis decreases this inhibitory effect leading to increased bone degradation. Roughly 50% of all individuals aged 70 years or more suffer from acute osteoporosis.

Apart from a genetic predisposition, the main causes of *secondary osteoporosis* are *lack of exercise*, diminished production of *calcitriol* (because of a lack of UV light or vitamin D) and a *calcium-deficient diet*. Moreover, an increased *corticosteroid* levels because of *Cushing disease* or treatment with certain drugs can also lead to osteoporosis and cause fractures.

Primary osteoporosis due to lack of sex hormones can be treated by giving **steroid hormones** or steroid receptor modulators (specific estrogen receptor modulators, **SERM**). **Bisphosphonate drugs** are also in use. These compounds are taken up by osteoclasts and inhibit their action. Bisphosphonates with the basic bonding pattern $-P-C-P-$ are analogous to diphosphate $-P-O-P-$ but cannot be cleaved by *alkaline phosphatase*.

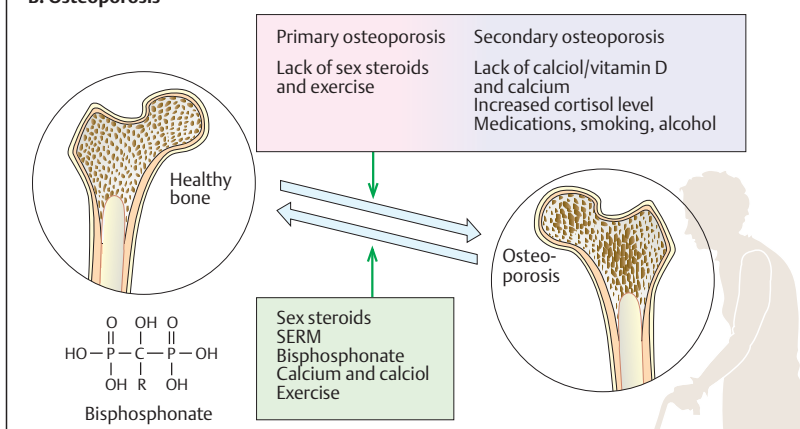
In every case of osteoporosis, an adequate intake of calcium and vitamin D (or endogenous biosynthesis of calcitriol) is important, together with physical exercise.

A. Diseases of the extracellular matrix

Congenital disorders (examples)		
Disease	Molecular cause	Clinical picture
Osteogenesis imperfecta	Various defects in the gene for collagen I	Disorder of bone formation, bone fragility
Ehlers–Danlos syndrome	Various disorders of collagen biosynthesis due to defects of modifying enzymes	Elastic skin, over-extensible joints, deformation of the spine, defective blood vessels
Mucopolysaccharidoses type A–VII	Disorders of proteoglycans degradation; many different enzymes can be affected	Growth disorders, skeletal deformation, disorder of brain development
Epidermolysis bullosa	Various defects of ECM proteins involved in anchoring	Blistering of the skin after mechanical stress
Marfan syndrome	Genetic defects in the fibrillin-1 gene	Tall stature, spider fingers, lens changes, aneurysms, aortic rupture
Glanzmann disease	Genetic defect of the fibrinogen receptor integrin on platelets	Clotting disorder, bleeding tendency
Congenital muscular dystrophy	Defective laminin 2, disorder of the connection between the cytoskeleton of muscle cells with the ECM	Weakened muscles

Acquired disorders (examples)		
Osteoporosis	Reduction of bone mass, deterioration of microarchitecture	Skeletal shrinkage, bone pain, fractures
Scurvy	Ascorbic acid deficiency, disorder of collagen hydroxylation	Defects of connective tissue, bleeding, tooth loss
Rheumatoid arthritis	Joint inflammation (immune reaction), increase in cytokines	Inflammatory swelling of the joints

B. Osteoporosis



Signal Transmission in the Central Nervous System

A. Structure of nerve cells

Nerve cells (neurons) are excitable cells that produce electrical signals and can react to such signals as well. Their structure is markedly different from that of other types of cell. Numerous branching processes project from their **cell body** (soma). Neurons are able to receive signals via **dendrites** and to pass them on via **axons**. The axons, which can be up to 1 m long, are usually surrounded by *Schwann cells*, which cover them with a lipid-rich myelin sheath to improve their electrical insulation. The transfer of stimuli occurs at the **synapses**, which link the individual neurons to each other as well as linking neurons functionally to muscle fibers. *Neurotransmitters* (p. 362) are stored in the axonal nerve endings. These signaling substances are released in response to electrical signals to excite neighboring neurons (or muscle cells). It is estimated that each of the more than 10^{11} neurons in the brain is in contact via synapses with more than 10^3 other neurons and that there is a total of roughly 10^{15} synapses.

There is a noticeably high proportion of lipids in the composition of nerve cells, representing about 50% of their dry weight. In particular, there is a very wide variety of sphingolipids (p. 42).

B. Neurotransmitters and neurohormones

Neurosecretions are classed into two groups: **neurotransmitters** are released into the *synaptic cleft* to influence neighboring cells (C). They have a short range and a short lifespan. By contrast, **neurohormones** are released into the blood, allowing them to cover larger distances. However, the distinction between the two groups is a fluid one; some neurotransmitters simultaneously function as neurohormones.

C. Synaptic signal transmission

All chemical synapses function according to a similar principle. In the area of the synapse, the surface of the signaling cell (*presynaptic membrane*) is separated from the surface of the receiving cell (*postsynaptic membrane*) only by a narrow *synaptic cleft*. When an **action potential** (p. 360) reaches the presynaptic membrane (1), *voltage-gated Ca^{2+} channels* integrated into the membrane open (2) and trig-

ger **exocytosis** of the neurotransmitter stored in the presynaptic cell (3; for details, see p. 214).

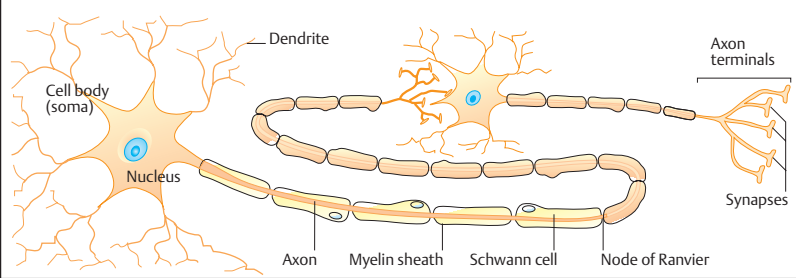
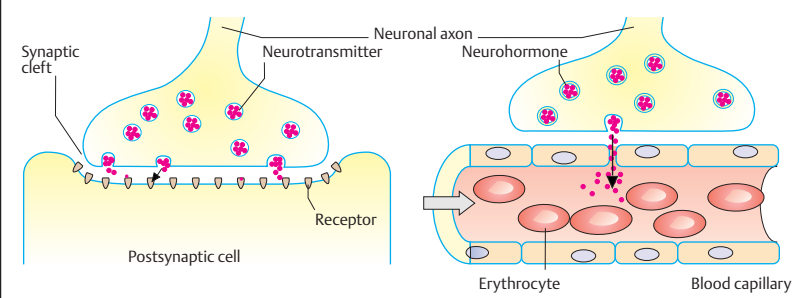
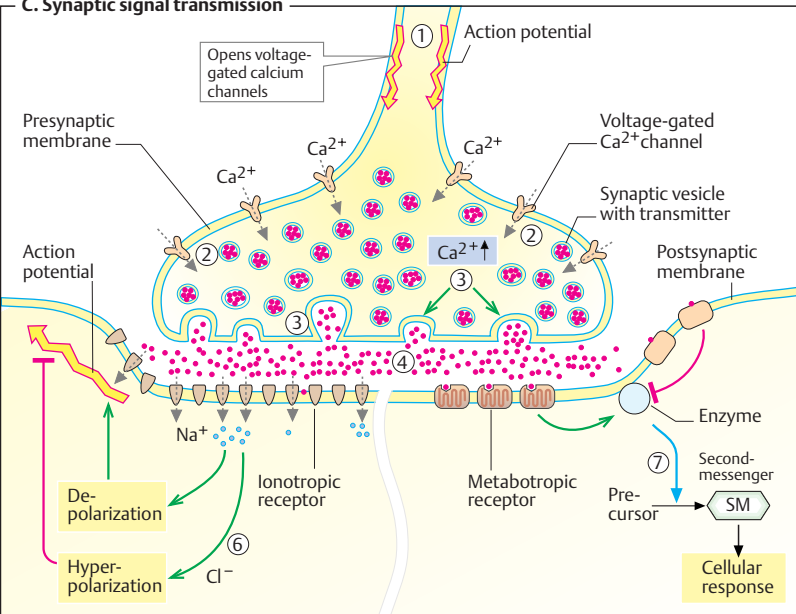
Each neuron usually releases *only one type of neurotransmitter* (4). Neurons that release dopamine are referred to as “dopaminergic,” for example, while those that release acetylcholine are “cholinergic,” etc. The transmitters that are released diffuse through the synaptic cleft and bind on the other side to **receptors** on the postsynaptic membrane. These receptors are integral membrane proteins that have binding sites for neurotransmitters on their exterior (p. 364).

The receptors for neurotransmitters are divided into two large groups according to the effect produced by binding of the transmitter (for details, see p. 364).

Ionotropic receptors (lower left) are *ligand-gated ion channels*. When they open as a result of the transmitter's influence, ions flow in due to the membrane potential (p. 108). If the inflowing ions are cations (e.g., Na^+ , K^+ , Ca^{2+}), **depolarization** of the membrane occurs and an action potential is triggered on the surface of the postsynaptic cell (5). This is the way in which stimulatory transmitters work (e.g., acetylcholine and glutamate). By contrast, if anions flow in (mainly Cl^-), the result is **hyperpolarization** of the postsynaptic membrane, which makes the production of a postsynaptic action potential more difficult (6). The action of inhibitory transmitters such as glycine and GABA is based on this effect.

A completely different type of effect is observed in **metabotropic receptors** (lower right). After binding of the transmitter, these receptors interact on the inside of the postsynaptic membrane with *G-proteins* (p. 404), which in turn activate or inhibit the synthesis of **second messengers**. Finally, second messengers activate or inhibit **protein kinases**, which phosphorylate cellular proteins and thereby alter the behavior of the postsynaptic cells (*signal transduction*; p. 398ff.).

Neurotransmitter action is terminated very quickly following their release as they are degraded or taken up again into the presynaptic cell (p. 363, B).

A. Structure of nerve cells**B. Neurotransmitters and neurohormones****C. Synaptic signal transmission**

Resting Potential and Action Potential

A. Resting potential

A characteristic property of living cells is the uneven distribution of positive and negative ions on the inside and outside of the plasma membrane. This gives rise to a **membrane potential** (p. 108)—i.e., there is electrical voltage between the two sides of the membrane, which can only balance out when *ion channels* allow the unevenly distributed ions to move.

At rest, the membrane potential (more accurately, the membrane voltage) in most cells is -60 to -90 mV. It mainly arises from the activity of *Na⁺/K⁺ transporting ATPase* (“Na⁺/K⁺-ATPase”), which is found in practically all animal cells. Using up ATP, this P-type ATPase (p. 212) “pumps” 3 Na⁺ ions out of the cell in exchange for 2 K⁺ ions. Some of the K⁺ ions, following the concentration gradient, leave the cell again through *potassium channels*. As the protein anions that predominate inside the cell cannot follow them, and inflow of Cl⁻ ions from the outside is not possible, the result is an excess of positive charges outside the cell, while negatively charged anions predominate inside it.

An **equilibrium potential** (p. 108) exists for each of the ions involved. This is the value of the membrane potential at which there is no *net* inflow or outflow of the ions concerned. For K⁺ ions, the resting potential lies in the range of the membrane potential, while for Na⁺ ions it is much higher at $+70$ mV. At the first opportunity, Na⁺ ions will therefore spontaneously flow into the cell. The occurrence of action potentials is based on this (see **B**).

Nerve cell membranes contain **ion channels** for Na⁺, K⁺, Cl⁻, and Ca²⁺ (p. 402). These channels are usually closed and open only briefly to let ions pass through. They can be divided into channels that are regulated by membrane potentials (“*voltage-gated*,” e.g., fast Na⁺ channels) and those regulated by ligands (“*ligand-gated*,” e.g., nicotinic acetylcholine receptors; pp. 364, 402).

B. Action potential

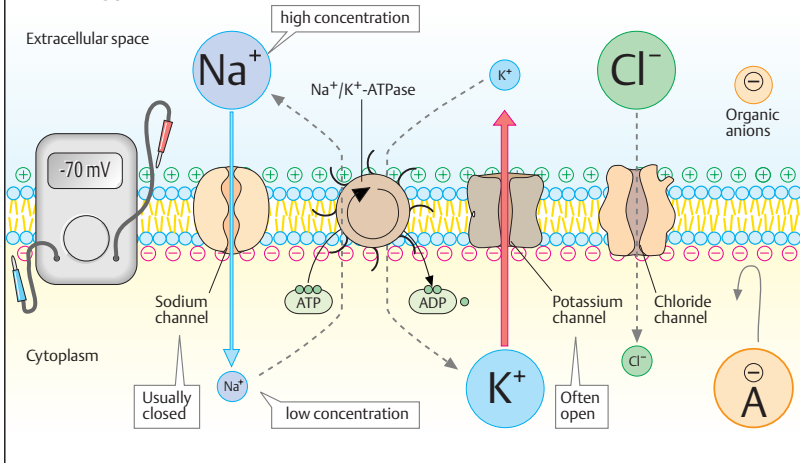
Action potentials are special signals that are used to transmit information in the nervous system. They are triggered by chemical stimuli (or more rarely electrical stimuli). The binding of a neurotransmitter to an ionotropic receptor (p. 358) results in a brief local increase in the

membrane potential from -70 mV to about $+30$ mV. Although the membrane potential quickly returns to the initial value within a few milliseconds (ms) at its site of origin, the depolarization is propagated because neighboring membrane areas are activated during this time period.

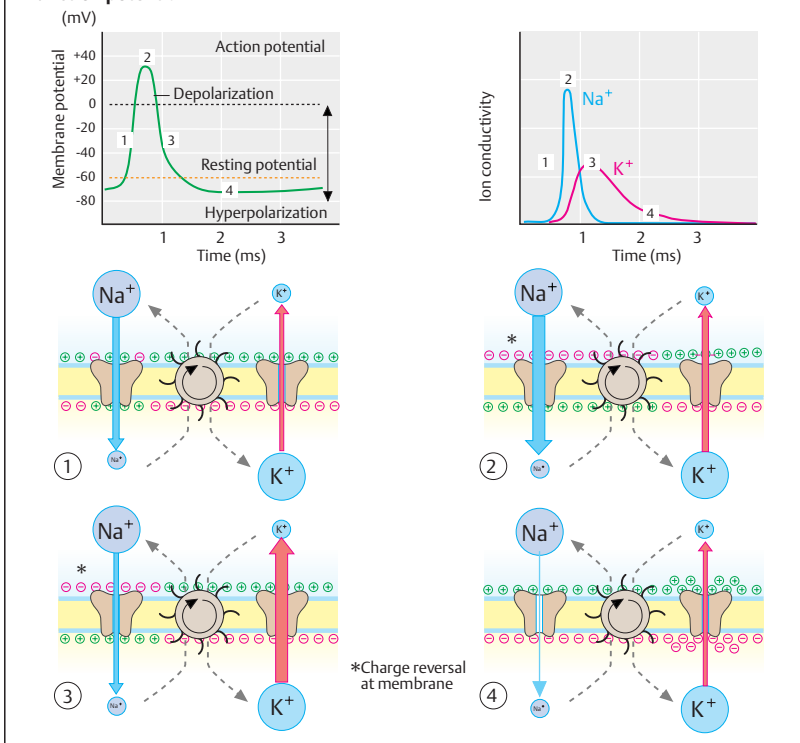
1. The process starts with the opening of voltage-gated Na⁺ channels (p. 402). Due to their high equilibrium potential (see **A**), Na⁺ ions flow into the cell and reverse the local membrane potential (**depolarization**).
2. The Na⁺ channels immediately close again, so that the inflow of positive charges is only very brief.
3. Due to the increase in the membrane potential, voltage-dependent K⁺ channels open and K⁺ ions flow out. In addition, Na⁺ /K⁺ ATPase pumps the Na⁺ ions that have entered back out again. This leads to **repolarization** of the membrane.
4. The two processes briefly lead to the charge even falling below the resting potential (**hyperpolarization**). The K⁺ channels also close after a few milliseconds. The nerve cell is then ready for re-stimulation.

Generally, it is always only a very small part of the membrane that is depolarized during an **action potential**. The process can therefore be repeated when the nerve cell is stimulated again. Conduction of the action potential on the surface of the nerve cell is based on the fact that the local increase in the membrane potential causes neighboring voltage-gated ion channels to open, so that the membrane stimulation spreads over the whole cell in the form of a *depolarization wave*.

A. Resting potential



B. Action potential



Neurotransmitters

Neurotransmitters are compounds that are produced by neurons, stored in the synapses, and released into the synaptic cleft in response to a stimulus. At the postsynaptic membrane, they bind to special receptors and affect their activity (p. 358). *Cotransmitters* are signaling substances that act simultaneously with neurotransmitters and modulate their effect.

A. Important neurotransmitters and cotransmitters

The table lists the most important of more than 100 compounds that function as neurotransmitters or cotransmitters. Among them are amino acids, amino acid derivatives, peptides, as well as acetylcholine and purine derivatives.

Acetylcholine (ACh), the acetic acid ester of the cationic alcohol *choline* (p. 40) acts at neuromuscular junctions, where it triggers muscle contraction (p. 342), in ganglia of the autonomic nervous system, and in cholinergic neurons in the brain and spinal cord. The metabolism of ACh is described on the next page.

Several **amino acids** (p. 50f.) have neurotransmitter effects. A particularly important one is *glutamate*, which acts as the main stimulatory transmitter in the CNS. More than half of the synapses in the brain are glutamatergic. The metabolism of glutamate and that of GABA synthesized from it (see below) are discussed in more detail on page 366. By contrast, *glycine* acts as an inhibitory neurotransmitter in the spinal cord and parts of the brain.

Biogenic amines arise from amino acids by decarboxylation (p. 52). This group includes *GABA* (γ -aminobutyric acid), which is formed from glutamate and is the most important inhibitory transmitter in the CNS. The *catecholamines* dopamine, norepinephrine, and epinephrine (p. 434) are derived from tyrosine, *serotonin* and *melatonin* from tryptophan, and *histamine* from histidine. These signal substances are neurotransmitters, and some of them additionally also act as mediators and/or hormones.

Peptides make up the largest group among the neurosecretions with transmitter function. Some are peptide hormones at the same time—e.g., thyrotropin-releasing hormone (TRH) and angiotensin II. Most neuropeptides are short (3–30 amino acid residues). At their N-terminus, many of them have a glutamate residue that has been cyclized to form *pyroglutamate*

(5-oxoproline, <G), while the C-terminus is often an uncharged acid amide group ($-\text{CONH}_2$) which better protects the peptide against breakdown by peptidases.

Endorphins, *dynorphins*, and *enkephalins* are a particularly interesting group of neuropeptides. They act as “endogenous opioids” by exerting analgesic, sedative, and euphoriant effects in extreme situations. Drugs such as morphine and heroin activate the receptors for these peptides.

Purine derivatives with cotransmitter function are all derived from adenine-containing nucleotides or nucleosides. ATP is released along with acetylcholine and other transmitters, and among other functions it regulates the emission of transmitters from its synapse of origin. The stimulatory effect of *caffeine* (1,3,7-trimethylxanthine) is mainly based on the fact that it binds to and blocks adenosine receptors.

B. Fate of the neurotransmitters

The following events are typically involved in neurotransmitter action:

1. **Biosynthesis** in the cytoplasm of presynaptic nerve endings from readily available precursors (often an amino acid) by specific enzymes.
2. Active **uptake into storage vesicles** by H^+ -dependent transport. Storage sometimes together with cotransmitters.
3. **Release** of vesicle contents by Ca^{2+} -regulated exocytosis into the synaptic cleft (pp. 214, 358).
4. **Action** by binding to specific receptors for neurotransmitters on the postsynaptic membrane (p. 364).
5. Rapid **termination of the action** by reuptake into presynaptic nerve endings and glial cells, by diffusion away or by enzymatic inactivation in the synaptic cleft (ACh, neuropeptides).
6. **Degradation** by enzymes (p. 434).

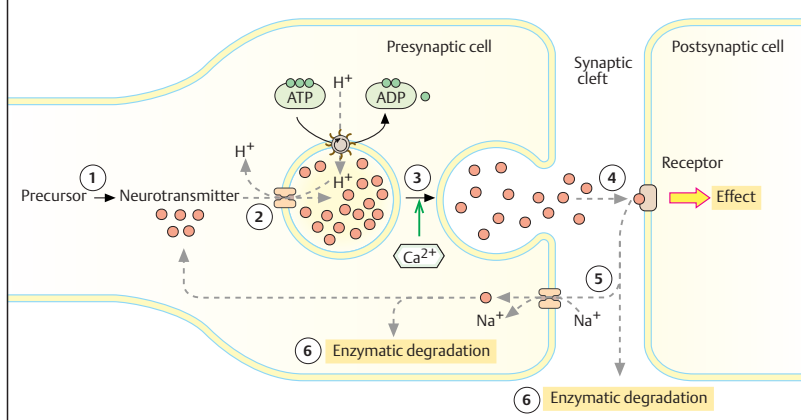
Drugs can intervene at different sites in this system by acting:

- on the supply of **precursors** (1)
- as **agonists** that imitate neurotransmitter action on receptors (4)
- as **antagonists** that competitively block neurotransmitter action on receptor (4)
- as **reuptake inhibitors** (5)
- as **inhibitors of enzymatic degradation** (6).

A. Important neurotransmitters and cotransmitters

Transmitter	Precursor	Functions	Inactivated by
Acetylcholine	Acetyl-CoA and choline	Often excitatory NT, control of neurons, glands, and muscle contraction	ACh esterase, choline reuptake (p. 365)
Amino acids			
Glutamate	—	Most important excitatory NT	Reuptake
Aspartate	—	Rare excitatory NT	Reuptake
Glycine	—	Inhibitory NT	Reuptake
Biogenic amines			
GABA	Glutamate	Most important inhibitory NT	Reuptake, GABA shunt (p. 366)
Dopamine	Tyrosine	Inhibition of neurons	Reuptake, MAO, COMT (p. 435)
Norepinephrine	Tyrosine	NT, stress adaptation hormone, blood pressure increase	Reuptake, MAO, COMT, conjugate formation (p. 435)
Epinephrine	Tyrosine	Stress adaptation hormone, blood pressure increase, control of metabolism; NT	Reuptake, MAO, COMT, conjugate formation (p. 435)
Serotonin	Tryptophan	NT and mediator (p. 437)	Reuptake, COMT, MAO
Melatonin	Tryptophan via serotonin	Hormone and NT, regulates circadian rhythm	Reuptake, hydroxylation, conjugate formation
Histamine	Histidine	NT and mediator (p. 437)	Reuptake, COMT, MAO, DAO
Peptides			
Endorphins Dynorphins Enkephalins	Proopiomelanocortin	Hormones and NT, endogenous opioids	Peptide cleavage
Other peptides	Amino acids	Cotransmitter, many special functions	Peptide cleavage
Purines			
ATP, adenosine	Nucleotides	Cotransmitter	Hydrolysis, reuptake

COMT: catechol-O-methyltransferase; DAO: diamine oxidase; GIT: gastrointestinal tract; MAO: monoamine oxidase; NT: neurotransmitter; CNS: central nervous system

B. Fate of the neurotransmitters

Receptors for Neurotransmitters

The receptors for neurotransmitters are proteins that are integrated into the membrane of the postsynaptic cell, where they regulate ion flow or signal transduction processes (p. 398ff).

A. Receptors for neurotransmitters

A considerable number of receptors for neurotransmitters are already known. The table only lists important examples. They are classified into two large groups according to their mode of action.

Ionotropic receptors are *ligand-gated ion channels* (left half of the table; see also p. 402). The receptors for stimulatory (excitatory) transmitters are indicated in the table by a "+". They mediate the inflow of cations, mainly Na⁺. When these receptors open after binding of the transmitter, local *depolarization* of the postsynaptic membrane occurs. By contrast, inhibitory neurotransmitters such as GABA and glycine allow Cl⁻ to flow in. This increases the membrane's negative resting potential and hinders the action of stimulatory transmitters by *hyperpolarization*.

Signal transduction at ionotropic receptors is fast, while **metabotropic receptors** (right half of the table) are slower. They are coupled to G-proteins (p. 404), through which they influence the *synthesis of second messengers*. Receptors that work with type G_q proteins increase the cAMP level in the postsynaptic cell, while those that activate type G_i proteins reduce it. Via type G_q proteins, other receptors increase the intracellular concentration of Ca²⁺ ions. Glutamate, GABA, acetylcholine, serotonin, and ATP can activate both ionotropic and metabotropic receptors.

There are several **receptor subtypes** for most neurotransmitters. These are distinguished numerically (e.g., D₁ to D₅) or are named after their agonists—i.e., after molecules experimentally found to activate the receptor. For example, one specific subtype of glutamate receptors also reacts to NMDA (*N*-methyl-D-aspartate), while another subtype reacts to the compound AMPA, etc.

B. Acetylcholine receptors

Acetylcholine (ACh) binds to two types of receptor. The **nicotinic ACh receptor** also responds to the alkaloid *nicotine* contained in tobacco. Many of the physiological effects of

nicotine are based on this fact. The nicotinic receptor is ionotropic. When acetylcholine binds to it, the ion channel opens and allows Na⁺ ions to diffuse into the postsynaptic cell and K⁺ ions to diffuse out (see A).

The **muscarinic ACh receptors** (of which there are at least five subtypes), on the other hand, are metabotropic. Their name is derived from the alkaloid *muscarine*, which is found in the fly agaric mushroom (*Amanita muscaria*), for example. Like ACh, muscarine is bound to the receptor, but in contrast to ACh it is not broken down and therefore causes permanent stimulation of muscle.

The muscarinic ACh receptors influence the cAMP level in the postsynaptic cells. Subtypes M₁, M₃, and M₅ increase it, while subtypes M₂ and M₄ reduce it (see A).

C. Metabolism of acetylcholine

Acetylcholine is synthesized enzymatically from acetyl-CoA and choline in the cytoplasm of the presynaptic axon [1] and is stored in **synaptic vesicles**, each of which contains around 1000–10000 ACh molecules. After it is released by exocytosis (p. 214), the transmitter travels by diffusion to the receptors on the postsynaptic membrane.

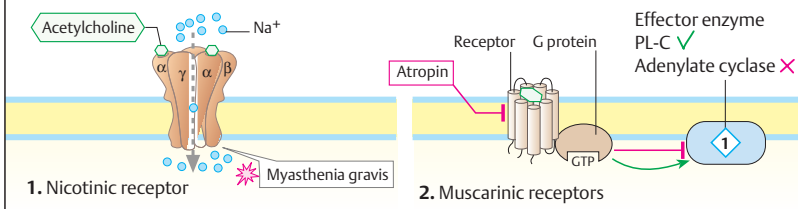
Inactivation of ACh by hydrolysis to acetate and choline immediately starts in the synaptic cleft. The reaction is catalyzed by *acetylcholin esterase* [2] which degrades ACh within a few milliseconds after release. The cleavage product **choline** is taken up again by the presynaptic neuron and glial cells, and reused for ACh synthesis [3].

Inhibitors. Substances that block the serine residue in the active center of *acetylcholin esterase* [2]—e.g., the neurotoxin E605 and other *organophosphates*—prevent ACh degradation and thus cause spastic stimulation of the postsynaptic muscle cells. Unlike this, *curare*, a paralyzing arrow-poison used by South American Indians, competitively inhibits binding of ACh to nicotinic receptors while *atropine* inhibits ACh binding to muscarinic receptors. Muscle relaxation occurs as a result.

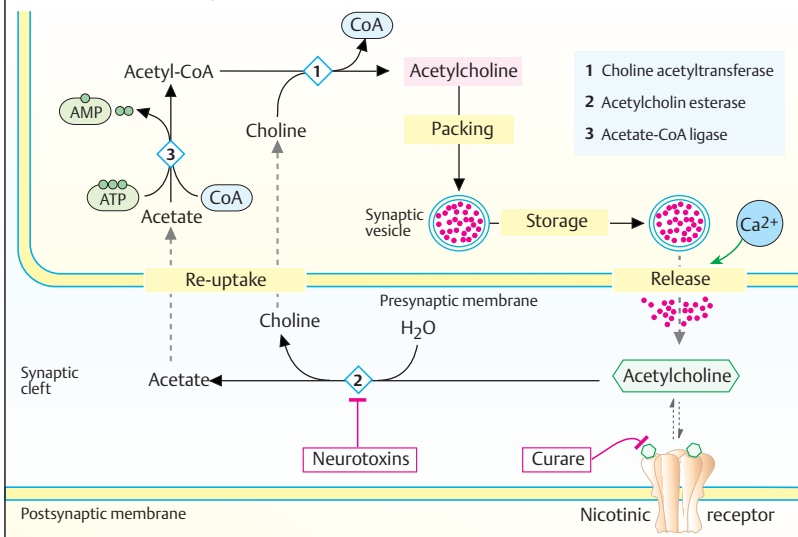
A. Receptors for neurotransmitters

Ionotropic				Metabotropic			
Transmitter	Receptor	Ion(s)	Effect	Transmitter	Receptor	Second-Messenger	Effect
Acetylcholine	Acetylcholine (nicotinic)	Na ⁺	+	Acetylcholine	Acetylcholine (muscarinic) M1, M3, M5, M2, M4	[Ca ²⁺] [cAMP]	+ -
Serotonin	5HT ₃	Na ⁺	+	Serotonin	5HT ₁ 5HT ₂ 5HT ₄	[cAMP] [cAMP] [cAMP]	+ + -
Glutamate	AMPA NMDA Kainic acid	Na ⁺ , K ⁺ Na ⁺ , K ⁺ , Ca ²⁺ Na ⁺ , K ⁺	+ + +	Norepinephrine	α ₁ α ₂ β ₁ , β ₂ , β ₃	[Ca ²⁺] [cAMP] [cAMP]	+ + -
GABA	GABA _A	Cl ⁻	-	Dopamine	D ₁ , D ₅ D ₂ , D ₃ , D ₄	[cAMP] [cAMP]	+ -
Glycine	Glycine	Cl ⁻	-	Opioid peptides	δ, κ, μ	[cAMP]	-

B. Acetylcholine receptors



C. Metabolism of acetylcholine



Metabolism of the Brain

The brain and other areas of the CNS have high ATP requirements. Although the brain only represents about 2% of the body's mass, it consumes around 20% of the metabolized oxygen and ca. 60% of the glucose. The neurons' high energy requirements are mainly due to ATP-dependent ion pumps (particularly Na^+/K^+ -ATPase) and other active transport processes that are needed for nerve conduction.

A. Energy metabolism of the brain

Glucose is normally the only energy source from which the brain is able to obtain adequate amounts of ATP. Lipids are unable to pass the **blood–brain barrier**, and amino acids are also only available in the brain in limited quantities (see B). Glucose is degraded by glycolysis, first to pyruvate and lactate and then by terminal oxidation to CO_2 and H_2O . In addition glial cells produce lactate from glucose and supply it to the surrounding neurons.

As brain cells only have minor glycogen reserves, they are dependent on a constant supply of glucose from the blood. A severe drop in the blood glucose level (hypoglycemia)—as can occur after insulin overdosage in diabetics, for example—rapidly leads to a drop in the ATP level in the brain. This results in loss of consciousness and neurological deficits that can lead to death.

Oxygen deficiency (*hypoxia*; p. 126) also affects the brain first. The effects of a brief period of hypoxia are still reversible, but as time progresses irreversible damage increasingly occurs and finally complete loss of function (“*brain death*”).

As glucose consumption is a measure of brain activity, active areas can be localized with imaging methods after administration of ^{18}F -*deoxyglucose* (FDG).

During periods of starvation, the brain after a few days acquires the ability to degrade **ketone bodies** (p. 318) in addition to glucose for ATP synthesis. In the first weeks of a starvation period, there is a strong increase in the activities of the enzymes required for ketone body utilization in the brain.

The use of ketone bodies by the brain saves glucose (which is degraded only to lactate in this case) and thereby reduces the breakdown of muscle protein that maintains gluconeogenesis in the liver during starvation. After a few weeks therefore loss of muscle protein declines to one-third of the initial value (p. 378).

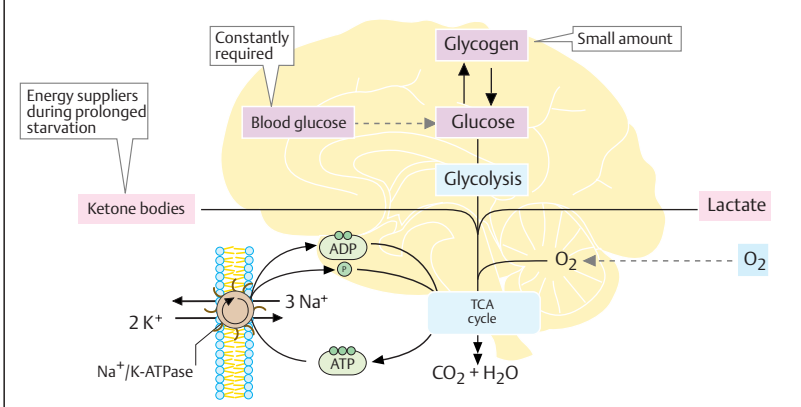
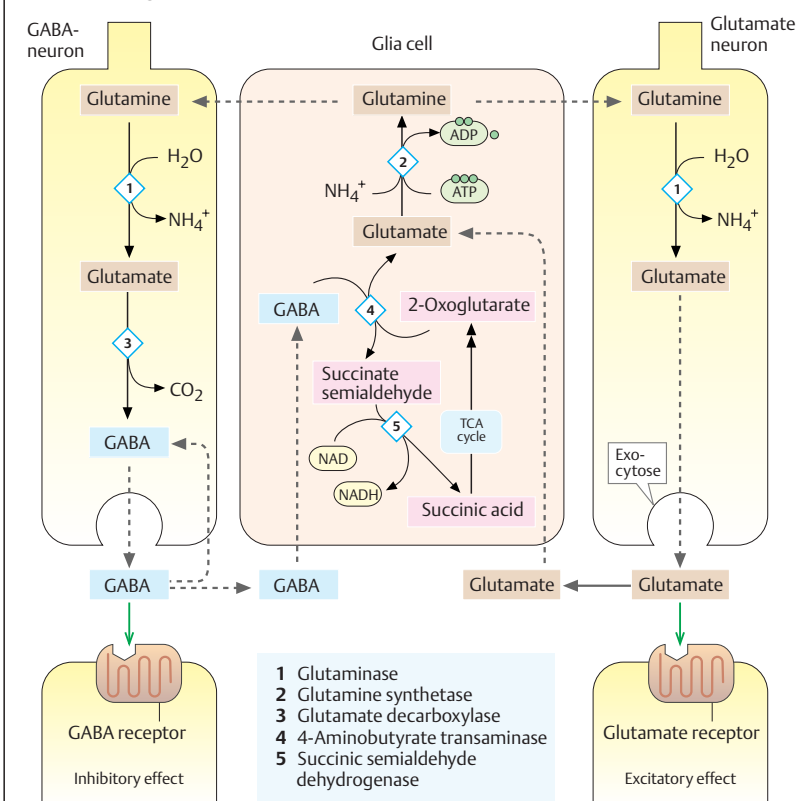
However, in the CNS, ketone bodies cannot fully replace glucose. Babies utilize ketone bodies more efficiently so they also tolerate a lower glucose level.

B. Glutamate, glutamine, and GABA

The proteinogenic amino acid **glutamate** (Glu) and the biogenic amine **4-aminobutyrate** (GABA) derived from it are among the most important **neurotransmitters** in the brain (p. 362). They are both synthesized in the brain itself. In addition to the **neurons**, which use Glu or GABA as transmitters, **glia cells** are also involved in the metabolism of these substances. Since glutamate and GABA as transmitters must not appear in the extracellular space in an unregulated way, the cells of the neuroglia (center) supply “glutamatergic” and “GABAergic” neurons with the precursor **glutamine** (Gln), which they produce from glutamate with the help of *glutamine synthetase* [1].

GABA neurons (left) and glutamate neurons (right) initially hydrolyze glutamine with the help of *glutaminase* [2] to form glutamate again. The glutamate neurons store this in vesicles and release it when stimulated. The GABA neurons continue the degradation process by using *glutamate decarboxylase* [3] to convert glutamate into the transmitter GABA. Both types of neuron take up their transmitter again. Some of it also returns to the neuroglia, where glutamate is amidated back into glutamine. Glutamate can also be produced again from GABA. The reaction sequence needed for this, known as the **GABA shunt**, is characteristic of the CNS. A *transaminase* [4] first converts GABA and 2-oxoglutarate into glutamate and succinate semialdehyde ($-\text{OOC}-\text{CH}_2-\text{CH}_2-\text{CHO}$). In an NAD^+ -dependent reaction, the aldehyde is oxidized to succinic acid [5], from which 2-oxoglutarate can be regenerated again via TCA cycle reactions.

The function of glutamate as a stimulatory transmitter in the brain is the cause of what is known as the “Chinese restaurant syndrome” as glutamate is often used as a flavor enhancer.

A. Energy metabolism of the brain**B. Glutamate, glutamine, and GABA**

Sight

Two types of photoreceptor cell are found in the human retina—*rods* and *cones*. The roughly 1.2×10^8 rods are sensitive to low levels of light, while the 6×10^6 cones are responsible for color vision at higher light intensities.

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

A. Light-induced signal cascade

Rods are structured by internal membrane discs, into which the 7-helix receptor **rhodopsin** is integrated (p. 400). In contrast to other receptors in the 7-helix class, rhodopsin is a light-sensitive *chromoprotein*. Its protein part, **opsin**, contains the aldehyde **retinal** (p. 392)—an isoprenoid which is bound to the ϵ -amino group of a lysine residue as an *aldimine*. The light absorption of rhodopsin is in the visible range, with a maximum at ~ 500 nm. The absorption properties of the visual pigment are thus optimally adjusted to the spectral distribution of sunlight.

Absorption of a photon triggers isomerization of the bound retinal from the **11-*cis* form** to the **all-*trans* form** (top). Within milliseconds, this *photochemical process* triggers an allosteric conformational change in rhodopsin. The resulting active conformation, **rhodopsin*** (metarhodopsin II), binds the G-protein **transducin** (p. 404) and activates it. The *signal cascade* that now follows causes the rod cells to release less neurotransmitter (glutamate) at their synapses. The adjoining bipolar neurons transmit this change to the brain as a signal for light.

There are several different rhodopsins in the **cones**. All of them contain retinal molecules as light-sensitive components, the absorption properties of which are modulated by the different types of opsin they contain, in such a way that certain colors can be perceived.

Dark (bottom left)

Rod cells that are not exposed to light contain relatively high concentrations ($70 \mu\text{M}$) of the cyclic nucleotide **cGMP** (3',5'-cyclicGMP; p. 406), which is synthesized by a *guanylate cyclase* [2]. The cGMP binds to an ion channel in the rod membrane (bottom left) and thus keeps it open. The inflow of cations (Na^+ , Ca^{2+}) depolarizes the membrane and leads to release of the neurotransmitter glutamate at the synapse.

Light (bottom right)

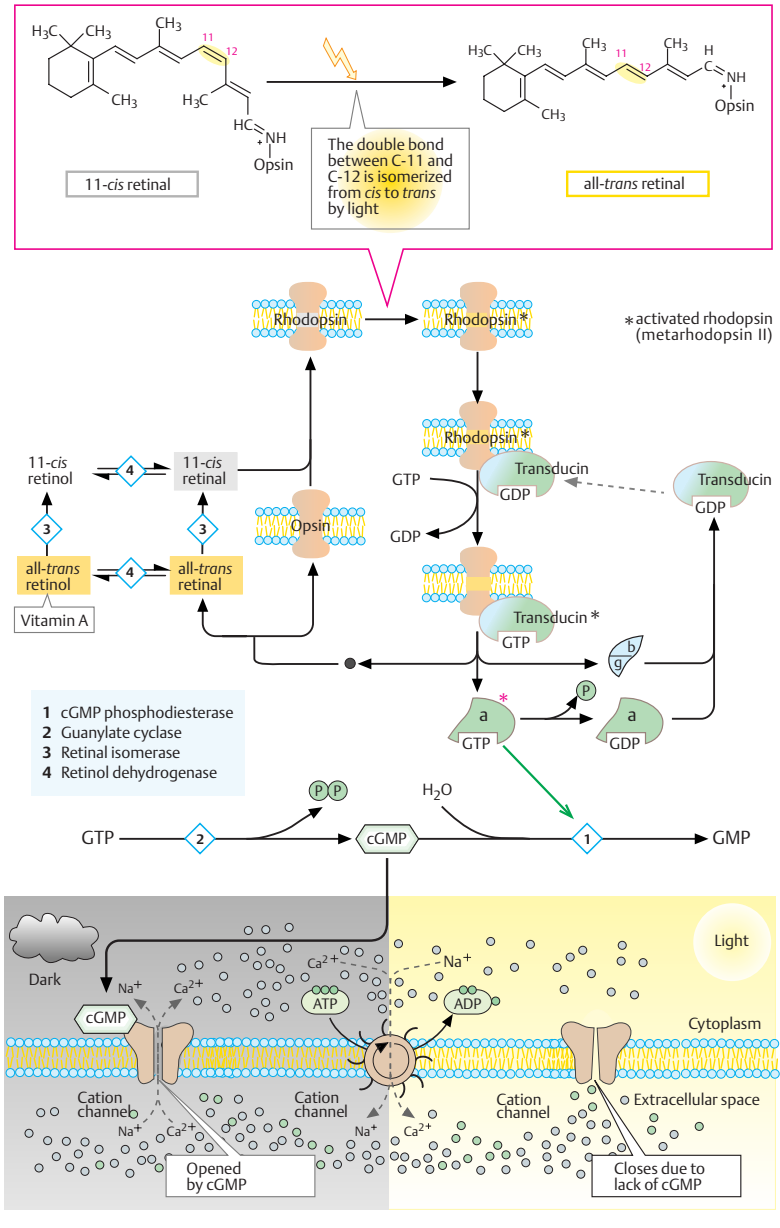
When the G-protein transducin binds to light-activated rhodopsin*, this leads to the GDP being exchanged for GTP. In transducin* that has been activated in this way, the GTP-containing α -subunit breaks off from the rest of the G molecule and activates a membrane *cGMP phosphodiesterase* [1]. This enzyme rapidly hydrolyzes cGMP to GMP and thus reduces the level of free cGMP within milliseconds. As a consequence, the cGMP bound at the cation channels dissociates off and the channels close. As cations are constantly being pumped out of the cell, the membrane potential falls and **hyperpolarization** of the cell occurs, which interrupts glutamate release.

Regeneration

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

1. The α -subunit of transducin* inactivates itself by GTP hydrolysis and associates again with the $\beta\gamma$ -subunit (p. 404). This terminates the activation of cGMP phosphodiesterase [1].
2. The reduced Ca^{2+} concentration causes activation of guanylate cyclase, which increases the cGMP level until the cation channels reopen.
3. Opsin is phosphorylated by rhodopsin kinase and binds the protein arrestin. All-*trans*-retinal is now released and regenerated to 11-*cis*-retinal. This takes place either directly through an *isomerase* [3] or by reduction to all-*trans*-retinol [4], conversion to the 11-*cis*-form, and subsequent oxidation to 11-*cis*-retinal. This can then bind again to free opsin and is available for the next cycle.

A. Light-induced signal cascade



Pathobiochemistry

A. Diseases of the nervous system (examples)

The table lists only a few important diseases of the many that affect neurons and their signal transduction. *Parkinson disease* is discussed on p. 434 with catecholamine metabolism.

B. Alzheimer disease

This neurodegenerative disease is characterized histologically by **Alzheimer plaques**, i.e., extracellular fibrillary protein deposits in the brain. These consist of **β -amyloid ($A\beta$)**, a small peptide of ~40 amino acids, which combines into larger $A\beta$ aggregates with a β pleated sheet structure. The plaques interfere with nerve function and induce inflammatory processes.

$A\beta$ is produced by enzymes that degrade a transmembrane protein named **β -amyloid protein precursor (APP)**, the function of which is unknown. Normal degradation of APP takes place sequentially via two proteinases, α - and γ -secretase, which cleave soluble peptides from the insoluble membrane protein. In Alzheimer patients, APP is attacked by a β -secretase instead of α -secretase, which cleaves at a different site and produces the precursor for insoluble $A\beta$.

A hyperphosphorylated structural protein in fiber-form is also deposited intracellularly in the brain of Alzheimer patients (not shown). In the normal situation, this **τ (tau) protein** stabilizes the microtubules of neurons while in fibril form it obstructs intracellular transport. The connection between τ protein and $A\beta$ is not clear. However, in both cases, *incorrect folding of a protein* is the predominant biochemical abnormality.

The primary cause of Alzheimer disease is still unknown, although there are various hypotheses (a transport defect, disorders of calcium metabolism, involvement of Cu or Al ions). The slowly progressive and ultimately fatal disease occurs mostly at an advanced age. Its incidence increases from about 2% to over 20% between the ages of 65 and 85 years. Up to now, it can only be diagnosed by neuropsychological tests.

C. Prion diseases

This group of neurodegenerative diseases is also due to incorrect protein folding. It includes **Creutzfeldt-Jakob disease (CJD)** in humans, **scrapie** in dogs and sheep, and **BSE** (bovine spongiform encephalopathy, “mad cow disease”) in cattle. All are caused by infectious proteins, the **prions** (PrP), which can occur in two different conformations. Noninfectious prions (**PrP^C**, C for cellular) are present in nerve cells as normal proteins in cytoplasmic and membrane-associated forms. PrP^C can transform spontaneously into isoforms that are more protease-resistant and less soluble (**PrP^{Sc}**, Sc for scrapie). The two forms have an identical primary structure but their secondary and tertiary structures differ: PrP^C contain more α -helices, whereas PrP^{Sc} have more β -pleated sheet structures. The special property of prions is that PrP^{Sc} molecules can serve as crystallization nuclei for the conformational change of PrP^C to PrP^{Sc} (“infection”). This is the reason for the *pathogenicity of PrP^{Sc}*. Conversion of PrP^C to PrP^{Sc} takes place slowly over years. PrP^{Sc} then aggregate into fibers (amyloids) and cause the death of infected cells by apoptosis. This results in neuronal deficits.

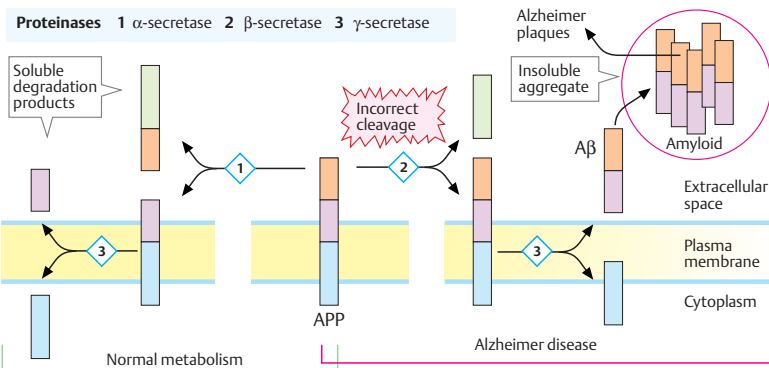
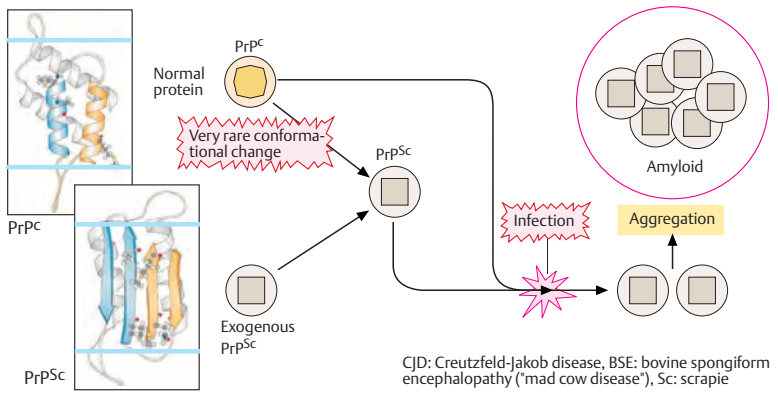
Prions can be transmitted in food. As the prions of cattle and humans are very similar, it is suspected that humans can contract the disease from BSE-infected beef.

Myasthenia gravis. This **autoimmune disease** is caused by antibodies against the nicotinic **acetylcholine receptor** (p. 364) in skeletal muscle. The antibodies bind to the receptors, thereby triggering their endocytosis and lysosomal degradation. Patients with myasthenia gravis therefore have fewer functional receptors for ACh. This leads to an inability of muscles to react appropriately to repeated stimulation by acetylcholine. Normal muscle excitability usually returns at rest. *Acetylcholinesterase inhibitors* (p. 364) and *immunosuppressant drugs* are used to treat the disease.

Lambert-Eaton syndrome is a similar autoimmune disease. In this case, the voltage-gated calcium channels of the presynaptic membrane of the motor endplate are affected.

A. Diseases of the nervous system (examples)

Disease	Cause	Consequences
Kernicterus	Hyperbilirubinemia in neonates with still incompletely closed blood-brain barrier	Brainstem damage due to bilirubin
Multiple sclerosis	Relapsing demyelination of the white matter of the CNS due to local autoimmune damage to the myelin sheath	Perception disorders, psychological changes, muscle weakness
Ion channel diseases	Rare genetic defects of ion channels	Epilepsy, ataxia, paralysis, familial forms of migraine
Myasthenia gravis	Autoimmune disease with antibodies against the nicotinic acetylcholine receptor of the neuromuscular synapses	Load-dependent inactivation of skeletal muscle
Alzheimer disease	Neurodegenerative disease (see B)	Forgetfulness, orientation disturbances, physical decay
Prion diseases	Neurodegenerative disease (see C)	Increasing neuronal deficits
Parkinson disease	Neurodegenerative disease (p. 435)	Akinesia, rigidity, tremor (p. 435)

B. Alzheimer disease

C. Prion diseases


Integration of Metabolism I

Glucose is at the heart of carbohydrate metabolism (**B**). It is the only energy source that can be used by all cells and is available to the tissues in an almost constant concentration as “blood sugar.”

The role of the body's **blood sugar regulation** systems is to ensure the organs' glucose supply, which varies within broad limits (**C**), without allowing the blood sugar level to drop significantly. The brain in particular reacts very sensitively to a decrease in blood glucose. On the other hand, dietary glucose can be available in large quantities in the absorptive phase (p. 376) and this has to be converted rapidly into storage forms so that the glucose level does not rise too much. Because of the kidney's limited capacity for glucose reabsorption (p. 334), elevated glucose levels would lead to losses. Moreover, glucose in high concentration damages blood vessels in the long term, which can result in disease (p. 430). The processes described here are regulated by several hormones, the functions of which are summarized in **C**.

A. Fuel reserves in adults

The body has considerable “fuel” reserves, i.e., metabolites that can be used for ATP production. **Triacylglycerols**, stored in adipose tissue, act as a long-term reserve; these have a caloric value of 500 MJ (1 megajoule = 1000 kJ), accounting for over 80% of reserves (p. 332). Fats are ideal reserve substances. They can be stored as insoluble and thus osmotically inactive droplets. They also have the highest energy density of all metabolites (39 kJ/g). **Muscle proteins** constitute the second biggest store that can be mobilized. During starvation, glucogenic amino acids (p. 168f.) formed by proteolysis act as gluconeogenesis precursors and can therefore sustain the blood sugar level for a prolonged period, unlike the fatty acids. **Glycogen** deposited in muscle and liver acts as a rapidly available source of glucose. Its contribution to the reserves is insignificant at less than 2%.

B. Blood sugar level

The blood sugar level is about $5 \text{ mmol} \cdot \text{L}^{-1}$ ($90 \text{ mg} \cdot \text{dL}^{-1}$) in the fasting state. Postprandially (directly after a meal), it can increase briefly to $7\text{--}8 \text{ mmol} \cdot \text{L}^{-1}$ (p. 376), while it dips to a level of $4 \text{ mmol} \cdot \text{L}^{-1}$ during sustained star-

vation (p. 378). Unlike temporary hyperglycemia, a markedly reduced glucose level (*hypoglycemia*), represents an acute danger for the organism. Only one hormone (*insulin*) reduces blood sugar, in contrast to a range of hormones that increase it (*glucagon*, *epinephrine*, *cortisol*, etc., see **D**).

C. Glucose turnover in organs

The basal glucose consumption in adults is normally around $10 \text{ g} \cdot \text{h}^{-1}$. The *brain* constantly accounts for about 60% and the *erythrocytes* for 10%–15%. Both rely on glucose as energy substrate and take it up *independent of insulin*. Other major consumers are *muscle* and *adipose tissue*, though their requirements fluctuate greatly. Glucose uptake into both tissues is stimulated by *insulin* via the transporter *Glut-4* (p. 428). As muscle is not capable of releasing glucose derived from glycogen (p. 136), the *liver* is the most important supplier of glucose. It normally releases an amount that exactly covers the basal consumption of $10 \text{ g} \cdot \text{h}^{-1}$, but it can increase the release of glucose several fold for a short period under the influence of glucagon or epinephrine.

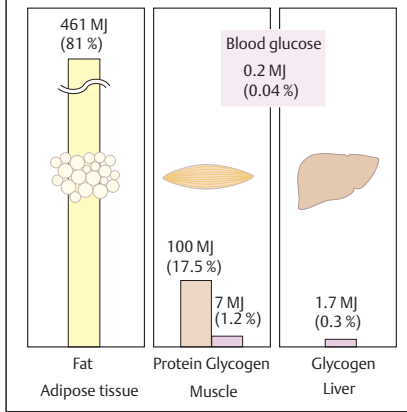
D. Hormonal effects on organ metabolism

The table gives an overview of the effects of important metabolic hormones in the liver, muscle, and adipose tissue.

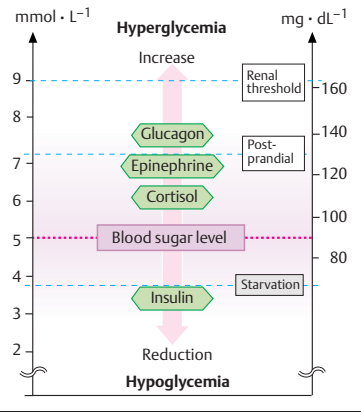
Insulin, the predominant **anabolic hormone**, promotes those metabolic pathways and transport processes that *reduce* the blood sugar, inhibits lipolysis and promotes the biosynthesis of storage fat and muscle protein.

The **catabolic hormones** *glucagon*, *epinephrine*, and *cortisol* act as insulin antagonists; they release glucose and fatty acids from their stores and stimulate gluconeogenesis. *Thyroxine* and the growth hormone *somatotropin* (not shown) also increase the blood sugar level by activating gluconeogenesis.

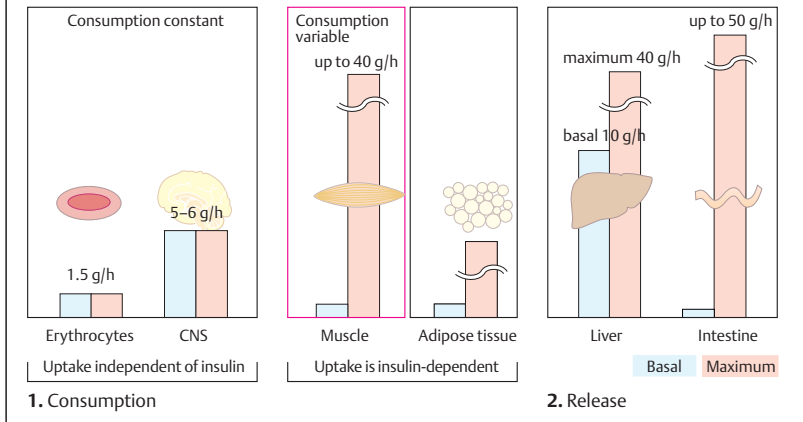
A. Fuel reserves in adults



B. Blood sugar level



C. Glucose turnover in organs



D. Hormonal effects on organ metabolism

Hormone	Liver				Muscle		Adipose tissue		
	Glucose uptake	Gluconeogenesis	Glycogen synthesis	Glycogen degradation	Glucose uptake	Protein synthesis	Glucose uptake	Lipogenesis	Lipolysis
Insulin	↑ a)	↓	↑	↓	↑ b)	↑	↑ b)	↑	↓
Glucagon	—	↑	↓	↑	—	—	—	—	↑
Epinephrine	—	↑	—	↑	—	—	—	—	↑
Cortisol	—	↑	↑	—	↓	↓ c)	—	—	↑
Thyroxine	—	↑	—	—	—	↑	—	—	—

a) via glucokinase b) via Glut-4 c) in extrahepatic tissues

Integration of Metabolism II

The contributions of the individual tissues to amino acid and lipid metabolism are very varied. The blood supplies the organs with metabolic precursors, distributes their products in the body, and transports end products to the liver and kidneys for excretion.

A. Organ cooperation in amino acid metabolism

Amino acids not required for protein synthesis are degraded in the organ itself to obtain energy or are converted to **glutamine** or **alanine** for transport in the blood. *Glutamine* is the most important *transport molecule for amino nitrogen*, with a plasma concentration of up to 0.8 mM. In addition, it acts as a preferred energy substrate for cells with a high energy requirement such as enterocytes, leukocytes, and renal tubular cells.

To form glutamine, the amino group of the amino acid being degraded is first transferred to 2-oxoglutarate (2-OG), with formation of *glutamate* [4]. The γ -carboxylate group of the glutamate formed is then amidated by *glutamine synthetase* [5], p. 106) to the acid amide group, with NH_4^+ acting as nitrogen donor. *Alanine* is produced from pyruvate in one step by *alanine transaminase* [3], with the amino group coming from glutamate.

In the liver and kidney, alanine and glutamine are used especially for *gluconeogenesis* (p. 134). Glutamine is first deaminated hydrolytically by *glutaminase* to glutamate [2], oxidative deamination of which by *glutamate dehydrogenase* [1] again provides 2-oxoglutarate (p. 172). Alanine is converted back to pyruvate by transamination (p. 172).

Muscle specializes in the degradation of *branched-chain amino acids*. As described above, their amino nitrogen is incorporated in glutamine and alanine via glutamate. Some of the *lactate* produced in anaerobically working muscle is also transported to the liver in the form of alanine (after oxidation to pyruvate and transamination). There it is resynthesized to glucose again. Functionally, this “*alanine cycle*” is similar to the Cori cycle (p. 344).

The **liver** is the only organ that can incorporate amino nitrogen into *urea* for excretion (*urea cycle*; p. 172). It also utilizes excess amino acids for gluconeogenesis (the most important precursor is *alanine*) or degradation.

The **kidney** prefers *glutamine* as a gluconeogenesis precursor. At the same time glutamine

degradation provides ammonia (NH_3) to buffer H^+ ions in the urine (p. 338). The kidney also provides other organs with *arginine* and *serine* (p. 338).

The enterocytes in the **intestine** meet their energy needs mainly by degradation of glutamine and glutamate, the nitrogen of which they release into the blood as alanine.

B. Lipid transport

Two types of complexes are involved in lipid transport: *proteins with lipid binding sites* (e.g., albumin, which transports free fatty acids) and *lipoproteins*. Lipoprotein synthesis and function are discussed in detail on page 282f.

The **intestine** absorbs monoacylglycerols and free fatty acids and synthesizes triacylglycerols from them, which are packed in *chylomicrons* and released into the lymph (p. 274). They are broken down in the periphery to fatty acids and glycerol, with the aid of *lipoprotein lipase* [6].

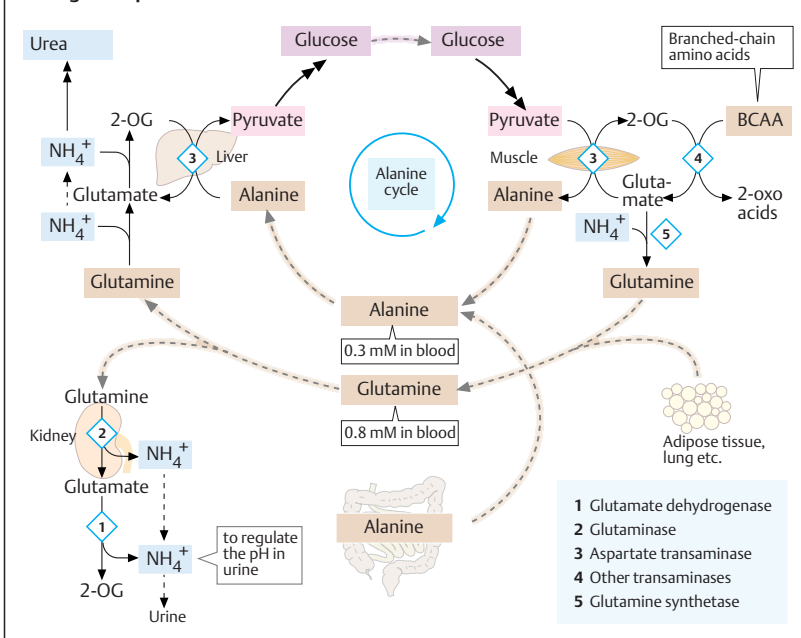
The **liver** converts fatty acids to fats and exports them in the form of VLDL (p. 282). When the supply of fatty acids is plentiful, the liver produces *ketone bodies* (C) and provides these to other organs (muscle, CNS; p. 378).

Adipose tissue releases fatty acids from chylomicrons and VLDL and converts them to storage fat. When necessary, it releases fatty acids again with the aid of *hormone-sensitive lipase* [7], which are utilized by muscle, the liver, and other organs.

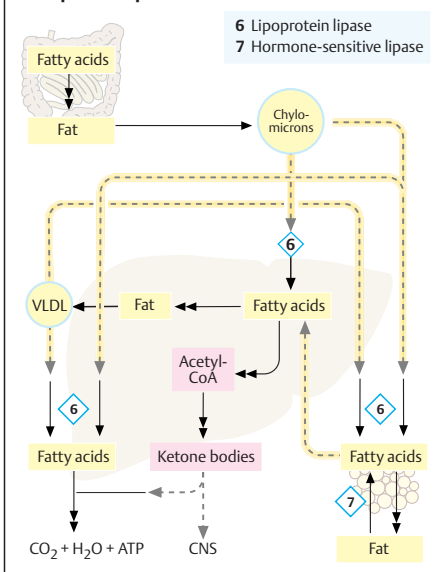
C. Ketone body degradation

The most important ketone body quantitatively is *3-hydroxybutyrate* (p. 378). To enter metabolism, it is first oxidized to *acetoacetate* [8]. This is activated by transfer of CoA from succinyl-CoA to *acetoacetyl-CoA* [9], which is further broken down to *acetyl-CoA* by a reaction of β -oxidation.

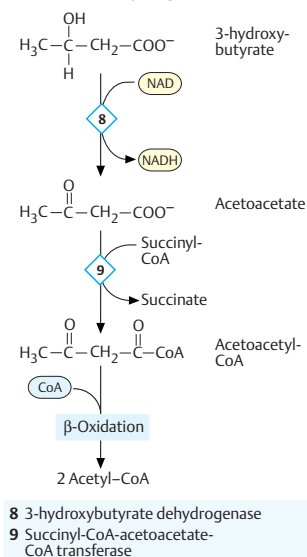
A. Organ cooperation in amino acid metabolism



B. Lipid transport



C. Ketone body degradation



Integration of Metabolism III

The periods during and immediately after consumption of a meal are called the *absorptive phase*. This phase in organ metabolism is dominated by transport processes and metabolic pathways that serve to incorporate metabolites absorbed from the intestine into short-term and long-term stores (glycogen, proteins, fat). The characteristic metabolic hormone in the absorptive phase is *insulin*, secretion of which from the pancreas increases 5–10-fold after a meal (C), while secretion of its antagonist *glucagon* is reduced by up to 50% (B).

A. Absorptive phase

The diagram gives a summary of processes that predominate during the absorptive phase. Processes activated by insulin are labeled “I” on a green background.

The **intestine** (top) releases *sugars* (mainly glucose) and *amino acids* into the blood during the absorptive phase. Most of these reach the liver via the portal vein. Fats reach the blood stream via lymph as components of *chylomicrons*.

In intermediary metabolism, the **liver** works together with adipose tissue as a balancing, *buffering*, and *storage organ*. In the absorptive phase it initially stores the arriving glucose in the form of *glycogen*. When the glycogen stores have been filled, glucose is broken down through glycolysis to acetyl-CoA. *Fat* is synthesized from this via fatty acids and exported as a component of VLDL. Glycogen synthesis and the conversion of glucose to fat are stimulated by *insulin*. Amino acids are converted by the liver into *glucose*, mainly by gluconeogenesis, as soon as the insulin level has fallen again.

Adipose tissue releases fatty acids from lipoproteins (chylomicrons and VLDL) with the aid of *lipoprotein lipase* (LPL) and stores them in the form of triacylglycerols. The glycerol necessary for fat synthesis is derived from glucose degradation (p. 330). *Insulin* increases glucose uptake via Glut-4 (pp. 212, 430) and promotes degradation of lipoproteins and synthesis of fat stores by activation of LPL.

Muscle also takes up more glucose under the influence of insulin in the absorptive phase and incorporates it in *glycogen*. In addition, amino acids are used by muscle for *protein synthesis*. This process is also promoted by insulin.

Glucose consumption by the **CNS** and **erythrocytes** is independent of the metabolic situation

and remains unchanged even in the absorptive phase (p. 372).

B. Blood sugar level after a meal

In healthy individuals, the blood glucose level increases by up to 50% after a meal (p. 372). The pancreas reacts within minutes by releasing stored *insulin*, while the *glucagon* level decreases. This first phase of the insulin effects is followed after about 30 minutes by a second phase, which is due to the secretion of newly synthesized insulin. Synthesis of insulin in the β cells is stimulated by tissue hormones from the intestine, including gastrin, secretin, and the peptide hormone *GLP-1* (glucagon-like peptide 1).

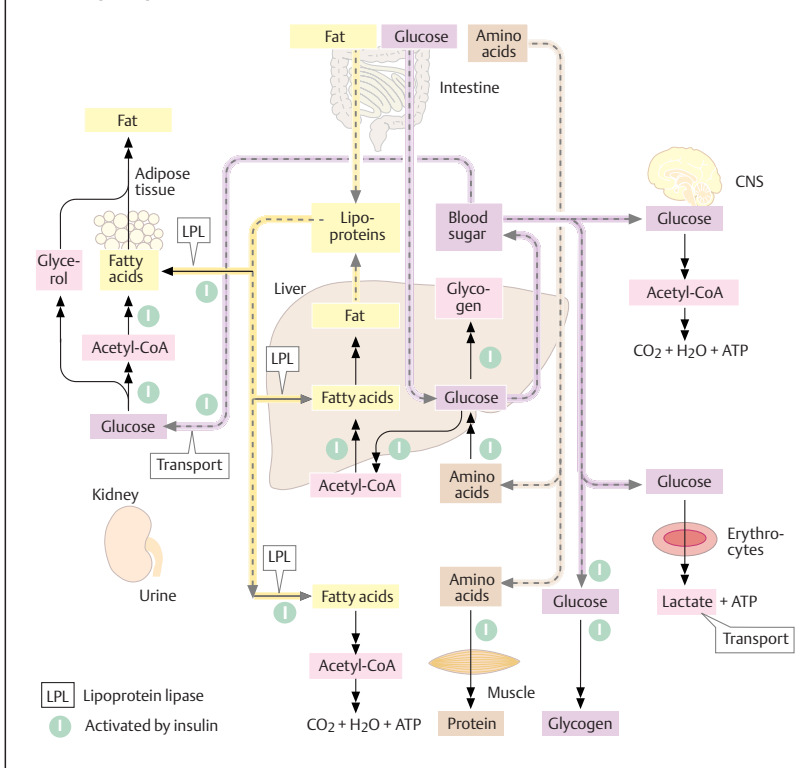
After an *oral glucose tolerance test* (OGTT), in which ca. 100 g of easily digestible carbohydrates are given, the blood sugar level increases and in healthy persons, it decreases again to the initial level after 2–3 hours. In patients with *diabetes mellitus* (p. 430), the blood glucose shows a higher initial increase and then decreases much more slowly.

C. Regulation of insulin secretion

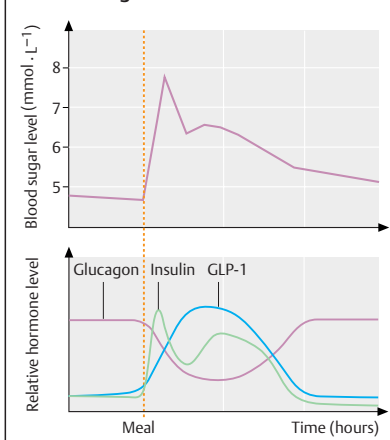
The most important signal for the secretion of insulin from the β cells of the pancreas (p. 430) is a *rise in the glucose in the blood*. This increases glucose uptake via the low-affinity *Glut-2 transporter* (p. 212), which in turn stimulates glucose degradation. The ATP produced closes an outward-directed K^+ *channel* (1) in the plasma membrane, thereby causing membrane *depolarization* (p. 360). This in turn opens *voltage-gated Ca^{2+} channels* (2) and leads via influx of Ca^{2+} to *exocytosis* (p. 214) of the stored insulin. *Gastrointestinal hormones* (B) and some *amino acids* also stimulate insulin secretion (p. 428).

Insulin secretion can be increased pharmacologically with *oral antidiabetic drugs* such as the *sulfonylureas*.

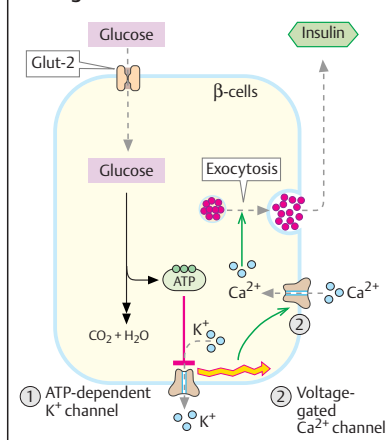
A. Absorptive phase



B. Blood sugar level after a meal



C. Regulation of insulin secretion



Integration of Metabolism IV

A few hours after the last meal, the *post-absorptive phase* begins, which continues into the *starvation phase* after a few days if there is no food intake. Massive changes take place in intermediary metabolism, which serve primarily to maintain the blood sugar level. This is important for survival as the brain, erythrocytes, and other tissues rely on a constant supply of glucose (p. 372).

In the post-absorptive phase, the blood sugar comes mainly from degradation of *glycogen* and later from *gluconeogenesis* (B). With more prolonged starvation, only gluconeogenesis is still available, supplied by degradation of proteins and fats. The dominant hormone in the post-absorptive and starvation phases is *glucagon* from the α cells of the pancreas, the blood concentration of which doubles while the *insulin* level decreases greatly.

A. Metabolism during starvation

The illustration shows the state of intermediary metabolism after a starving period of several weeks. At this time, glycogen has long been unavailable (B); by contrast, the adjustment of the brain to utilization of ketone bodies is complete (see below). In the figure the sites of action of *glucagon* and *cortisol* are labeled with the letters "G" and "C" on a green background.

In **adipose tissue**, *lipolysis* by *hormone-sensitive lipase* (p. 330) is fully activated because of the high glucagon level. In this situation, about 180 g of fat are broken down per day. Approximately 20% of the released fatty acids are used in muscle and other tissues for energy production, while 80% are converted in the liver to ketone bodies. The glycerol released during lipolysis is used for gluconeogenesis.

During starvation, the **liver** is the most important source of glucose, which it produces from amino acids, lactate, and glycerol by gluconeogenesis. The stated amount of $80 \text{ g} \cdot \text{d}^{-1}$ is a fraction of the amount of glucose that is released with normal nutrition or in the post-absorptive phase (basal: $10 \text{ g} \cdot \text{h}^{-1} = 240 \text{ g} \cdot \text{d}^{-1}$; p. 372). On the other hand, the formation of ketone bodies by the liver is enormously increased. One-third of the total amount of $150 \text{ g} \cdot \text{d}^{-1}$ is used by muscles, while two-thirds serves to supply the brain (see below).

A lot of **muscle** protein is broken down initially in the post-absorptive phase, to provide precursors for gluconeogenesis in the liver. Since

such intensive proteolysis would impair muscle function in the long term, it is reduced greatly as soon as the brain has switched to utilizing ketone bodies.

The **brain** meets its energy needs in starvation mainly by degradation of ketone bodies (inset). After prolonged starvation, it reduces its glucose consumption by two thirds and then uses mainly 3-hydroxybutyrate and some acetoacetate (p. 374).

During starvation, the **kidneys** also contribute to gluconeogenesis, but the precursors are mainly glutamine and lactate.

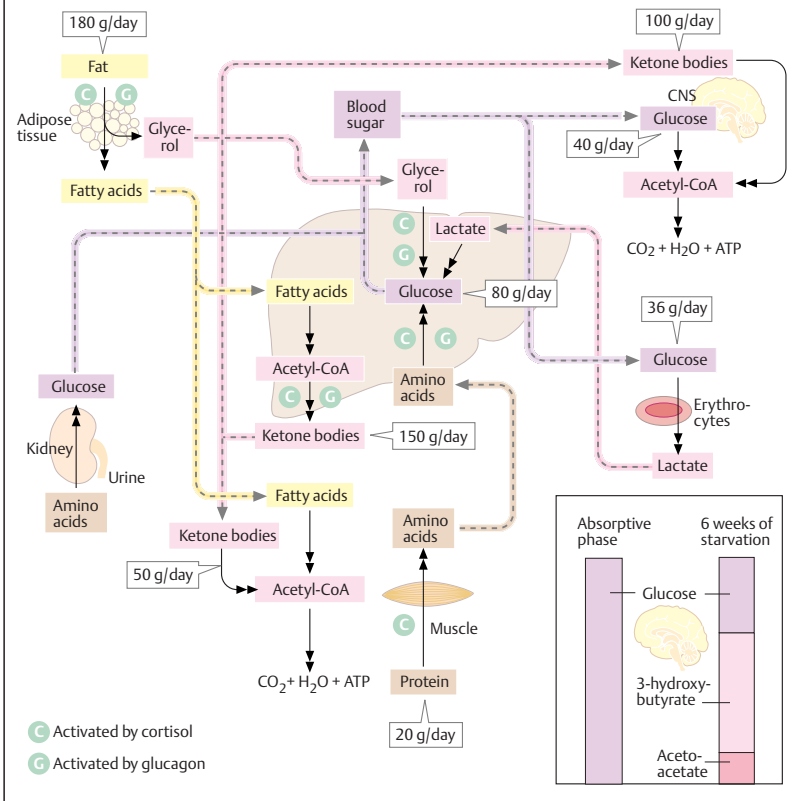
B. Origin of blood glucose

The illustration shows the time course of glucose consumption, starting with the last intake of food. When the dietary glucose has been consumed, glycogen degradation supplies most of the glucose for 8–12 hours. At the same time, gluconeogenesis is activated and this takes over the entire glucose supply after 1–2 days. As mentioned above, gluconeogenesis is reduced after about a week of starvation to save muscle protein.

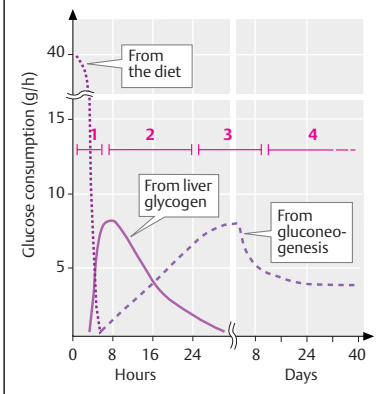
C. Metabolite levels

The change in metabolism during starvation is also apparent from the time course of metabolite concentrations in the blood. The blood sugar level falls in the first days of the starvation phase to a constant value of $3.5\text{--}4 \text{ mmol} \cdot \text{L}^{-1}$. Nitrogen excretion in the urine (blue curve) also decreases continuously because proteolysis in muscle is reduced more and more. On the other hand, the plasma level of ketone bodies increases dramatically, and after 3–4 weeks it reaches twice the glucose concentration.

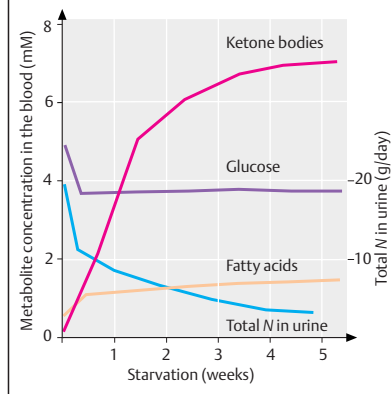
A. Metabolism during starvation



B. Origin of blood glucose



C. Metabolite levels



Nutrition

Organic Substances

A balanced human diet must contain a large number of different components. These include *proteins, carbohydrates, fats, minerals, water, and vitamins*. These substances can occur in widely varying amounts and proportions, depending on the type of diet. As several components of the diet are *essential for life*, they have to be regularly ingested with food. Recommended daily minimums for nutrients have been published by the World Health Organization (WHO) and several national expert committees.

A. Energy requirement

The amount of energy required by a human is expressed in *kilojoules* per day ($\text{kJ} \cdot \text{d}^{-1}$). An older unit for energy is the kilocalorie (kcal; $1 \text{ kcal} = 4.187 \text{ kJ}$). The figures given are recommended values for adults with a normal body weight. However, actual requirements are influenced by age, sex, body weight, and in particular by physical activity. In those involved in competitive sports, for example, requirements can increase from 12000 to 17000 $\text{kJ} \cdot \text{d}^{-1}$.

It is recommended that about half of the energy intake should be in the form of carbohydrates, a third at most in the form of fat, and the rest as protein. When the daily energy intake is calculated, the fact that *alcoholic beverages* can make a major contribution is often overlooked. Ethanol has a caloric value of about $30 \text{ kJ} \cdot \text{g}^{-1}$ (p. 326).

When energy balance is positive because of overnutrition, nutrients are converted to endogenous storage substances (glycogen, fats, and proteins), and when energy balance is negative, the body consumes these storage substances.

B. Nutrients

Proteins provide the body with amino acids for endogenous protein biosynthesis. Excess amino acids are broken down to provide energy (p. 160ff.). Most amino acids are *glucogenic*—i.e., they can be converted into glucose (p. 168).

Proteins are indispensable components of the diet, as they provide **essential amino acids** that the human body is not capable of producing on its own (p. 174). Some amino acids are able to substitute for each other in the diet. For example, humans can form tyrosine, which is

actually essential, by hydroxylation from phenylalanine, and cysteine from methionine.

The *minimum daily requirement* of protein is 37 g for men and 29 g for women, but the recommended amounts are about twice these values. Requirements in pregnant and breast-feeding women are even higher. Not only the quantity, but also the biological quality of protein is important. Proteins that lack several essential amino acids or only contain small quantities of them are considered to be of low value, and larger quantities of them are therefore needed. For example, pulses only contain small amounts of methionine, while wheat and corn proteins are poor in lysine. In contrast to vegetable proteins, most animal proteins are high-value (with exceptions such as collagen and gelatin).

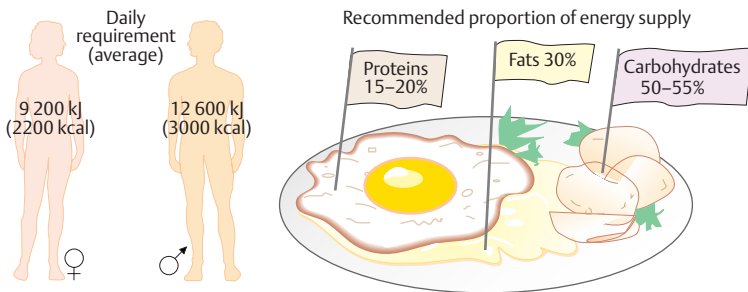
Carbohydrates serve as a general and easily available energy source but are not essential. In the diet, they are present as *monosaccharides* in honey and fruit, or as *disaccharides* in milk (which contains lactose) and in all foods sweetened with sugar (sucrose). Metabolically usable *polysaccharides* are found in vegetable and animal products (starch and glycogen, respectively). Carbohydrates represent a substantial proportion of the body's energy supply, but they are not essential.

Fats are primarily important energy suppliers in the diet. Per gram, they provide, roughly, twice as much energy as proteins and carbohydrates. Fats are essential as suppliers of *fat-soluble vitamins* (p. 392) and as sources of *polyunsaturated fatty acids* (p. 38), which are needed to biosynthesize membrane lipids and eicosanoids (p. 438). Besides fats, there are other dietary lipids (p. 36ff.), including cholesterol.

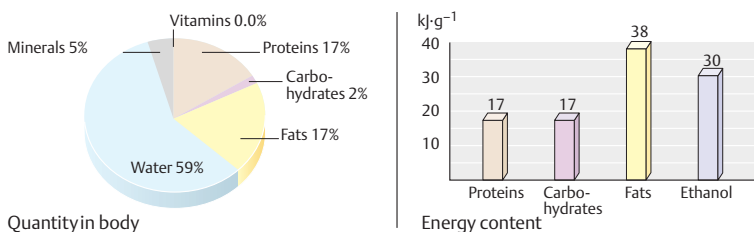
The **mineral substances and trace elements** are a very heterogeneous group of essential nutrients. They are divided into *macrominerals* and *microminerals* (p. 384).

Vitamins are also indispensable components of the diet. The animal body requires them in very small quantities to synthesize coenzymes and signaling substances (p. 392ff.).

A. Energy requirement



B. Nutrients



	Quantity in body (kg)	Energy content kJ·g ⁻¹ (kcal·g ⁻¹)	Daily requirement (g) a b c	General function in metabolism	Essential constituents
Proteins	10	17 (4.1)	♂ 37 55 92 ♀ 29 45 75	Supplier of amino acids Energy source	Essential amino acids: Val, Leu, Ile, Lys, Phe, Trp, Met, Thr, His
Carbohydrates	1	17 (4.1)	0 390 240–310	General source of energy (glucose) Energy reserve (glycogen) Roughage (cellulose) Supporting substances (bones, cartilage, mucus)	Non essential nutritional constituent
Fats	10–15	38 (9.3)	10 80 130	General energy source Most important energy reserve Solvent for vitamins Supplier of essential fatty acids	Polyunsaturated fatty acids 10 g/day: Linoleic acid Linolenic acid Arachidonic acid
Water	35–40	0	2400 – –	Solvent Cellular building block Dielectric Reaction partner Temperature regulator	
Minerals	3	0		Building blocks Electrolytes Cofactors of enzymes	Macrominerals Microminerals (trace elements)
Vitamins	–	–		Often components of enzymes Precursors for signaling substances Redox protection	Lipid-soluble vitamins Water-soluble vitamins

a: Minimum daily requirement b: Recommended daily intake c: Actual daily intake in developed countries

Minerals and Trace Elements

A. Minerals

Water is quantitatively the most important essential inorganic nutrient in the diet. In adults, the body has a daily requirement of 2–3 L of water, which is supplied from drinks, water contained in solid foods, and from the *oxidation water* produced in the respiratory chain (p. 120). The special role of water for living processes is discussed in more detail elsewhere (p. 22f.).

The elements essential for life can be divided into **macroelements** (daily requirement > 100 mg) and **microelements** (*trace elements*, daily requirement < 100 mg). The macroelements include the **electrolytes** *sodium* (Na), *potassium* (K), *calcium* (Ca), and *magnesium* (Mg), and the nonmetals *chlorine* (Cl), *phosphorus* (P), and *sulfur* (S).

The essential microelements are only required in trace amounts (p. 2). This group includes *iron* (Fe), *zinc* (Zn), *manganese* (Mn), *copper* (Cu), *cobalt* (Co), *chromium* (Cr), *selenium* (Se), *molybdenum* (Mo), and *iodine* (I). Fluorine (F) is not essential for life, but does promote healthy bones and teeth (p. 348). The list of essential microelements probably has to be extended to include *tin* and *vanadium*. The importance of *nickel*, *bromine*, *arsenic*, *cadmium*, *barium*, *strontium*, *silicon*, and *arsenic* for life processes is still unclear.

The second column in the table lists the average **amounts** of mineral substances in the body of an adult weighing 65 kg. The **daily requirements** listed in the fourth column also apply to an adult, and are *average values*. Children, pregnant and breast-feeding women, and those who are ill generally have higher mineral requirements relative to body weight than men.

As the human body is able to store many minerals, deviations from the daily ration are balanced out over a given period of time. Minerals stored in the body include *water*, which is distributed throughout the whole body; *calcium*, stored in the form of apatite in the bones (p. 348); *iodine*, stored as thyroglobulin in the thyroid (p. 426); and *iron*, stored in the form of ferritin and hemosiderin in the bone marrow, spleen, and liver (p. 388). The storage site for many trace elements is the liver. In many cases, the metabolism of minerals is regulated by *hormones*—e.g., the uptake and excretion of H_2O , Na^+ , Ca^{2+} , and phosphate (p. 334), and storage of Fe^{2+} and I^- .

Absorption of the required mineral substances from food usually depends on the body's requirements, and in several cases also on the composition of the diet. One example of dietary influence is *calcium* (p. 386). Its absorption as Ca^{2+} is promoted by lactate and citrate, but phosphate, oxalic acid, and phytol inhibit calcium uptake from food by forming complexes and insoluble salts.

Mineral deficiencies are not uncommon and can have quite a variety of causes—e.g., an unbalanced diet, absorption disturbances, and diseases (p. 390). *Calcium deficiency* can lead to rickets, osteoporosis, and other disturbances. *Chloride deficiency* is observed as a result of severe Cl^- losses due to vomiting. Due to the low content of iodine in food in many regions of central Europe, *iodine deficiency* is widespread there and can lead to goiter (p. 426). *Magnesium deficiency* can be caused by digestive disorders or an unbalanced diet—e.g., in alcoholism. Trace element deficiencies often result in a disturbed blood picture—i.e., forms of anemia.

The last column in the table lists some of the functions of minerals. It should be noted that almost all of the **macroelements** in the body function either as *nutrients* or *electrolytes*. Iodine (as a result of its incorporation into iodothyronines; p. 426) and calcium (p. 408) act as *signaling substances*. Most **microelements** (trace elements) are *cofactors for proteins*, especially for enzymes. Particularly important in quantitative terms are the *iron proteins* hemoglobin, myoglobin, and the cytochromes. Zinc occurs in more than 300 different *zinc proteins*. *Copper* is a component of oxidases that transfer the electrons to oxygen. This group includes cytochrome c oxidase, superoxide dismutase, monoamine oxidase, tyrosinase, and lysyloxidase.

A. Minerals

Mineral	Content* (g)	Major source	Daily requirement (g)	Functions/Occurrence
Water	35 000–40 000	Drinks Water in solid foods From metabolism (300g)	1200 900	Solvent, cellular building block, dielectric, coolant, medium for transport, reaction partner
Macroelements (daily requirement >100 mg)				
Na	100	Table salt	1.1–3.3	Osmoregulation, membrane potential, mineral metabolism
K	150	Vegetables, fruit, cereals	1.9–5.6	Membrane potential, mineral metabolism
Ca	1300	Milk, milk products	0.8	Bone formation, blood clotting, signal molecule
Mg	20	Green vegetables	0.35	Bone formation, cofactor for enzymes
Cl	100	Table salt	1.7–5.1	Mineral metabolism
P	650	Meat, milk, cereals, vegetables	0.8	Bone formation, energy metabolism, nucleic acid metabolism
S	200	S-containing amino acids (Cys and Met)	0.2	Lipid and carbohydrate metabolism, conjugate formation
Microelements (trace elements)			(mg)	
Fe	4–5	Meat, liver, eggs, vegetables, potatoes, cereals	10	Hemoglobin, myoglobin, cytochromes, Fe/S clusters
Zn	2–3	Meat, liver, cereals	15	Zinc finger proteins, insulin storage, zinc enzymes
Mn	0.02	Found in many foodstuffs	2–5	Enzymes
Cu	0.1–0.2	Meat, vegetables, fruit, fish	2–3	Oxidases
Co	<0.01	Meat	Traces	Vitamin B ₁₂
Cr	<0.01		0.05–0.2	Not clear
Mo	0.02	Cereals, nuts, legumes	0.15–0.5	Redox enzymes
Se		Vegetables, meat	0.05–0.2	Selenium enzymes
I	0.03	Seafood, iodized salt, drinking water	0.15	Thyroxin
Requirement not exactly known				■ Metals ■ Non-metals
F		Drinking water (fluoridated), tea, milk	0.0015–0.004	Bones, dental enamel

* Content in the body of a 65 kg adult

Calcium Metabolism

A. Functions of calcium

The human body contains 1–1.5 kg Ca^{2+} , most of which (~99%) is located in the mineral substance of bone and teeth (p. 348).

In addition to this role as an inorganic **bone component**, Ca^{2+} ions act as **second messengers** in signal transduction pathways (p. 408). They thus regulate specific cell functions such as *exocytosis* of neurotransmitters (or *muscle contraction* (pp. 214, 342, 358)). As **enzyme cofactors**, Ca^{2+} ions are indispensable in *blood coagulation* (Ca^{2+} = factor IV; p. 294). Many *metabolic enzymes* are also activated by Ca^{2+} , e.g., isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase and PDH phosphatase. Extracellular Ca^{2+} also acts as a **membrane potential stabilizer**. The intracellular and extracellular concentrations of Ca^{2+} are strictly regulated to make these functions possible (see **C** and p. 408).

Proteins bind Ca^{2+} as a **Ca^{2+} complex** via oxygen ligands, particularly carboxylate groups and carbonyl groups of peptide bonds.

B. Bone remodeling

Deposition of Ca^{2+} in bone (*mineralization*) and Ca^{2+} *mobilization* from bone are regulated by at least 15 hormones and hormone-like signaling substances. These mainly influence the maturation and activity of bone cells.

Osteoblasts deposit collagen, as well as Ca^{2+} and phosphate, and thereby create new bone matter, while the **osteoclasts**, which are derived from macrophages, secrete H^+ ions and collagenases that locally dissolve bone (*bone remodeling*). Osteoblasts and osteoclasts mutually activate each other by releasing **cytokines** (p. 440) and **growth factors** (p. 436). This helps to keep bone formation and bone breakdown in balance.

The Ca^{2+} -selective hormones calcitriol, parathyroid hormone, and calcitonin influence this interaction in the bone cells. **Parathyroid hormone** promotes Ca^{2+} release by promoting the release of cytokines by osteoblasts. In turn, the cytokines stimulate the development of mature osteoclasts from precursor cells (bottom). **Calcitonin** inhibits this process. At the same time, it promotes the development of osteoblasts (top). Estrogens normally inhibit the stimulation of osteoclast differentiation by osteoblasts. If the effects of estrogen decline, the osteoclasts predominate and excess bone removal occurs (*osteoporosis*, p. 356).

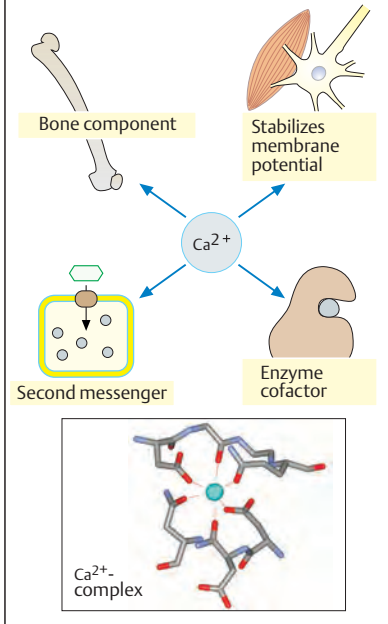
The effects of the steroid hormone **calcitriol** (p. 46) in bone are complex. On the one hand, it promotes bone formation by stimulating osteoblast differentiation (top). This is particularly important in small children, in whom calcitriol deficiency can lead to mineralization disturbances (**rickets**; p. 390). On the other hand, calcitriol increases blood Ca^{2+} levels through increased Ca^{2+} mobilization from bone. An overdose of vitamin D (cholecalciferol), the precursor of calcitriol, can therefore have unfavorable effects on the skeleton similar to those of vitamin deficiency (*hypervitaminosis*, p. 392).

C. Calcium homeostasis

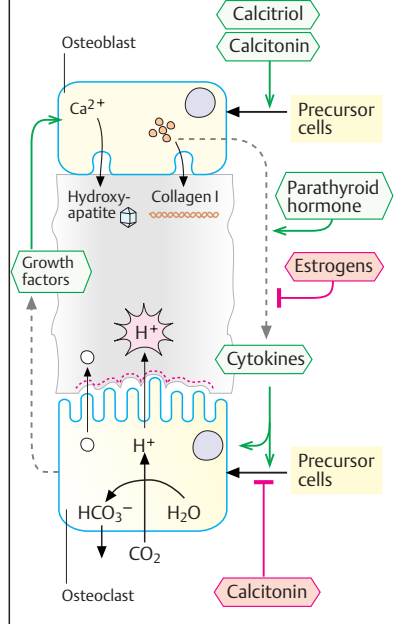
Ca^{2+} metabolism is balanced in healthy adults. Approximately 1 g Ca^{2+} is taken up per day, about 300 mg of which is absorbed. The same amount is also excreted again. The amounts of Ca^{2+} released from bone and deposited in it per day amount to roughly 500 mg. Growing children and pregnant or lactating women have an increased calcium requirement. Milk and milk products, especially cheese, are particularly rich in calcium.

Calcitriol and parathyroid hormone, on the one hand, and calcitonin on the other, ensure a more or less constant level of Ca^{2+} in the blood plasma and in the extracellular space (80–110 $\text{mg} \cdot \text{L}^{-1}$; 2.0–2.6 mM). Just under half of this is the biochemically active ionized (free) Ca^{2+} . The peptide **parathyroid hormone** (PTH; 84 amino acid residues [AA]) and the steroid **calcitriol** (p. 46) directly or indirectly promote processes that raise the Ca^{2+} level in blood. Calcitriol increases Ca^{2+} absorption in the intestines and kidneys by inducing transporters. Parathyroid hormone supports these processes by stimulating calcitriol biosynthesis in the kidneys. In addition, it directly promotes absorption of Ca^{2+} in the kidneys (p. 334) and Ca^{2+} release from bone (see **B**). The PTH antagonist **calcitonin** (32 AA) counteracts these processes.

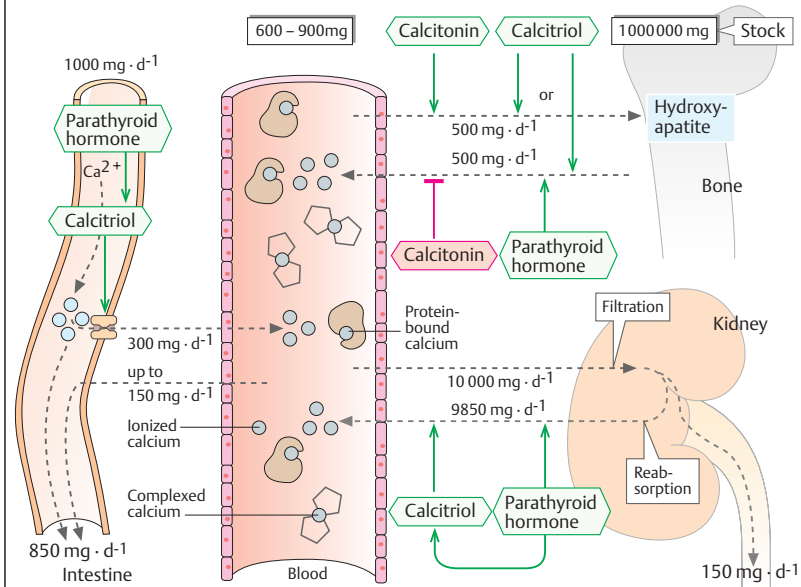
A. Functions of calcium



B. Bone remodeling



C. Calcium homeostasis



Iron Metabolism

A. Distribution of iron

Iron (Fe) is quantitatively the most important trace element (p. 384). The human body contains 4–5 g iron, which is about as much as an ordinary nail contains. In the body, iron is present almost exclusively in protein-bound form. Approximately 73% is found in **heme proteins** (pp. 86, 188), mainly hemoglobin (66%, top left), myoglobin (6%), and in heme enzymes (just under 1%). Another 1% of the iron is bound in **iron-sulfur clusters** (top right), which function as cofactors in the respiratory chain, in photosynthesis, and in other redox chains (p. 122). The remaining 26% consists of iron in **transport** and **storage proteins** (e.g., transferrin and ferritin; see B).

B. Iron metabolism

Absorption. Iron metabolism is regulated by absorption (p. 390). Iron is present in the diet as protein-bound iron or as free Fe^{3+} ions. The recommended daily iron intake is 10 mg, or 15 mg in women before menopause, but only some 10%–15% of this is absorbed. Only Fe^{2+} ions can be absorbed. For this, a *ferrereductase* on the surface of intestinal epithelial cells reduces the Fe^{3+} to Fe^{2+} . Reducing agents in the diet such as ascorbate (p. 394) therefore promote iron absorption. In the enterocytes, Fe^{2+} is bound to *mobilferrin* and **ferritin** (details on p. 390). The Fe^{2+} reaches the bloodstream through transporters, where it is oxidized to Fe^{3+} by *ferroxidase* (ceruloplasmin) and then bound by *transferrin*.

Heme-bound iron can be absorbed completely from the small intestine and thus contributes to iron absorption. Several concomitant substances in the diet bind iron irreversibly.

Transport. The β -globulin **transferrin**, with a mass of 80 kDa, serves to transport iron in the blood. The monomeric protein consists of two similar domains, each of which can bind a Fe^{3+} ion very tightly. Normally, only about half of transferrin's binding sites are saturated with iron.

Similar iron transport proteins, known as *lactoferrins* (bottom right) are found in secretions such as saliva, tears and milk. Transferrin and the lactoferrins keep the concentrations of *free* iron in body fluids below $10^{-10} \text{ mol} \cdot \text{L}^{-1}$, as higher iron concentrations are toxic (p. 288). This low level also prevents the multiplication in the body of bacteria that require free iron as a growth factor.

Transferrin and the lactoferrins, like LDL (p. 282f.), are taken up into the cells with the aid of *transferrin receptors* by *receptor-mediated endocytosis* (p. 214).

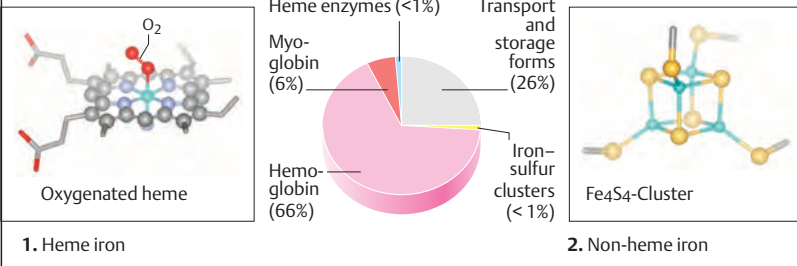
Erythropoiesis. The majority of the absorbed iron is used in the bone marrow to make red blood cells. The Fe^{2+} is incorporated by *ferrochelatase* in the previously synthesized tetrapyrrole scaffolding only in the final step of heme biosynthesis (p. 188).

Recycling. As much as 2.5 to 3 g of iron is bound to hemoglobin (*heme iron*) and circulates in the blood as a component of erythrocytes. However, over time the erythrocyte ages and its flexibility diminishes owing to damage of the membrane lipids and cytoskeleton. Aged erythrocytes are taken up by macrophages of the spleen and other organs and degraded after about 120 days. The organic part of the heme is oxidized to bilirubin (p. 190), while the iron passes back into the plasma pool. The amount of iron obtained daily from heme degradation far exceeds the amount absorbed from the intestine.

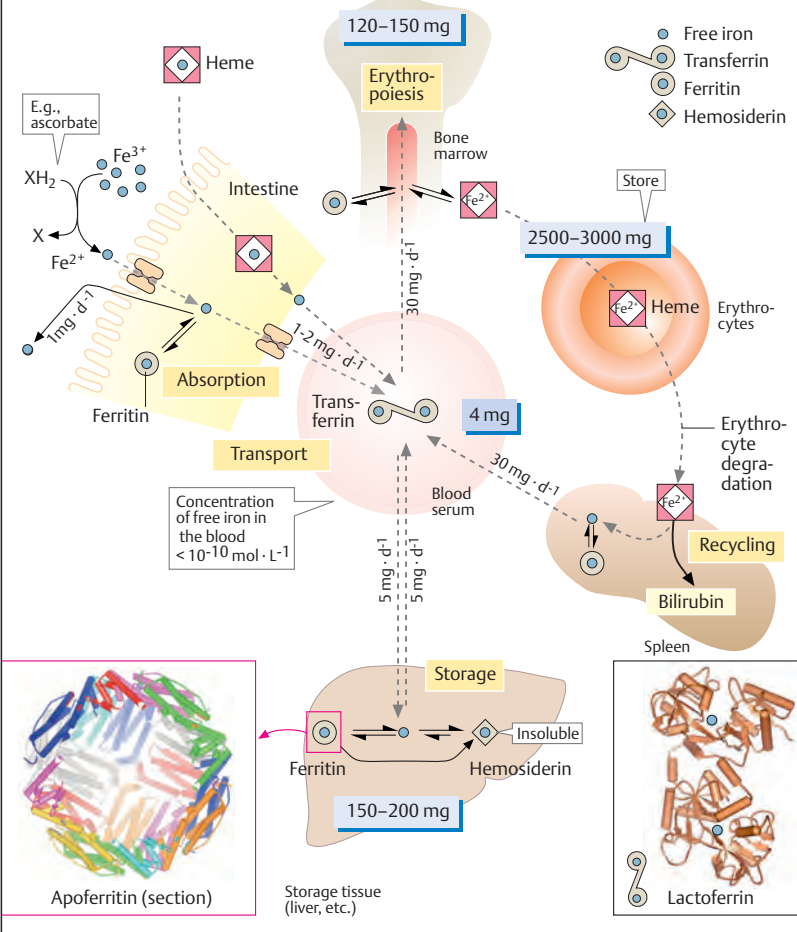
Storage. Excess iron is incorporated into ferritin and stored in this form in the liver, spleen, and bone marrow. The **apoferritin** molecule consists of 24 subunits and has the shape of a hollow sphere. Apoferritin (bottom left) takes up Fe^{2+} ions, which are oxidized to Fe^{3+} and then deposited inside the sphere in the form of *ferrihydrate*. In this way, each ferritin molecule can store several thousand iron ions. Excess iron is passed on by ferritin to **hemosiderin**, another storage molecule, from which it can be mobilized with difficulty.

Excretion (not shown). Iron is excreted from the body via the feces (bile, intestinal cells) and also in urine and sweat and by skin shedding. When open wounds bleed, the body loses about 0.6 mg iron with each milliliter of blood. This is why women of reproductive age have increased iron requirements because of their regular menstrual bleeding.

A. Distribution of iron



B. Iron metabolism



Pathobiochemistry

Diseases due to malnutrition are common. *Undernutrition* continues to be a global problem and the cause of many secondary diseases. In developed countries, however, it is not *malnutrition* but rather *overnutrition* (p. 332) that is the greater problem.

A. Deficiency diseases

There is a range of known mineral deficiency diseases.

Calcium deficiency can result in *osteoporosis* (p. 356), *rickets* in children and *osteomalacia* in adults. The central cause of all three diseases is a disorder of the hormone system or vitamin metabolism.

Selenium deficiency leads to various diseases, all of which are due to lack of selenocysteine (p. 52).

Iodine deficiency is common in Central Europe, where inadequate production of thyroid hormones (p. 426) causes *goiter*.

Fluoride deficiency contributes to the development of *caries* (p. 348).

Menkes disease is a rare hereditary condition in which a copper transport protein (Menkes protein) on the basolateral membrane of mucosal cells is deficient. Copper ions can therefore not be released into the blood. **Copper deficiency** is the result.

Iron deficiency can have different causes. It is usually due to iron loss because of *bleeding*, and more rarely to *inadequate iron supply and absorption*. An *increased iron requirement* during growth and pregnancy can also lead to iron deficiency states. *Vegetarians* are often affected, as a vegetarian diet contains less iron, and iron absorption is often made more difficult by some plant substances. In severe cases of iron deficiency, the diminished hemoglobin synthesis leads to *iron deficiency anemia*. The erythrocytes in these patients are smaller and contain less hemoglobin. As the membrane is also altered, it is eliminated prematurely in the spleen.

B. Iron absorption and hemochromatosis

Disorders due to **increased iron concentrations** are rarer. These conditions, known as hemochromatoses, can have *genetic* causes or may be due to repeated administration of *blood transfusions*. As the body has hardly any means of increasing iron excretion, more and more

stored iron is deposited in the organs over many years in patients with hemochromatosis, mainly **hemosiderin** (p. 388), ultimately leading to severe disturbances of organ function, e.g., *liver cirrhosis* or *diabetes mellitus*. The simplest form of treatment to reduce the iron concentration is *venesection*, as most of the iron is found in the erythrocytes as hemoglobin (p. 388).

Iron absorption in the intestine is complex. Fe^{2+} from the diet produced by enzymatic reduction (p. 388) is transported into intestinal epithelial cells by the *divalent metal transporter (DMT-1)*, where it binds to the transport protein **mobilferrin**. When the iron supply is sufficient, mobilferrin transfers the iron to the storage protein **ferritin** (p. 388). Since the lifespan of enterocytes is only a few days, iron bound in this way in mucosal cells is lost again rapidly in the feces. However, if iron is needed, the iron in the enterocytes is released into the blood by the transporter **ferroportin** working together with the protein **hephaestin**, and it is then bound in the blood to **transferrin**, as described on page 388. Thus, the transport activity of ferroportin determines the rate of iron absorption.

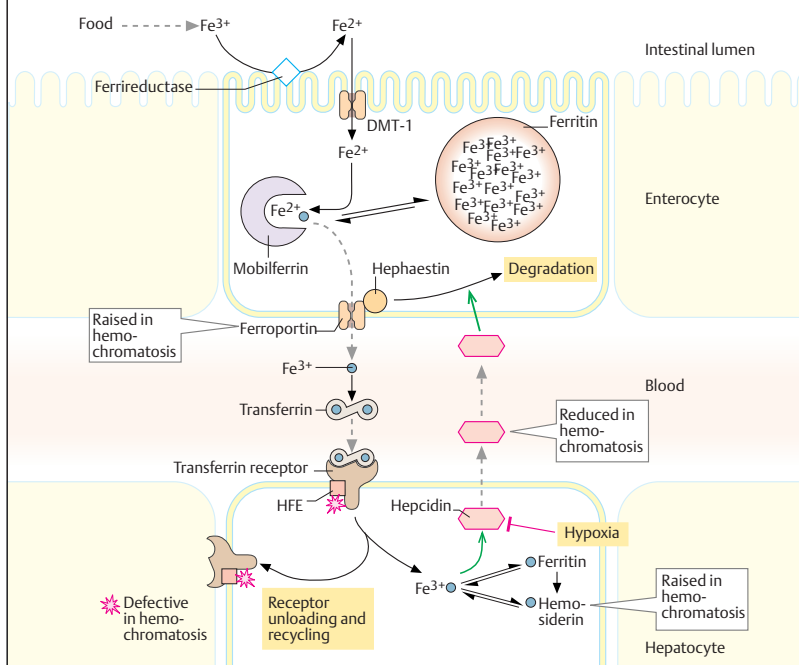
The number of ferroportin molecules in the intestinal cell membrane is regulated by **hepcidin**, a protein produced in the liver and released into the blood when sufficient iron is present. Hepcidin attaches to ferroportin and triggers its internalization and lysosomal *degradation*. Hecpudin thereby reduces iron absorption into the body. *Hypoxia*, on the other hand, inhibits hepcidin secretion. Because of this, more iron is absorbed and additional erythrocytes can be produced.

Hemochromatosis is usually caused by a mutation in the gene for the **HFE** protein, which is involved in the binding and internalization of transferrin by the **transferrin receptor**. The defect in the HFE protein signals iron deficiency to the liver, resulting in a low hepcidin level. This leads to a reduction in binding of hepcidin to ferroportin. As a result more iron than necessary is constantly absorbed.

A. Deficiency diseases

Disease	Molecular cause	Clinical picture
Rickets (in children) Osteomalacia (in adults)	<i>Calcium deficiency</i> caused by absence of vitamin D (calcio) or UV radiation for endogenous calcio synthesis; also resistance to calcio	Bone softening, tooth defects, muscle hypotonia and seizures due to calcium deficiency
Selenium deficiency diseases	In <i>selenium-deficient</i> diet, no synthesis of selenoproteins because of absence of selenocysteine (p. 52)	Juvenile cardiomyopathy, joint cartilage degeneration, neurodegeneration, myopathies
Goiter	Hypertrophy of the thyroid caused by <i>iodine deficiency</i> (p. 426)	Enlargement of the thyroid
Menkes disease	<i>Copper deficiency</i> caused by a deficient copper transport protein in the intestine. Reduced activity of copper enzymes	Depigmentation of the skin, fragile, tortuous hairs
Caries	Chemical lability of dental enamel caused by <i>fluoride deficiency</i>	Tooth decay, demineralization of dentine
Iron deficiency anemia	<i>Iron deficiency</i> because of inadequate dietary intake, because of bleeding, increased iron requirement. Fall in hemoglobin concentration	Reduced energy, fatigue

B. Iron absorption and hemochromatosis



Vitamins I

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

A. Vitamin supply

A healthy diet usually covers average daily vitamin requirements. Another source of vitamins is the intestinal flora, though this production takes place in a region where absorption is poor. Malnutrition, malnourishment (e.g., an unbalanced diet in older people, in alcoholics, ready meals), or absorption disturbances can lead to an inadequate supply of vitamins from which **hypovitaminosis**, or in extreme cases **avitaminosis**, can result. Medical treatments that kill the intestinal flora—e.g., antibiotics—can also lead to vitamin deficiencies (K, B₁₂, H) due to the absence of bacterial vitamin synthesis.

Since only a few vitamins can be stored (A, D, E, B₁₂), a lack of vitamins in the diet quickly leads to **deficiency diseases**. These often affect the skin, blood cells, and nervous system. The causes of vitamin deficiencies can be treated by improving nutrition and by administering vitamins in tablet form. An overdose of vitamins leads to **hypervitaminoses**, with toxic symptoms, only in the case of vitamins A, B₆, and D. Normally, excess vitamins are rapidly excreted in the urine.

B. Lipid-soluble vitamins

Vitamins are classified as either lipid-soluble or water-soluble. The lipid-soluble vitamins include vitamins A, D, E, and K, all of which belong to the isoprenoids (p. 44).

Vitamin A (retinol) is the parent substance of the **retinoids**, which include **retinal** and **retinoic acid**. The retinoids can also be synthesized by enzymatic cleavage from the provitamin β -carotene. Retinoids are found in meat-containing diets, whereas β -carotene occurs in fruits and vegetables (particularly carrots). **Retinal** is involved in visual processes as the pigment of the chromoprotein **rhodopsin**

(p. 368). **Retinoic acid**, like the steroid hormones, influences the transcription of genes in the cell nucleus (p. 419). It acts as a differentiation factor in growth and development processes. Vitamin A deficiency can result in **night blindness**, **visual impairment**, and **growth disturbances**.

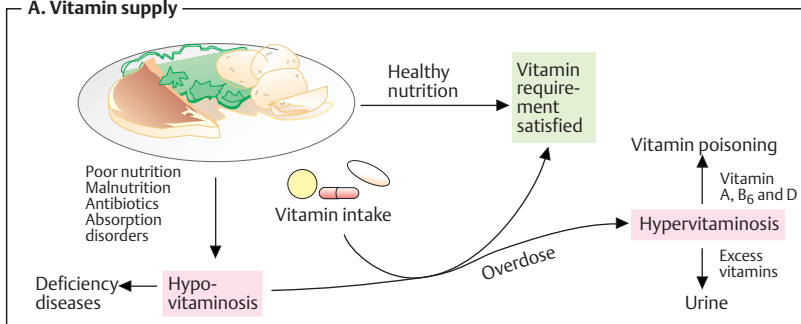
Vitamin D (calcitriol, cholecalciferol) is the precursor of the hormone **calcitriol** (1 α ,25-dihydroxycholecalciferol). Together with two other hormones (parathyroid hormone and calcitonin), calcitriol regulates the calcium metabolism (p. 386). Calcitriol can be synthesized by a photochemical reaction in the skin from 7-dehydrocholesterol, an intermediate of cholesterol biosynthesis (p. 156). Vitamin D deficiencies only occur when the skin receives insufficient exposure to ultraviolet light and vitamin D is lacking in the diet. Deficiency is observed in the form of **rickets** in children and **osteomalacia** in adults. In both cases, bone mineralization is disturbed (p. 348).

Vitamin E (tocopherol) and related compounds only occur in plants (e.g., wheat germ). They contain what is known as a **chroman ring**. Vitamin E is mainly located in the lipid phase of biological membranes, where as an **antioxidant** it protects unsaturated lipids against ROS (p. 288) and other radicals.

Vitamin K (phyloquinone) and similar substances with modified side chains are involved in the γ -carboxylation of glutamate residues of proteins in the liver and osteoblasts (pp. 62, 296). The **coagulation factors II, VII, IX, X, proteins C and S**, and also **osteocalcin** are among the affected proteins. The form that acts as a cofactor for γ -carboxylase is derived from the vitamin by enzymatic reduction.

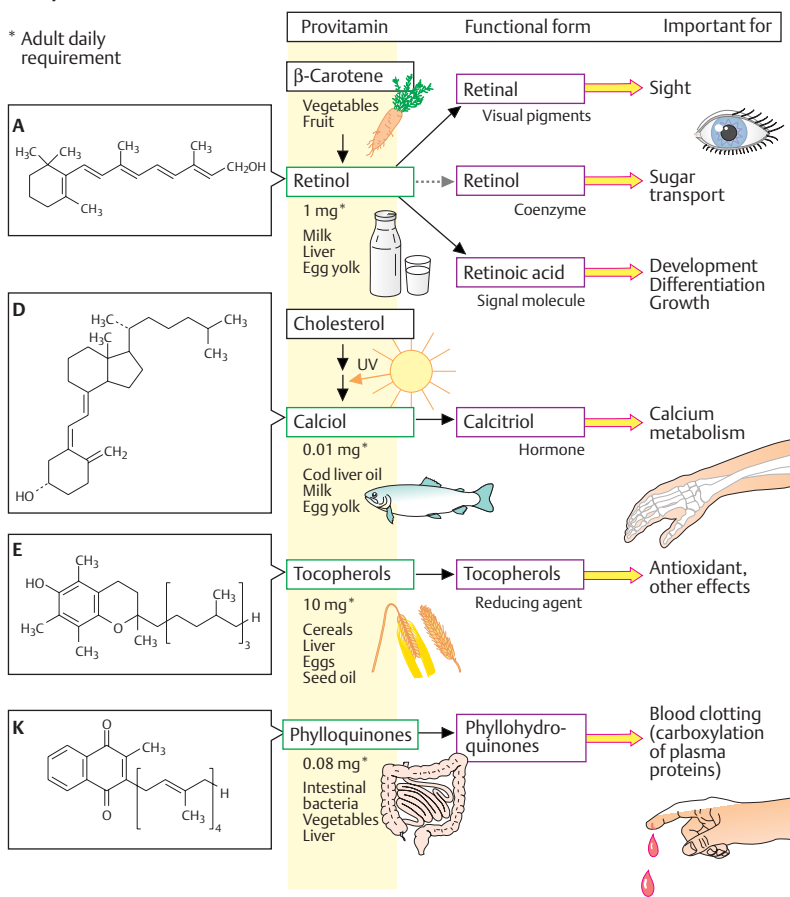
Vitamin K antagonists (e.g., **coumarin** derivatives) inhibit this reduction and consequently carboxylation as well. This fact is used to inhibit blood coagulation in **thrombosis prophylaxis**. Vitamin K deficiency occurs only rarely. The vitamin is formed by bacteria of the intestinal flora. However, as this takes place in the colon in humans, not much can be absorbed.

A. Vitamin supply



B. Lipid-soluble vitamins

* Adult daily requirement



Vitamins II

A. Water-soluble vitamins

The water-soluble vitamins include the B group of vitamins along with vitamins C and H. **Vitamin B₁ (thiamine)** contains a *pyrimidine* and a *thiazole ring*, which are joined by a methylene group. Its active form is **thiamine diphosphate** ("thiamine pyrophosphate," TPP; p. 88), which contributes as a coenzyme to the transfer of hydroxyalkyl residues (active aldehyde groups: oxidative decarboxylation of 2-oxoacids [p. 112] and the transketolase reaction [p. 132]). Thiamine deficiency leads to *beriberi*, a disease with symptoms that include neurological disturbances, cardiac insufficiency, and muscular atrophy.

Vitamin B₂ is a complex of several vitamins.

Riboflavin serves as a component of the redox coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD; p. 86). As *prosthetic groups*, **FMN** and **FAD** are cofactors for various oxidoreductases. No specific vitamin deficiency disease is known.

Folate, the anion of folic acid, is made up of a *pteridine derivative*, *4-aminobenzoate*, and one or more *glutamate* residues (p. 90). After reduction to tetrahydrofolate (**THF**), folate serves as a coenzyme in C1 metabolism. Folate deficiency leads in particular to disturbances in nucleotide biosynthesis and thus cell proliferation (*megaloblastic anemia*).

Nicotinate and **nicotinamide**, ("niacin") are required for biosynthesis of the coenzymes nicotinamide adenine dinucleotide (**NAD⁺**) and nicotinamide adenine dinucleotide phosphate (**NADP⁺**) (pp. 86, 204). The animal organism is able to convert *tryptophan* into nicotinate, but only with a poor yield. Vitamin deficiency manifests in the form of skin damage (*pellagra*), digestive disturbances, and depression.

Pantothenic acid, an acid amide consisting of β -alanine and 2,4-dihydroxy-3,3'-dimethylbutyrate (pantoic acid) is the precursor of *coenzyme A* (pp. 8, 88) and *acyl carrier protein* (ACP; p. 150). Deficiency diseases are rare.

Vitamin B₆ consists of substituted pyridines—**pyridoxal**, **pyridoxol**, or **pyridoxamine**. The illustrated pyridoxal carries an aldehyde group ($-\text{CHO}$) at C-4. Pyridoxol is the corresponding alcohol ($-\text{CH}_2\text{OH}$), and pyridoxamine the amine ($-\text{CH}_2\text{NH}_2$).

The active form of vitamin B₆, **pyridoxal phosphate**, is the most important coenzyme in amino acid metabolism (pp. 88, 166ff.). *Glycogen phosphorylase* (p. 136) also contains pyri-

doxal phosphate as a cofactor. Vitamin B₆ deficiency is rare.

Vitamin B₁₂ (cobalamin) contains a tetrapyrrole system (*corrin*), with cobalt as the central atom (p. 90). It is exclusively synthesized by microorganisms. Cobalamin can only be absorbed in the small intestine when the gastric mucosa secretes what is known as *intrinsic factor*—a glycoprotein that binds cobalamin (the *extrinsic factor*) and protects it from degradation. The liver is able to store vitamin B₁₂ in amounts sufficient to last for several months. Deficiency is usually due to an absence of intrinsic factor and the resulting absence of absorption. This leads to a disturbance in blood formation known as *pernicious anemia*. Derivatives of cobalamin are involved in rearrangement reactions.

Vitamin C is **L-ascorbic acid** (chemically: 2-oxogulonolactone; p. 86). Humans, apes, and guinea pigs require vitamin C because they lack the enzyme *L-gulonolactone oxidase*, which catalyzes the final step in the conversion of glucose into ascorbate. Vitamin C is heat-labile and is particularly abundant in fruit and vegetables. Many soft drinks and foodstuffs also have ascorbic acid added to them as an antioxidant and flavor enhancer. In the body, ascorbic acid serves as a reducing agent (p. 86) in *collagen synthesis*, *tyrosine degradation*, *catecholamine synthesis*, and *bile acid synthesis*. Vitamin deficiency becomes evident in the form of *scurvy*, with connective-tissue damage, bleeding, and tooth loss.

Vitamin H (biotin) (p. 88) is covalently attached via its side chain to lysine residues of enzymes that catalyze carboxylation reactions. Biotin-dependent carboxylases include *pyruvate carboxylase* (and *acetyl-CoA carboxylase* (p. 150)). Biotin binds with high affinity ($K_d = 10^{-15}$ M) and specificity to *avidin*, a protein found in egg white.

Signaling Systems

Signal Transduction

For a multicellular organism to be capable of life, its cells must be able to communicate with one another. This is achieved mainly with the assistance of extracellular **signaling molecules**, which can act over great or short distances by binding to **receptors**. Most cells are able both to send out and to receive signals.

The mechanisms of signal transduction in organisms follow shared principles and the involved molecules form families that have a degree of kinship. The principles of intracellular signaling were obviously established early in the course of evolution.

A. Signal transduction

There are hundreds of different signals that can affect cellular activity and they can be very different in nature. *Hormones, neurotransmitters, mediators, odorants, flavors, metabolites, and membrane components of other cells are chemical signals.* Specialized cells can also perceive **physical signals**, e.g., *light, electrical impulses, or mechanical stimuli.* Perception of light, for example, enables animals to see (p. 368). Mechanoreception is involved in hearing and pressure regulation. Ion channels that react to action potentials (p. 360) are receptors for electrical impulses.

The great majority of signaling substances are **hydrophilic molecules**. They bind to **receptor proteins** (p. 400) on the plasma membrane of their target cells. This activates a signal pathway in which various **intracellular signaling proteins** take part. These ultimately regulate the activity of **effector proteins**, thereby altering the behavior of the cell. Effector proteins can be *transcription factors, metabolic enzymes, components of the cytoskeleton, or ion channels.* While regulation of the cells by altering their gene expression takes hours to become effective, the adjustment of metabolism and the control of motions and ion concentrations proceed much faster (in seconds to minutes).

Small **lipophilic signaling molecules** (left) can penetrate into the target cells and bind there to intracellular receptors which are usually ligand-gated **transcription factors** (p. 418).

The components of signal transduction chains are linked together in a variety of ways. This allows *amplification, splitting of the signal, formation of networks, offsetting of several signals, positive and negative feedback, adaptation to signal strength, and switching-off the signal.*

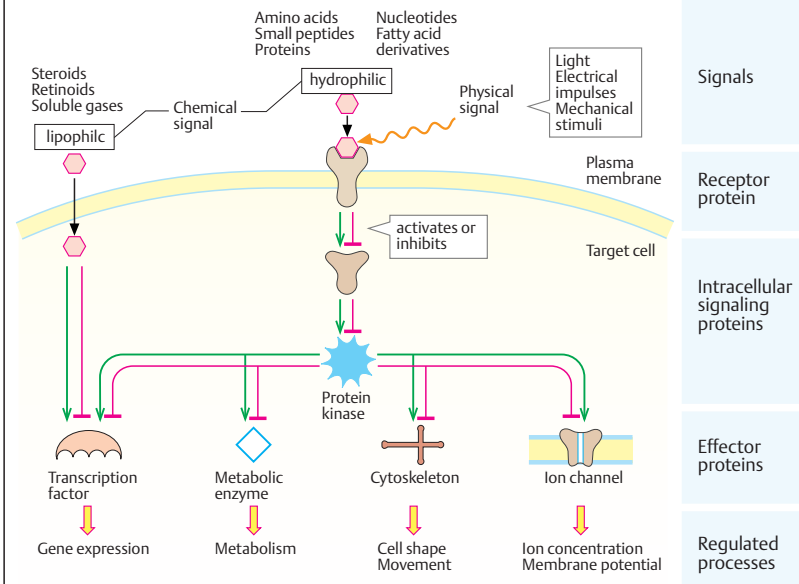
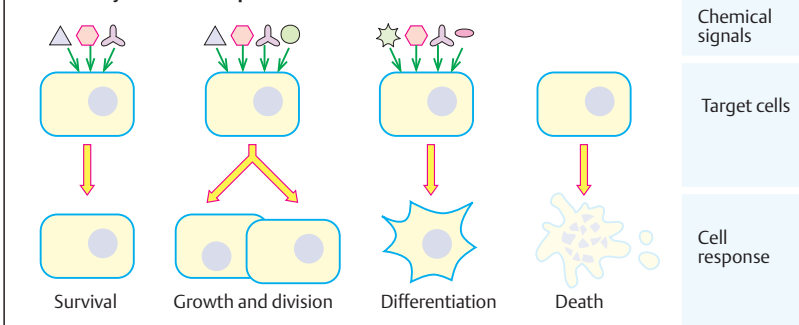
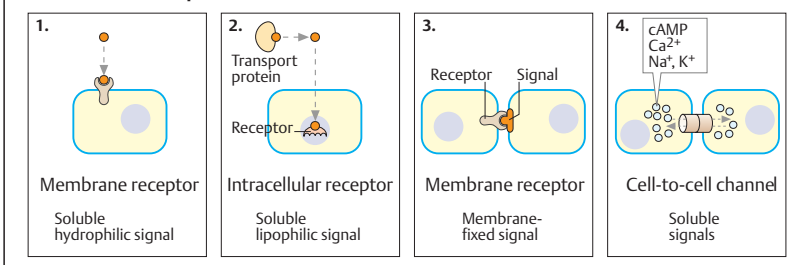
B. Diversity of the cell response

Cells can only react to a signal if they possess the appropriate receptor. They are then "**target cells**". However, the response to the signal can be very different depending on the cell type. For example, a liver cell responds to the hormone epinephrine (p. 434) with an increase in glycogenolysis, whereas an adipocyte responds with an increase in lipolysis, and a cardiac muscle cell with an increase in the frequency of contraction.

Moreover, the cells are exposed to a large number of different signals simultaneously, which can influence one another. Cells can respond to a given signaling environment in a very differentiated way, which is typical for each cell type. It can range from *survival, through growth, division and differentiation, to cell death by apoptosis.*

C. Communication possibilities

The most frequent form of communication through signals involves **membrane receptors**, as most signaling molecules are polar and cannot penetrate the cell membrane (1). Small lipophilic signals such as steroids, retinoic acid and the gases NO and CO, on the other hand, exert their effect through **intracellular receptors**, as they can penetrate the cell membrane more easily (2). In immune system communication (p. 306) and in developmental processes, the signal molecules are often firmly bound to a cell membrane. A **direct contact** between the signaling cell and the target cell is then necessary (3). Finally, cells can also exchange small signaling molecules such as cAMP and inorganic ions through **gap junctions** (4). These junctions are water filled channels made up from the protein *connexin*.

A. Signal transduction**B. Diversity of the cell response****C. Communication possibilities**

Membrane Receptors

To receive and transmit chemical or physical signals, cells are equipped with **receptor proteins**. Most of these are integrated in the plasma membrane, where they receive signals from their surroundings and transmit them to the interior of the cell. Other receptor proteins are located in intercellular membranes. By contrast, receptors for lipophilic hormones are mostly located in the nucleus or sometimes in the cytoplasm (p. 418).

In the human genome, there are more than 1500 genes for receptors.

A. Membrane receptors

Cell-surface receptors transmit signals into the interior of the target cell. In this group there are three different types of receptor.

1. Ion channel-coupled receptors (*ligand-gated ion channels, ionotropic receptors*, p. 402). They occur at synapses of electrically excitable nerve and muscle cells and allow very fast signal transduction. Their ligands are usually *neurotransmitters* (p. 362).

2. G-protein-coupled receptors (*GPCR; 7-helix membrane receptors, serpentine receptors*). These control membrane enzymes or ion channels indirectly. Their effect is transmitted by trimeric GTP-binding proteins (*G-proteins*, p. 404).

3. Enzyme-coupled receptors (*1-helix membrane receptors*). These are either *ligand-activated enzymes* themselves or they attach to an enzyme and activate it. Receptors of this type bind the ligand on the outside of the membrane, which activates a catalytic center on the inside. Usually, this is a *protein kinase* (p. 410), which then phosphorylates and thus controls the receptor itself and also other proteins (**3a**). Phosphorylated protein domains are often binding sites for special *adapter proteins*, which can trigger a signal cascade (**3b**; p. 412).

Mechanism of receptor action. Membrane receptors are made up from several domains with different functions. The **receptor domain** on the outside of the cell contains binding sites that are complementary to the *ligand* which is bound with *high specificity* and *high affinity* ($K_d \leq 10^{-8} \text{ mol} \cdot \text{L}^{-1}$).

As the **effector domain** on the inside is usually separated from the receptor part by a membrane, a mechanism for *signal transfer* between the domains is needed. *Conformational changes* induced by ligand-binding play a crucial part here. Some receptors *dimerize* after

binding of the ligand, thereby bringing the effector domains into mutual contact (p. 440).

B. G-protein-coupled receptor

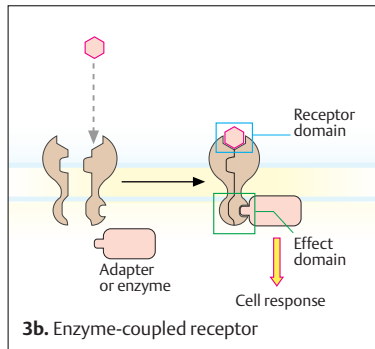
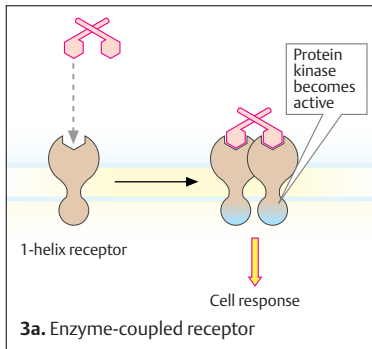
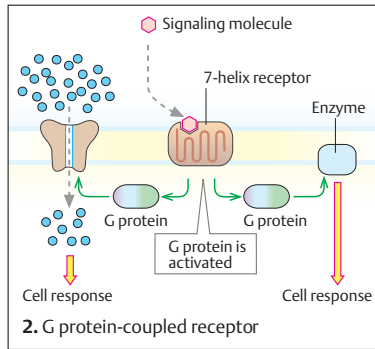
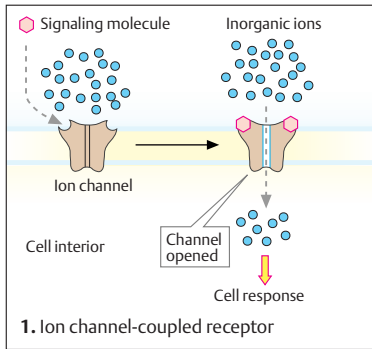
A large group of receptors, known as GPCRs, act through G-proteins (p. 404). They span the membrane with α -helices seven times (*7-helix receptors*). After activation by ligands, they bind and activate G-proteins on the inside of the cell, which then exchange bound GDP for GTP. This converts the G-proteins to the active state. The activated G-protein then binds to an ion channel or, more frequently, to enzymes which generate secondary signaling molecules (**second messengers**; p. 406ff.).

G-proteins can have an activating or inhibitory effect, depending on their type. The illustration shows the complex of the light receptor **rhodopsin** (red), with the associated G-protein **transducin** (green-blue-purple). Both proteins play a part in the visual process (p. 368). The GTP-binding α -subunit (green) and the γ -subunit (violet) of transducin are anchored in the membrane via lipids (p. 210).

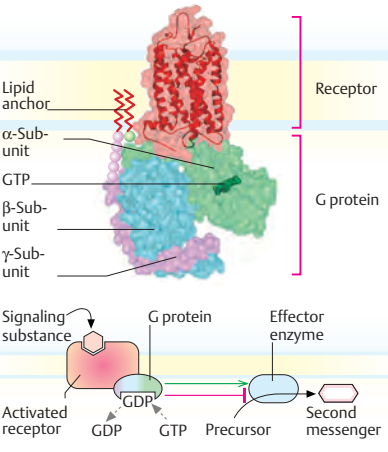
C. Enzyme-coupled receptor

The receptor for the hormone insulin (p. 428) belongs to the family of **enzyme-coupled receptors** (*1-helix receptors*). These proteins span the membrane with only one α -helix. The subunits of the dimeric receptor (red and blue) each consist of two polypeptides (α and β) linked by disulfide bonds. The α -chains together bind insulin, while the β -chains contain the transmembrane helix and, at the C-terminus, domains with **tyrosine kinase** activity. In the activated state, the *receptor tyrosine kinases* (RTKs) phosphorylate themselves and mediator proteins (*receptor substrates*) that set in motion cascades of further phosphorylations (p. 428).

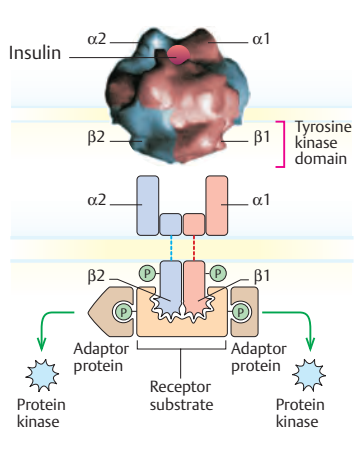
A. Membrane receptors



B. G-protein-coupled receptor



C. Enzyme-coupled receptor



Ion Channels

A. General description

The passage of inorganic ions through membranes is made possible by **ion channels**, which permit passive transport, and **ion pumps**, which move ions against a concentration gradient, usually with consumption of ATP (p. 210f.).

Ion channels are *membrane proteins* that allow inorganic ions to pass rapidly through the cell membranes (*facilitated diffusion*, p. 210). They are classified according to the ion for which they are preferentially permeable. The most important ions for which there are ion channels are the cations **Na⁺**, **K⁺**, and **Ca²⁺** and the anion **Cl⁻**. Ion channels for H⁺ are rare and Mg²⁺ ions usually employs ion channels for other ions. Other *classification criteria* for ion channels are the nature of the *stimulus* responsible for opening the ion channel, its *function*, and its *pharmacological properties*.

In its center, the ion channel has a water-filled **pore** that allows the passage of ions. In the resting state, ion channels are closed (bottom). They open only rarely for a few milliseconds. On average, only about 0.1% of ion channels are open at a given moment. Opening of the pore is usually—but not always—regulated. Depending on the ion channel type, it is triggered either by a change in voltage at the membrane (**“voltage-gated” ion channels**), by ligands (**“ligand-gated” ion channels**), by mechanical stretching, or by a temperature change. The variety of ligand-gated ion channels is particularly great. Their ligands are often neurotransmitters (p. 362) or second messengers (p. 406f.).

The transported ions diffuse *passively* through the ion channels. When the channel is open, ion flow is driven by the ion's *concentration difference* and the *electrical voltage of the membrane*. The balance of this **electrochemical gradient** determines the direction and influences the speed of ion flow. Up to 10⁷ ions per second can pass a single channel, which corresponds to an electrical current of 0.5 to 10 picoamperes. Despite this high permeability, ion channels are usually very *selective*. For example, a K⁺ channel lets through only 1 Na⁺ ion per 10 000 K⁺ ions. This selectivity is achieved by the fact that the inside of the pore is very narrow and specifically interacts with the ion (see D).

B. Composition

The ion channels are typical *membrane proteins* (p. 212), which are located in the cytoplasmic membrane and in the membranes of some organelles. They have a symmetrical ring-shaped structure and consist of four, five, or six structurally similar domains, which are either repeated units of a single protein or separate proteins. Each domain contains two to six transmembrane helices.

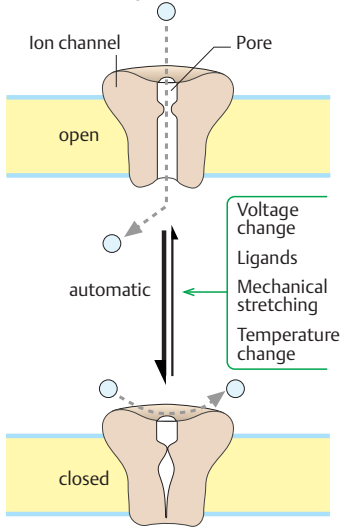
C. Nicotinic ACh receptor

Many receptors for neurotransmitters function as ligand-gated channels for Na⁺, K⁺, and/or Ca²⁺ ions. The nicotinic receptors for **acetylcholine** (ACh, p. 364) are known in greatest detail. They consist of five separate but structurally closely-related subunits. Each forms four transmembrane helices, and the second of each of them is involved in the central pore. In the motor endplate (p. 342), the subunit order αβγαδ is found. ACh binds to the α subunits in the interior of the structure, thereby opening the pore for a short time (1–2 ms). In it, negatively charged residues are arranged in a ring in three groups. They are responsible for the ion specificity of the channel. It is assumed that binding the neurotransmitter alters the position of the subunits in such a way that the pore widens.

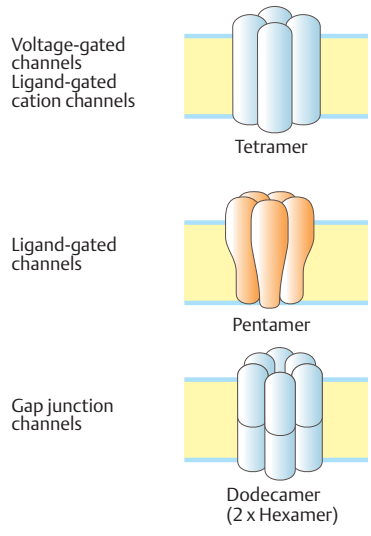
D. K⁺ channel

The illustration shows the tetrameric structure of a K⁺ channel from the bacterium *Streptomyces lividans*. Different α-helices can be identified on the side view of the subunits. An outer helix creates contact with the lipids of the membrane, an inner helix forms the internal space of the channel, and another is involved in formation of the pore. The selectivity of the ion channel is guaranteed by a *selectivity filter*. Here the K⁺ ions interact with several carboxyl groups of the peptide chain without a hydrated shell. Negative charges at the inlet and outlet of the channel attract K⁺ ions.

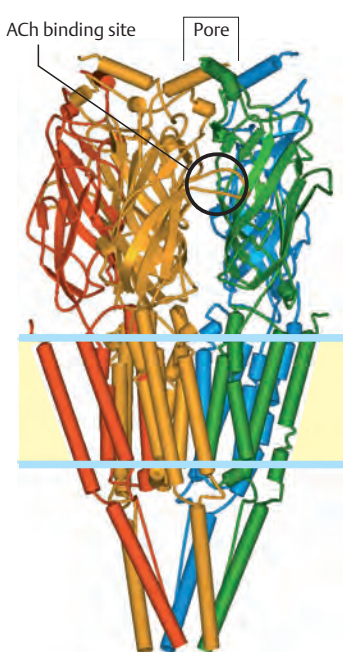
A. General description



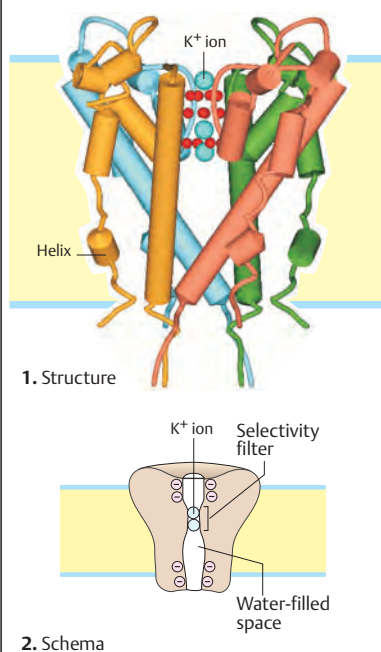
B. Composition



C. Nicotinic ACh receptor



D. K⁺ channel



GTP-Binding Proteins

GTP-binding proteins function as molecular switches. They can be divided into two groups. The large, **heterotrimeric GTP-binding proteins** ("G-proteins") transmit signals from *G-protein-coupled receptors* (GPCRs; p. 400). Small **monomeric GTPases** are involved in the signal transduction of other cell surface receptors. These include *Ras*, *Ras-like* GTPases and members of the *Rho* family. Both forms of GTP-binding proteins are also found as regulatory elements for cellular transport, translation, and cell division.

A. Activation and inactivation of GTP-binding proteins

When they have bound GTP, the GTP-binding proteins are active and transmit signals. As they possess an intrinsic *GTPase* activity, they hydrolyze the bound GTP slowly to GDP, thus switching themselves off after some time. Proteins in a complex with GDP then return to the resting state. The *GTPase* activity of G-proteins is enhanced by **GTPase-activating proteins** (GAPs; also **RGS** for *regulator of G-protein signaling*). To be switched on again, the GTP-binding proteins have to exchange their GDP for GTP from the cytoplasm. This is aided by **guanine nucleotide exchange factors**.

B. Signal transduction by G-proteins

Large **G-proteins** transfer signals from **GPCRs** (*7-helix receptors*) to membrane enzymes or ion channels (p. 400). They are heterotrimers consisting of three different subunits (α , β , and γ). The α -subunit can bind GDP or GTP (hence the name "G-protein") and has *GTPase* activity.

1. In the resting state, G-proteins contain a GDP molecule. When a **GPCR** is stimulated by binding of a **ligand**, the receptor changes its conformation in such a way that the corresponding G-protein can attach on the inside. This causes the α -subunit of the G-protein to exchange the bound **GDP** for **GTP**. The G-protein then separates from the receptor and dissociates into the α and $\beta\gamma$ subunits (the β and γ subunits remain connected to one another). Further G-proteins can be activated as long as the receptor is carrying its ligand.

2. The components of the G-protein bind to other membrane proteins and alter their activity: *ion channels* are opened or closed, and *enzymes* are activated or inactivated. For example, in the case of the β_2 *catecholamine receptor*,

the active α -subunit of the G_s -protein, by binding to **adenylate cyclase**, causes enzymatic production of a **second messenger**, **cAMP**. This activates *protein kinase A* (PK-A), which in turn activates or inhibits further proteins by phosphorylation (p. 410).

3. The internal *GTPase* activity of the α -subunit hydrolyzes the bound GTP to GDP within a period of seconds to minutes, and thereby terminates the action of the α -subunit on adenylyl cyclase. Auxiliary proteins are involved in the process (*regulators of G-protein signaling*, RGS = GAP, see A).

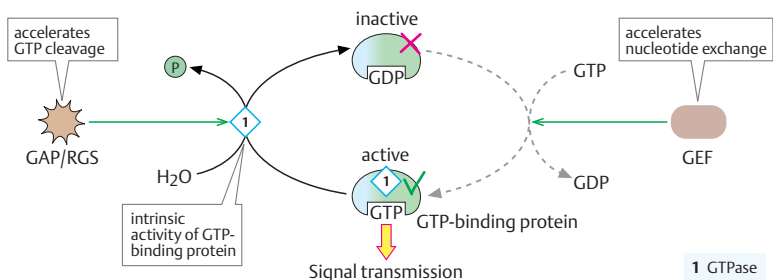
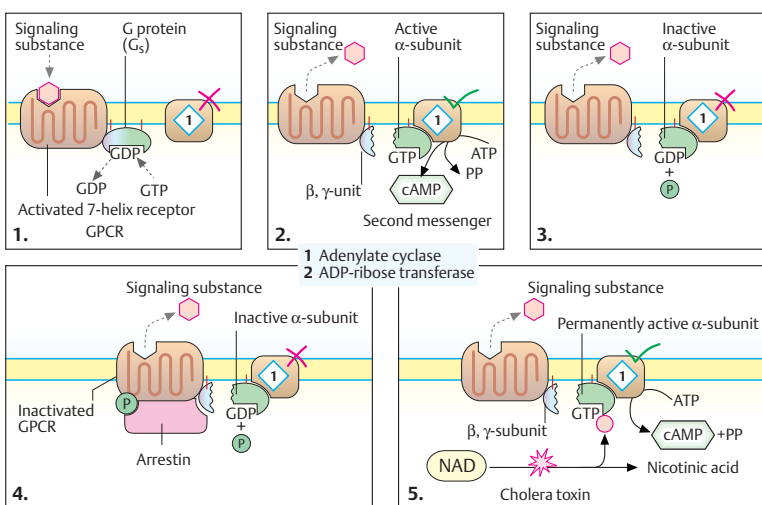
4. The $\beta\gamma$ subunit of the G-protein stimulates a *protein kinase* (β ARK, not shown) to phosphorylate the receptor. This reduces its affinity for the ligand and leads to binding of the blocking protein **arrestin**.

5. G-proteins of the types G_q and G_i are targets for bacterial toxins. **Cholera toxin** is an enzyme produced by the intestinal bacterium *Vibrio cholerae* which penetrates into the cells of the intestinal epithelium. There it transfers an ADP-ribose residue from NAD to the α -subunit of G_q , so that its *GTPase* activity is blocked. The persistently high level of cAMP results in major loss of water and Cl^- ions, causing diarrhea.

Pertussis toxin from the *Bordetella pertussis* bacterium, which causes whooping cough, catalyzes ADP-ribosylation of the α -subunit of G_i (not shown). This prevents interaction with the GPCR and thus the inhibitory action of the G-protein.

C. Effect of trimeric G-proteins

Several types of G-protein can be distinguished according to their mode of action. *Stimulatory G-proteins* (G_s) are widespread. They activate adenylyl cyclases (see below) or influence ion channels. *Inhibitory G-proteins* (G_i) inhibit adenylyl cyclase, while G-proteins of the G_q family activate phospholipase C- β , which hydrolyzes phosphatidylinositol bisphosphate into inositol trisphosphate (InsP_3) and diacylglycerol (DAG) (p. 406).

A. Activation and inactivation of GTP-binding proteins**B. Signal transduction by G-proteins****C. Effect of trimeric G-proteins**

G class	Direct effects	Intracellular effect
G_s	Activates adenylate cyclases and Ca^{2+} channels	cAMP $\uparrow$, $Ca^{2+}\uparrow$
G_{olf}	Activates adenylate cyclases in the olfactory system	cAMP $\uparrow$
G_i	Inhibits some adenylate cyclases and calcium channels, activates cGMP-specific phosphodiesterase, activates inward K^+ channels	cAMP $\downarrow$, $Ca^{2+}\downarrow$, cGMP $\downarrow$, $K^+\uparrow$
G_o	Activates K^+ channels, inactivates Ca^{2+} channels, activates phosphorylase C	$K^+\uparrow$, $Ca^{2+}\downarrow$, InsP $_3$ and DAG $\uparrow$
G_t	Activates cGMP-specific phosphodiesterase	cGMP $\downarrow$
G_q	Activates phospholipase C	InsP $_3$ and DAG $\uparrow$
$G_{12/13}$	Activates phospholipase A_2 and other effectors	Complex effect

Second Messengers I

Second messengers are *intracellular* chemical signals, the concentration of which is regulated by hormones, neurotransmitters, and other extracellular signals (p. 398). They arise from easily available substrates and only have a short half-life. The most important second messengers are cAMP, cGMP, inositol triphosphate (InsP₃), diacylglycerol (DAG), and nitrogen monoxide (NO). They will be discussed on this page and the next.

A. Cyclic AMP

The second messenger cyclic AMP (cAMP) has been known the longest. It was first identified as an intracellular signaling substance in the regulation of glycogen metabolism (p. 136).

Metabolism. The nucleotide **cAMP** (adenosine 3',5'-cyclic monophosphate) is synthesized by membrane-bound **adenylate cyclases** [1] on the inside of the plasma membrane. The **adenylate cyclases** are a family of enzymes that cyclize ATP to cAMP with cleavage of diphosphate (PP_i). The hydrolytic degradation of cAMP to AMP is catalyzed by a **phosphodiesterase** that is specific for cAMP [2]. This enzyme ensures that cAMP is quickly inactivated after biosynthesis. **Methylxanthines** such as caffeine, inhibit phosphodiesterase. By contrast, **insulin** activates the enzyme and so reduces the cAMP level (p. 430).

The concentration of cAMP at any time depends primarily on the activity of **adenylate cyclase**. It is regulated by **G-proteins**, which in turn are controlled by extracellular signals via **G-protein-coupled receptors**. G_s stimulates **adenylate cyclase** while G_i is inhibitory (p. 404). The complex of Ca²⁺ and **calmodulin** (p. 408) also activates specific **adenylate cyclases**.

Mode of action. cAMP is an allosteric effector of **protein kinases of the A type** (PK-A, [3]). It attaches to the regulatory subunits of the enzyme, where it causes a conformation change, which leads to the release of active enzyme (p. 410). Most effects of cAMP are mediated via PK-A. In olfactory neurons, cAMP also controls ion channels directly.

B. Examples of cAMP-mediated hormone effects

The table lists some hormones, the effects of which are mediated by cAMP.

Like cAMP, **cGMP** also functions as a second messenger. In mammals, it does not have the

same significance as cAMP, but it is involved in vision (p. 368) and in the signal transduction of NO and atrial natriuretic peptide (p. 408).

C. Inositol 1,4,5-trisphosphate and diacylglycerol

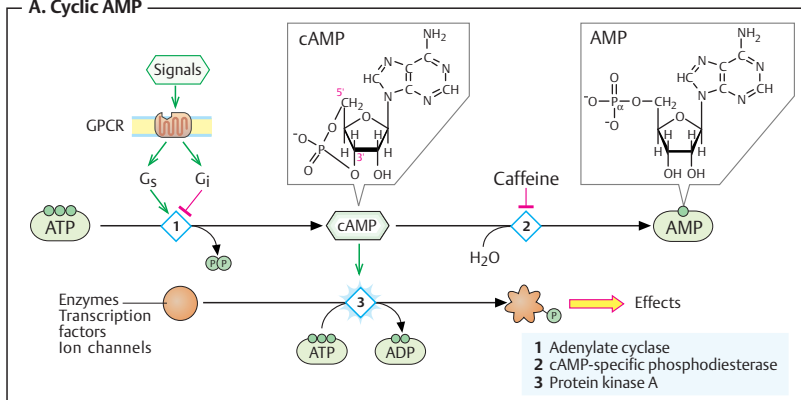
Other second messengers arise not from a nucleotide but from a membrane lipid. For this to happen, G-proteins of type G_q activate membrane-bound **phospholipase C-β** (PLC-β, [4]). This enzyme produces two second messengers by hydrolysis from the double-phosphorylated membrane phospholipid **phosphatidylinositol bisphosphate** (PIInsP₂), i.e., inositol 1,4,5-trisphosphate (InsP₃), which is hydrophilic, and the hydrophobic **diacylglycerol (DAG)**. InsP₃ migrates through the cytosol to the endoplasmic reticulum (ER), where it opens InsP₃-gated Ca²⁺ channels that allow Ca²⁺ to flow into the cytoplasm from the ER reserve (p. 386). This causes the intracellular calcium level to rise abruptly. Ca²⁺ then acts through **calmodulin** as a third messenger or activates enzymes like protein kinase C (p. 408f.). The action of InsP₃ can be terminated by one of the following processes: 1) InsP₃ is dephosphorylated by a specific phosphatase to InsP₂; 2) alternatively, InsP₃ is phosphorylated to InsP₄ by a special kinase; 3) the Ca²⁺ level is reduced again by ion pumps (p. 408).

Unlike InsP₃, DAG, which is strongly lipophilic, remains in the membrane, where it can activate type C **protein kinases** (PK-C, p. 408f.), which pass on the signal by phosphorylating certain proteins in the presence of Ca²⁺ ions. Hydrolysis of DAG can also supply arachidonic acid which is itself a signaling substance and the origin of the eicosanoids (p. 438).

D. Examples of actions mediated by InsP₃ and DAG

The table lists signaling substances that produce InsP₃ and DAG by activating phospholipase C-β.

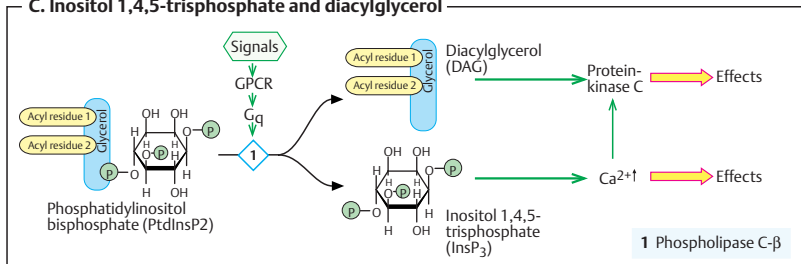
A. Cyclic AMP



B. Examples of cAMP-mediated hormone effects

Hormone	Target tissue	Response
Thyroid stimulating hormone, TSH	Thyroid	Thyroxine synthesis and secretion, p. 426
	Adipose tissue	Hydrolysis of triglycerides, p. 144
Adrenocorticotrophic hormone, ACTH	Adrenal cortex	Cortisol synthesis and secretion, p. 420
Luteinizing hormone, LH	Ovary	Progesterone synthesis and secretion, p. 422
Epinephrine	Muscle	Glycogenolysis, p. 136
	Heart	Heart rate ↑, contractility ↑
	Adipose tissue	Hydrolysis of triglycerides, p. 144
Parathyroid hormone, PTH	Bone	Bone resorption, p. 386
Glucagon	Liver	Glycogenolysis, p. 136
	Adipose tissue	Hydrolysis of triglycerides, p. 144
Vasopressin	Kidney	Water absorption, p. 334

C. Inositol 1,4,5-trisphosphate and diacylglycerol

D. Examples of actions mediated by InsP₃ and DAG

Signal	Target tissue	Response
Acetylcholine	Pancreas	Promotes amylase secretion
	Smooth muscle	Muscle contraction, p. 342
Thrombin	Platelets	Platelet aggregation, p. 294
Vasopressin	Liver	Glycogenolysis, p. 136

Second Messengers II

A. Calcium ions

Calcium level. The Ca^{2+} ion (p. 386) is a signaling substance. Its concentration in the cytoplasm is normally very low (10–100 nM), as it is kept down by ATP-driven Ca^{2+} pumps and $\text{Na}^+/\text{Ca}^{2+}$ exchangers. In addition, many proteins in the cytoplasm and organelles bind calcium and thus act as Ca^{2+} buffers.

Specific signals (e.g., an action potential, mechanical traction, or a second messenger such as InsP_3 or cAMP) can trigger a sudden increase in the cytoplasmic Ca^{2+} level to 500–1000 nM by opening Ca^{2+} channels in the plasma membrane or in the membranes of the *endoplasmic* or *sarcoplasmic reticulum*. One is a calcium channel in the cell membrane, which is opened by change of the membrane potential. It was named “dihydropyridine receptor” [1] because dihydropyridines bind to the receptor and act as antagonists. Another such channel in the ER is called the **ryanodine receptor** [2] since it can be experimentally blocked by *ryanodine*, a plant alkaloid. The physiological ligands for this receptor are other calcium channels or Ca^{2+} itself which thereby amplifies its own release from the ER.

Due to opening of Ca^{2+} channels, the Ca^{2+} level in the cytoplasm, only rises very briefly (Ca^{2+} “spikes”), and Ca^{2+} “oscillations” occur. When the external signal is increased, it is not the height but rather the frequency of the spikes that increases. High Ca^{2+} concentrations in the cytoplasm have cytotoxic effects in the long term.

Calcium effects. The biochemical effects of Ca^{2+} in the cytosol are mediated by special Ca^{2+} -binding proteins (“calcium sensors”), which include *calmodulin*, the *annexins*, and *troponin C* in muscle (p. 342). The most important of these is **calmodulin**, a relatively small protein (17 kDa) that occurs in all animal cells. Binding of four Ca^{2+} ions (light blue) converts it into a versatile **regulatory element**. Via a dramatic conformational change (cf. **2a** and **2b**), Ca^{2+} -calmodulin enters into interaction with other proteins and modulates their properties. Using this mechanism, Ca^{2+} ions regulate the activity of enzymes, ion pumps, and components of the cytoskeleton. In some cases, calmodulin is a fixed component of the regulated protein.

B. Nitrogen monoxide as a mediator

Nitrogen monoxide (**NO**) is a short-lived radical that functions as a locally acting mediator

and second messenger. Neurons use NO more often as a signaling substance. Macrophages and neutrophils produce large amounts of NO in inflammatory processes to kill invading bacteria.

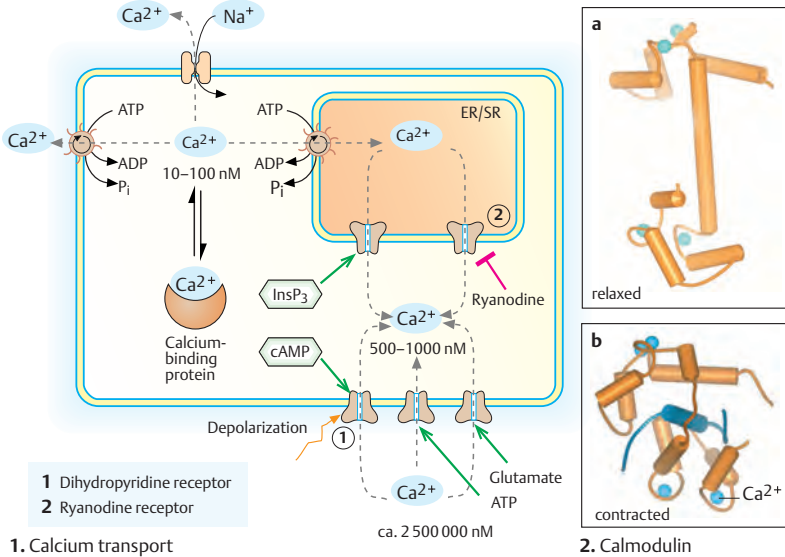
Biosynthesis (left). In a complex reaction, NO arises from arginine in the endothelial cells of the blood vessels [1]. This reaction is catalyzed by *NO synthase* (NOS), of which there are various forms, eNOS in endothelial cells, nNOS in neurons and muscle cells, and iNOS in macrophages. eNOS and nNOS are expressed constitutively and regulated by Ca^{2+} -calmodulin (p. 386), which forms when the Ca^{2+} level increases. By contrast, iNOS is induced only by external signals.

Degradation. The half-life of NO is very short, at 5–10 s. It is inactivated by reaction with oxygen and water.

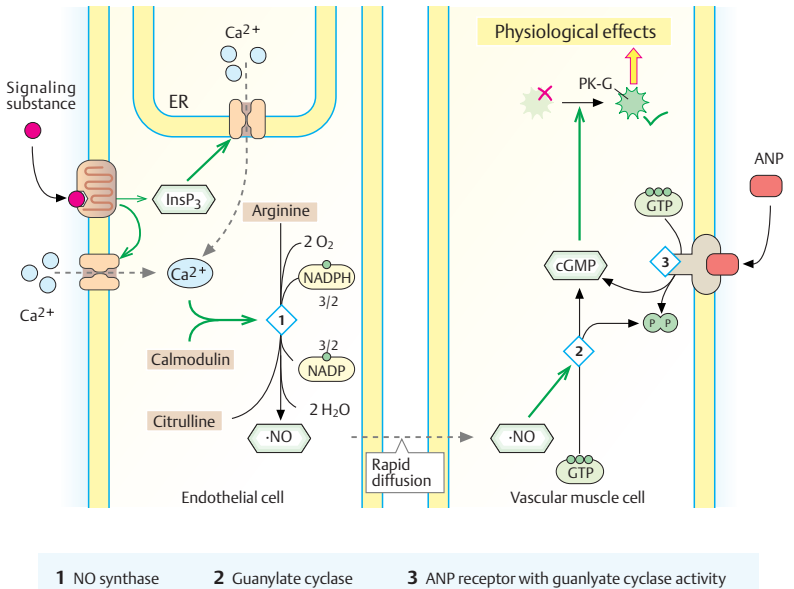
Effects (left). In the vascular endothelium, NO produced by eNOS diffuses into the underlying muscle cells, where it leads, as a result of activation of *guanylate cyclase* [2], to the formation of the second messenger **cGMP** (p. 406). Finally, by activating a special protein kinase (**PK-G**), cGMP triggers relaxation of the smooth muscle and thus dilation of the vessels. The effects of atrial natriuretic peptide (**ANP**; p. 336) in reducing blood pressure are also mediated by cGMP-induced vasodilatation. In this case, cGMP is formed directly by the guanylate cyclase activity [3] of the ANP receptor.

Medical aspects. The drug *nitroglycerin* (glyceryl trinitrate), which is used in the treatment of *angina pectoris*, releases NO in the bloodstream and thereby leads to a reduced work load and better perfusion of cardiac muscle. Erection during sexual arousal is also triggered by local release of NO. The cGMP concentration in the penis is increased by NO, thereby causing relaxation of a muscle, which allows blood flow into the penis. As arousal declines, the cGMP is quickly degraded by *cGMP-specific phosphodiesterase*. *Sildenafil* (Viagra) and related drugs inhibit degradation of cGMP, thus stimulating and maintaining the erection.

A. Calcium ions



B. Nitrogen monoxide as a mediator



Protein Kinases and Phosphatases

A. Protein phosphorylation and dephosphorylation

The most important mechanism for the *interconversion* of proteins (p. 100) is esterification with a phosphate group. **Phosphorylation** is frequently employed for regulating the activity, structure, and location of proteins. The introduction of negative charges with the phosphate residue leads in many cases to a *conformational change* of the target protein. Moreover, the phosphorylated region can become a *new binding site* for other proteins, e.g., molecules with a SH2 domain (p. 412).

Proteins can be phosphorylated on *serine*, *threonine*, and more rarely on *tyrosine* residues. Enzymes that catalyze phosphorylation of these residues with consumption of ATP are called **protein kinases (PKs, [1])**. In the human genome there are over 500 genes for PKs, which can phosphorylate approximately one-third of all proteins.

Removal of the phosphate groups is just as important for the reversible regulation of proteins. This is performed by *protein phosphatases* (or more accurately, **phosphoprotein phosphatases (PPs [2])**, for which there are about 200 different genes in the genome. With PKs and PPs, proteins in the cell can be switched on and off reversibly. The activity of PKs and PPs is therefore strictly regulated.

B. Protein kinase classification (important examples)

Protein kinases are classified according to the type of the phosphorylated residue. Among the PKs, the *serine/threonine-specific PKs* represent the biggest group. They are involved in signal transduction in the cytoplasm and are often named after their activators. The *tyrosine-specific PK* group is much smaller. Some of its representatives are membrane receptors ("*receptor tyrosine kinases*"), which are activated by external ligands. In addition, there are also "*nonreceptor tyrosine kinases*" in the cytoplasm, which are regulated by phosphorylation/dephosphorylation. Some PKs can phosphorylate both Ser/Thr and Tyr ("*PK with double specificity*"). The PPs are classified similarly. PKs can phosphorylate proteins at several amino acid residues. Some PKs phosphorylate themselves (*autophosphorylation*) and thus become permanently activated.

C. Protein kinase A (PK-A)

cAMP-dependent protein kinase (PK-A) has been known the longest. It mediates the effect of the second messenger *cAMP* (p. 406). Enzymes of the PK-A family in the inactive state are heterotetramers (C_3R_1), the *catalytic subunits (C)* of which are blocked by *regulatory units (R)* (autoinhibition). When *cAMP* binds to the regulatory units, the C-units separate from the R-units and become enzymatically active. A total of four *cAMP* molecules are necessary to activate one PK-A molecule completely. Active PK-A phosphorylates the *serine* and *threonine residues* of more than 100 different protein substrates. The activities of enzymes, transcription factors and ion channels are regulated in this way.

Many of the effects of *cAMP* take effect by transcriptional control. Genes regulated by *cAMP* have a **cAMP response element (CRE)** in their promoter region. **CRE-binding protein (CREBP)** can attach to this element when it is phosphorylated by PK-A, and thus activated as a transcription factor (p. 242).

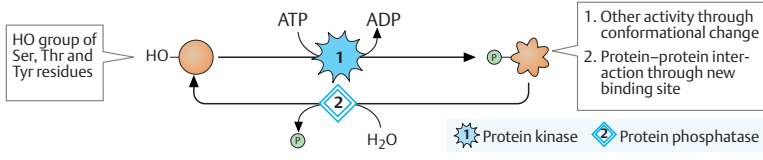
D. Protein kinase C (PK-C)

This PK [4] is *Ca²⁺-dependent*, hence the designation *PK-C*. It is present in the cytoplasm as an inactive enzyme and is activated by the second messengers **InsP₃** and **DAG** (p. 406). This happens when **InsP₃** increases the intracellular Ca^{2+} concentration and DAG acts as a docking site in the membrane. PK-C becomes active only when bound with *Ca²⁺-phospholipid complexes* in the membrane. It has a large number of substrates.

Some PKs are integral membrane proteins, which are activated from the outside by extracellular signals such as insulin or epidermal growth factor ("*receptor tyrosine kinases*"). They are discussed on p. 400.

Since protein kinases can be deregulated, especially in the case of cancer, they are important targets for the pharmacological treatment of tumors.

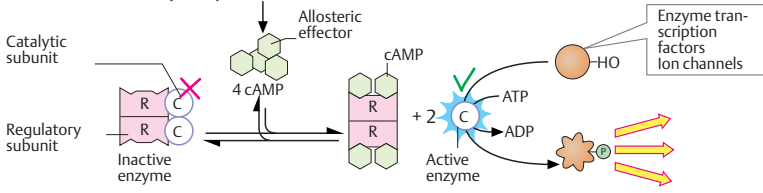
A. Protein phosphorylation and dephosphorylation



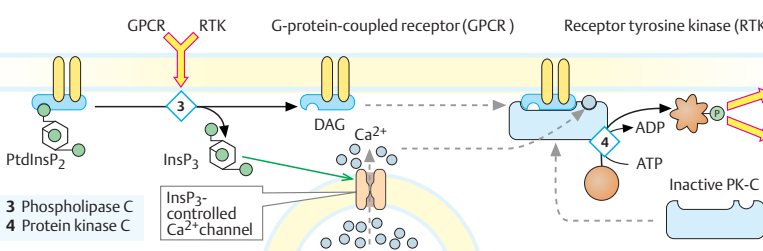
B. Protein kinase classification (important examples)

Name	Activated by
Serine/threonine protein kinases	
Protein kinase A (PK-A)	cAMP
Protein kinase B (PK-B)	PtdInsP ₃ (phosphatidylinositol 3,4,5-trisphosphate)
Protein kinase C (PK-C)	DAG (diacylglycerol)
Ca ²⁺ /calmodulin-dependent protein kinase A (PK-CaM)	Ca ²⁺ /calmodulin, phosphorylation
Protein kinase G (PK-G)	cGMP
AMP-dependent protein kinase A (AMPK)	AMP
Mitogen-activated PK	Phosphorylation
Tyrosine protein kinases	
Insulin receptor	Insulin
Receptor for platelet growth factor	Platelet growth factor
Src kinase	Phosphorylation
Janus kinase	Phosphorylation
Protein kinases with dual specificity	
MEK1	Phosphorylation
Serine/threonine protein phosphatases	
Protein phosphatase 1 (PP-1)	Phosphorylation
Threonine protein phosphatases	

C. Protein kinase A (PK-A)



D. Protein kinase C (PK-C)



Signal Cascades

Signal transduction proceeds as a cascade of reactions connected in series (p. 398).

A. Interaction domains

To bring partners for *protein–protein interaction* together, many signal proteins have **interaction domains**. With these *coupling elements*, signal proteins can bind to specific structural elements of other proteins or lipids. For example, *Src homology 2 (SH2) domains* and *phosphotyrosine-binding (PTB) domains* bind to phosphorylated tyrosine residues of activated receptors and intracellular signaling proteins (top left). By contrast, *Src homology 3 (SH3) domains* bind to short proline-rich peptide sequences (top center). *Pleckstrin homology (PH) domains* attach to charged head groups of *inositol phosphatides* (p. 40), which are formed in the plasma membrane as a result of an extracellular signal. Proteins with this domain therefore associate with the plasma membrane (top right). In addition to the examples given, there are further coupling elements.

When a signal protein consists only of interaction domains, with which it brings other signal proteins together, it is called an **adapter protein**.

The lower part of the illustration shows the composition of two type-C phospholipases (PLCs) and an adapter protein (IRS) as examples of the modular structure of signaling proteins. **PLC-β** is regulated by a G-protein-coupled receptor (GPCR) via G-proteins and it therefore has an interaction domain for *G-proteins* (p. 406). **PLC-γ**, by contrast, is activated by *receptor tyrosine kinases* (p. 410). It therefore has two *SH2 domains*, with which it can bind to phosphotyrosine residues of the activated receptor. *Insulin receptor substrate (IRS)*, p. 428) binds to the phosphotyrosine groups of the activated insulin receptor through the *PTB domain* and, by means of its phosphotyrosine residues, acts itself as a docking site for other signaling proteins.

Proteins that assemble large signal complexes through interaction domains and confer a structure on them are called **scaffolding proteins**. They ensure the specificity of multifunctional enzymes and prevent *cross-talk* between signaling chains.

B. Parallel signaling pathways

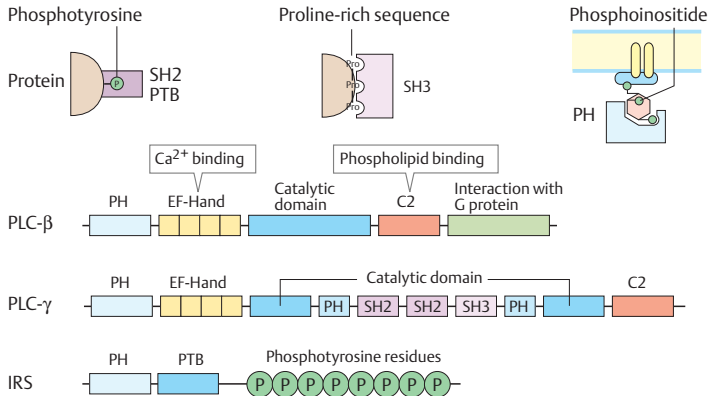
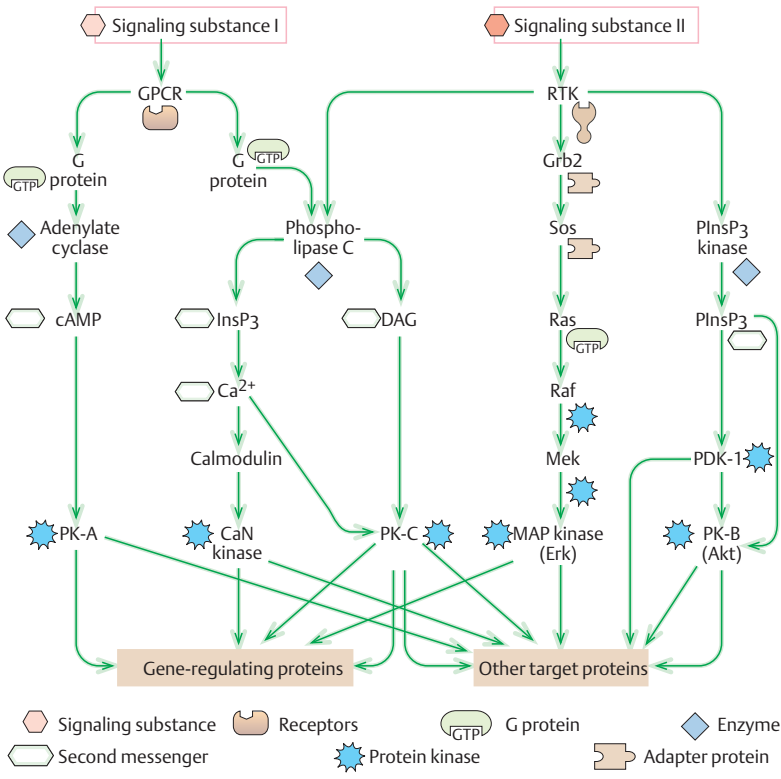
To visualize the variety and interlinking of signaling pathways, the illustration shows five parallel intracellular signaling pathways schematically. They are activated by ligands that act through a G-protein-coupled receptor (GPCR; left) and a receptor tyrosine kinase (RTK; right).

The signaling pathway of **cAMP** is shown on the extreme left (p. 406), and beside it is the pathway of **Ca²⁺/calmodulin**. The pathway via the second messenger **DAG** (p. 406) is shown in the center.

To the right of this, the **MAP kinase pathway** can be seen. This important signal cascade starts on the outside of the cell with the binding of a signaling substance (often a *growth factor*, or a *mitogen*) to a *receptor tyrosine kinase* (RTK). The receptor phosphorylates itself at tyrosine residues. The *adapter protein Grb2* then binds to the phosphotyrosine residues via SH2. With its SH3 domains, Grb2 recruits the *guanine nucleotide exchange factor* (GEF, p. 404) **Sos**, which activates the *small GTPase Ras* by GDP/GTP exchange. This recruits and activates a *protein kinase module*, which consists of three protein kinases (PKs) in series, known as **Raf**, **Mek**, and **Erk**. Erk, the third of the three PKs, is also called **MAP kinase** (*mitogen-activated protein kinase*). This then phosphorylates different proteins, including other PKs and also, after transfer into the nucleus, regulators of transcription involved in *cell division*. There are different isoforms of Ras, Raf, Mek, and Erk so that there are several parallel MAPK pathways.

The pathway on the extreme right proceeds through a *phosphatidylinositol 3-kinase (PIns-3-kinase)*, which phosphorylates membrane lipids instead of proteins. Its substrates are phosphoinositol lipids. The resulting *phosphatidylinositol 3,4,5-trisphosphate (PInsP₃)* is a docking site for signaling proteins with PH interaction domains. A *phosphoinositide-dependent protein-kinase 1 (PDK-1)*, for example, is activated there and this then phosphorylates *protein kinase B (PK-B = act)*.

All of the signaling pathways end by activating specific protein kinases (PK) that then phosphorylate and thereby activate transcription factors and other gene-regulatory proteins as well as further target proteins.

A. Interaction domains**B. Parallel signaling pathways**

Basics

Hormones are *chemical signaling substances* (p. 398). They are synthesized in specialized cells that are often associated to form *endocrine glands*. Hormones are released into the blood and transported with the blood to their *effector organs*. In the organs, the hormones carry out physiological and biochemical regulatory functions. In contrast to these so-called **endocrine hormones**, **tissue hormones** are only active in the immediate vicinity of the cells that secrete them.

The distinctions between hormones and other signaling substances (mediators, neurotransmitters, and growth factors) are fluid. **Mediators** (p. 436) is the term used for signaling substances that do not derive from special hormone-forming cells, but are from many cell types. They have hormone-like effects in their immediate surroundings. *Histamine* (pp. 362, 436) and *prostaglandins* (p. 438) are important examples of these substances. **Neurohormones** and **neurotransmitters** are signaling substances that are produced and released by nerve cells (p. 362). **Growth factors** and **cytokines** mainly promote cell proliferation and cell differentiation (pp. 436, 440).

A. Hormones: overview

The animal organism contains more than 100 hormones and hormone-like substances, which can be classified either according to their structure or according to their function. In chemical terms, most hormones are *amino acid derivatives*, *peptides* or *proteins*, or *steroids*. Hormones regulate the following processes:

- **Growth and differentiation of cells, tissues, and organs**

These processes include cell proliferation, embryonic development, and sexual differentiation—i.e., processes that require a prolonged time period and involve de-novo protein synthesis. For this reason, mainly steroid and thyroid hormones, which function via transcriptional regulation, are active in this field (p. 418).

- **Metabolic pathways**

The regulation of metabolism requires rapidly acting mechanisms. Many of the hormones involved mediate the *interconversion* of enzymes (p. 102). The main processes subject to hormonal regulation are the uptake and degradation of storage substances (glycogen, fat), metabolic pathways for bio-

synthesis and degradation of central metabolites (glucose, fatty acids, etc.), and the supply of metabolic energy.

- **Digestive processes**

Digestive processes are usually regulated by peptides acting locally (paracrine; p. 416), but mediators, biogenic amines, and neuropeptides are also involved.

- **Homeostasis**

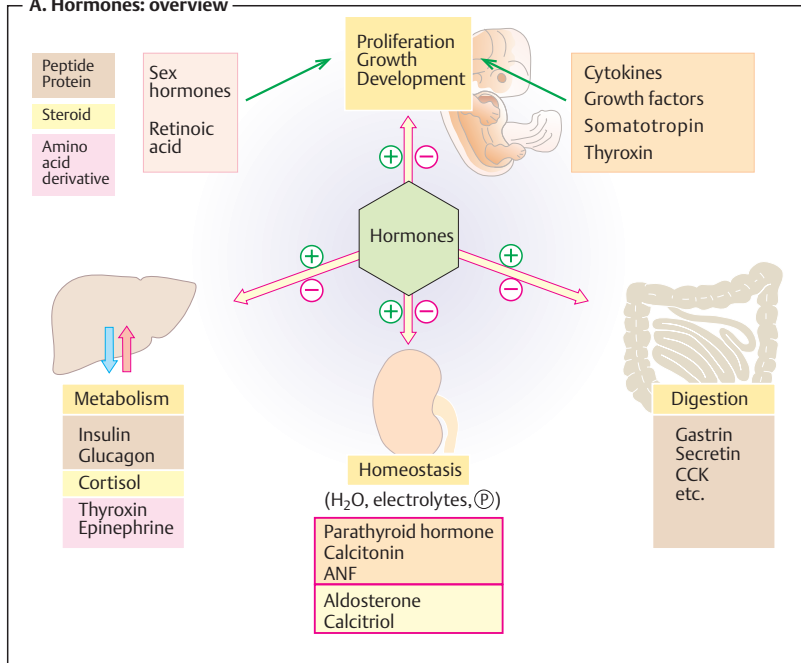
The concentrations of Na^+ , K^+ , Ca^{2+} , and Cl^- in body fluids, and the physiological variables dependent on these (e.g., blood pressure, pulse), are subject to strict regulation. The principal site of action of the hormones involved is the kidneys, where hormones increase or reduce the reabsorption of ions and recovery of water (p. 334ff.). The concentrations of Ca^{2+} and phosphate are also precisely regulated.

Many hormones influence the above processes only indirectly by regulating the synthesis and release of other hormones (*hormonal hierarchy*; p. 416).

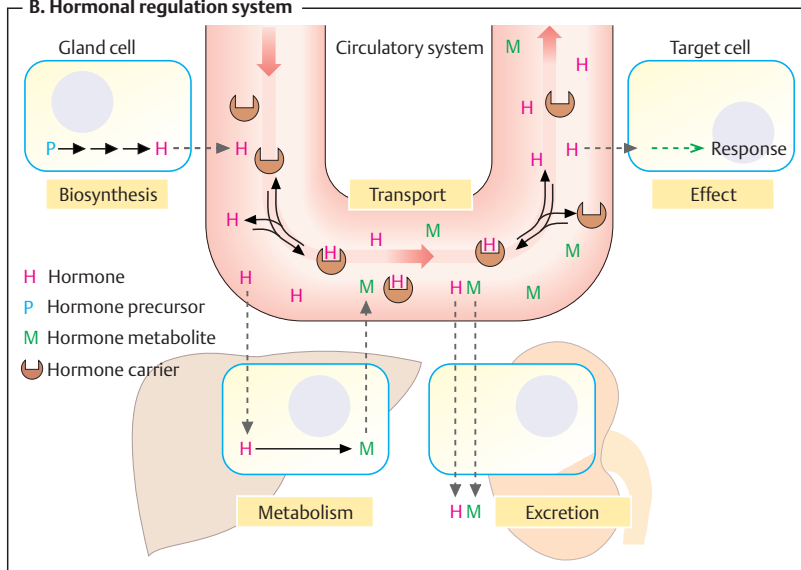
B. Hormonal regulation system

Hormones are at the center of a hormonal regulation system. Specialized glandular cells synthesize them from precursors, store them in many cases, and release them into the bloodstream when needed (**biosynthesis**). For **transport**, the poorly water-soluble lipophilic hormones are bound to plasma proteins known as hormone carriers. To stop the effects of the hormones again, they are inactivated by enzymatic reactions, most of which take place in the liver and bloodstream (**metabolism**). Finally, the hormones and their metabolites are eliminated via the excretory system, usually in the kidney (**excretion**). All of these processes affect the concentration of a hormone and thus contribute to regulation of the hormonal signal. Synthesis and secretion usually have the greatest influence on hormone levels. In the effector organs, target cells receive the hormone's message. These cells have hormone receptors for the purpose, which bind the hormone (pp. 400, 418.). Binding of a hormone passes information to the cell and triggers a response (**effect**).

A. Hormones: overview



B. Hormonal regulation system



Plasma Levels and Hormone Hierarchy

A. Endocrine, paracrine, and autocrine hormone effects

Hormones transfer signals by migrating from their site of synthesis to their site of action. They are usually transported in the blood. In this case, they are said to have an **endocrine effect** (1; example: *insulin*). By contrast, *tissue hormones*—the target cells for which are in the immediate vicinity of the glandular cells that produce them—are said to have a **paracrine effect** (2; example: *gastrointestinal tract hormones*). When signaling substances also act upon the cells that synthesize them, they are said to have an **autocrine effect** (3; example: *prostaglandins*). Autocrine effects are often found in tumor cells, which stimulate their own proliferation in this way (p. 452).

Insulin, which is formed in the B cells of the pancreas, has both endocrine and paracrine effects. As a hormone with endocrine effects, it regulates glucose and fat metabolism (p. 428f.). Via a paracrine mechanism, it inhibits the synthesis and release of *glucagon* from the neighboring A cells.

B. Plasma level dynamics

Hormones circulate as signaling substances in the blood at very low concentrations (10^{-12} to between 10^{-7} mol·L⁻¹). These values change periodically in rhythms that depend on the time of day, month, or year, or on physiological cycles.

The first example shows the **circadian rhythm** of the cortisol level. As an activator of gluconeogenesis (p. 134f.), cortisol is mainly released in the early morning, when the liver's glycogen stores are declining. During the day, the plasma cortisol level declines.

Other hormones are released into the blood in a spasmodic and irregular manner. In this case, their concentrations change in an **episodic** or **pulsatile** fashion. This applies, for instance, to luteinizing hormone (LH, lutropin).

Concentrations of other hormones are **event-regulated**. For example, the body responds to increased blood sugar levels after meals by releasing *insulin* (p. 376). Regulation of hormone synthesis, release, and degradation allows the blood concentrations of hormones to be precisely adjusted. This is based either on simple

feedback control or on hierarchically structured regulatory systems.

C. Regulatory circuit

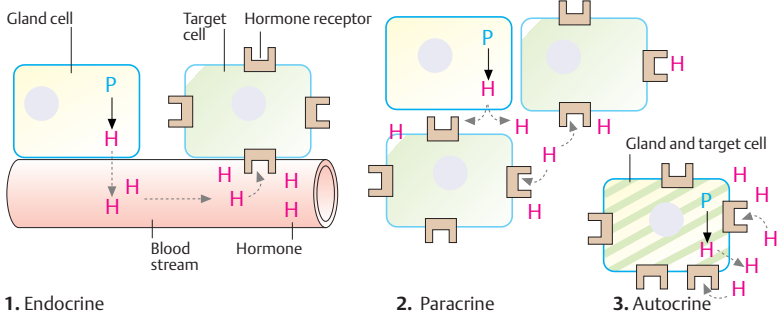
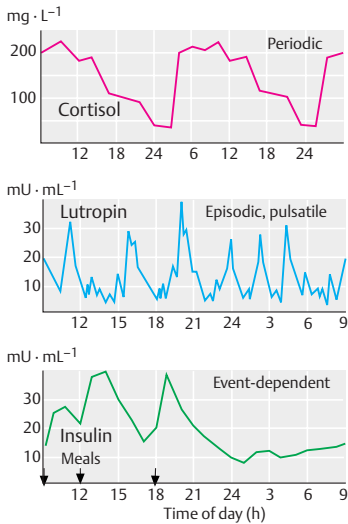
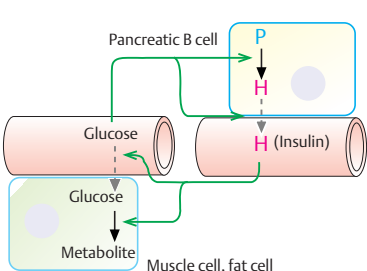
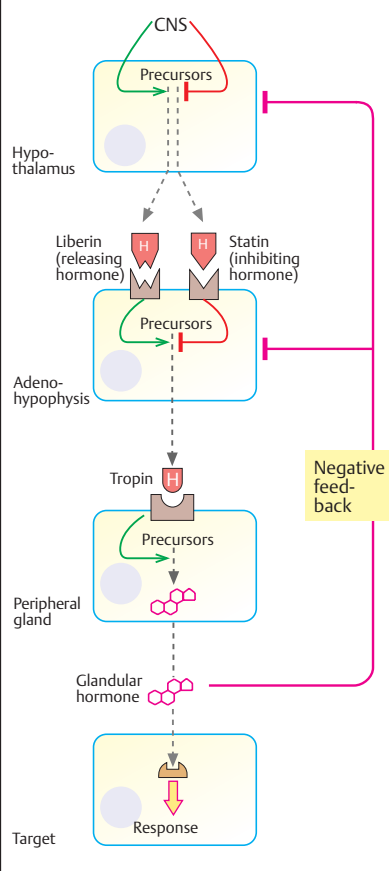
The biosynthesis and release of *insulin* by the pancreatic B cells is stimulated by high blood glucose levels (> 5 mM; p. 376). The insulin released then stimulates increased uptake and utilization of glucose by the cells of the muscle and adipose tissues. As a result, the blood glucose level falls back to its normal value, and further release of insulin stops.

D. Hormone hierarchy

Hormone systems are often linked to each other, giving rise in some cases to a hierarchy of higher-order and lower-order hormones. A particularly important example is the *pituitary-hypothalamic axis*, which is controlled by the central nervous system (CNS).

Nerve cells in the hypothalamus react to stimulatory or inhibitory signals from the CNS by releasing activating or inhibiting factors, which are known as **liberins** ("releasing hormones") and **statins** ("inhibiting hormones"). These neurohormones reach the adenohypophysis by short routes through the bloodstream. In the adenohypophysis, they stimulate (liberins) or inhibit (statins) the biosynthesis and release of tropins. **Tropins** (*glandotropic hormones*) in turn stimulate peripheral glands to synthesize glandular hormones. Finally, the **glandular hormone** acts on its target cells in the organism. In addition, it passes effects back to the higher-order hormone systems. This (usually *negative*) **feedback** influences the concentrations of the higher-order hormones, creating a feedback loop.

Many steroid hormones are regulated by this type of axis—e.g., thyroxine, cortisol, estradiol, progesterone, and testosterone. In the case of the *glucocorticoids*, the hypothalamus releases *corticotropin-releasing hormone* (CRH or corticoliberin, a peptide consisting of 41 amino acids), which in turn releases *corticotropin* (ACTH, 39 amino acid residues) in the pituitary gland. Corticotropin stimulates synthesis and release of the glandular steroid hormone *cortisol* in the adrenal cortex.

A. Endocrine, paracrine, and autocrine hormone effects

B. Plasma level dynamics

C. Regulatory circuit

D. Hormone hierarchy


Mechanism of Action

Lipophilic substances that act in the *cell nucleus*, include the steroid hormones, calcitriol, iodothyronines (T_3 and T_4), retinoic acid, and also certain lipid metabolites (see list in **B**). These signaling substances bind to *intracellular receptors*, which function as *ligand-gated transcription factors* and—supported by other proteins—regulate gene transcription.

A. Mechanism of action of lipophilic signaling substances

Only free signaling substances, i.e., those not bound to transport proteins, in the blood are able to penetrate the plasma membrane and enter the cell. Most lipophilic hormones encounter their **receptors** in the cell nucleus, some already in the cytoplasm. Usually receptors for lipophilic hormones undergo conformational changes after ligand binding and thereby acquire the ability to bind as dimers to *DNA control elements* in the promoters of specific genes, from where they can influence the transcription of the affected genes—i.e., they act as *transcription factors*.

The illustration shows the mechanism of action of **cortisol**, which is unusual in the sense that the ligand–receptor complex already arises in the cytoplasm. The free glucocorticoid receptor (GR) is present in the cytoplasm as a monomer in complex with the chaperone **hsp90** (p. 222). Binding of cortisol to the GR leads to an *allosteric conformational change* in the receptor, which is then released from the hsp90 and becomes capable of *dimerization* and DNA binding.

In the nucleus, the activated dimeric receptor binds to nucleotide sequences known as **hormone response elements** (HREs). These are short palindromic DNA segments that usually promote transcription as enhancer elements (p. 242). The illustration shows the HRE for glucocorticoids (GRE; “n” stands for any nucleotide). Each receptor only recognizes its “own” HRE and therefore only influences the transcription of genes that have the respective HRE in their promoter. Recognition between the receptor and the HRE is based on interactions between amino acid residues in the DNA-binding domain (**C**) of the receptor and the relevant bases in the HRE (emphasized in color in the structure illustrated).

As discussed on page 242, the receptor does not interact directly with RNA polymerase, but rather—along with other transcription factors—with a *coactivator/mediator complex* that processes incoming signals and passes them on to the polymerase. In this way, hormonal effects lead within a period of minutes to hours to altered levels of mRNAs for key proteins in cellular processes (“cellular response”). However, some rapid effects of lipophilic hormones (taking effect in seconds to minutes) are mediated not by gene regulation but by controlling protein kinases and other signal transduction enzymes.

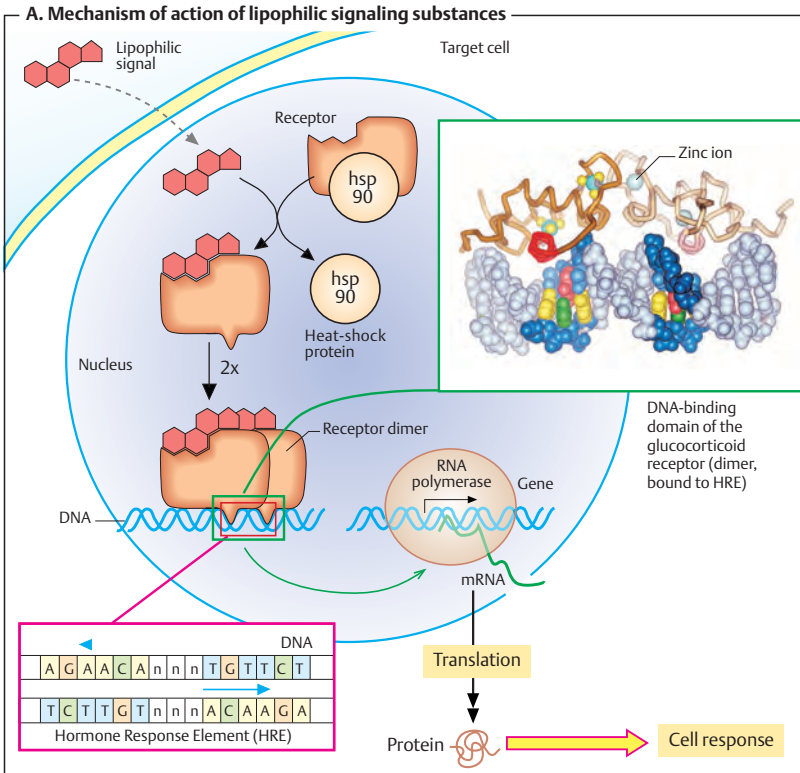
B. Nuclear receptors and their ligands

The receptors for lipophilic signaling substances belong to the *protein superfamily* of transcription factors. The family includes receptors for *lipophilic hormones* and *lipid metabolites*. The table lists important representatives. In the activated state, the receptors form dimers with themselves (homodimers) or with other receptors (heterodimers). Some receptor proteins, for which there is no known ligand, are called “*orphan receptors*.”

C. Modular structure of nuclear receptors

Nuclear receptors are constructed in a modular fashion from **domains** with various lengths and functions. Reading from the N- to the C-terminus, these are: the *transcription-activating domain*, the *DNA-binding domain*, a *nuclear localization sequence* (p. 204), and the *ligand-binding domain*.

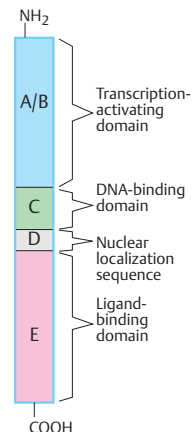
The homology among receptors is particularly great in the area of the DNA-binding domain. The proteins have cysteine-rich sequences here that coordinatively bind zinc ions (**A**, Cys shown in yellow, Zn^{2+} in light blue). These centers, known as “zinc fingers” or “**zinc clusters**,” stabilize the domains and support their dimerization, but do not take part in DNA binding directly. As in other transcription factors (p. 242), “recognition helices” are responsible for that.



B. Nuclear receptors and their ligands

Nuclear receptors for	Acronym	Important ligands
Lipophilic hormones		
Glucocorticoids	GR	Cortisol
Mineralocorticoids	MR	Aldosterone
Androgens	AR	Testosterone
Estrogens	ER	Estradiol
Gestagens	PR	Progesterone
Vitamin D hormone	VDR	Calcitriol
Thyroid hormone	T3R	Triiodothyronine
Retinoic acids	RAR RXR	all-trans-retinoic acid 9-cis-retinoic acid
Lipid metabolites		
Fatty acids and fatty acid derivatives	PPAR	Palmitic acid Prostaglandins
Oxysterols	LXR	Hydroxycholesterol
Bile acids	FXR	Cholic acid
Bile acid conjugates	PXR	Glycocholic acid

C. Modular structure of nuclear receptors



Corticosteroids

The *adrenal cortex* (AC) produces two major types of steroid hormones, *glucocorticoids* and *mineralocorticoids*, but also some androgens.

A. Cortisol

Biosynthesis. The glucocorticoid **cortisol** is produced from cholesterol in the *zona fasciculata* of the AC. *Pregnenolone* and *progesterone* are intermediates (p. 424). Like the other steroid hormones, cortisol is secreted into the blood by exocytosis immediately after synthesis, where it binds to the transport protein *transcortin*.

Glucocorticoid biosynthesis is under the control of **CRH** (corticoliberin) released from the hypothalamus and the tropic hormone **ACTH** (corticotropin) from the *hypothalamic-pituitary axis* (p. 416). It shows a circadian rhythm, with the greatest activity in the early morning. Cortisol can be converted by reversible dehydrogenation to the hormonally inactive **cortisone**, which has an oxo instead of a hydroxyl group at C-11.

Biochemical effects. Cortisol helps the body to adapt to long-term stress and extreme situations. In particular, it allows the body to survive long periods of starvation by stimulating protein degradation (**proteolysis**, p. 162) in extrahepatic tissues, especially muscle, and by promoting conversion of the resulting amino acids to glucose in the liver (**gluconeogenesis**, p. 134). To do this, cortisol induces several key enzymes of amino acid and carbohydrate metabolism in the liver (pp. 138, 372). In adipose tissue, cortisol promotes the effects of other hormones on the release of fatty acids (**lipolysis**, p. 330).

The catabolic effect on extrahepatic **protein metabolism** results in a negative nitrogen balance.

In higher concentrations, glucocorticoids have *immunosuppressive* and *anti-inflammatory* effects, properties which are used pharmacologically. Since cortisol is an antagonist of insulin with regard to glucose metabolism (p. 102), glucocorticoids given therapeutically can also cause *glucose intolerance* and *diabetes mellitus* as unwanted side effects. Persistently high hormone concentrations inhibit the activity of osteoblasts in bone. Corticosteroids also exert a slight mineralocorticoid effect.

B. Aldosterone

The two *mineralocorticoids* produced by the AC are **aldosterone** and the less active **11-deoxycorticosterone**. The aldehyde group at C-18 can form a hemiacetal with the neighboring hydroxyl group at C-11.

Biosynthesis. Like cortisol synthesis, that of aldosterone takes place in the *zona glomerulosa* of the AC with pregnenolone and progesterone as intermediates. After hydroxylations at C-21, C-18, and C-11, then C-18 is oxidized to an aldehyde group. After secretion into the bloodstream, aldosterone is predominantly bound to albumin.

Biosynthesis of aldosterone is stimulated by **angiotensin II**, a central component of the *renin-angiotensin system* (pp. 338, 432) which is triggered by hypovolemia or hyponatremia.

Effects. Aldosterone acts on the proximal and distal *renal tubules* (p. 336). There, aldosterone induces the expression of *sodium channels*, *Na⁺/K⁺-ATPase* and several *metabolic enzymes*. In this way, aldosterone promotes **Na⁺ reabsorption** and increases excretion of K⁺, H⁺, and NH₄⁺. Aldosterone thus causes the Na⁺ level and extracellular volume to increase, so that blood pressure rises. In sweat and salivary glands Na⁺ excretion is also slowed by aldosterone.

Pathobiochemistry. Overproduction of cortisol is the cause of **Cushing syndrome**, which is usually due to microadenomas in the pituitary producing increased amounts of ACTH.

In **Conn syndrome**, aldosterone synthesis is increased. The cause can be a benign adenoma of the adrenal cortex.

By contrast, **Addison syndrome** is characterized by underproduction of cortisol. The cause is usually destruction of adrenocortical cells by an *autoimmune disease*.

The **adrenogenital syndrome** (AGS) a genetic disorder leading to the masculinization of female fetuses, is usually due to mutations in the *21-hydroxylase* gene that block cortisol synthesis (see A). The lack of cortisol stimulates ACTH secretion, so that cortisol precursors accumulate in the AC from which androgens are eventually produced.

Sex Steroids and the Menstrual Cycle

Androgens represent the family of male sex hormones, while **estrogens** (follicular hormones) and **gestagens** (*progestins*; corpus luteum hormones) constitute the family of female sex hormones.

A. Androgens

The most important androgen is **testosterone**. In men, it is produced in the *Leydig cells of the testes*. Small amounts of **5 α -dihydrotestosterone** (DHT) and estradiol are also produced there. The adrenal cortex in both men and women can also synthesize androgens, but these have little biological importance under normal circumstances.

Biosynthesis. The biosynthesis of testosterone proceeds from cholesterol via progesterone (p. 424). It is stimulated in the testis by *luteotropic hormone* (LH), a tropin from the pituitary (p. 416).

DHT is produced from testosterone by reduction with *5 α -reductase* [1]. This takes place both in the testes and in the periphery. Both androgens are bound in the blood to *sex hormone-binding globulin* (SHBG), and partly also to albumin.

Effects. Androgens are necessary for the formation, maintenance and function of the male sex organs, i.e., the development of the genitals, sperm development in the Sertoli cells, function of the accessory glands, and maturation of secondary sex characteristics. Apart from these *genital effects*, androgens also have *extragenital effects*. These include an *anabolic* effect on muscle and bone and a stimulation of biosynthetic metabolism (protein synthesis, N-retention).

B. Estrogens

The most important estrogens are **estradiol** along with *estrone* and *estriol*. Their characteristic feature is the aromatic A-ring and the phenolic hydroxyl group at C-3 (p. 46).

Biosynthesis. In women, estrogens are synthesized jointly by the *theca cells* and *granulosa cells* of the ovarian follicle and in the *corpus luteum*. Small amounts are produced in the adrenal cortex and adipose tissue. Biosynthesis starts with cholesterol, and proceeds via pregnenolone and testosterone (p. 424), which is converted to estradiol by means of *aromatase* [2]. Like testosterone, biosynthesis is stimu-

lated by *luteinizing hormone* (LH). Aromatase activity is promoted by *follicle-stimulating hormone* (FSH).

During transport in the blood, estradiol binds to *albumin* and *SHBG*.

Effects. As with the androgens, the effects of estrogens can be classified as *genital* and *extragenital*. They promote the development and function of tissues involved in reproduction, especially the vagina and uterus. Women's *secondary sex characteristics* are also under the control of estrogens. Together with the gestagens, they are involved in the *menstrual cycle*. Estrogens have an *anabolic effect* on general metabolism: synthesis of fats in the liver and adipose tissue and incorporation of calcium in bone.

C. Gestagens

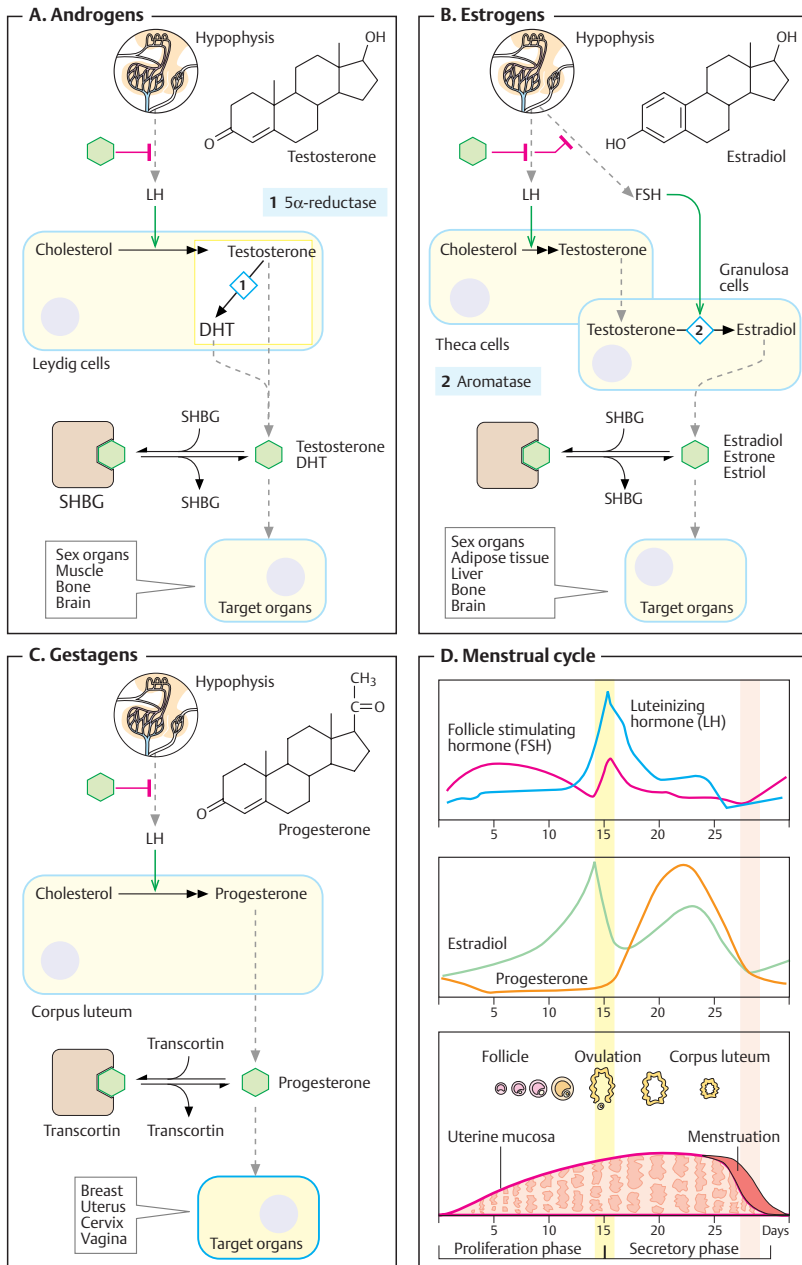
Progesterone is the only natural gestagen in humans.

Biosynthesis. Progesterone is produced from cholesterol under LH control (p. 424), and it is synthesized in the *corpus luteum* of the ovary and in the *placenta* during pregnancy. In the blood, progesterone is bound to **transcortin**.

Effects. Together with the estrogens, progesterone is involved in the *menstrual cycle*. As the pregnancy hormone, it prepares the uterine mucosa for implantation of the fertilized egg and provides for maintenance of the pregnancy. If fertilization does not take place, progesterone synthesis and action fall again by a feedback effect on the hormonal axis (p. 416).

D. Menstrual cycle

The menstrual cycle in women is marked by characteristic concentration profiles of the two steroid hormones **estradiol** and **progesterone** and of the pituitary tropins, **LH** and **FSH**. In the *proliferation phase* (follicular phase), the concentration of estradiol increases. By contrast, the *secretory phase* (luteal phase) is dominated by progesterone. In the *ovulatory phase* around day 15 of the cycle, the secretion of LH and FSH markedly increases. This results in ovulation and the transition of the cycle from the first phase to the second.



Metabolism of Steroid Hormones

A. Biosynthesis of steroid hormones

The steroid hormones are synthesized in steroidogenic glandular cells where they are produced from **cholesterol** in a stepwise fashion. The cholesterol required is obtained from various sources. It is either taken up into the glandular cells as a constituent of low-density lipoproteins (LDL; p. 282f.), or synthesized by glandular cells themselves from acetyl-CoA (p. 156). Excess cholesterol is stored in the form of esters with fatty acids. Hydrolysis allows mobilization of the cholesterol from these again.

Regulation. The biosynthesis of the steroid hormones is stimulated by **gladotropic hormones**, which activate protein kinase A (PK-A) via GPCR, G-proteins, adenylate cyclase, and cAMP (p. 400ff.). PK-A then activates *cholesterol ester hydrolase* and *StAR protein* (Steroidogenic acute regulatory protein), by which cholesterol enters the mitochondria. This initial transfer is the rate-determining step of steroid hormone biosynthesis. Which steroid is then produced depends on the enzymatic equipment of the glandular cells (see B).

In this way, steroids from six **steroid hormone families** are produced in steroid hormone-producing cells. These are the *gestagens* (lead substance: progesterone), the *glucocorticoids* (cortisol), the *mineralocorticoids* (aldosterone), the *androgens* (testosterone), the *estrogens* (estradiol) and the *D hormones* (calcitriol).

B. Reactions

Among the reactions involved, *hydroxylations* are particularly numerous. These are catalyzed by specific *monooxygenases* ("hydroxylases") of the cytochrome P450 family (p. 324). In addition, there are *hydrogenations* and *dehydrogenations*, as well as *cleavage* and *isomerization reactions*. The estrogens have a special place among the steroid hormones, as they are the only ones that contain an aromatic A-ring. This structure is formed by a special "*aromatase*."

Pregnenolone (C_{21}) is the first important intermediate in the biosynthesis of most steroid hormones. It is produced from cholesterol (C_{27}) by two hydroxylations and oxidative cleavage of the side chain. Subsequent dehydrogenation of the hydroxyl group at C-3 and shifting of the double bond from C-5 to C-4 results in **progesterone** (C_{21}).

With the exception of calcitriol, all steroid hormones are derived from pregnenolone or pro-

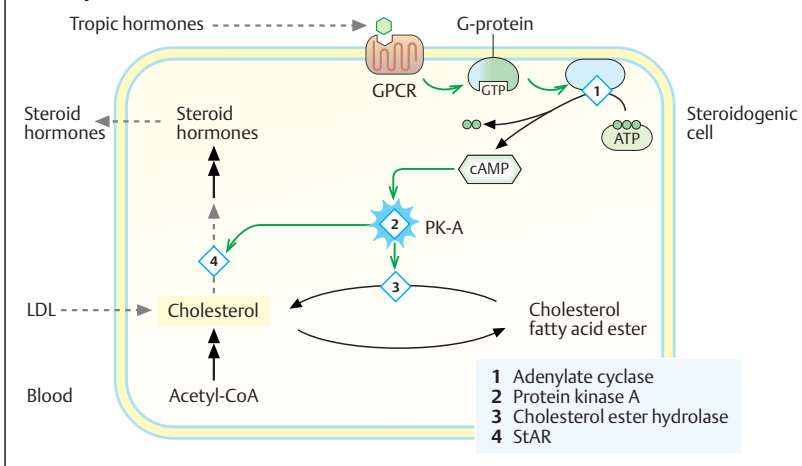
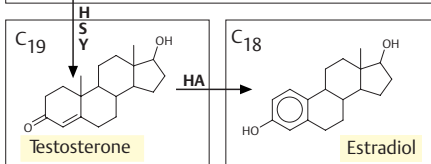
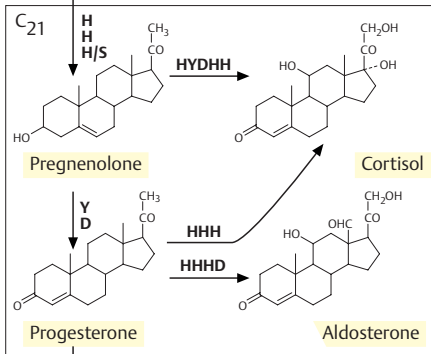
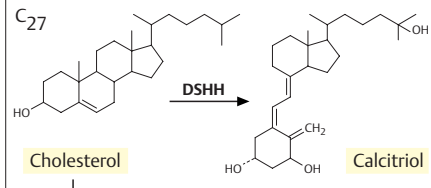
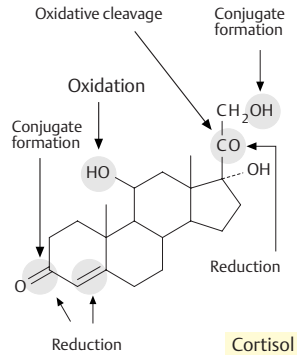
gesterone. Three hydroxylations at C-17, C-21, and C-11 of progesterone lead to the glucocorticoid **cortisol** (C_{21}). Hydroxylation at C-17 is omitted during synthesis of **aldosterone** (C_{21}). Instead, the angular methyl group (C-18) is oxidized to the aldehyde group. During synthesis of **testosterone** (C_{19}) from progesterone, the side chain is completely removed by oxidation. Aromatization of the A-ring, as mentioned above, finally leads from testosterone to **estradiol** (C_{18}).

The biosynthesis of **calcitriol** (C_{27} ; vitamin D hormone; p. 386) from cholesterol follows a different pathway. First, an additional double bond is introduced in the B-ring of cholesterol. Under the influence of UV light on the skin, the B-ring is then photochemically cleaved, and the secosteroid *cholecalciferol* arises (calcitriol = vitamin D₃; p. 392). Two Cyt P450-dependent hydroxylations in the liver at C-25 and in the kidneys at C-1 finally produce the active vitamin D hormone.

Transport. The blood provides transport proteins for the sparingly soluble steroid hormones (p. 280). They are all bound by **albumin** in a rather nonselective way. On the other hand, the binding of testosterone and estradiol to **SHBG** (sex hormone-binding globulin), of progesterone and cortisol to **transcortin** and of D hormones to **vitamin D-binding protein** is specific.

C. Inactivation

The steroid hormones are mainly inactivated in the liver, by enzymes of *biotransformation* (p. 322). The reactions include **hydroxylations**, **oxidation** of OH groups and **reduction** of oxo groups. Finally, the steroid metabolites are conjugated with *glucuronic acid* or *sulfate* for excretion (p. 322). A combination of several inactivation reactions gives rise to many different steroid metabolites. These are excreted with the *urine* and also partly via the *bile*. Evidence of steroids and steroid metabolites in the urine is used to investigate hormone metabolism.

A. Biosynthesis of steroid hormones**B. Reactions****C. Inactivation**

H: Hydroxylation
D: Dehydrogenation
Y: Hydrogenation
S: Cleavage
A: Aromatization

Thyroid Hormones

A. Thyroxine (T_4)

The thyroid hormones—thyroxine and its metabolites—are derived from the aromatic amino acid *tyrosine*. The basic component of thyroxine is called *thyronine*. In thyroxine, it carries four iodine atoms on the two aromatic rings and is therefore called **3,5,3',5'-tetraiodothyronine** (T_4 , **thyroxine**). The thyroxine metabolite **3,5,3'-triiodothyronine** (T_3) is the more potent form of the two thyroid hormones. The illustration shows the structural formula of T_4 on the left, with a rod and sphere model (iodine atoms purple) on the right and as a van der Waals representation below. T_4 and T_3 are lipophilic hormones with the same mechanism of action as the steroid hormones (p. 418). Nuclear receptors for T_3 are found in many tissues of the body.

B. Biosynthesis of thyroid hormones

Thyroxine and triiodothyronine are produced in the follicular cells of the **thyroid gland**. Their biosynthesis is reminiscent of the biosynthesis of peptide hormones.

The follicular cells synthesize **thyroglobulin**, a large dimeric glycoprotein (660 kDa), and secrete it into the lumen of the follicle which is filled with thyroglobulin. Some of the protein's tyrosine residues are then iodinated. For this to happen, the follicular cells take up **iodide** (I^-) from the blood by cotransport with two Na^+ ions. In this way iodide is concentrated by several orders of magnitude. On the luminal side of the cells, iodide is oxidized to **iodonium** (I^+) by **thyroperoxidase** [1] with H_2O_2 as the oxidant. The same enzyme then catalyzes a reaction which substitutes hydrogens at the 3' and 5' positions of tyrosine residues of thyroglobulin with iodine (**organification of iodide**). The transfer of iodinated phenolic groups from one iodinated tyrosine residue to another is also ascribed to thyroperoxidase. In the form of this macromolecular **precursor** thyroxine is stored in the follicles.

When T_4 is required, the follicular cells take up thyroglobulin from the colloid by endocytosis and cleave it by lysosomal degradation. This results in iodinated thyronines, including T_3 and T_4 , which are released into the blood. The cells of the thyroid gland secrete roughly 80–100 μg of hormone per day ($T_4:T_3$ roughly 2:1). Also within the follicular cells, the iodine atoms of mono- and diiodotyrosine residues are salvaged by **deiodases**. In the periphery, **deio-**

dases also convert T_4 to T_3 , which is the more active form of the thyroid hormones.

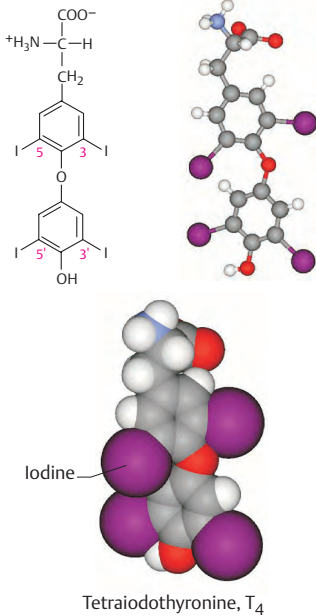
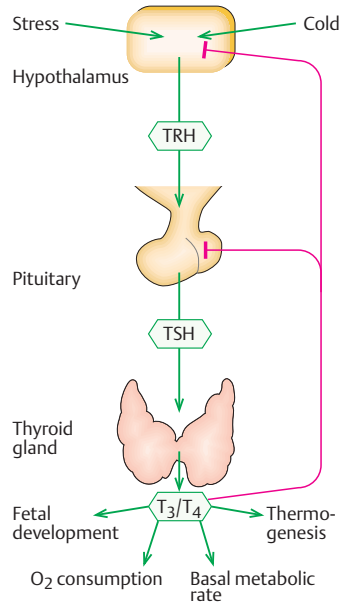
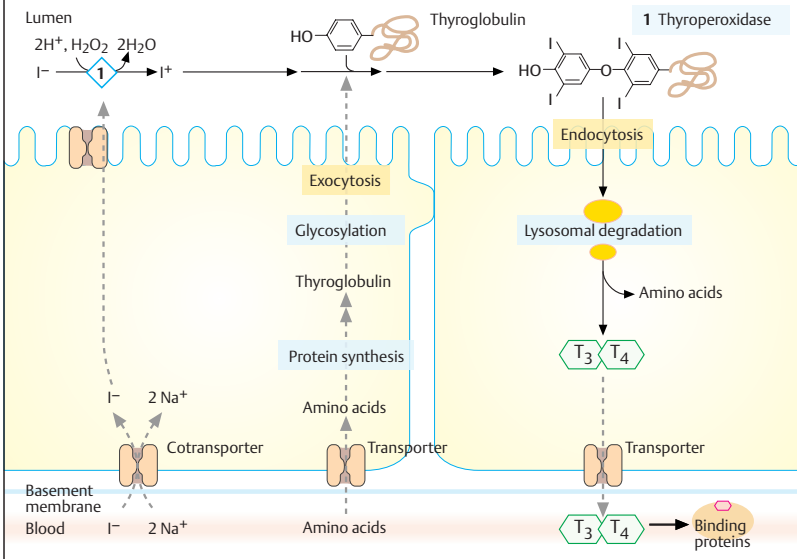
In the blood, T_4 and T_3 are more than 99% bound to transport proteins. Only 0.025% of the T_4 and 0.5% of the T_3 are free in the blood (ft_4 and ft_3) and active as hormones. The most important binding protein in the blood plasma is **thyroxine-binding globulin** (TBG), but the hormones are also bound by **transthyretin** (prealbumin) and **albumin** (p. 280). Binding of the thyroid hormones to proteins ensures an unusually long half-life of about seven days for T_4 and about one day for T_3 .

For inactivation, iodine is removed from T_4 and T_3 in the liver and kidneys. Some **deiodases** belong to the interesting group of **selenium-containing enzymes** (p. 52).

C. Regulation of thyroid activity

The thyroid is subject to regulation by the **hypothalamic–pituitary axis** (p. 420). Stimuli such as cold and stress are registered by the brain in the hypothalamus. In reaction, certain neurons secrete the hormone **thyrotropin** (thyrotropin-releasing hormone, **TRH**), a small neuropeptide consisting of only three amino acids. TRH stimulates specific cells in the anterior pituitary lobe to produce and secrete the peptide hormone **thyrotropin** (thyroid-stimulating hormone, **TSH**). TSH, a polypeptide consisting of two subunits (28 kDa), regulates the activity of the thyroid cells. It stimulates practically all processes involved in thyroid hormone biosynthesis (see **B**).

Effects. Thyroid hormones regulate **development** of the fetus, so they are classical differentiation hormones. They promote **oxygen consumption** by stimulating the respiratory chain, they raise the **basal metabolic rate**, and they stimulate **thermogenesis**, in part by inducing Na^+/K^+ -ATPase.

A. Thyroxine T₄**C. Regulation of thyroid activity****B. Biosynthesis of thyroid hormones**

Insulin

Insulin is the most important *anabolic* hormone of mammals. It regulates metabolism together with its antagonists *glucagon* and *epinephrine*, and also *cortisol*, *thyroxine*, and *growth hormone* (p. 372). The structure, biosynthesis, and mechanism of action of insulin are discussed here. Its numerous effects (see also pp. 132, 138ff., 144) are summarized on the next page in the discussion of diabetes mellitus.

A. Structure and biosynthesis

1. Primary structure. Insulin is a *peptide hormone* made up of 51 amino acids (AA). It consists of two chains, an **A chain** (21 AA, yellow) and a **B chain** (30 AA, orange), which are linked to each other by two *disulfide bonds*. A third disulfide bond stabilizes the structure of the A chain.

2. Biosynthesis. Insulin is produced by B cells of the *Langerhans islets* of the **pancreas**. As is usual with secretory proteins, the hormone precursor, **preproinsulin** (1), has a **signal peptide** of 24 AA (green), which guides the peptide chain into the interior of the ER (p. 220). **Proinsulin** (2) is produced there after cleavage of the signal peptide and formation of the disulfide bonds. Apart from the later A chain (yellow) and the later B chain (orange), this still contains the **C peptide** with 35 AA (gray). Proinsulin then enters the Golgi apparatus and is converted there to mature **insulin** (3) by removal of the C peptide catalyzed by *prohormone convertase* (limited proteolysis). Finally, the insulin is stored in the form of zinc-containing hexamers in vesicles, the β -granules, together with the cleaved C peptide (4). It is secreted when necessary.

The exocytosis of insulin from the B cells is stimulated by *glucose* (p. 416). Branched-chain AAs and gastrointestinal hormones like *GIP* and *GLP-1 (incretins)*; pp. 376, 436) also have a stimulatory effect.

B. Signal transduction

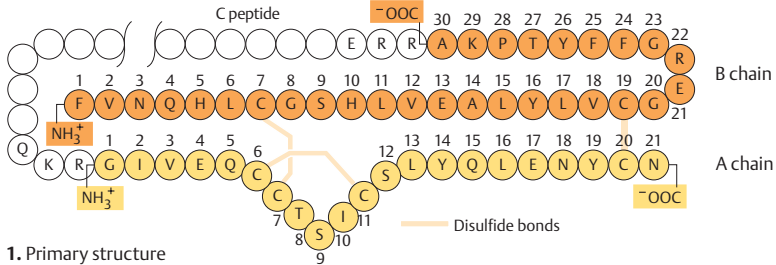
The diverse effects of insulin (see p. 160) are triggered by binding of the hormone to the insulin receptor.

The **insulin receptor** is an $\alpha_2\beta_2$ tetramer that has internal, potentially active, tyrosine kinase domains (*receptor tyrosine kinase*; p. 400). Binding of the hormone on the outside of the target cell activates the intracellular tyrosine

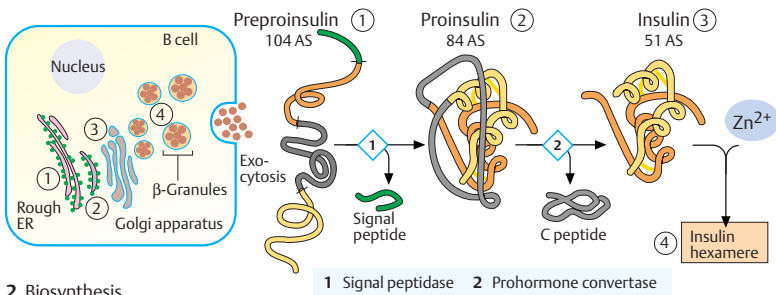
kinase activity of the receptor, which then phosphorylates itself and other proteins (receptor substrates) at various tyrosine residues. **Adaptor proteins**, which conduct the signal further, bind to the phosphotyrosine residues. The long-term effects of insulin are mediated by transcriptional control (left part of the illustration). The adaptor proteins **Grb-2** and **Sos** ("son of sevenless") bind to the phosphorylated **IRS** (insulin-receptor substrate) and stimulate the G-protein **Ras** (p. 404). Ras activates the protein kinase **Raf** and this sets in motion a phosphorylation cascade that leads via the kinases **Mek** and **Erk** (also known as MAPK, "mitogen-activated protein kinase"; p. 412) to the phosphorylation of transcription factors in the nucleus.

The faster effects of insulin on carbohydrate and lipid metabolism (right part of the illustration) are possible without protein synthesis. In addition to Grb-2, another dimeric adaptor protein can also bind to phosphorylated IRS. This adaptor protein thereby acquires *phosphatidylinositol-3-kinase* activity (**Plns3K**) and, in the membrane, phosphorylates phospholipids from the phosphatidylinositol group (p. 406) at position 3. Protein kinase **PDK-1** binds to these reaction products, becoming activated itself and in turn activating protein kinase B (**PK-B**). This has several effects. Via the auxiliary protein AA160, PK-B ensures that vesicles that contain the glucose transporter, Glut-4, fuse with the plasma membrane. This results in inclusion of Glut-4 in the membrane and thus to increased glucose uptake into the muscles and adipose tissue (p. 212). In addition, PK-B inhibits glycogen synthase kinase 3 (**GSK-3**) by phosphorylation. As GSK-3 in turn inhibits glycogen synthase by phosphorylation (p. 140), its inhibition by PK-B leads to *increased* glycogen synthesis. Protein phosphatase-1 (**PP-1**) activated by PK-B converts glycogen synthase into its active form by dephosphorylation (p. 140). PK-B inactivates the Foxo transcription factor by phosphorylation, thereby inhibiting the transcription of PEP-CK (pp. 102, 242).

A. Structure and biosynthesis

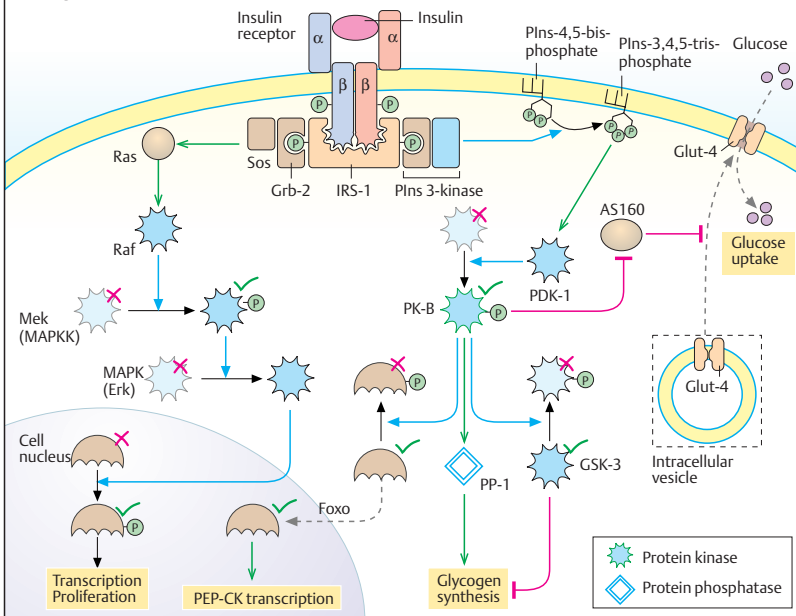


1. Primary structure



2. Biosynthesis

B. Signal transduction



Diabetes Mellitus

Diabetes mellitus is a common metabolic disease that is caused by absolute or relative **insulin deficiency**. The lack of this peptide hormone (p. 428) mainly affects carbohydrate and lipid metabolism.

Diabetes mellitus occurs in two forms. In **type 1 diabetes** (*insulin-dependent diabetes mellitus*, IDDM), the insulin-forming cells are destroyed in young individuals by an autoimmune reaction, probably virus-induced. The less severe **type 2 diabetes** (*noninsulin-dependent diabetes mellitus*, NIDDM) usually has its onset at a later age. The causes are associated with *insulin resistance* but the details have not yet been clarified (p. 332).

A. Effects of insulin deficiency

The effects of insulin on **carbohydrate metabolism** are discussed on page 138ff. In simplified terms, they can be described as *stimulation of glucose utilization* and *inhibition of gluconeogenesis*. In addition, the transport of glucose from the blood into many tissues by Glut-4 is also insulin-dependent (p. 212; exceptions to this include the liver, CNS, and erythrocytes).

The **lipid metabolism** of adipose tissue is also influenced by the hormone. In these cells, insulin stimulates the conversion of glucose into fatty acids. This is mainly based on activation of *acetyl-CoA carboxylase* (p. 144) and increased availability of NADPH due to increased pentose-phosphate pathway activity (p. 132). On the other hand, insulin inhibits the degradation of fat by *hormone-sensitive lipase* (p. 144) and reduces the breakdown of muscle protein.

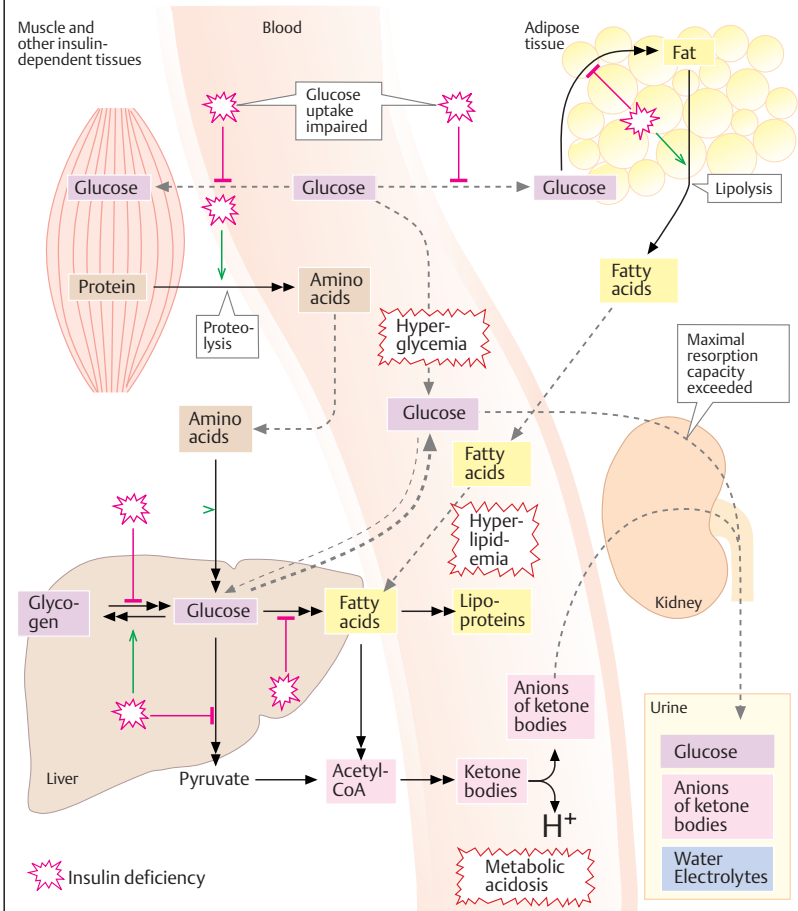
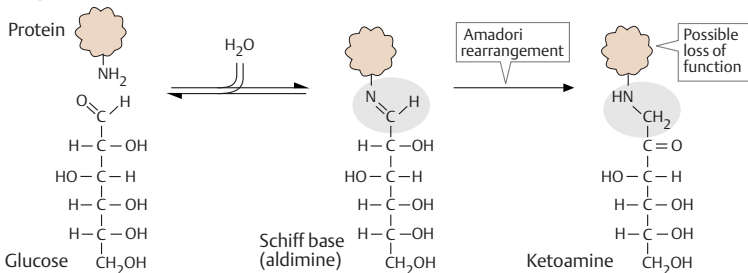
The effects of *insulin deficiency* on metabolism are indicated by asterisks in the illustration. Particularly noticeable is the increase in the glucose concentration in the blood from 5 mM (90 mg · dL⁻¹) to 9 mM (160 g · dL⁻¹) or more (**hyperglycemia**, elevated blood glucose level). In *muscle* and *adipose tissue*—the important glucose consumers—glucose uptake and glucose utilization are impaired by insulin deficiency. Glucose utilization in the *liver* is also impaired. At the same time, gluconeogenesis is stimulated, partly due to increased proteolysis in the muscles. This increases the blood sugar level further. When the capacity of the *kidneys* to reabsorb glucose is exceeded (at plasma concentrations of 9 mM or more), glucose is increasingly excreted in the urine (**glucosuria**).

The increased degradation of fat that occurs in type 1 diabetes has serious consequences. Some of the fatty acids that accumulate in large quantities are taken up by the liver and used for lipoprotein synthesis (**hyperlipidemia**), and the rest are broken down into acetyl-CoA. As the tricarboxylic acid cycle is not capable of taking up such large quantities of acetyl-CoA, the excess is used in the liver to form **ketone bodies** (*acetoacetate* and *3-hydroxybutyrate*; p. 318). As H⁺ ions are released in this process, severe **metabolic acidosis** can occur in type 1 diabetes (diabetic coma). The *acetone* that is also formed gives the breath of these patients a characteristic odor. In addition, large amounts of ketone body anions appear in the urine (**ketonuria**).

The metabolic disorders caused by insulin deficiency can lead, in acute cases, to **diabetic coma** and death.

B. Glycation

Diabetes mellitus can have major complications if it is not well controlled. Raised blood sugar levels lead in the long term to changes in the blood vessels (*diabetic angiopathy*), kidney damage (*nephropathy*), and damage to the nervous system (*neuropathy*), as well as to *cataracts* in the eyes. The main cause of these **late diabetic complications** is probably a nonenzymatic reaction of the aldehyde group of glucose with amino groups of proteins, lipids, and nucleic acids ("*glycation*" or "*glycosylation*"). Even in healthy individuals, 4%–6% of hemoglobin A is present in glycated form, i.e., as **HbA1c**. This percentage increases in untreated diabetics and serves as a measure of glucose level control. The illustration shows reversible formation of a **Schiff base** between an aldehyde and an amino group, and the subsequent Amadori rearrangement to a glycated product. If long-lived proteins are glycated, they can be rearranged further to end products known as advanced glycosylation end products (**AGE**).

A. Effects of insulin deficiency**B. Glycation**

Other Hormones

The number of water-soluble hormones in the human body is very great (> 100). Most of these are **peptide hormones**.

A. Peptide hormones (further examples)

Some peptide hormones have been mentioned already, including **parathyroid hormone** and **calcitonin** (p. 386), **angiotensin II** (p. 338) **insulin** (p. 428), **glucagon** (see below), **leptin** (p. 330), **growth hormone** (see below), **lutropin** (LH), **folliotropin** (FSH) (p. 422), **thyrotropin** (TSH, p. 426) and **corticotropin** (ACTH, p. 420). The table lists some other peptide hormones. **Vasopressin** and **oxytocin** are closely related cyclic nonapeptides that are secreted by the neurohypophysis. **Atrial natriuretic peptide** (ANP) comes from the heart and regulates sodium and water balance (pp. 336, 408). During pregnancy, **prolactin** prepares the woman for lactation and **chorionic gonadotropin** (hCG) maintains the pregnancy. As hCG is relatively small at 28 kDa, it appears in the urine, where it can be used as an **indicator of pregnancy**.

B. Glucagon

Glucagon, a peptide hormone of 29 amino acids, is an important antagonist of insulin. It is synthesized by the A cells of the *Langerhans islets* of the *pancreas*. As in the case of insulin, a **preprohormone** is produced, from which **proglucagon** and then **glucagon** are released by limited proteolysis and stored in vesicles. Exocytosis of glucagon is inhibited by **glucose**, **insulin**, and **somatostatin**. A drop in the glucose concentration to below 2.8 mM, as occurs with a lack of food, triggers glucagon secretion. Its half-life in the blood is in the range of minutes. The liver is glucagon's most important **target organ**. There, it binds specifically to a **7-helix receptor** (GPCR; p. 400) and causes formation of cAMP via a **G_s-protein** (p. 406). An increase in the cAMP concentration leads via **protein kinase A** activation to accelerated **glycogenolysis** and reduced **glycogen synthesis** (p. 136ff.). The higher cAMP concentration also triggers increased degradation of **fructose 2,6-bisphosphate** (p. 139). This halts **glycolysis** while **gluconeogenesis** is accelerated (p. 134). This reversal of metabolism takes place quickly (in minutes). More slowly, glucagon brings about the **induction of enzymes** of gluconeogenesis via cAMP, which activates the transcription factor **CREB** in the nucleus (pp. 242, 410).

Overall, glucagon ensures that the liver releases glucose instead of taking it up. The glucose level is normalized as a result, as long as the liver has adequate energy reserves (glycogen and substrates of gluconeogenesis). The extent to which glucagon is involved in lipid metabolism has not been clarified.

Preproglucagon is also synthesized in the intestine and brain. However, in these locations, it does not give rise to glucagon but to two tissue hormones with a different primary structure and action, namely, **glucagon-like peptides**, **GLP-1** and **GLP-2** (pp. 376, 436).

C. Growth hormone

Growth hormone (GH, somatotropin, STH) is produced in the **adenohypophysis** under the control of the hypothalamic hormones, **somatoliberein** (GHRH) and somatostatin. The peptide hormone ghrelin from the gastric mucosa has a stimulatory effect on GH formation. Deep sleep and physical activity increase the secretion of GH via increased GHRH.

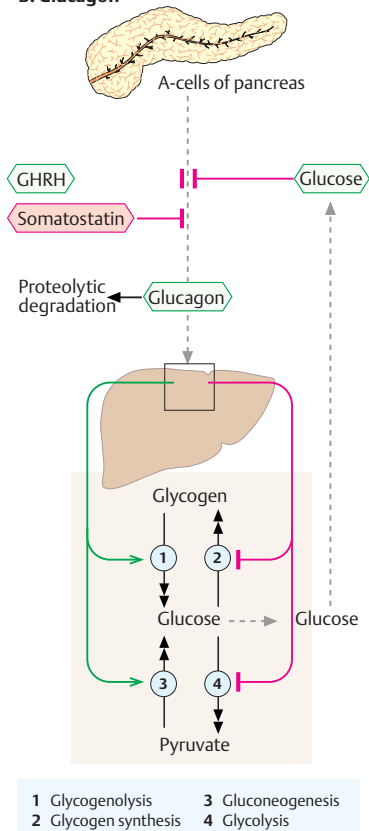
GH is an **anabolic hormone** that promotes **growth** and regulates **metabolism**. Some of its actions are mediated by **insulin-like growth factors** (somatomedins), **IGF-1** and **IGF-2**, the formation of which, particularly in the liver, is induced by GH. However, during prenatal development, these two hormones act independently of GH.

Postnatally, GH and IGF stimulate longitudinal bone growth until puberty. Thereafter, osteoblasts and osteoclasts are still activated by GH/IGF to adapt the bone to changing conditions throughout life. In **muscle**, GH/IGF produces an enlargement of muscle mass. The **internal organs** are also under the growth control of these hormones. In metabolism, GH acts as an insulin antagonist: it inhibits glucose uptake and promotes gluconeogenesis and lipolysis.

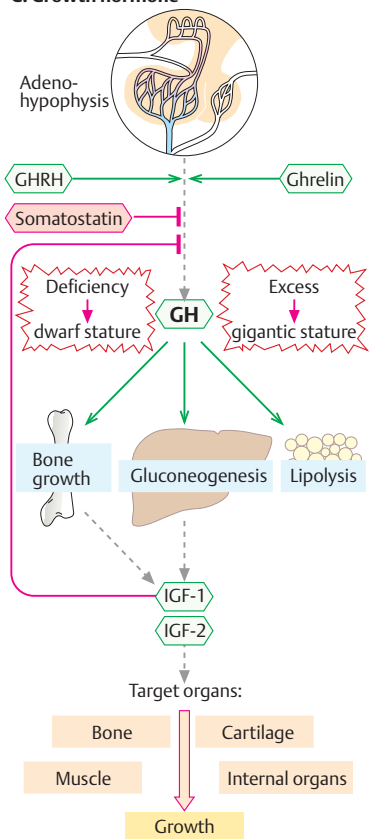
A. Peptide hormones (further examples)

Peptide hormone	Site of synthesis	Effect	Target organs
Vasopressin (antidiuretic hormone, ADH)	Neurohypophysis	Blood pressure↑, reabsorption of water	Muscle of small blood vessels, kidney
Oxytocin	Neurohypophysis	Labor↑, milk ejection↑, contraction of seminiferous tubules↑	Uterus, breast, testes
Atrial natriuretic hormone (ANP)	Cardiac atrium	Diuresis↑, natriuresis↑, blood pressure↓, renin secretion↓	Kidney, arteriolar smooth musculatur
Prolactin	Adenohypophysis	Lactation↑	Breast
Chorionic gonadotropin (hCG)	Placenta during pregnancy	Biosynthesis of progesterone and estradiol↑	Ovary

B. Glucagon



C. Growth hormone



Catecholamines

The catecholamines dopamine, norepinephrine, and epinephrine belong to the “*biogenic amines*.” They all contain a *o*-dihydroxyphenyl (“catechol”) group and act as *neurotransmitters* (p. 362). Norepinephrine, and in particular epinephrine are also *hormones*.

A. Biosynthesis

The biosynthesis of the catecholamines takes place in the *adrenal medulla* and in neurons of the central and peripheral nervous system. It originates from the amino acid **tyrosine**.

- [1] Hydroxylation of the aromatic ring initially produces **dopa** (3,4-dihydroxyphenylalanine; p. 52). This reaction uses the unusual coenzyme *tetrahydrobiopterin* (THB).
- [2] Decarboxylation of dopa requires the coenzyme *pyridoxal phosphate* and yields the biogenic amine **dopamine**, an important transmitter in the CNS. In dopaminergic neurons, catecholamine synthesis stops at this point.
- [3] The adrenal gland and (nor)adrenergic neurons continue the synthesis by hydroxylating dopamine into **norepinephrine** (noradrenaline). *Ascorbic acid* (vitamin C; p. 86) acts as a hydrogen-transferring coenzyme here.
- [4] Finally, *N*-methylation of norepinephrine yields **epinephrine** (adrenaline). The coenzyme for this reaction is *S*-adenosylmethionine (SAM; p. 88).

Whether a neuron is dopaminergic, noradrenergic, or adrenergic therefore depends on its enzyme equipment. The adrenal medulla contains all four enzymes; norepinephrine and epinephrine are produced there in a ratio of 1:4. The biosynthesis of norepinephrine and epinephrine is stimulated neurally by *acetylcholine* and in a paracrine way by *cortisol*, and it is inhibited by the *end products*. After their biosynthesis, the catecholamines are stored in vesicles together with *ATP*, various *neuropeptides* and the protein *chromogranin*. They are released jointly by exocytosis (p. 358).

Epinephrine in the blood comes from the adrenal medulla while norepinephrine mainly stems from the endings of sympathetic nerves. The *half-life* of the catecholamines in the blood is only a few minutes. In the synaptic cleft, their effect is terminated even faster. This is achieved by reuptake into the presynaptic neu-

rons by means of a Na^+ -dependent *norepinephrine transporter* (NET).

B. Effects

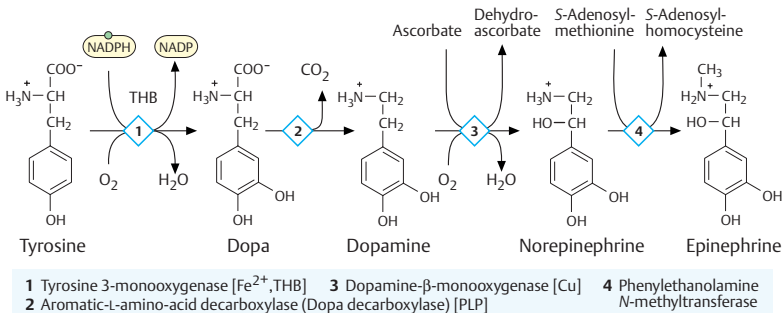
The catecholamines act by binding to membrane receptors of the GPCR type (p. 364). *Dopamine* is a neurotransmitter in the CNS where it is involved in numerous regulatory and control processes. *Norepinephrine* and *epinephrine* are both neurotransmitters of (nor)adrenergic neurons and also adrenal medullary hormones. Their effects can be regarded as a reaction to a threatening situation (“flight or fight”), including increase in heart rate, rise in blood pressure, increase in the blood levels of glucose and fatty acids, and higher muscle activity. They thus serve for adaptation to *acute stress*.

C. Parkinson disease

This disease is characterized by destruction of dopaminergic neurons in the *substantia nigra*, which are involved in the control of motor processes in the striatum. Sufficient *dopamine* is no longer released there. The consequences for the patients are *akinesia*, *rigidity*, and *tremor*. Parkinson disease can be treated by giving the dopamine precursor *L-dopa*, which unlike dopamine itself can pass the blood–brain barrier. *L-Dopa* is then converted to dopamine in the CNS by *dopa decarboxylase* (**A**, [2]).

D. Inactivation

The catecholamines and other biogenic amines are inactivated by enzymatic reactions both in neurons and in non-neural tissue, especially the liver. *Catechol-O-methyltransferase* (COMT) methylates them at the hydroxyl group. The coenzyme in the reaction is *S-adenosyl methionine*. Alternatively, the amino group can be oxidized to an aldehyde by a flavin-containing amine oxidase (*monoamine oxidase*, MAO, isotypes A and B). The resulting aldehyde is either oxidized further to the acid or reduced to the alcohol. Vanillylmandelic acid is the most important degradation product of epinephrine and norepinephrine. The end products of catecholamine metabolism appear mainly in the urine.

A. Biosynthesis of the catecholamines**B. Effects****Dopamine:**

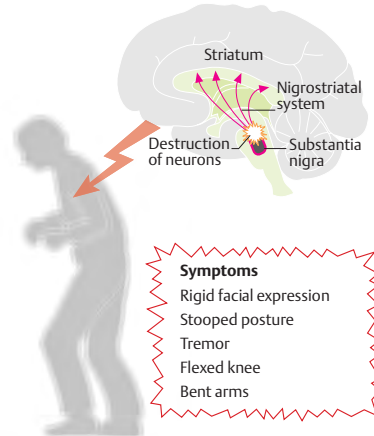
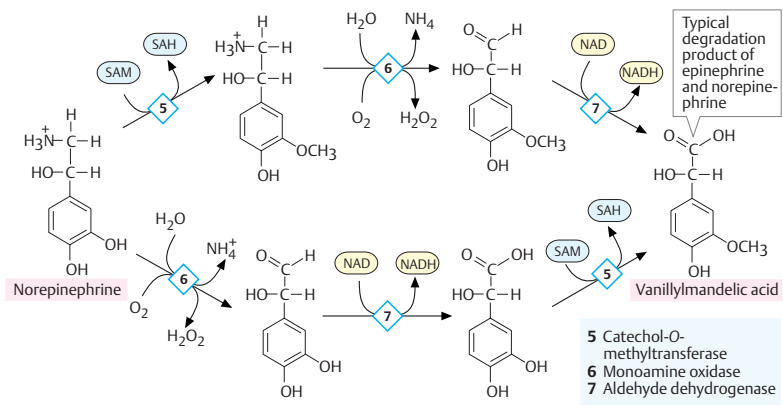
Transmitter: controls extrapyramidal motor function, involved in perception of pleasure and happiness and in generation of dependencies
Neurohormone: inhibits prolactin secretion (PIF)

Norepinephrine:

Transmitter: crucial neurotransmitter in postganglionic neurons of the sympathetic nervous system

Epinephrine/norepinephrine:**Hormone:**

Heart: increases contractility and rate, dilates coronary arteries
Periphery: increases blood pressure by vasoconstriction, relaxation of smooth muscle
Metabolism: mobilization of energy reserves by glycogenolysis, lipolysis, and gluconeogenesis, inhibition of insulin secretion

C. Parkinson disease**D. Inactivation**

Tissue Hormones and Mediators

The hormonelike signaling substances with an extracellular action comprise the **tissue hormones** and **mediators**. They are secreted by individual cells, and usually act locally, i.e., in the immediate vicinity of their production site, without being transported through the blood (*paracrine action*; p. 416).

A. Tissue hormones and mediators

The table lists important representatives of the tissue hormones and mediators, with their functions.

B. Gastrointestinal hormones

These peptide hormones of the gastrointestinal (GI) tract exert their effect mainly in a *paracrine* manner (p. 416). Together they form the *enteroendocrine system*.

Gastrin is produced in the *G cells* of the pyloric region of the stomach and stimulates the *ECL cells* of the stomach to secrete *histamine* (see below; also p. 270).

Cholecystokinin (CCK) is released from the *I cells* of the small intestine and stimulates the acinar cells of the pancreas to secrete *pancreatic enzymes* and HCO_3^- , and stimulates the *chief cells* of the stomach to secrete *pepsinogen* and causes the *gallbladder to contract*.

Secretin from the *S cells* of the small intestine stimulates the epithelial cells of the acini in the *pancreas* to release *pancreatic juice*, and stimulates the *chief cells* of the stomach to release *pepsinogen*.

Serotonin is produced in the *enterochromaffin* (EC) cells of the small and large intestine. It stimulates gastrointestinal smooth muscle motility.

Somatostatin secreted by *D cells* in many parts of the gastrointestinal tract inhibits the activity of other endocrine cells. It is also an important inhibitor of hormone secretion by the *pancreatic islets* and the *adenohypophysis*.

Glucagonlike peptides (GLP-1 and GLP-2; p. 432) are secreted by *L cells* of the intestinal mucosa in reaction to glucose as a signal for food intake to the body. **GLP-1** promotes the release of insulin in the pancreas ("*incretin effect*") and the *satiety sensation* in the brain (p. 376). **GLP-2** inhibits gastric juice secretion by the parietal cells and influences the motility and nutrient absorption of the small intestine.

C. Histamine

Histamine is a prominent representative of mediators.

Metabolism. Histamine is a *biogenic amine* produced by decarboxylation of histidine (p. 52). It is produced in *mast cells*, in *basophilic leukocytes*, in gastric *ECL cells* (p. 270) and in *histaminergic neurons* in the CNS. In mast cells, histamine is stored in secretory vesicles together with *heparin* and *neutral proteases* and is released in response to a stimulus.

Histamine is inactivated by *N*-methylation due to *histidine methyltransferase* and/or by oxidation to aldehyde and acid catalyzed by *monoamine oxidase* (MAO), *diamine oxidase* (DAO), or *aldehyde oxidase* (p. 362).

Effects. There are four different types of membrane receptors for histamine on its target cells (H_1 – H_4).

H₁ receptors mediate *allergic reactions* of the skin and respiratory tract. Depending on the location, this may lead to bronchoconstriction, vasoconstriction, or vasodilatation and an increase in vascular permeability.

H₂ receptors on the parietal cells of the gastric mucosa are involved in the control of *hydrochloric acid production* (p. 274).

H₃ receptors are found in the CNS and on peripheral neurons. They are involved in regulation of *brain functions* (sleeping and waking, learning, and memory) and also affect blood circulation of the heart as well as certain hormone axes.

H₄ receptors occur on mast cells and blood cells and help to regulate the *chemotaxis* of these cells.

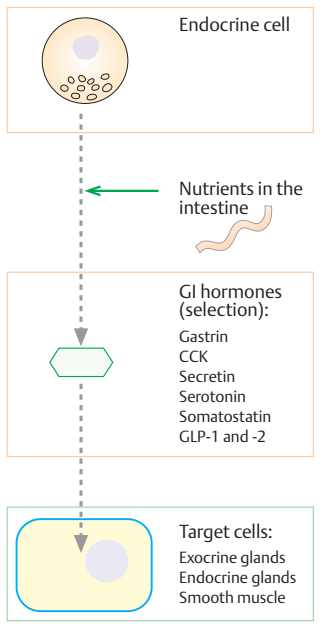
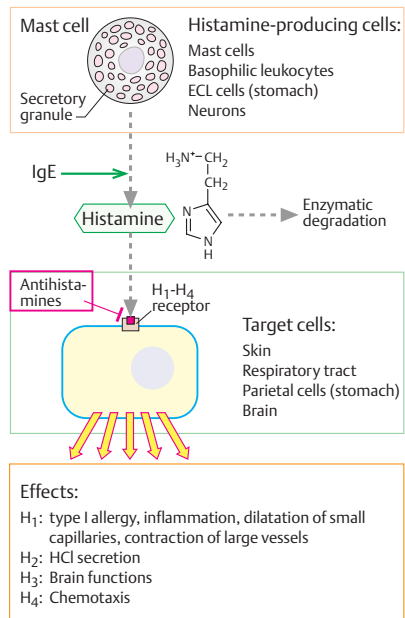
Pathobiochemistry. The IgE-mediated release of histamine from mast cells is crucially involved in triggering *type I allergic reactions*, including bronchial asthma, hay fever, and hives (p. 312).

The effects of histamine can be reduced with selective **antihistamines**. For instance, *hay fever* is treated with H_1 antagonists. *Allergic shock* is another important area of use for these drugs. *Excessive hydrochloric acid production* in the stomach can be inhibited by H_2 antagonists such as *cimetidine* and *ranitidine*. Because of the sedating effect of some antihistamines that can cross the blood–brain barrier, H_2 -blockers are also used as *hypnotics*.

A. Tissue hormones and mediators

Tissue hormones	Function	Mediators	Synthesis	Function
Cytokines	Signaling substances in the immune and hematopoietic systems, p. 444	Histamine	Biogenic amine of histidine, p. 52	Promotes inflammation, width and permeability of capillaries, type I allergy, HCl secretion, brain functions (NT)
Growth factors	Signals for cell growth, differentiation, division and apoptosis, p. 444f, 448	Serotonin	Biogenic amine of 5-hydroxy-tryptophan, p. 52	Promotes vasodilatation in skin and skeletal muscle, intestinal peristalsis, contraction of vascular and bronchial muscle, brain functions (NT)
Gastro-intestinal tract hormones	Local regulation of digestion	Eicosanoids	Arachidonic acid derivatives, p. 438	Varied effects
Kinins Bradykinin Kallidin	Promote dilatation and permeability of blood vessels, leukocyte migration. Inhibit platelet activation. Signal pain	Nitrogen monoxide	Product of arginine, p. 408	Lowers blood pressure. Inhibits platelet aggregation. Promotes learning processes (NT). Toxic at high concentrations

NT: neurotransmitter

B. Gastrointestinal hormones**C. Histamine**

Eicosanoids

The **eicosanoids** are a group of autocrine and paracrine signaling substances that arise from C₂₀ fatty acids (Greek *eicosa* = 20). As mediators, they influence a large number of physiological processes (see below). Eicosanoid metabolism is therefore an important drug target. **Biosynthesis.** Almost all of the body's cells can form eicosanoids. Membrane phospholipids that contain **arachidonic acid** (20:4; p. 38) or other polyunsaturated C₂₀ fatty acids provide the starting material.

Initially, **phospholipase A₂** (PLA₂ [1]) releases the arachidonate moiety from these phospholipids. The activity of PLA₂ is hormone-regulated, i.e., it is inhibited by **glucocorticoids** via the protein **lipocortin**. The arachidonate produced is a signaling substance itself. However, its metabolites are even more important.

Two different pathways lead from arachidonate to the eicosanoids. The key enzyme for the first pathway (**cyclooxygenase pathway**) is **prostaglandin-H₂ synthase** [2]. In a two-step reaction, it catalyzes the cyclization of arachidonate to prostaglandin H₂ (PGH₂). The first reaction step of PGH synthase is catalyzed by its cyclooxygenase (COX) activity and introduces oxygen into the fatty acid. The second step is a peroxidase reaction. Depending on further enzyme equipment, different **prostaglandins**, **prostacyclines**, and **thromboxanes** are produced from PGH₂. Collectively, these are known as **prostanoids**. An isocyclic five-membered ring is characteristic of the prostaglandins, while the prostacyclines have an additional oxygen-containing five-membered ring and thromboxanes have an oxygen-containing six-membered ring (see formulas).

The second pathway (**lipoxygenase pathway**) is catalyzed by **5-lipoxygenases** [3], which forms hydroxy fatty acids and hydroperoxy fatty acids from arachidonate, from which elimination of water and various conversion reactions give rise to the **leukotrienes**. These are open-chain fatty acid derivatives.

Mode of action. Eicosanoids act via membrane receptors in the immediate vicinity of their site of synthesis, both on the synthesizing cell itself (**autocrine** action) and on neighboring cells (**paracrine** action). Many of their effects are mediated via 7-helix receptors by second messengers, viz. cAMP, Ca²⁺, and inositol phosphates.

Eicosanoids can have a stimulatory or inhibitory effect on smooth-muscle contraction, blood pressure, respiration, and intestinal and

uterine activity. In the stomach, prostaglandins inhibit **HCl secretion** via G_i-proteins. At the same time, they promote mucus secretion, which protects the gastric mucosa against the acid (p. 270). In addition, prostaglandins are involved in **bone metabolism** and in the activity of the **sympathetic nervous system**. In the immune system, prostaglandins are important in the **inflammatory reaction**. Among other things, they attract leukocytes to the site of infection. Eicosanoids are also decisively involved in the development of pain and **fever**. The thromboxanes promote platelet aggregation and other processes involved in hemostasis (p. 294).

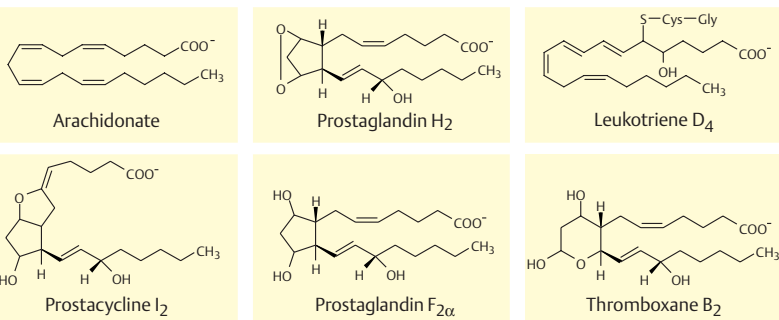
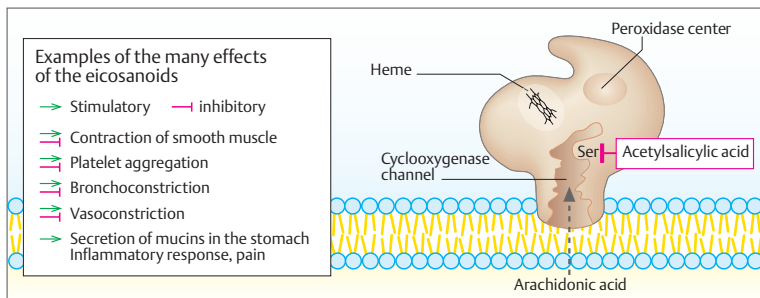
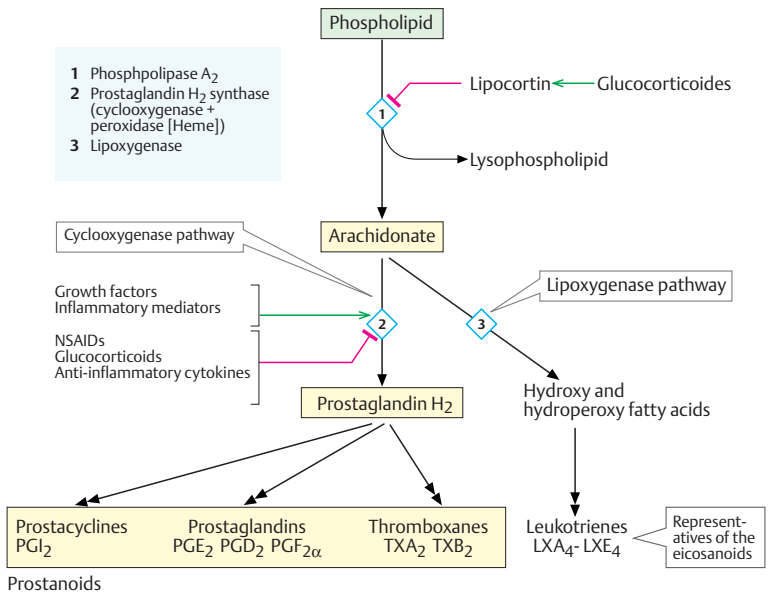
Metabolism. Eicosanoids are inactivated within a period of seconds to minutes. This takes place by enzymatic reduction of double bonds and dehydrogenation of hydroxyl groups. As a result of this rapid degradation, their range is very limited.

■ Further information

PGH synthase [2] occurs in a constitutive form (COX-I) and in a form that can be induced by inflammatory mediators (COX-II).

Acetylsalicylic acid and other **nonsteroidal anti-inflammatory drugs** (NSAIDs) inhibit the activity of one or both PGH synthases and consequently the synthesis of most eicosanoids. This explains their **analgesic**, **antipyretic**, and **antirheumatic effects**. Acetylsalicylic acid irreversibly acetylates a serine residue in the vicinity of the active center of PGH synthase so that access for substrates is blocked. Other NSAIDs act as competitive inhibitors. Side effects of NSAIDs also result from inhibition of eicosanoid synthesis. For example, they impair hemostasis because the synthesis of thromboxanes in platelets is inhibited. In the stomach, NSAIDs increase HCl secretion and at the same time inhibit the formation of protective mucus, which may lead to gastritis and ulcers.

A. Eicosanoids



Cytokines

A. Cytokines

Cytokines are hormonelike *peptides* and *proteins*, which are synthesized and released by cells of the immune system and other cell types. Their numerous biological functions operate in three areas: they regulate the *development and homeostasis of the immune system*; they control the *hematopoietic system*; and they are involved in *non-specific defense*, influencing inflammatory processes, blood coagulation, and blood pressure. In general, cytokines regulate the growth, differentiation, and survival of cells. They are also involved in regulating apoptosis (p. 448).

There is an extremely large number of cytokines; only the most important representatives are listed here. The cytokines include *interleukins* (ILs), *lymphokines*, *monokines*, *chemokines*, *interferons* (IFNs), and *colony-stimulating factors* (CSFs). Via interleukins, immune cells stimulate the proliferation and activity of other immune cells (p. 304). Interferons are used medically in the treatment of viral infections and other diseases.

Although cytokines rarely show structural homologies with each other, their effects are very similar. The cytokines differ from *hormones* (p. 414ff.) only in certain respects: they are released by many different cells, rather than being secreted by defined glands, and they regulate a wider variety of target cells than the hormones.

B. Signal transduction in the cytokines

The cytokines are **hydrophilic signaling substances** that act by binding to *receptors* on the cell surface (p. 400). Binding of a cytokine to its receptor (1) leads via several intermediate steps (2–5) to the activation of transcription of specific genes (6).

In contrast to the receptors for insulin and growth factors (p. 400), the **cytokine receptors** (with a few exceptions) have *no* tyrosine kinase activity. After binding of cytokine (1), they associate with one another to form homodimers, join together with other signal transduction proteins (STPs) to form dimers, or promote dimerization of other STPs (2). Class I cytokine receptors interact with three different STPs (gp130, β_c , and γ_c). The STPs themselves do not bind cytokines, but conduct the signal to tyrosine kinases (3). The fact that different cytokines can activate the same STP

via their receptors explains the overlapping biological activity of some cytokines.

As an example of the signal transduction pathway in cytokines, the illustration shows the way in which the **IL-6 receptor**, after binding its ligand **IL-6** (1), induces the dimerization of the STP **gp130** (2). The dimeric gp130 binds cytoplasmic **tyrosine kinases** of the Jak family ("**Janus kinases**," with two kinase centers) and activates them (3). The Janus kinases phosphorylate cytokine receptors, STPs, and various cytoplasmic proteins that conduct the signal further. In addition, they phosphorylate transcription factors known as **STATs** ("signal transducers and activators of transcription"). STATs are among the proteins that have an **SH2 domain** and are able to bind phosphotyrosine residues (p. 412). They therefore bind to cytokine receptors that have been phosphorylated by Janus kinases. When STATs are then also phosphorylated themselves (4), they are converted into their active form and become dimers (5). After transfer to the nucleus, they bind—along with auxiliary proteins as transcription factors—to the promoters of inducible genes and in this way regulate their transcription (6).

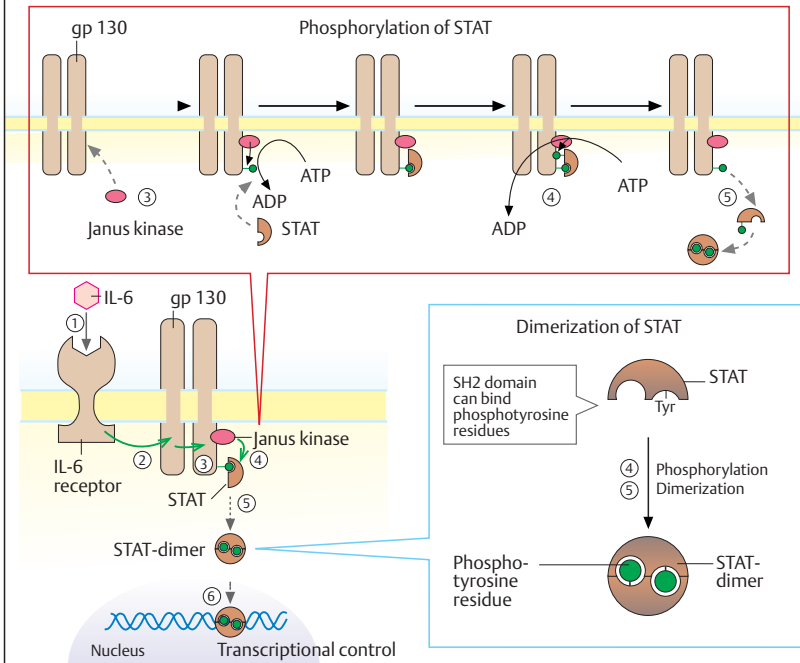
The activity of the cytokine receptors is terminated by **protein phosphatases**, which hydrolytically cleave the phosphotyrosine residues. Several cytokine receptors are able to lose their ligand-binding extracellular domain by proteolysis (not shown). The extracellular domain then appears in the blood, where it competes for binding to cytokine molecules. This reduces the effective cytokine concentration in the blood.

Anaphylaxis ("cytokine storm") is a severe overreaction of the immune system. In response to allergens, a massive secretion of histamine by mast cells and inflammatory cytokines by macrophages (especially IL-1, IL-6 and TNF) can cause a circulatory collapse and lead to multiorgan failure.

A. Cytokines

Cytokine	Effects
Proinflammatory	
Interleukin 1 (IL-1), IL-6, tumor necrosis factor α (TNF α)	Mediation of innate immune response in inflammation and injury: release of mediators, induction of acute phase proteins, pyrexia, stimulation of B and T cells
Anti-inflammatory	
IL-4, IL-6, IL-10, IL-11, IL-13	Restriction and local limitation of immune defense and inflammation
Immunomodulatory	
IL-2, IL-3, IL-4, IL-6, IL-7, IL-9	Promotion of T cell differentiation, increase in IgE production by B lymphocytes
Virostatic	
Interferon α (IFN α), IFN β , IFN γ	Inhibition of viral replication: induction of RNA degradation, halting of protein biosynthesis and triggering apoptosis of virus-infected cells
Chemotactic	
IL-8	Neutrophil attraction
Growth-promoting	
Erythropoietin (Epo)	Promotion of erythrocyte production
Granulocyte/macrophage colony-stimulating factor (GM-CSF)	Stimulation of granulocyte and macrophage production by myeloid stem cells

B. Signal transduction in the cytokines



Growth and Development

Cell Cycle I

All of the cells in the body arise by cell division. This process can be subdivided into growth of the cells, duplication of cell components such as DNA and organelles, and actual division. In humans, many millions of cells divide every second.

A. Cell cycle

Proliferating cells undergo a cycle of division (the cell cycle), which lasts about 24 hours in mammalian cells in cell culture. The cycle is divided into four different **phases**, which occur in the sequence **G₁** (gap 1), **S** (for synthesis), **G₂** (gap 2), and **M** (for mitosis). In the **G₁** phase, the duration of which can vary, the cell grows by de-novo synthesis of cell components. In the **S** and **G₂** phases, preparations are made for mitosis.

Fully differentiated cells only divide rarely or not at all. They have left the **G₁** phase and are in a resting state (the so-called **G₀ phase**), in which they can remain permanently. Under the influence of **mitogenic signals** (growth factors, cytokines, tumor viruses, etc.), some resting cells enter the **G₁ phase** again and after passing a **control point** (see below), they start a new cell cycle. DNA is replicated (p. 238) during the **S phase**, and new chromatin is formed. This is followed by the **G₂ phase**, where, for example, tubulin is synthesized for the spindle apparatus. Particularly remarkable in morphological terms is the short **M phase**, in which the chromatids separate (**mitosis**) and two daughter cells are formed (**cytokinesis**).

Together, the **G₁**, **G₀**, **S**, and **G₂** phases are referred to as the **interphase**, which alternates in the cell cycle with the short **M phase**.

In the body, the frequency of division of cells of individual tissues varies. *Nerve cells* and *muscle cells* normally do not divide at all, though there are exceptions. They remain permanently in the **G₀** phase. The cells of other tissues and organs divide rarely but can be stimulated to cell division by growth factors (e.g., the *hepatocytes*). By contrast, *spermatozoa* and *bone marrow cells*, like cells of the *skin* and *digestive tract*, divide rapidly and regularly.

There are **checkpoints** to ensure that the cell cycle takes place in an orderly fashion. At these, the cell decides whether or not the cell cycle will be continued. At the **start checkpoint** in the late part of the **G₁** phase, it is decided whether the cell cycle is initiated. The **G₂/M checkpoint** at the end of the **G₂** phase gives the

green light for mitosis, and the **metaphase/anaphase checkpoint** in the **M phase** allows cytokinesis to start.

B. Control of the cell cycle

The progression of the cell cycle is regulated by a family of **cyclin-dependent kinases (CDK1–6)**. These are special Ser/Thr-specific **protein kinases** which have to bind an activator protein from the cyclin family (**cyclin A–D**) to become active. While the CDKs are present constantly during the cell cycle, the concentration of individual cyclins is subject to major fluctuations (top left) because they are synthesized phase-specifically and are degraded again rapidly in proteasomes after being marked with ubiquitin (p. 162). Because of this, a CDK can become active only when the corresponding cyclin is available. Other mechanisms ensure additional regulation of the protein kinase activity of the cyclin–CDK complexes (p. 446).

Certain combinations of **cyclin–CDK complexes** are typical for individual phases of the cell cycle. Each of these complexes has specific proteins as substrates, the function and structure of which are controlled by phosphorylation/dephosphorylation (see table). The substrates of the cyclin–CDK complexes include proteins in the nucleus as well as transcription factors, inhibitors of transcription factors, and a range of nuclear enzymes that alter chromatin and DNA, in addition to proteins of the Golgi apparatus and centrosomes.

Active cyclin–CDK complexes allow the cycle to pass the three checkpoints. Otherwise, the cell cycle comes to a halt. This regulatory mechanism ensures that dividing cells are big enough, that their DNA is intact, and that the previous steps of the cycle have been completed in full.

A. Cell cycle**M phase**

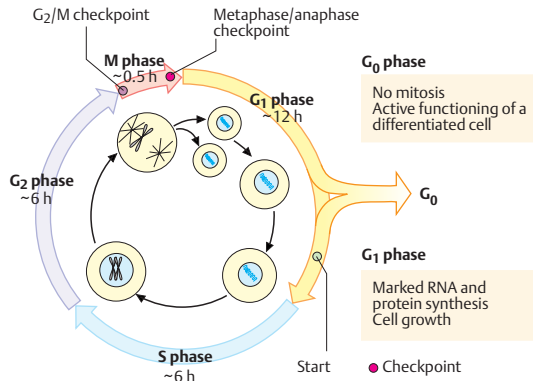
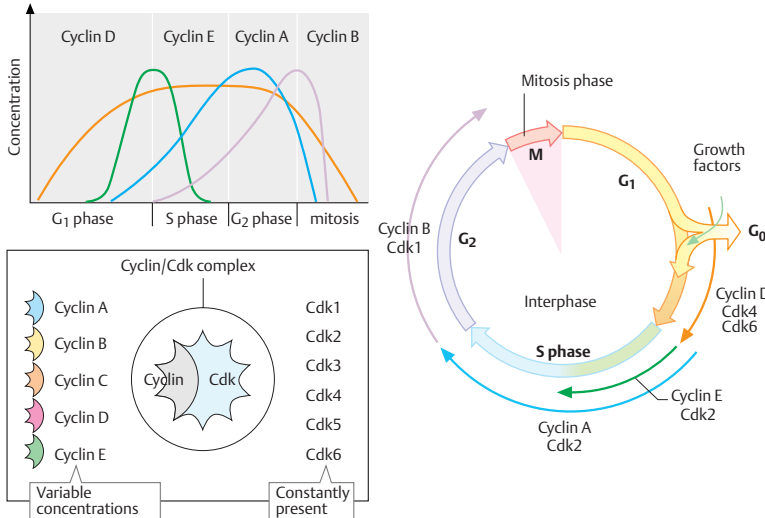
Mitosis
Cytokinesis
(cytoplasm division)

G₂ phase

Preparation for mitosis
Tubulin synthesis

S phase

DNA replication
Histone synthesis
Centrosome formed
Chromosome duplication
Reduced RNA and protein
synthesis

**B. Control of the cell cycle**

Cell cycle phase	Cdk	Cyclin family	Cyclin/Cdk complex substrates
G ₁	Cdk4 Cdk6	Cyclin D	Rb proteins
G ₁ /S	Cdk2	Cyclin E	Rb proteins, enzymes for modifying histones, DNA replication, DNA repair, centrosome maturation
S	Cdk2 Cdk1	Cyclin A	Transcription factors, enzymes for modifying histones, DNA replication, DNA repair, centrosome maturation
M	Cdk1	Cyclin B	Histones, laminins, proteins of the nuclear pore complex, Golgi complex, DNA replication and translation, microtubule-binding proteins

Cell Cycle II

Cell cycle regulation can be regarded as a network of positive and negative protein interactions by which cell division is triggered or continued when all the preconditions for this have been met.

A. Regulation of cyclin-dependent kinases

The *cyclin-dependent kinases* (**CDKs**) are at the heart of cell cycle control (p. 444). Their activity is regulated strictly and is controlled at various levels—by binding of their *activating subunits*, the **cyclins**, by negative regulators (**CDK inhibitors**, **CKIs**) by *phosphorylation/dephosphorylation*, and also by folding and their subcellular location.

The illustrated heterodimer complex of cyclin and CDK can be produced from a combination of different cyclins (A–D) and different CDKs (1–6), as described on the previous page. To become active, a protein kinase still has to be phosphorylated by a **CDK-activating kinase** (**CAK**, enzyme [3]). CAK is a particular cyclin–CDK complex (CDK7/cyclin H) which contains an additional protein (Mat1). On the other hand, phosphorylations by *protein kinases* named **Wee1** and **Myt1** [1] inactivate the CDKs. This inhibition can be abolished by *protein phosphatase* **Cdc25** [2].

The **CDK inhibitors** (**CKIs**) belong to two families. Representatives of the **INK4** family bind directly to CDKs and inhibit them. By contrast, representatives of the **Cip/Kip** family bind to the active cyclin–CDK complex.

The cyclin–CDK complex is finally inactivated by *ubiquitylation* of the cyclin [4] which initiates its degradation in proteasomes (p. 162). The inactive CDK remains behind for the next cycle. *Protein phosphatases* [5] then regain the upper hand in the cell and dephosphorylate all the proteins involved in that step of the cell cycle, including the CDKs.

B. Retinoblastoma protein

Rb-protein (pRb, *retinoblastoma protein*) is an important substrate of the G_1/S -CDK complexes. pRb goes through a phosphorylation–dephosphorylation cycle during the cell cycle. During the G_0 and G_1 phases, pRb is present in unphosphorylated form which binds to the **transcription factor E2F** and thereby blocks it. When the cycle moves into the S_1 phase, pRb becomes phosphorylated and releases E2F, al-

lowing it to activate its target genes. The corresponding proteins (e.g., DNA polymerase, dihydrofolate reductase, thymidine kinase, and S phase cyclins) are all needed for replication processes in the S phase.

C. Protein p53

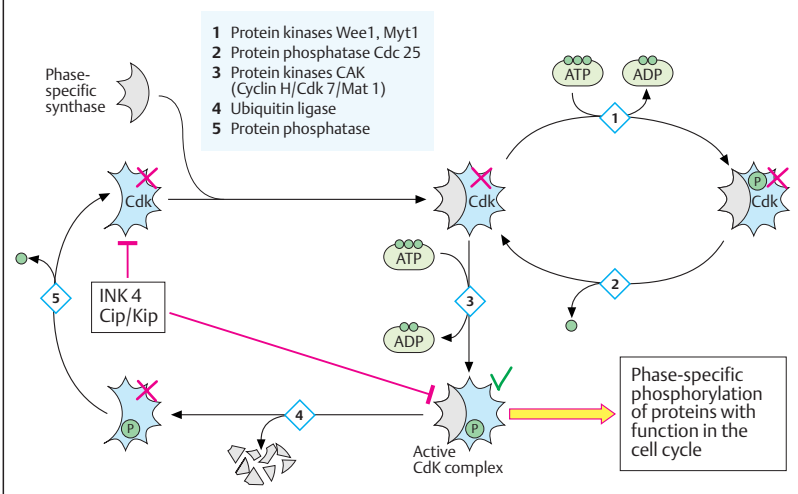
Protein p53 is called the “watchman of the genome” because it is activated by specific protein kinases (ATM or ATR) when *DNA damage* and *stress* occur. Phosphorylated p53 acts as a *transcription factor* and induces the synthesis of protein **p21**. This, in turn, acts as a **CDK inhibitor** (Cip/Kip family) and blocks the action of the G_1 -CDK complex, so that the cell cycle comes to a halt at the transition to the S_1 phase and the DNA can be repaired at leisure. When this has been successfully completed, p53 is dephosphorylated, ubiquitinated, and passed on to the proteasomes for degradation. Thus, p53 does not accumulate in normal cells.

If the DNA repair systems do not succeed in eliminating the DNA damage, a steady increase in the concentration of phosphorylated p53 finally drives the cell into apoptosis (p. 448).

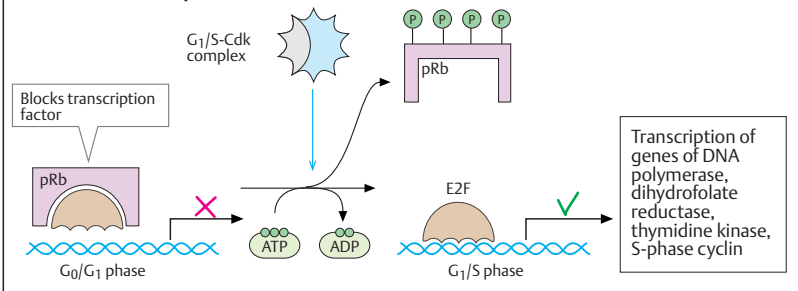
Proteins pRb and p53 are products of **tumor suppressor genes** (anti-oncogenes, p. 450). Complete absence of them, e.g., due to mutations, leads to accelerated cell division, a typical feature of tumors. In fact, *somatic mutations* in pRb and p53 have been found in more than half of all human tumors.

Human papilloma virus, which is responsible for the development of cervical tumors, codes for protein named E6, among others, which interacts with the *tumor suppressor protein p53* and promotes its degradation.

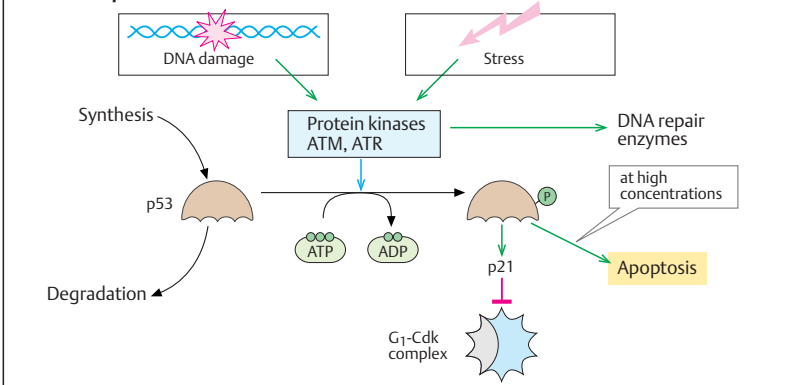
A. Regulation of cyclin-dependent kinases



B. Retinoblastoma protein



C. Protein p53



Apoptosis

A. Cell proliferation and apoptosis

The number of cells in any tissue is mainly regulated by two processes—cell **proliferation** and *physiological cell death*, **apoptosis**. Both of these processes are controlled by stimulatory and inhibitory factors that act in solute form (growth factors and cytokines) or are presented in bound form on the surface of neighboring cells (see below). A distinction is made between apoptosis and **necrosis** (not shown), in which cell death is usually attributable to physical or chemical damage.

Apoptosis is genetically programmed cell death, which leads to “tidy” breakdown and disposal of cells. Morphologically, apoptosis is characterized by shrinking of the cell, changes in the cell membrane (with the formation of small blebs known as “apoptotic bodies”), shrinking of the nucleus, chromatin condensation, and fragmentation of DNA. A biochemical feature is the occurrence of negatively charged *phosphatidylserine* on the outside of the cytoplasmic membrane. *Macrophages* and other phagocytic cells recognize this signal and remove apoptotic cells by *phagocytosis* without inflammatory phenomena developing.

Not only the growth of tissue (or, more precisely, the number of cells) is regulated by apoptosis. In addition, apoptosis removes unwanted or superfluous cells—e.g., during embryonic development or in the immune or nervous system. Diseased cells are also eliminated by apoptosis—e.g., tumor cells, virus-infected cells, and cells with irreparably damaged DNA. An everyday example of this is the peeling of the skin after sunburn.

B. Regulation of apoptosis

At the center of the apoptotic process are the **caspsases** (from cysteine **asp**artic **acid**-specific enzyme), a group of specialized *cysteine proteinases* (p. 162), which cleave proteins next to aspartate residues when activated. Together they constitute an *enzyme cascade*, similar to the blood clotting cascade. When a cell receives apoptotic signals (see below) their resting states (**procaspases**) are sequentially activated by limited proteolysis. **Initiator caspsases** (caspase-2, -8, -9, and -10) start the cascade. They activate **effector caspsases** (caspase-3, -6 and, -7), which then cleave important cellular proteins that eventually bring about cell death. Prominent substrates of effector caspsases include *laminins* in the nuclear envelope, *protein*

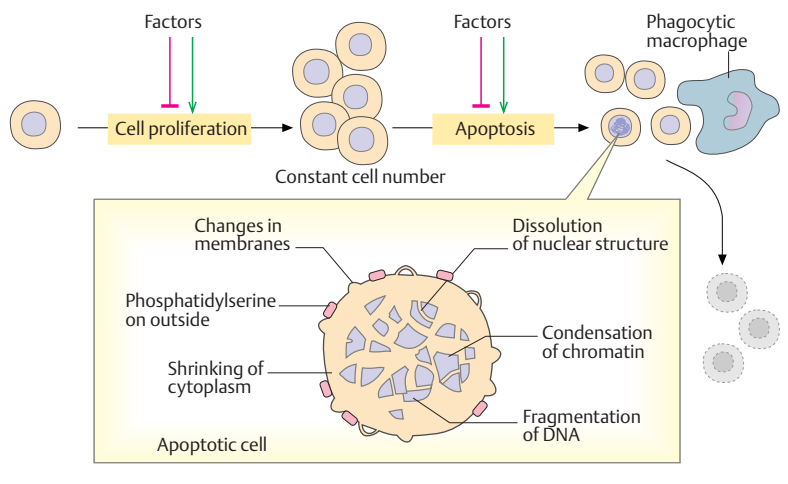
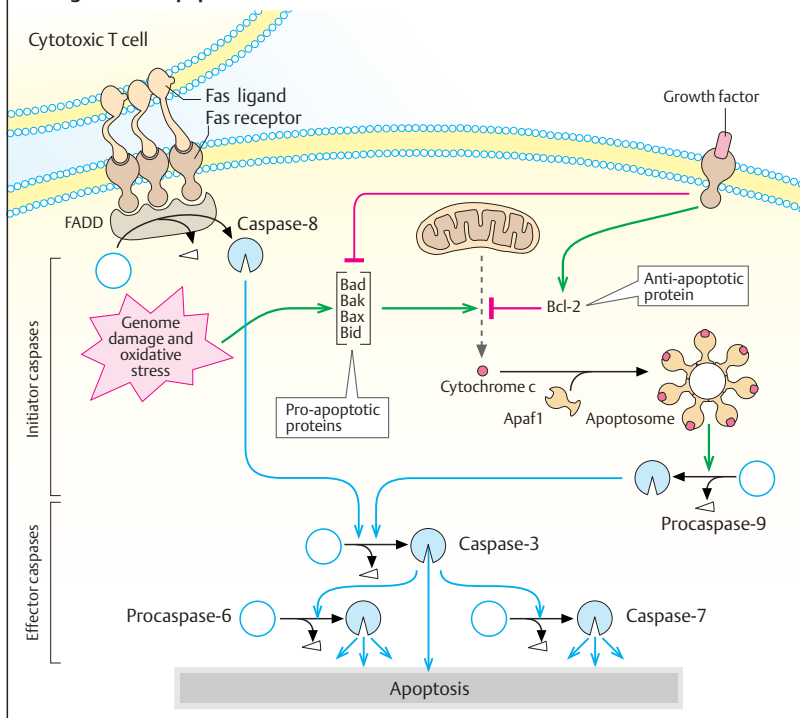
kinases, *transcription factors*, *snRP proteins* (p. 244), and *inhibitors of special DNases*, that are able to fragment the nuclear DNA.

Apoptosis is controlled by extracellular and intracellular signals. The *extrinsic pathway* for apoptosis is triggered on the cell surface by ligands that bind to receptors of the *tumor necrosis factor* family (TNFR, “death receptors”). These include **Fas receptors**, which are present on the plasma membrane of most cells in the body. When *Fas ligands* bind to a cell's Fas receptors, trimerization of the receptors takes place. Via the adapter protein FADD (“Fas-associated death domain”), this activates the initiator *caspsases* 8 and 10 inside the cell, setting in motion the apoptotic process.

The *intrinsic, mitochondrial pathway* is triggered by genotoxic (DNA damage, p. 254) or oxidative **stress** (p. 288). Aided by **Bcl proteins**, chemical stress makes the outer mitochondrial membrane leaky. As a result, mitochondrial proteins reach the cytoplasm. **Cytochrome c** in particular (p. 120) then triggers the caspase cascade by binding to the adapter protein **Apaf1** and promoting formation of an **apoptosome**, a wheel-shaped heptamer that recruits initiator procaspase-9 and activates it to *caspase-9*.

The **Bcl protein** family not only includes proapoptotic proteins (**Bad**, **Bak**, **Bax**, and **Bid**) but also proteins that inhibit apoptosis (including **Bcl-2**). The labile equilibrium between these Bcl proteins is shifted toward apoptosis by **stress**. Cleavage of Bid to **tBid** by caspase-8 also activates Bcl-2. In this way, the intrinsic signaling pathway for triggering apoptosis is linked with the extrinsic pathway and can reinforce it. Extracellular **growth factors** ensure inactivation of Bad or replication of Bcl-2, thus preventing apoptosis.

Finally, the activated initiator caspsases 8, 9, and 10 cleave **procaspase-3** and thereby set off the degradation of cellular components.

A. Cell proliferation and apoptosis**B. Regulation of apoptosis**

Oncogenes

Oncogenes are cellular genes that can trigger uncontrolled cell proliferation if their sequence is altered or their expression is incorrectly regulated. They were first discovered as *viral (v-) oncogenes* in retroviruses that cause tumors (tumor viruses). Viruses of this type (see p. 404) sometimes incorporate host cell genes into their own genome. If these genes are reincorporated into the host DNA of another cell again during later infection, tumors can then be caused in rare cases. Although virus-related tumors are rare, research into them has made a decisive contribution to our understanding of oncogenes and their functioning.

A. Proto-oncogenes: biological role

The cellular form of oncogenes (known as *c-oncogenes* or **proto-oncogenes**) code for proteins involved in controlling growth and differentiation processes. They only become oncogenes if their sequence has been altered by *mutations* (p. 254), *deletions*, or other processes, or when excessive amounts of the gene products have been produced as a result of *overexpression*.

Overexpression can occur when **amplification** leads to numerous functional copies of the respective gene, or when the gene falls under the influence of a highly active promoter (p. 242). If the control of oncogene expression by **tumor suppressor genes** (anti-oncogenes; p. 446) is also disturbed, **transformation** and unregulated proliferation of the cells can occur. A single activated oncogene does not usually lead to a loss of growth control. It only occurs when over the course of time mutations and regulation defects accumulate in one and the same cell. If the immune system does not succeed in eliminating the transformed cell, this can over the course of months or years grow into a macroscopically visible **tumor**.

B. Oncogene products: biochemical functions

A feature common to all oncogenes is the fact that they code for proteins involved in *signal transduction processes* that control the **cell cycle** (p. 444f.) or **apoptosis** (p. 448). Oncogenes are designated using three-letter abbreviations that usually indicate the origin of the viral gene and are written in italics (e.g., *myc* for myelocytomatosis, a viral disease in birds). On-

cogene products can be classified into the following groups according to their functions.

1. Ligands of receptors, e.g., *growth factors* and *cytokines*, which promote cell proliferation.

2. Membrane receptors of the 1-helix type with tyrosine kinase activity (RTK), which can bind growth factors and hormones (p. 400).

3. GTP-binding proteins and adapter proteins. This group includes the G-proteins in the strict sense and related proteins such as Ras (p. 404), the product of the oncogene *c-ras*.

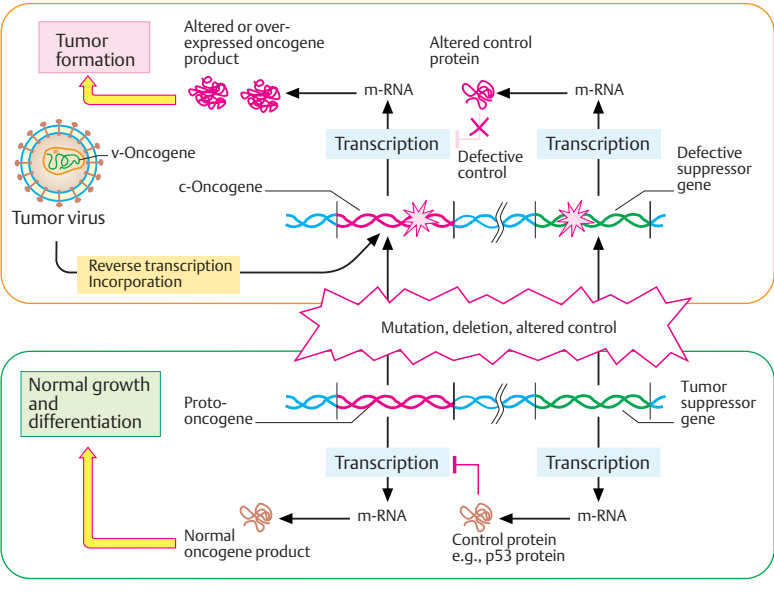
4. Receptors for lipophilic hormones mediate the effects of steroid hormones and related signaling substances. They regulate the transcription of specific genes (p. 418). The products of several oncogenes (e.g., *erbA*) belong to this superfamily of *ligand-gated transcription factors*.

5. Nuclear tumor suppressors inhibit return to the cell cycle in fully differentiated cells. The genes that code for these proteins are referred to as *anti-oncogenes* due to this function. On the role of p53 and pRb, see page 446.

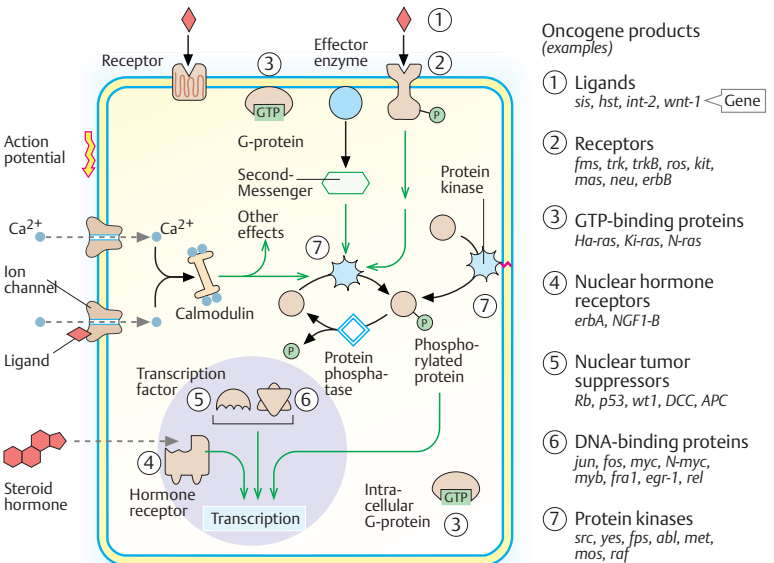
6. DNA-binding proteins. A whole series of oncogenes code for *transcription factors*. Particularly important for cell proliferation are *myc*, as well as *fos* and *jun*. The protein products of the latter two genes form the transcription factor AP-1 as a heterodimer.

7. Protein kinases play a central role in intracellular signal transduction. By phosphorylating proteins, they bring about alterations in biological activity that can only be reversed again by the effects of *protein phosphatases* (p. 410). The interplay between protein phosphorylation by protein kinases and dephosphorylation by protein phosphatases (*interconversion*) serves to regulate the cell cycle (p. 444f.) and other important processes. The protein kinase Raf is also involved in the signal transduction of insulin (p. 428).

A. Proto-oncogenes: biological role



B. Oncogene products: biochemical functions



Tumors

A. Division behavior of cells

The body's cells are normally subject to strict "social" control. They only divide until they come into contact with neighboring cells; cell division then ceases due to *contact inhibition*. Exceptions to this rule include embryonic cells (tissue growth), cells of the intestinal epithelium (constantly being replaced), cells in the bone marrow (where formation of blood cells takes place), and **tumor cells**. *Uncontrolled cell proliferation* is an important indicator of the presence of a tumor. While normal cells in cell culture only divide 20–60 times, tumor cells are potentially immortal and are not subject to contact inhibition. A further characteristic is their *resistance to apoptosis*.

In medicine, a distinction is made between *benign* and *malignant tumors*. Benign tumors consist of slowly growing, largely differentiated cells. By contrast, malignant tumors show rapid, invasive growth and tend to form *metastases* (dissemination of daughter lesions). The 200 or so different types of tumor that exist are responsible for more than 20% of deaths in Europe and North America.

B. Transformation

The transition of a normal cell into a tumor cell is referred to as **transformation**.

Normal cells have all the characteristics of fully differentiated cells specialized for a particular function. Their division is inhibited and they are usually in the G₀ phase of the cell cycle (p. 444). Their external shape is variable and is determined by a strongly structured cytoskeleton.

In contrast, *tumor cells* divide without inhibition and are often de-differentiated—i.e., they have acquired some of the properties of embryonic cells. The surface of these cells is altered, and this is particularly evident in a disturbance of contact inhibition by neighboring cells. The cytoskeleton of tumor cells is also restructured and often reduced, giving them a rounded shape. The nuclei of tumor cells can be atypical in terms of shape, number, and size.

Tumor markers are clinically important for detecting certain tumors. These are proteins that are formed with increasing frequency by tumor cells (group 1) or are induced by them in other cells (group 2). Group 1 tumor markers include *tumor-associated antigens*, secreted

hormones, and enzymes. The table lists a few examples.

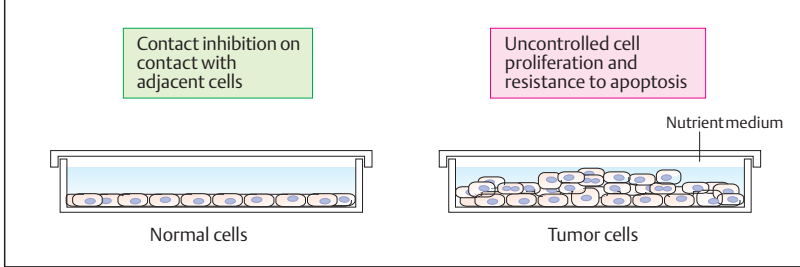
The transition from a normal to a transformed state is a process involving several steps.

1. Tumor initiation. The development of almost every tumor begins with damage to the DNA of an individual cell. The genetic defect is almost always caused by environmental factors. These can include *tumor-inducing chemicals* (*carcinogens*—e.g., components of tar from tobacco), *physical processes* (e.g., UV light, X-ray radiation; p. 254), or in rare cases *tumor viruses* (p. 456). Most of the estimated 10¹⁴ cells in the human body probably suffer this type of DNA damage during the average lifespan, but it is usually repaired again (p. 254). It is mainly defects in **proto-oncogenes** (p. 450) that are relevant to tumor initiation; these are the decisive cause of *transformation*. Loss of an **anti-oncogene** (a tumor-suppressor gene) can also contribute to tumor initiation.

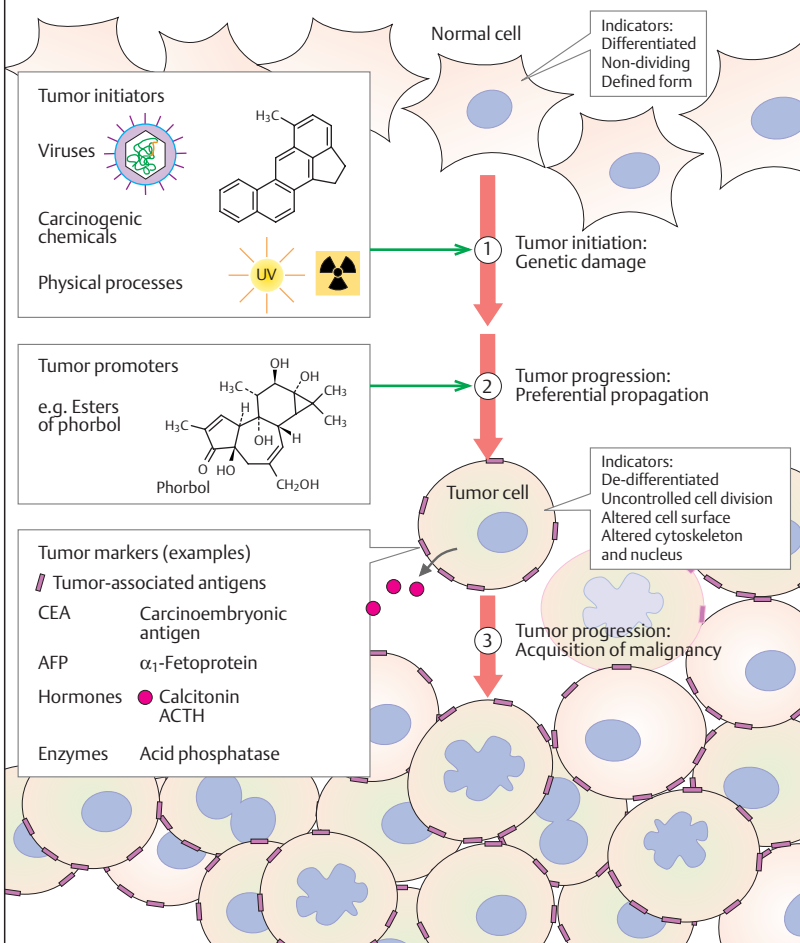
2. Tumor promotion is preferential proliferation of a cell damaged by transformation. It is a very slow process that can take many years. Certain substances are able to strongly accelerate it—e.g., *phorbol esters*. These occur in plants (e.g., *Euphorbia* species) and act as activators of protein kinase C (p. 410).

3. Tumor progression finally leads to a macroscopically visible tumor as a result of growth. When solid tumors of this type exceed a certain size, they form their own vascular network that supplies them with blood (*angiogenesis*). *Collagenases* (matrix metalloproteinases, MMPs; p. 354) play a special role in the *metastatic process*, by loosening surrounding connective tissue and thereby allowing tumor cells to disseminate and enter the bloodstream. New approaches to combating tumors have been aimed at influencing tumor angiogenesis and metastatic processes. Certain forms of leukemia are now treated with specific tyrosine kinases.

A. Division behavior of cells



B. Transformation



Cytostatic Drugs

Tumors (p. 452) arise from degenerated (transformed) cells that grow in an uncontrolled way as a result of genetic defects. Most transformed cells are recognized by the immune system and eliminated (p. 304). If endogenous defense is not sufficiently effective, rapid tumor growth can occur. Attempts are then made to inhibit growth by physical or chemical treatment.

A frequently used procedure is targeted **irradiation** with γ -rays, which block cell reproduction due to their mutagenic effect (p. 254). Another approach is to inhibit cell growth by **chemotherapy**. The growth-inhibiting substances used are known as **cytostatic drugs**. Most cytostatic agents directly or indirectly inhibit DNA replication in the S phase of the cell cycle (see p. 394). The first group (**A**) lead to chemical changes in cellular DNA that impede transcription and replication. A second group of cytostatic agents (**B**) inhibit the synthesis of DNA precursors. Unfortunately, neither radiotherapy nor chemotherapy act selectively—i.e., they damage normal cells as well, and are therefore often associated with severe side effects.

A. Alkylating agents, anthracyclines

Alkylating agents are compounds capable of reacting covalently with DNA bases. If a compound of this type contains *two* reactive groups, intramolecular or intermolecular *crosslinking* of the DNA double helix and “bending” of the double strand occurs. Examples of this type shown here are **cyclophosphamide** and the inorganic complex **cisplatin**. Anthracyclines such as **doxorubicin** (adriamycin) insert themselves noncovalently between the bases and thus lead to local alterations in the DNA structure.

B. Antimetabolites

Antimetabolites are enzyme inhibitors (p. 82) that selectively block metabolic pathways. The majority of clinically important cytostatic drugs act on **nucleotide biosynthesis** (p. 184). Many of these are modified nucleobases or nucleotides that *competitively* inhibit their target enzymes (p. 82). Many are also incorporated into the DNA, thereby preventing replication.

The cytostatic drugs administered (indicated by a syringe in the illustration) are often not active themselves but are only converted into the actual active agent in the metabolism. This also applies to the adenine analogue **6-mercaptopurine**, which is initially converted to the mononucleotide tIMP (thioinosine monophosphate) [1]. Via several intermediate steps, tIMP gives rise to tdGTP, which is incorporated into the DNA and leads to cross-links and other anomalies in it. The second effective metabolite of 6-mercaptopurine is S-methylated tIMP, an inhibitor of *amidophosphoribosyl transferase* ([3]; p. 182).

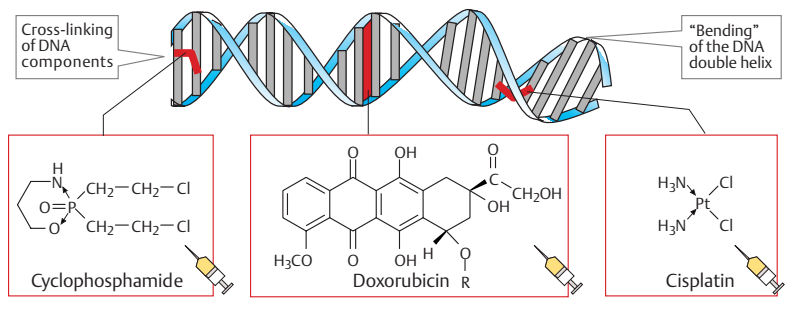
Hydroxyurea selectively inhibits *ribonucleotide reductase* (p. 184). As a radical scavenger, it removes the tyrosine radicals that are indispensable for the functioning of the reductase. Two other important cytostatic agents target the synthesis of DNA-typical thymine, which takes place at the level of the deoxymononucleotide. The deoxymononucleotide formed by **5-fluorouracil** or the nucleoside 5-fluorodeoxyuridine inhibits *thymidylate synthase* [5]. This is based on the fact that the fluorine atom in the pyrimidine ring cannot be substituted by a methyl group. In addition, the fluorine analogue is also incorporated into the DNA.

Dihydrofolate reductase acts as an auxiliary enzyme for thymidylate synthase. It is involved in the regeneration of the coenzyme N^5,N^{10} -methylene-THF, initially reducing DHF to THF with NADPH as the reductant (p. 184). The folic acid analogue **methotrexate**, a frequently used cytostatic agent, is an extremely effective competitive inhibitor of dihydrofolate reductase. It leads to the depletion of N^5,N^{10} -methylene-THF in the cells and thus to cessation of DNA synthesis.

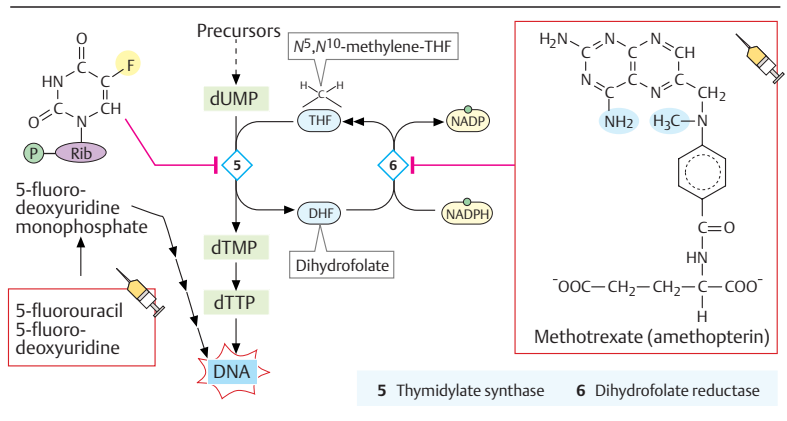
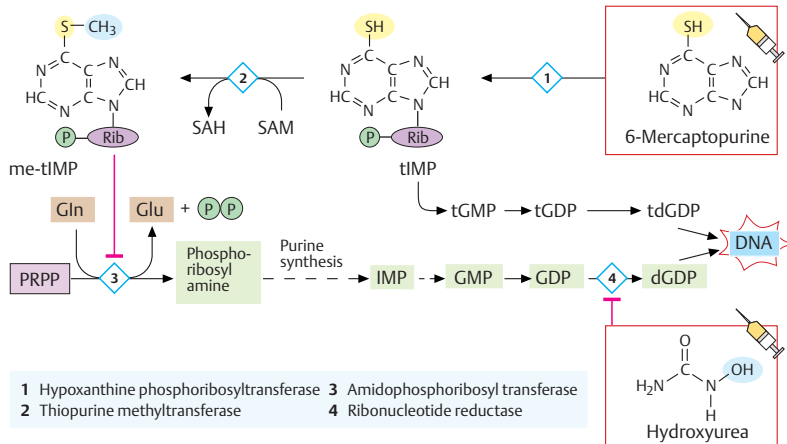
■ Further information

To reduce the side effects of cytostatic agents, new approaches are currently being developed on the basis of **gene therapy** (p. 262). Attempts are being made, for example, to administer drugs in the form of precursors (known as prodrugs), which only become active in the tumor itself (“tumor targeting”).

A. Alkylating agents, anthracyclines



B. Antimetabolites



Viruses

Viruses are *parasitic nucleoprotein complexes*. In the simplest case, they consist of a single nucleic acid molecule (DNA or RNA, never both) and a protein coat. Viruses have *no metabolism of their own*, and can therefore only replicate themselves with the help of host cells. They are therefore not regarded as independent organisms. Viruses that damage the host cell when they replicate are *pathogens*. Diseases caused by viruses include AIDS, rabies, poliomyelitis, measles, German measles, smallpox, influenza, and the common cold.

A. Viruses: examples

Only a few examples from the large number of known viruses are illustrated here. They are all shown on the same scale.

Viruses that only replicate in bacteria are known as **bacteriophages** (or “phages” for short). An example of a phage with a simple structure is **M13**. It consists of a single-stranded DNA (ssDNA) molecule made up of about 7000 bp with a coat made up of 2700 helically arranged protein subunits. The coat of a virus is referred to as a *capsid*, and the complete structure as a *nucleocapsid*. In genetic engineering, M13 has been used as a *vector* for foreign DNA (see p. 258).

The phage **T4** (bottom left), one of the largest viruses known, has a much more complex structure with around 170 000 base pairs (bp) of double-stranded DNA (dsDNA) contained within its “head.” The “legs” bind to the host bacterium, into which the phage injects its DNA.

The **tobacco mosaic virus** (center right), a plant pathogen, has a structure similar to that of M13, but contains ssRNA instead of DNA. The **poliovirus**, which causes poliomyelitis, is also an RNA virus. In the **influenza virus**, the pathogen that causes viral flu, the nucleocapsid is additionally surrounded by a *coat* derived from the plasma membrane of the host cell (top right). The coat carries viral proteins that are involved in the infection process.

B. Capsid of the rhinovirus

Rhinoviruses cause the common cold. In these viruses, the capsid is shaped like an *icosahedron*—i.e., an object made up of 20 equilateral triangles. Its surface is formed from three different proteins, which associate with one another to form pentamers and hexamers.

C. Life cycle of human immunodeficiency virus

The *human immunodeficiency virus (HIV)* causes the immunodeficiency disease known as **AIDS** (*acquired immune deficiency syndrome*). The structure of this virus is similar to that of the influenza virus (A).

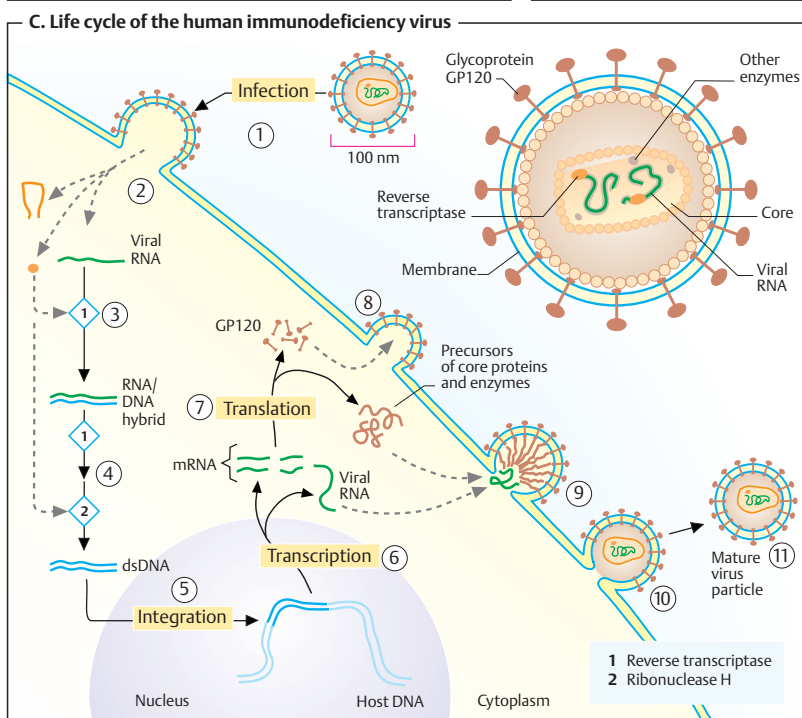
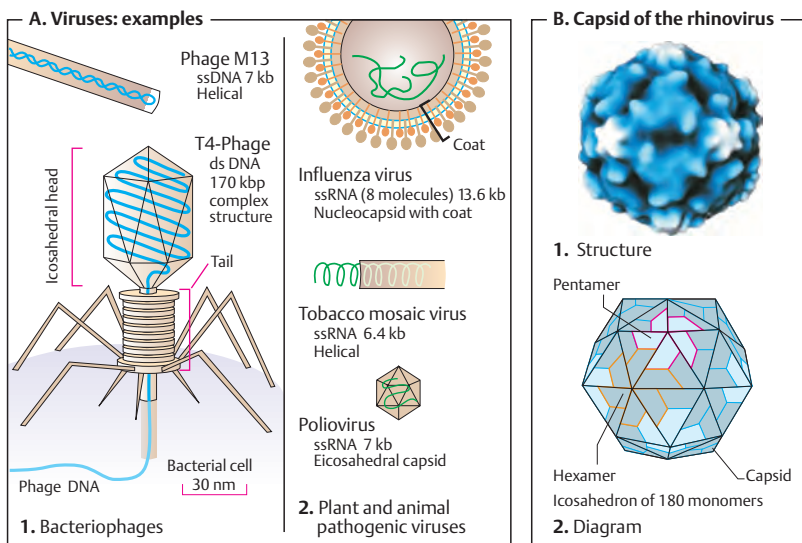
The HIV genome consists of two molecules of ssRNA (each 9.2 kb). It is enclosed by a double-layered capsid and a protein-containing coating membrane. HIV mainly infects T helper cells (p. 304) and can thereby lead to failure of the immune system in the longer term.

During infection (1), the virus's coating membrane fuses with the target cell's plasma membrane, and the core of the nucleocapsid enters the cytoplasm (2). In the cytoplasm, the viral RNA is initially transcribed into an RNA/DNA hybrid (3) and then into dsDNA (4). These reactions are catalyzed by *reverse transcriptase*, an enzyme deriving from the virus, with involvement of RNase H [2]. The dsDNA formed is integrated into the host cell genome (5), where it can remain in an inactive state for a long time.

When viral replication occurs, the DNA segment corresponding to the viral genome is first transcribed by host cell enzymes (6). This gives rise not only to viral ssRNA, but also to transcription of mRNAs for precursors of the viral proteins (7). These precursors are integrated into the plasma membrane (8, 9) before undergoing proteolytic modification (10). The cycle is completed by the release of new virus particles (11).

The group of RNA viruses to which HIV belongs are called **retroviruses**, because DNA is produced from RNA in their replication cycle—the reverse of the usual direction of transcription (DNA → RNA).

Nowadays, the development of AIDS in HIV-infected patients can be suppressed for a long period by combined chemotherapy. The actions of the drugs employed include inhibition of *reverse transcriptase* and *HIV proteinase*, which catalyzes modification of the viral proteins.



Appendix

Abbreviations for amino acids, p. 51

For bases and nucleosides, p. 65

For monosaccharides, p. 31

AA	Amino acid	fMet	N-formylmethionine
ACE	Angiotensin-converting enzyme (peptidyl-dipeptidase A)	FMN	Flavin mononucleotide
ACP	Acyl carrier protein	Fp	Flavoprotein (containing FMN or FAD)
ACTH	Adrenocorticotrophic hormone (corticotropin)	GABA	γ -Aminobutyric acid
ACTase	Aspartate carbamyltransferase	GDP	Guanosine 5'-diphosphate
ADH	Antidiuretic hormone (adiuretin, vasopressin)	Glut	Glucose transporter
ADP	Adenosine 5'-diphosphate	GMP	Guanosine 5'-monophosphate
AIDS	Acquired immunodeficiency syndrome	GSH	Reduced glutathione
ALA	5-Aminolevulinic acid	GSSG	Oxidized glutathione
AMP	Adenosine 5'-monophosphate	GTP	Guanosine 5'-triphosphate
ANF	Atrial natriuretic factor	h	hour
ANP	Atrial natriuretic peptide (= ANF)	Hb	Hemoglobin
AP	Alkaline phosphatase	HBDH	Hydroxybutyrate dehydrogenase
ATP	Adenosine 5'-triphosphate	HDL	High-density lipoprotein
APC	Antigen-presenting cell	HIV	Human immunodeficiency virus
AVP	Arginine vasopressin	HLA	Human leukocyte-associated antigen
b	Base	HMG-CoA	3-Hydroxy-3-methylglutaryl-CoA
bp	Base pair	HMP	Hexose monophosphate pathway
BPG	2,3-Bisphosphoglycerate	hnRNA	Heterogeneous nuclear ribonucleic acid
cAMP	3',5'-Cyclic AMP	HPLC	High-performance liquid chromatography
CAP	Catabolite activator protein	hsp	Heat-shock protein
CD	Cluster of differentiation	IDL	Intermediate-density lipoprotein
CDK	Cyclin-dependent protein kinase (in cell cycle)	IF	Intermediary filament
cDNA	Complementary DNA	IFN	Interferon
CDP	Cytidine 5'-diphosphate	Ig	Immunoglobulin
cGMP	3',5'-Cyclic GMP	IL	Interleukin
CIA	Chemoluminescence immunoassay	InsP ₃	Inositol 1,4,5-trisphosphate
CK	Creatine kinase	IPTG	Isopropylthiogalactoside
CMP	Cytidine 5'-monophosphate	IRS	Insulin-receptor substrate
CoA	Coenzyme A	kDa	Kilodalton (10 ³ atomic mass units)
CoQ	Coenzyme Q (ubiquinone)	K _m	Michaelis constant
CSF	Colony-stimulating factor	LDH	Lactate dehydrogenase
CTP	Cytidine 5'-triphosphate	LDL	Low-density lipoprotein
d	Deoxy-	M	Molarity (mol · L ⁻¹)
Da	Dalton (atomic mass unit)	MAP	Microtubule-associated protein
DAG	Diacylglycerol	MAP	
dd	Dideoxy-	kinase	Mitogen-activated protein kinase
DH	Dehydrogenase	MHC	Major histocompatibility complex
DNA	Deoxyribonucleic acid	MPF	Maturation-promoting factor
dsDNA	Double-stranded DNA	mRNA	Messenger ribonucleic acid
EA	Ethanolamine	MTOC	Microtubule-organizing center
EIA	Enzyme-linked immunoassay	N	Nucleotide with any base
ECM	Extracellular matrix	NAD ⁺	Oxidized nicotinamide adenine dinucleotide
ER	Endoplasmic reticulum	NADH	Reduced nicotinamide adenine dinucleotide
ETF	Electron-transferring protein	NADP ⁺	Oxidized nicotinamide adenine dinucleotide phosphate
FAD	Flavin adenine dinucleotide	NADPH	Reduced nicotinamide adenine dinucleotide phosphate
Fd	Ferredoxin	NeuAc	N-acetylneuraminic acid
FFA	Free fatty acid	nm	Nanometer (10 ⁻⁹ m)
		ODH	2-Oxoglutarate dehydrogenase

PAGE	Polyacrylamide gel electrophoresis	S	Svedberg (unit of sedimentation coefficient)
Pan	Pantetheine	SAH	S-adenosyl L-homocysteine
PAPS	Phosphoadenosine phosphosulfate	SAM	S-adenosyl L-methionine
PCR	Polymerase chain reaction	SDS	Sodium dodecylsulfate
PDH	Pyruvate dehydrogenase	sER	Smooth endoplasmic reticulum
PEG	Polyethylene glycol	sn	Stereospecific numbering
PEP	Phosphoenolpyruvate	snRNA	Small nuclear ribonucleic acid
PEP-CK	Phosphoenolpyruvate carboxy-kinase	SR	Sarcoplasmic reticulum
pH	pH value	ssDNA	Single-stranded DNA
P _i	Inorganic phosphate	STR	Short tandem repeat
PK	Protein kinase	TBG	Thyroxine-binding globulin
PKU	Phenylketonuria	THB	Tetrahydrobiopterin
PLP	Pyridoxal phosphate	THF	Tetrahydrofolate
PP	Protein phosphatase	TLC	Thin-layer chromatography
PP _i	Inorganic diphosphate	TPP	Thiamine diphosphate
PPP	PPP Pentose phosphate pathway	TRH	Thyrotropin-releasing hormone (thyroliberin)
PQ	Plastoquinone	Tris	Tris(hydroxymethyl)amino-methane
PRPP	5-Phosphoribosyl 1-diphosphate	tRNA	Transfer ribonucleic acid
PTH	Parathyroid hormone	TSH	Thyroid-stimulating hormone (thyrotropin)
Q	Oxidized coenzyme Q (ubiquinone)	UCP	Uncoupling protein
QH ₂	Reduced coenzyme Q (ubiquinol)	UDP	Uridine 5'-diphosphate
R	Gas constant	UMP	Uridine 5'-monophosphate
rER	Rough endoplasmic reticulum	UTP	Uridine 5'-triphosphate
RES	Reticuloendothelial system	UV	Ultraviolet radiation
RFLP	Restriction fragment length polymorphism	V _{max} , V	Maximal velocity (of an enzyme)
RIA	Radioimmunoassay	VLDL	Very-low-density lipoprotein
RNA	Ribonucleic acid		
ROS	Reactive oxygen species		
rRNA	Ribosomal ribonucleic acid		

1. SI base units

Quantity	SI unit	Symbol	Remarks
Length	Meter	m	1 yard (yd) = 0.9144 m 1 inch (in) = 0.0254 m 1 Å = 10 ⁻¹⁰ m = 0.1 nm
Mass	Kilogram	kg	1 pound (lb) = 0.4536 kg
Time	Second	s	
Current strength	Ampere	A	
Temperature	Kelvin	K	°C (degree Celsius) = K – 273.2 Fahrenheit: °C = 5/9 × (°F – 32)
Light	Candela	Cd	
Amount of substance	Mole	mol	

2. Derived units

Quantity	Unit	Symbol	Derivation	Remarks
Frequency	Hertz	Hz	s ⁻¹	
Volume	Liter	L	10 ⁻³ · m ³	1 US gallon (gal) = 3.785 L
Force	Newton	N	kg · m · s ⁻²	
Pressure	Pascal	Pa	N · m ⁻²	1 bar = 10 ⁵ Pa 1 mmHg = 133.3 Pa
Energy, work, heat	Joule	J	N · m	1 calorie (cal) = 4.1868 J
Power	Watt	W	J · s ⁻¹	
Electrical charge	Coulomb	C	A · s	
Voltage	Volt	V	W · A ⁻¹	
Concentration	Molarity	M	mol · L ⁻¹	
Molecular mass	Dalton	Da	1.6605 × 10 ⁻²⁴ g	
Molar mass	–	–	g	
Molecular weight	–	M _r	–	Nondimensional
Reaction rate		v	mol · s ⁻¹	
Catalytic activity	Katal	kat	mol · s ⁻¹	1 unit (U) = 1.67 × 10 ⁻⁸ kat

Specific activity	–	–	kat · (kg enzyme) ⁻¹	Usually: U · (mg enzyme) ⁻¹
Sedimentation coefficient	Svedberg	S	10 ⁻¹³ s	
Radioactivity	Becquerel	Bq	Decays · s ⁻¹	1 Ci (Ci) = 3.7 × 10 ¹⁰ Bq

3. Multiples and fractions

Factor	Prefix	Symbol	Example
10 ⁹	Giga	G	GHz = 10 ⁹ hertz
10 ⁶	Mega	M	MPa = 10 ⁶ pascal
10 ³	Kilo	k	kJ = 10 ³ joule
10 ⁻³	Milli	m	mM = 10 ⁻³ mol · L ⁻¹
10 ⁻⁶	Micro	μ	μV = 10 ⁻⁶ volt
10 ⁻⁹	Nano	n	nkat = 10 ⁻⁹ katal
10 ⁻¹²	Pico	p	pm = 10 ⁻¹² meter

4. Important constants

Constants

General gas constant, R	R = 8.314 J · mol ⁻¹ · K ⁻¹
Loschmidt (Avogadro) number, N (number of particles per mol)	N = 6.0225 × 10 ²³
Faraday constant F	F = 96480 C · mol ⁻¹

Individual graphic elements in the following plates are based on the following sources, used with the kind permission of the authors and publishers concerned.

Page	Figure	Source
55	A	Goodsell DS. Trends Biochem Sci 1993;18:65–68
141	A	Yañez AJ, et al. FEBS Lett 2004;577:54–158
151	A	Asturias FJ, et al. Nature Structural & Molecular Biology 2005;12:225–232
201	C	MB Omary, et al. Trends Biochem Sci 2006;31:383–394
227	A	RCSB, Protein Data Bank, Molecule of the Month, http://www.rcsb.org/pdb/static.do?p=education_discussion/molecule_of_the_month/pdb57_1.html
233	C	http://en.wikipedia.org/wiki/Human_genome
283	B	Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990, p. 306, Fig. 11-47
305	A	Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990, p. 1097, Fig. 34-13
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379	B,C	Cahill GF. Annu Rev Nutr 2006;26:1–22
377	B	http://commons.wikimedia.org/wiki/File%3AVerlauf_Insulin-Glucagon-GLP1_nach_Habener.svg
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447	A	Malumbres M, Barbacid M. Trends Biochem Sci 2005;30:630–641, Fig. 1

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