Digestion: overview

Most components of food (see p. 360) cannot be resorbed 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 resorption of the resulting products.

A. Hydrolysis and resorption of food components ●

Following mechanical fragmentation of food during chewing in the mouth, the process of enzymatic degradation 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 (see p. 268). Almost all digestive enzymes are *hydrolases* (class 3 enzymes; see p. 88); they catalyze the cleavage of composite bonds with the uptake of water.

Proteins are first denatured by the stomach's *hydrochloric acid* (see 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 resorbed by the intestinal mucosa in cotransport with Na^+ ions (see p. 220). There are separate transport systems for each of the various groups of amino acids.

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 (see p. 220). In addition, monosaccharides also have 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 resorbed by the intestinal wall in the region of the jejunum.

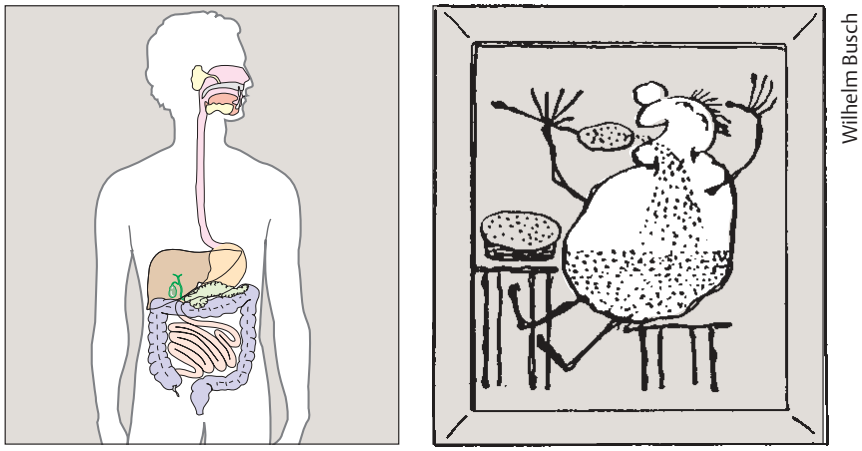
Lipids are a special problem for digestion, as they are not soluble in water. Before enzymatic breakdown, they have to be emulsified by *bile salts* and *phospholipids* in the bile (see p. 314). At the water–lipid interface, *pancreatic lipase* then attacks triacylglycerols with the help of *colipase* (see p. 270). The cleavage products include *fatty acids, 2–monoacylglycerols, glycerol,* and *phosphate* from phospholipid breakdown. After resorption into the epithelial cells, fats are resynthesized from fatty acids, glycerol and 2–monoacylglycerols and passed into the lymphatic system (see p. 272). The lipids in milk are more easily digested, as they are already present in emulsion; on cleavage, they mostly provide short-chain fatty acids.

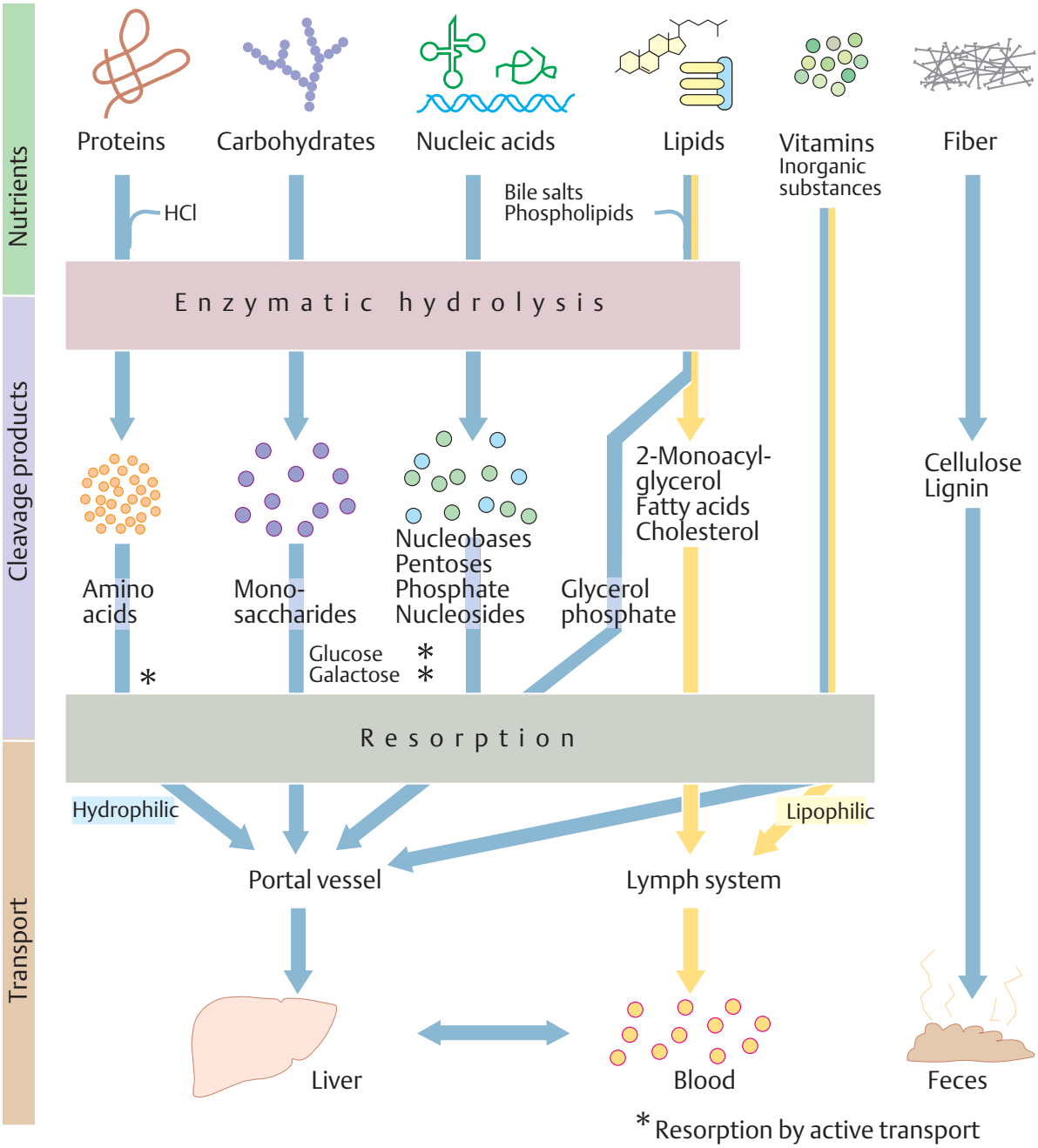
Inorganic components such as water, electrolytes, and *vitamins* are directly absorbed by the intestine.

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 resorbed 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* (see p. 278) into the *lymph system,* and only reach the blood via the thoracic duct.

A. Hydrolysis and resorption of dietary constituents





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* attacks polysaccharides, and a lipase hydrolyzes a small proportion of the neutral fats. α–Amylase and *lysozyme,* a murein-cleaving enzyme (see p. 40), probably serve to regulate the oral bacterial flora rather than for digestion (see p. 340).

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; see p. 270). 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* (see pp. 176, 270). In addition, the gastric mucosa secretes what is known as *"intrinsic factor"*—a glycoprotein needed for resorption of vitamin B_{12} ("extrinsic factor") in the bowel.

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 HCO_3^- content, the buffer capacity of which is suf cient 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 optimums in the neutral or weakly alkaline range. Many of them are formed and secreted as proenzymes and are only activated in the bowel lumen (see p. 270).

Trypsin, chymotrypsin, and *elastase* are endopeptidases that belong to the group of serine proteinases (see p. 176). Trypsin hydrolyzes specific peptide bonds on the C side of the basic amino acids Arg and Lys, while chymotrypsin prefers peptide bonds of the apolar amino acids Tyr, Trp, Phe, and Leu (see p. 94). 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 exopeptidases cleave individual amino acids from the C–terminal end of the peptides (see p. 176).

~–Amylase, the most important endoglycosidase in the pancreas, catalyzes the hydrolysis of α1→4 bonds in the polymeric carbohydrates starch and glycogen. This releases maltose, maltotriose, and a mixture of other oligosaccharides.

Various pancreatic enzymes hydrolyze lipids, including *lipase* with its auxiliary protein *colipase* (see p. 270), *phospholipase* A_2, 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* (see p. 314), *phospholipids, bile pigments,* 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). Resorption of fat-soluble vitamins would also be affected.

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.

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 (3.2.1.1)	Cleave starch ↓
Lysozyme (3.2.1.17)	Attacks bacterial cell walls ↓

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

↓ Endoenzyme
-◂ Exoenzyme

Compo-nents | Function or substrate

Gastric juice

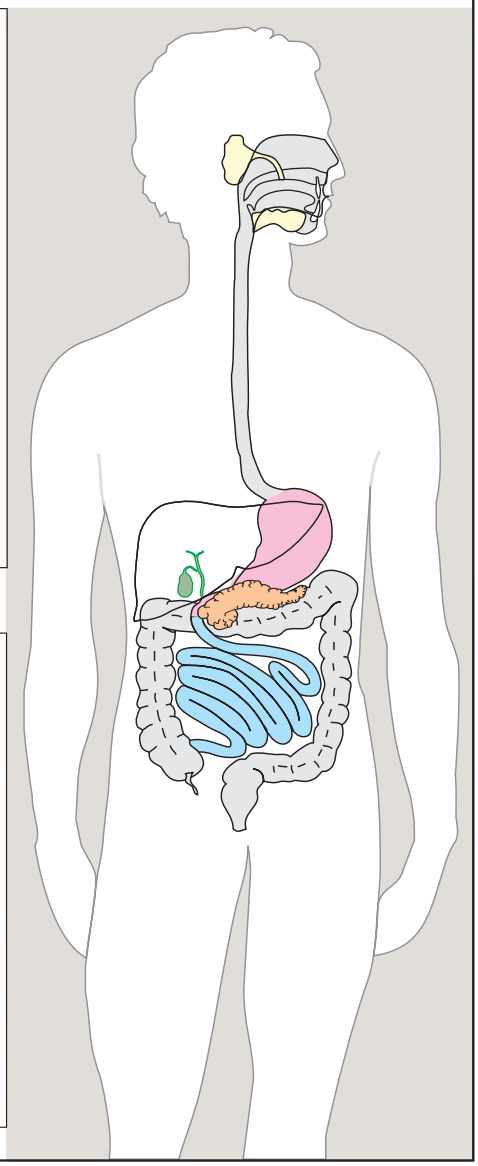
Daily secretion 2–3 l
pH 1

Water	
Salts	
HCl	Denatures proteins, kills bacteria
Mucus	Protects stomach lining
Pepsins (3.4.23.1-3)	Cleave proteins ↓
Chymosin (3.4.23.4)	Precipitates casein ↓
Triacylglycerol lipase (3.1.1.3)	Cleaves fats
Intrinsic factor	Protects vitamin B_{12}

Pancreatic secretions

Daily secretion 0.7–2.5 l
pH 7.7 (7.5–8.8)

Water	
HCO_3^-	Neutralizes gastric juice
Trypsin (3.4.21.4)	Proteins ↓
Chymotrypsin (3.4.21.1)	Proteins ↓
Elastase (3.4.21.36)	Proteins ↓
Carboxypeptidases (3.4.n.n)	Peptides -◂
α-Amylase (3.2.1.1)	Starch and glycogen ↓
Triacylglycerol lipase (3.1.1.3)	Fats
Co-Lipase	Cofactor for lipase
Phospholipase A_2 (3.1.1.4)	Phospholipids
Sterol esterase (3.1.1.13)	Cholesterol esters
Ribonuclease (3.1.27.5)	RNA
Deoxyribonuclease I (3.1.21.1)	DNA



Secretions of the small intestine

Daily secretion unknown
pH 6.5–7.8

Aminopeptidases (3.4.11.n)	Peptides -◂
Dipeptidases (3.4.13.n)	Dipeptides
α-Glucosidase (3.2.1.20)	Oligosaccharides ↓
Oligo-1,6-glucosidase (3.2.1.10)	Oligosaccharides ↓
β-Galactosidase (3.2.1.23)	Lactose
Sucrose α-glucosidase (3.2.1.48)	Sucrose
α, α-Trehalase (3.2.1.28)	Trehalose
Alkaline phosphatase (3.1.3.1)	Phosphoric acid esters
Polynucleotidases (3.1.3.n)	Nucleic acids, nucleotides ↓
Nucleosidases (3.2.2.n)	Nucleosides -◂
Phospholipases (3.1.n.n)	Phospholipids

Digestive processes

Gastric juice is the product of several cell types. The *parietal cells* produce hydrochloric acid, *chief cells* release pepsinogen, and *accessory cells* form a mucin-containing mucus.

A. Formation of hydrochloric acid ◑

The secretion of **hydrochloric acid** (H^+ and Cl^-) by the parietal cells is an active process that uses up 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 [2]), 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* [1] (a transport ATPase of the P type; see p. 220). 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 pepsinogen to form pepsin (see below) and creates an optimal pH level for it to take effect. It also denatures food proteins so that they are more easily attacked by proteinases, and it kills micro-organisms.

Regulation. HCl secretion is stimulated by the peptide hormone *gastrin,* the mediator *histamine* (see p. 380), and—via the neurotransmitter *acetylcholine*—by the autonomous nervous system. The peptide *somatostatin* and certain *prostaglandins* (see p. 390) have inhibitory effects. Together with cholecystokinin, secretin, and other peptides, gastrin belongs to the group of **gastrointestinal hormones** (see p. 370). All of these are formed in the gastrointestinal tract and mainly act in the vicinity of the site where they are formed—i. e., they are paracrine hormones (see p. 372). While gastrin primarily enhances HCl secretion, *cholecystokinin* and *secretin* mainly stimulate pancreatic secretion and bile release.

B. Zymogen activation ◑

To prevent self–digestion, the pancreas releases most proteolytic enzymes into the duodenum in an inactive form as *proenzymes* (zymogens). Additional protection from the effects of premature activation of pancreatic proteinases is provided by *proteinase inhibitors* in the pancreatic tissue, which inactivate active enzymes by complex formation (right).

Trypsinogen plays a key role among the proenzymes released by the pancreas. In the bowel, it is proteolytically converted into active trypsin (see p. 176) by **enteropeptidase**, a membrane enzyme on the surface of the enterocytes. Trypsin then autocatalytically activates additional trypsinogen molecules and the other proenzymes (left).

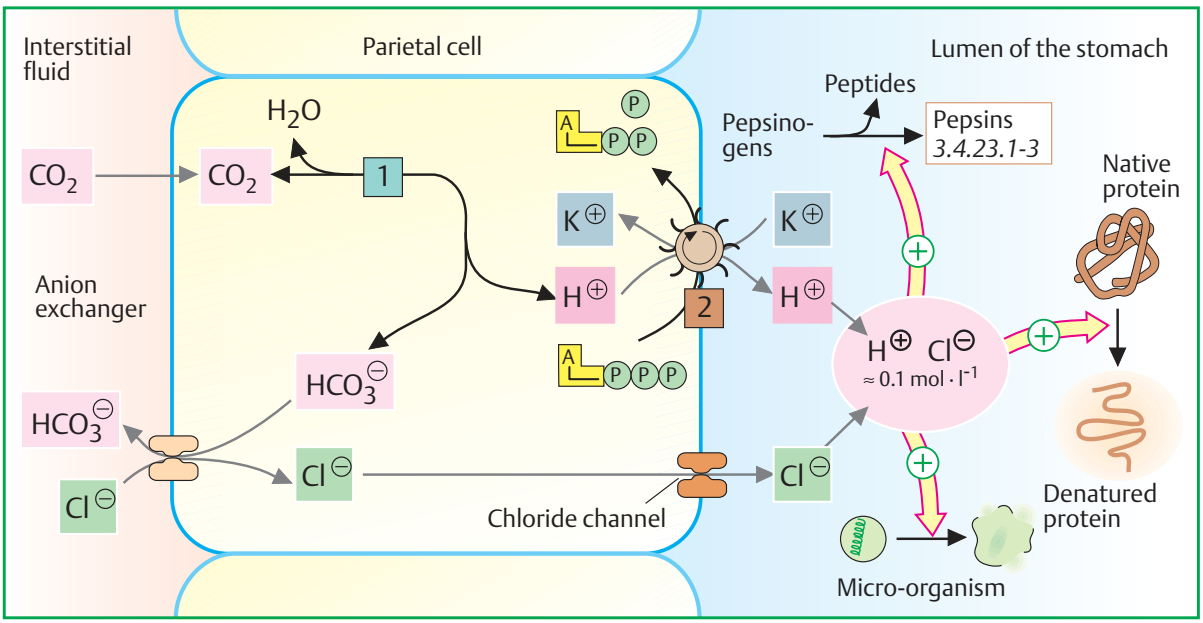
C. Fat digestion ◑

Due to the "hydrophobic effect" (see p. 28), water-insoluble neutral fats in the aqueous environment of the bowel lumen would aggregate into drops of fat in which most of the molecules would not be accessible to pancreatic lipase. The amphipathic substances in bile (bile acids, bile salts, phospholipids) create an **emulsion** in which they occupy the surface of the droplets and thereby prevent them from coalescing into large drops. In addition, the bile salts, together with the auxiliary protein colipase, mediate binding of *triacylglycerol lipase* [1] to the emulsified fat droplets. Activation of the lipase is triggered by a conformation change in the C-terminal domain of the enzyme, which uncovers the active center.

During passage through the intestines, the active lipase breaks down the triacylglycerols in the interior of the droplets into free fatty acids and amphipathic monoacylglycerols. Over time, smaller **micelles** develop (see p. 28), in the envelope of which monoacylglycerols are present in addition to bile salts and phospholipids. Finally, the components of the micelles are resorbed by the enterocytes in ways that have not yet been explained.

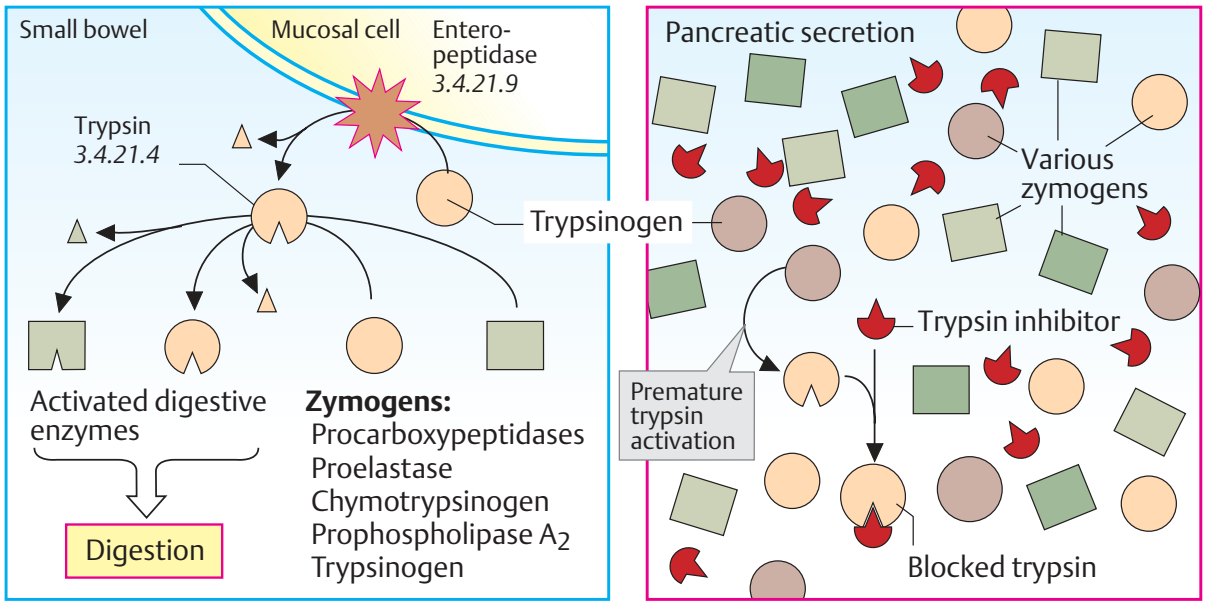
Monoacylglycerols and fatty acids are reassembled into fats again (see p. 272), while the bile acids return to the liver (enterohepatic circulation; see p. 314).

A. Formation of hydrochloric acid

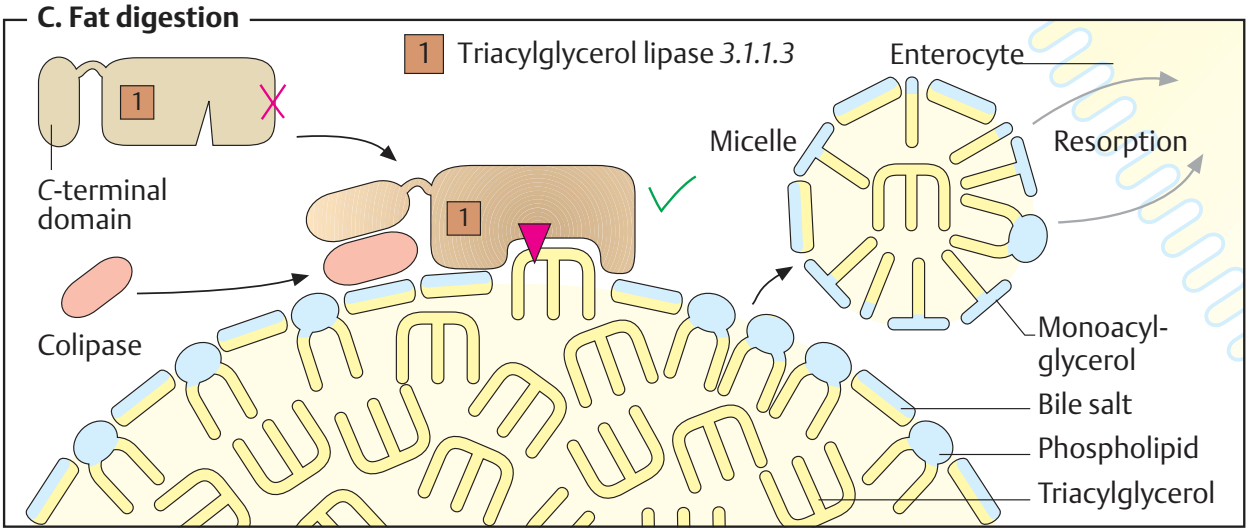


1 Carbonate dehydratase *4.2.1.1* [Zn^{2+}] 2 H^+/K^+-exchanging ATPase *3.6.1.36*

B. Zymogen activation



C. Fat digestion



Resorption

Enzymatic hydrolysis in the digestive tract breaks down foodstuffs into their resorbable components. *Resorption* of the cleavage products takes place primarily in the small intestine. Only ethanol and short–chain fatty acids are already resorbed to some extent in the stomach.

The resorption process is facilitated by the large inner surface of the intestine, with its brush–border cells. Lipophilic molecules penetrate the plasma membrane of the mucosal cells by simple diffusion, whereas polar molecules require *transporters* (facilitated diffusion; see p. 218). In many cases, carrier-mediated cotransport with Na^+ ions can be observed. In this case, the difference in the concentration of the sodium ions (high in the intestinal lumen and low in the mucosal cells) drives the import of nutrients against a concentration gradient (secondary active transport; see p. 220). Failure of carrier systems in the gastrointestinal tract can result in diseases.

A. Monosaccharides ◑

The cleavage of polymeric carbohydrates by *~–amylase* [1] leads to **oligosaccharides**, which are broken down further by *exoglycosidases* (oligosaccharidases and disaccharidases [2]) on the membrane surface of the brush border. The monosaccharides released in this way then pass with the help of various *sugar–specific transporters* into the cells of intestinal epithelium. *Secondary active transport* serves for the uptake of **glucose** and **galactose**, which are transported against a concentration gradient in cotransport with Na^+. The Na^+ gradient is maintained on the basal side of the cells by *Na^+/K^+-ATPase* [3]. Another passive transporter then releases glucose and galactose into the blood. **Fructose** is taken up by a special type of transporter using facilitated diffusion.

Amino acids (not illustrated)

Protein degradation is initiated by *proteinases*—by pepsins in the stomach and by trypsin, chymotrypsin, and elastase in the small intestine. The resulting peptides are then further hydrolyzed by various *peptidases* into amino acids. Individual amino acid groups have *group–specific amino acid transporters,* some of which transport the amino acids into the enterocytes in cotransport with Na^+ ions (secondary active transport), while others transport them in an Na^+–independent manner through facilitated diffusion. Small peptides can also be taken up.

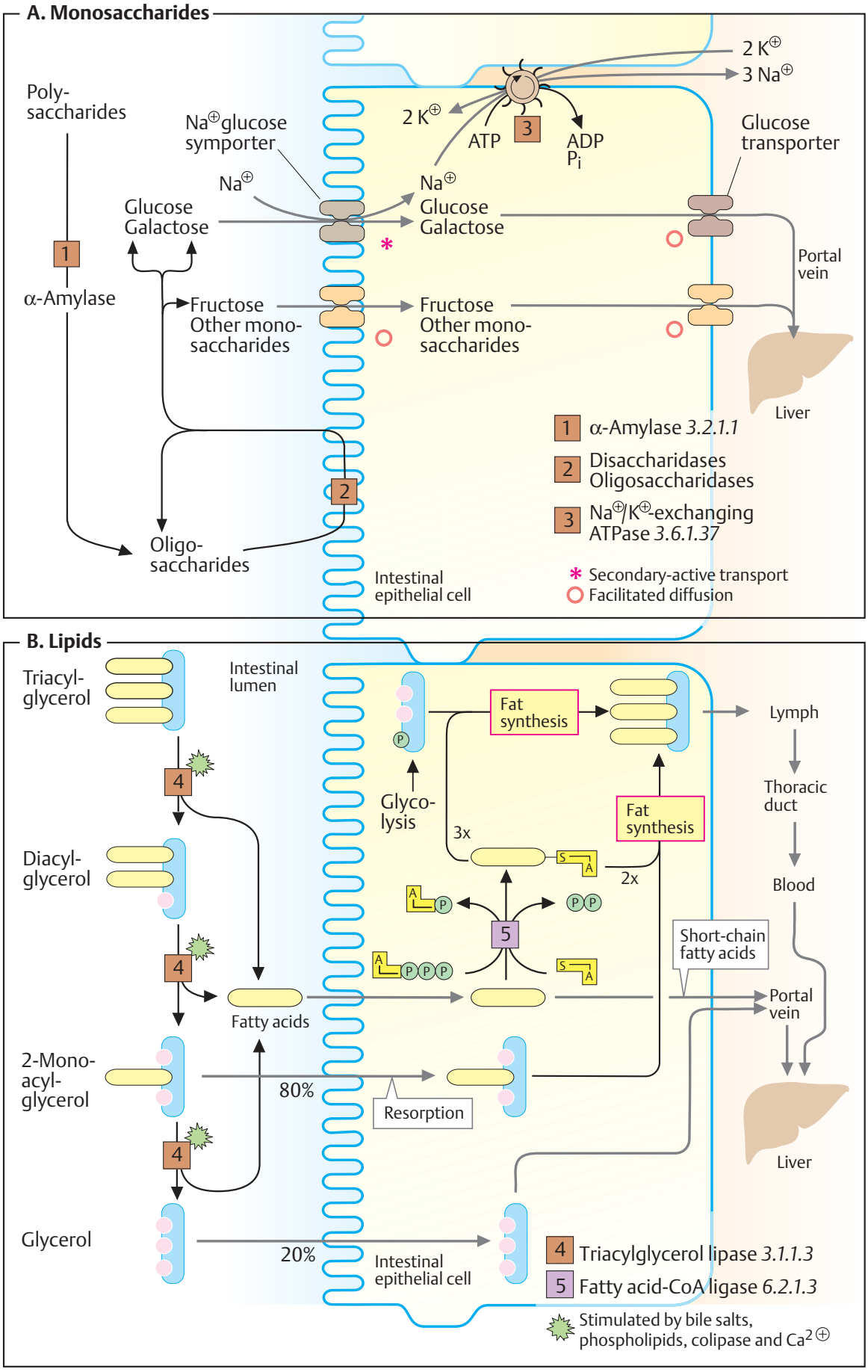
B. Lipids ◑

Fats and other lipids are poorly soluble in water. The larger the accessible surface is—i. e., the better the fat is emulsified—the easier it is for enzymes to hydrolyze it (see p. 270). Due to the special properties of milk, milk fats already reach the gastrointestinal tract in emulsified form. Digestion of them therefore already starts in the oral cavity and stomach, where lipases in the saliva and gastric juice are available. Lipids that are less accessible—e. g., from roast pork—are emulsified in the small intestine by *bile salts* and *bile phospholipids.* Only then are they capable of being attacked by *pancreatic lipase* [4] (see p. 270).

Fats (triacylglycerols) are mainly attacked by pancreatic lipase at positions 1 and 3 of the glycerol moiety. Cleavage of two fatty acid residues gives rise to **fatty acids** and **2-monoacylglycerols**, which are quantitatively the most important products. However, a certain amount of **glycerol** is also formed by complete hydrolysis. These cleavage products are resorbed by a non-ATP-dependent process that has not yet been explained in detail.

In the mucosal cells, *long-chain fatty acids* are resynthesized by an ATP-dependent ligase [5] to form acyl-CoA and then triacylglycerols (fats; see p. 170). The fats are released into the lymph in the form of **chylomicrons** (see p. 278) and, bypassing the liver, are deposited in the thoracic duct—i. e., the blood system. *Cholesterol* also follows this route.

By contrast, *short-chain fatty acids* (with chain lengths of less than 12 C atoms) pass directly into the blood and reach the liver via the portal vein. Resorbed glycerol can also take this path.



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 in order to transport them to the lungs, liver, and kidney for excretion. The blood also distributes *hormones* throughout the organism (see p. 370).

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 regulated by the blood in combination with the lungs, liver, and kidneys (see p. 288). The regulation of *body temperature* also depends on the controlled transport of heat by the blood.

Defense. The body uses both non-specific and specific mechanisms to defend itself against pathogens. The defense system includes the *cells of the immune system* and certain *plasma proteins* (see p. 294).

Self-protection. To prevent blood loss when a vessel is injured, the blood has systems for stanching blood flow and coagulating the blood (hemostasis; see p. 290). The dissolution of blood clots (fibrinolysis) is also managed by the blood itself (see p. 292).

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 pp. 280–285.

The **leukocytes** include various types of granulocyte, monocyte, and lymphocyte. All of these have immune defense functions (see p. 294). 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.

Thrombocytes are cell fragments that arise in the bone marrow from large precursor cells, the megakaryocytes. Their task is to promote hemostasis (see p. 290).

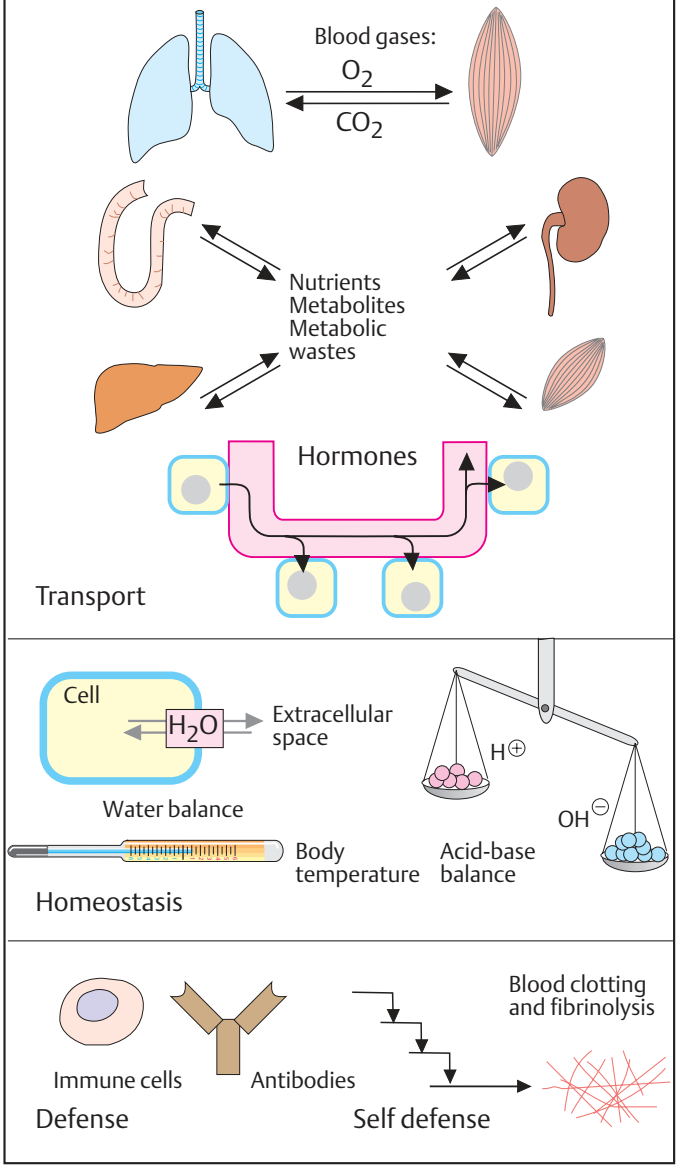
C. Blood plasma: composition ◑

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 (see p. 290).

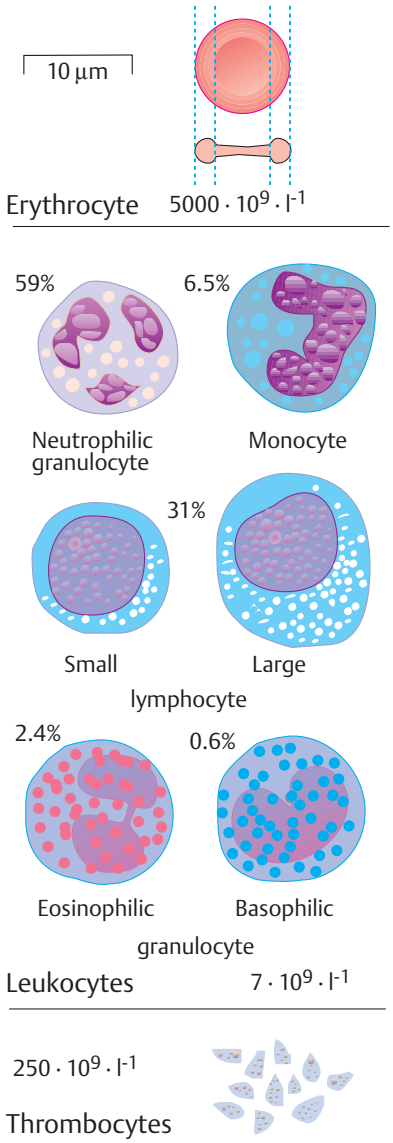
Laboratory assessment of the composition of the blood plasma is often carried out in clinical chemistry. Among the electrolytes, there is a relatively high concentration of Na^+, Ca^{2+}, and Cl^- ions in the blood in comparison with the cytoplasm. By contrast, the concentrations of K^+, Mg^{2+}, and 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 *"physiological saline"* (NaCl at a concentration of 0.15 mol L^{-1}) is almost isotonic with blood plasma.

A list of particularly important **metabolites** in the blood plasma is given on the right.

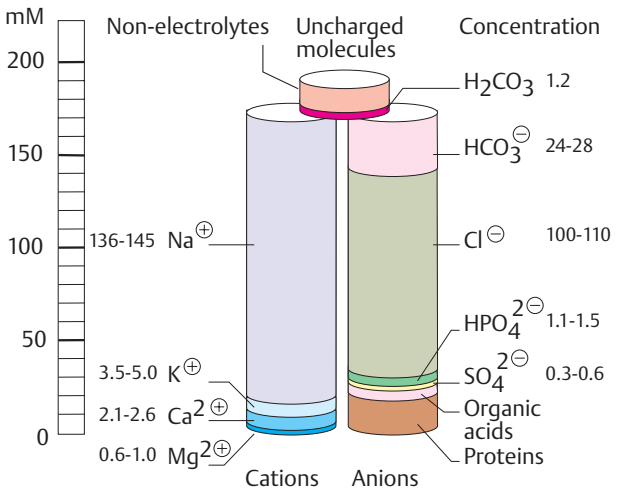
A. Functions of the blood



B. Cellular elements



C. Blood plasma: composition



Metabolite	Concentration (mM)
Glucose	3.6 – 6.1
Lactate	0.4 – 1.8
Pyruvate	0.07 – 0.11
Urea	3.5 – 9.0
Uric acid	0.18 – 0.54
Creatinin	0.06 – 0.13
Amino acids	2.3 – 4.0
Ammonia	0.02 – 0.06
Lipids (total)	5.5 – 6.0 $g \cdot l^{-1}$
Triacylglycerols	1.0 – 1.3 $g \cdot l^{-1}$
Cholesterol	1.7 – 2.1 $g \cdot l^{-1}$

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 frequent 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 apolar 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 Ca^{2+} and Mg^{2+} ions. 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.

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; see p. 278), hormones, vitamins, and metal ions. In addition, they provide coagulation factors, protease inhibitors, and the proteins of the complement system (see p. 298). Soluble antibodies (immunoglobulins; see p. 300) 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 (see p. 302) 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 (see p. 44). *N*-acetylneuraminic acid (sialic acid; see p. 38) 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.

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 (see p. 392), 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 proteins are altered in some diseases (known as *dysproteinemias*).

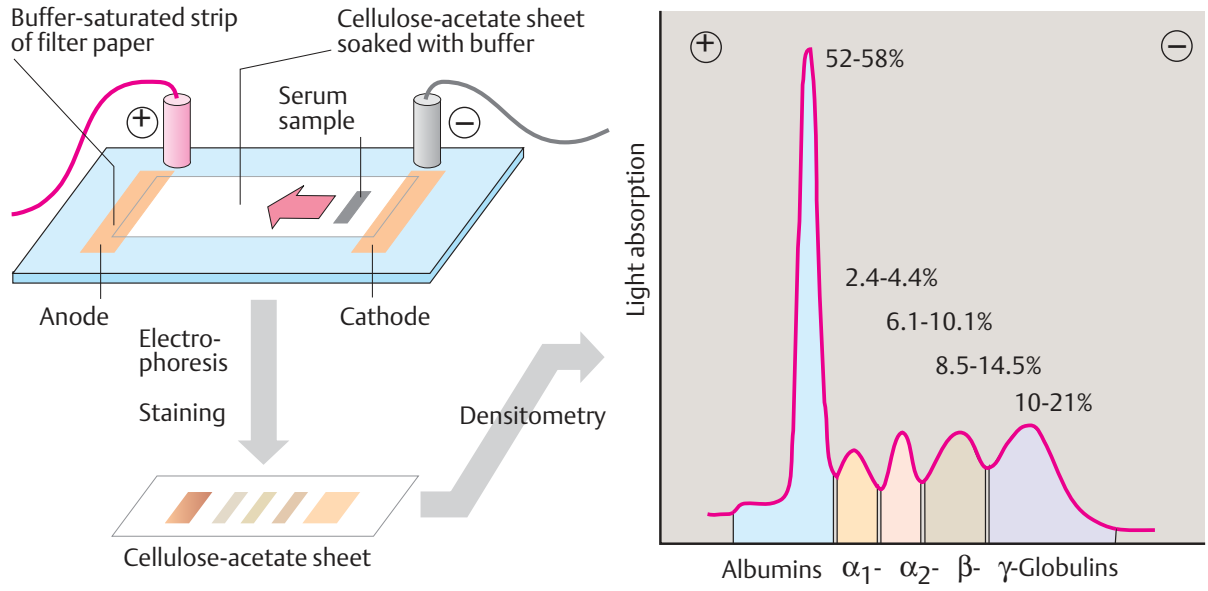
B. Carrier electrophoresis ◑

Proteins and other electrically charged macromolecules can be separated using electrophoresis (see also pp. 78, 262). Among the various procedures used, **carrier electrophoresis** on cellulose acetate foil (CAF) is particularly simple. Using this method, serum proteins—which at slightly alkaline pH values all move towards the anode, due to their excess of negative charges—can be separated into the five fractions mentioned. After the proteins have been stained with dyes, the resulting bands can be quantitatively assessed using densitometry.

A. Plasma proteins

Group	Protein	M_r in kDa	Function
Albumins:	Transthyretin	50-66	Transport of thyroxin and triiodothyronin
	Albumin: 45 g · l^{-1}	67	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
	Lipoprotein (HDL)	200-400	Transport of lipids
	Prothrombin	72	Coagulation factor II, thrombin precursor (*3.4.21.5*)
	Transcortin	51	Transport of cortisol, corticosterone and progesterone
	Acid glycoprotein	44	Transport of progesterone
	Thyroxin-binding globulin	54	Transport of iodothyronins
α_2-Globulins:	Ceruloplasmin	135	Transport of copper ions
	Antithrombin III	58	Inhibition of blood clotting
	Haptoglobin	100	Binding of hemoglobin
	Cholinesterase (3.1.1.8)	ca. 350	Cleavage of choline esters
	Plasminogen	90	Precursor of plasmin (*3.4.21.7*), 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 calciols
β-Globulins:	Lipoprotein (LDL)	2.000-4.500	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	162	Mucosa-protecting antibodies
	IgM	900	Early antibodies
	IgD	172	B-lymphocyte receptors
	IgE	196	Reagins

B. Electrophoresis



Lipoproteins

Most lipids are barely soluble in water, and many have amphipathic properties. In the blood, free triacylglycerols would coalesce into drops that could cause fat embolisms. By contrast, amphipathic lipids would be deposited in the blood cells' membranes and would dissolve them. Special precautions are therefore needed for lipid transport in the blood. While long-chain fatty acids are bound to albumin and short-chain ones are dissolved in the plasma (see p. 276), other lipids are transported in **lipoprotein complexes**, of which there several types in the blood plasma, with different sizes and composition.

A. Composition of lipoprotein complexes ◑

Lipoproteins are spherical or discoid aggregates of **lipids** and **apoproteins**. They consist of a nucleus of apolar lipids (triacylglycerols and cholesterol esters) surrounded by a single-layered shell approximately 2 nm thick of amphipathic lipids (phospholipids and cholesterol; the example shown here is LDL). 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 apolar lipids it contains—the lower its density is.

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

B. Transport functions

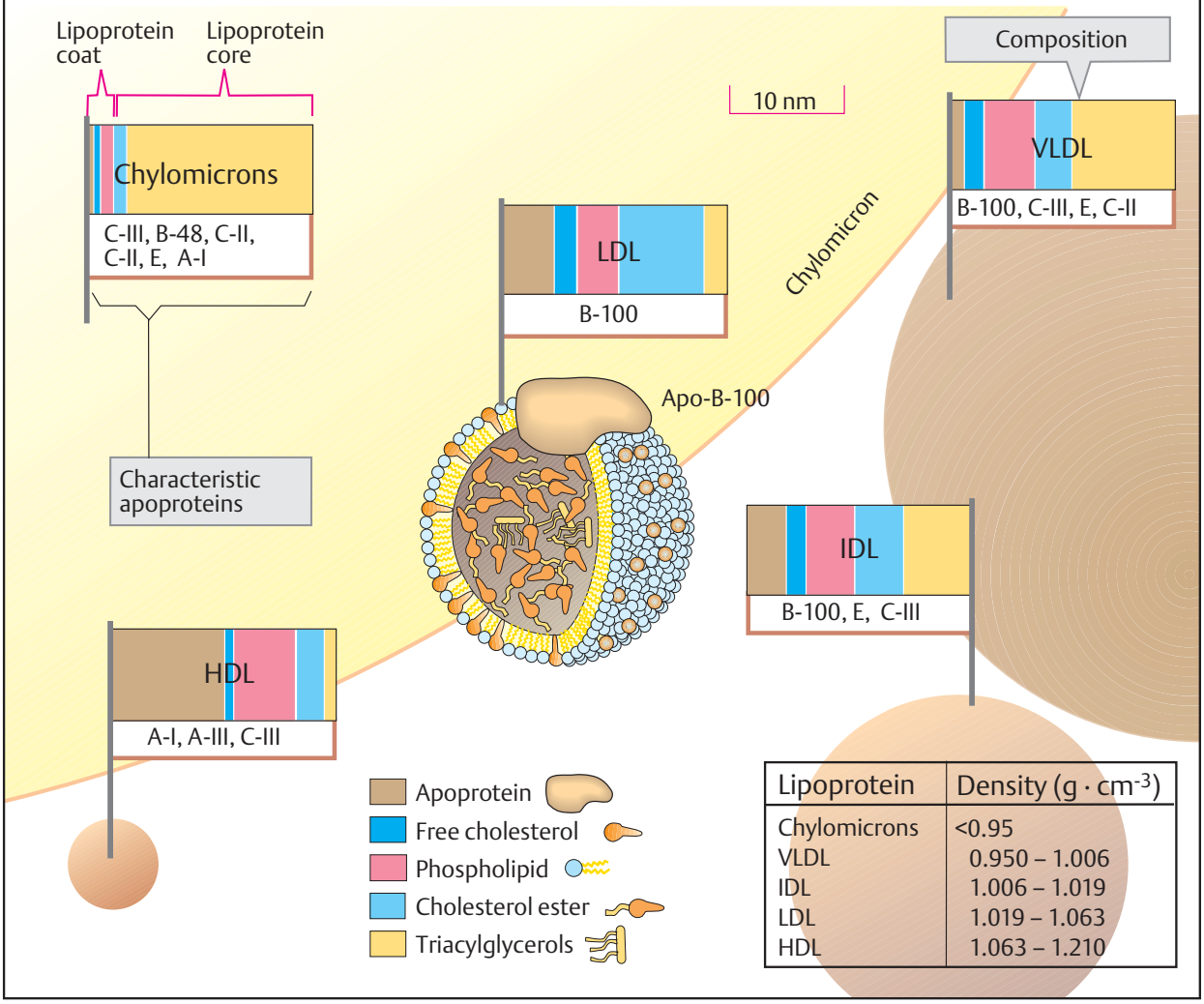
The classes of lipoproteins differ not only in their composition, but also in the ways in which they originate and function.

The **chylomicrons** take care of the transport of triacylglycerols from the intestine to the tissues. They are formed in the intestinal mucosa and reach the blood via the lymphatic system (see p. 266). In the peripheral vessels—particularly in muscle and adipose tissue—*lipoprotein lipase* [1] on the surface of the vascular endothelia hydrolyzes most of the triacylglycerols. Chylomicron breakdown is activated by the transfer of apoproteins E and C from HDL. While the fatty acids released and the glycerol are taken up by the cells, the chylomicrons gradually become converted into **chylomicron remnants**, which are ultimately removed from the blood by the liver.

VLDLs, **IDLs**, and **LDLs** are closely related to one another. VLDLs formed in the liver (see p. 312) transport triacylglycerols, cholesterol, and phospholipids to other tissues. Like chylomicrons, they are gradually converted into IDL and LDL under the influence of *lipoprotein lipase* [1]. This process is also stimulated by HDL. Cells that have a demand for cholesterol bind LDL through an interaction between their **LDL receptor** and ApoB-100, and then take up the complete particle through **receptor-mediated endocytosis**. This type of transport is mediated by depressions in the membrane (*"coated pits"*), the interior of which is lined with the protein *clathrin.* After LDL binding, clathrin promotes invagination of the pits and pinching off of vesicles (*"coated vesicles"*). The clathrin then dissociates off and is reused. After fusion of the vesicle with lysosomes, the LDL particles are broken down (see p. 234), and cholesterol and other lipids are used by the cells.

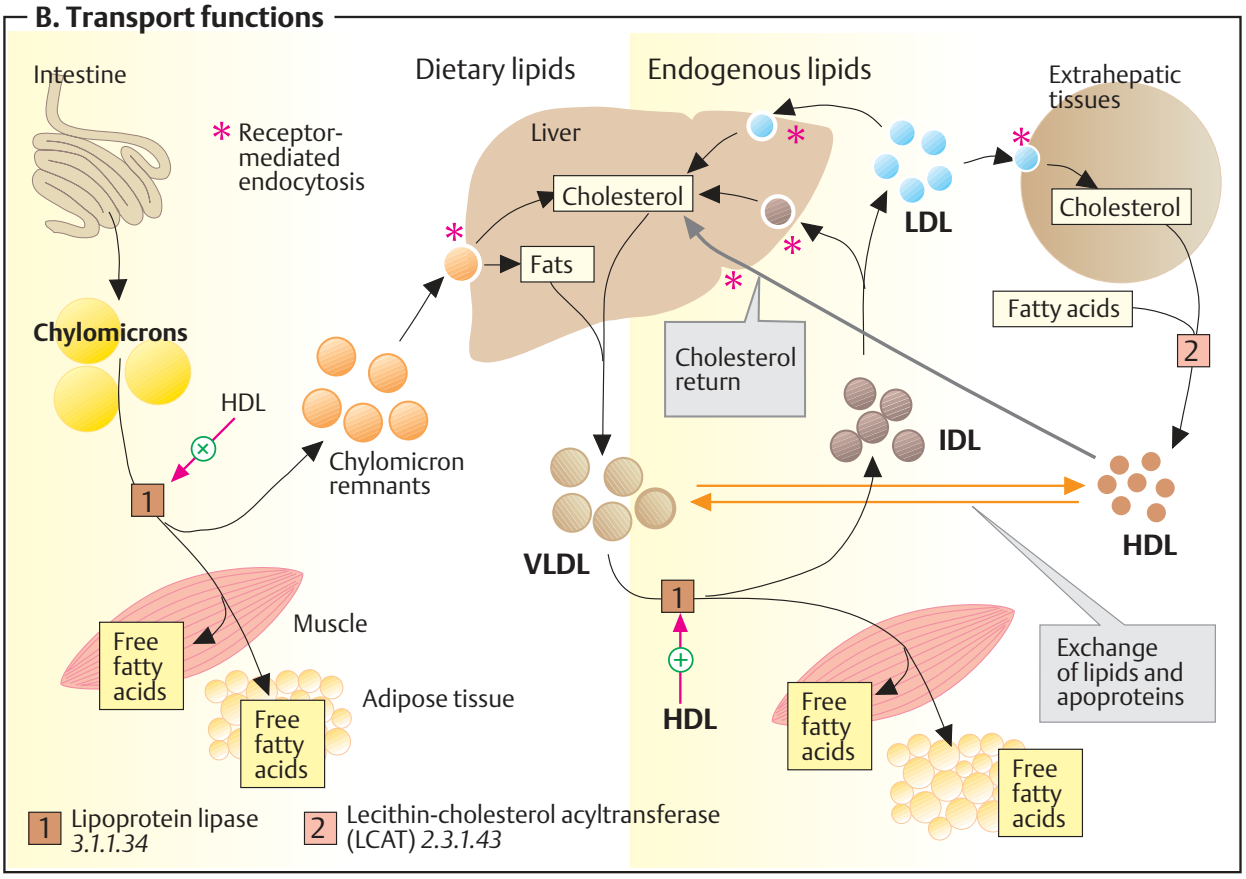
The **HDLs** also originate in the liver. They return the excess cholesterol formed in the tissues to the liver. While it is being transported, cholesterol is acylated by *lecithin cholesterol acyltransferase* (LCAT). The cholesterol esters formed are no longer amphipathic and can be transported in the core of the lipoproteins. In addition, HDLs promote chylomicron and VLDL turnover by exchanging lipids and apoproteins with them (see above).

A. Composition of lipoprotein complexes



Lipoprotein	Density ($g \cdot cm^{-3}$)
Chylomicrons	<0.95
VLDL	0.950 – 1.006
IDL	1.006 – 1.019
LDL	1.019 – 1.063
HDL	1.063 – 1.210

B. Transport functions



Hemoglobin

The most important task of the red blood cells (erythrocytes) is to **transport** molecular oxygen ($\mathbf{O_2}$) from the lungs into the tissues, and carbon dioxide ($\mathbf{CO_2}$) from the tissues back into the lungs. To achieve this, the higher organisms require a special transport system, since O_2 is *poorly soluble in water.* For example, only around 3.2 mL O_2 is soluble in 1 L blood plasma. By contrast, the protein **hemoglobin** (Hb), contained in the erythrocytes, can bind a maximum of 220 mL O_2 per liter—70 times the physically soluble amount.

The Hb content of blood, at 140–180 g L^{-1} in men and 120–160 g L^{-1} in women, is twice as high as that of the plasma proteins (50–80 g L^{-1}). Hb is therefore also responsible for the majority of the blood proteins' pH buffer capacity (see p. 288).

A. Hemoglobin: structure ◑

In adults, hemoglobin (**HbA**; see below) is a *heterotetramer* consisting of two α-chains and two β-chains, each with masses of 16 kDa. The α- and β-chains have different sequences, but are similarly folded. Some 80% of the amino acid residues form *α-helices,* which are identified using the letters A–H.

Each subunit carries a **heme group** (formula on p. 106), with a central bivalent **iron ion**. When O_2 binds to the heme iron (**Oxygenation** of Hb) and when O_2 is released (**Deoxygenation**), the oxidation stage of the iron does *not* change. Oxidation of Fe^{2+} to Fe^{3+} only occurs occasionally. The oxidized form, *methemoglobin,* is then no longer able to bind O_2. The proportion of Met-Hb is kept low by reduction (see p. 284) and usually amounts to only 1–2%.

Four of the six coordination sites of the iron in hemoglobin are occupied by the nitrogen atoms of the pyrrol rings, and another is occupied by a histidine residue of the globin (the *proximal histidine*). The iron's sixth site is coordinated with oxygen in oxyhemoglobin and with H_2O in deoxyhemoglobin.

B. Hemoglobin: allosteric effects ◑

Like aspartate carbamoyltransferase (see p. 116), Hb can exist in two different states (*conformations*), known as the T form and the R form. The **T form** (for *tense;* left) and has a much *lower O_2* af nity than the R form (for *relaxed;* right).

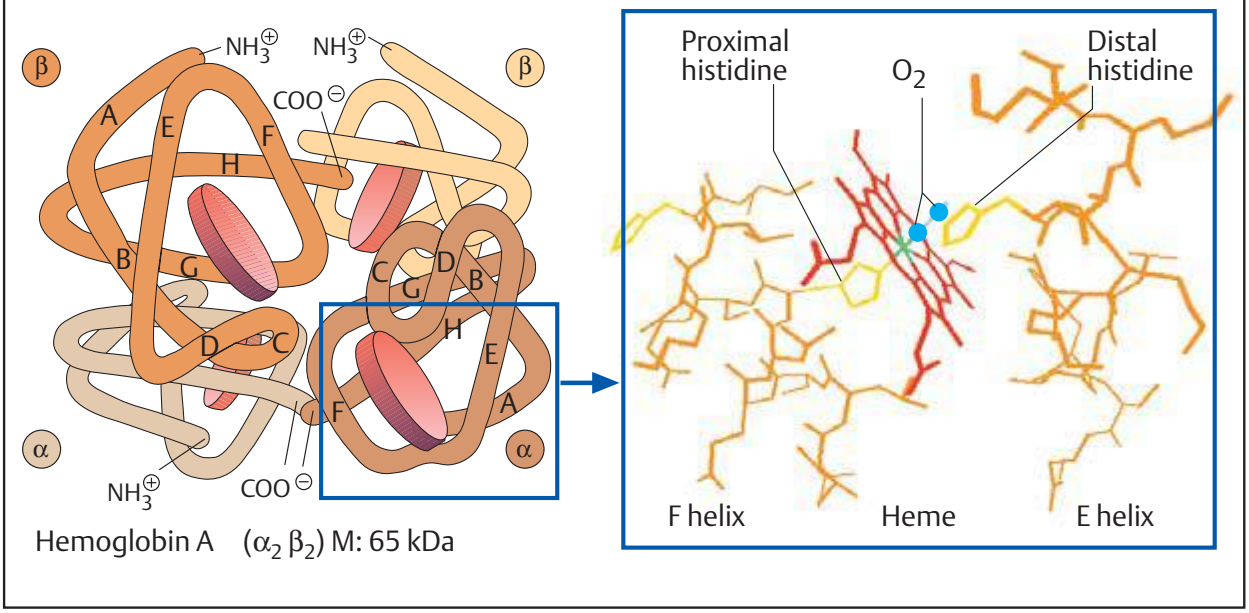
Binding of O_2 to one of the subunits of the T form leads to a local conformational change that weakens the association between the subunits. Increasing O_2 partial pressure thus means that more and more molecules convert to the higher–af nity R form. This **cooperative interaction** between the subunits increases the O_2 af nity of Hb with increasing O_2 concentrations—i. e., the O_2 saturation curve is **sigmoidal** (see p. 282).

Various **allosteric effectors** influence the equilibrium between the T and R forms and thereby regulate the O_2 binding behavior of hemoglobin (yellow arrows). The most important effectors are *CO_2, H^+,* and *2,3-bisphosphoglycerate* (see p. 282).

Further information

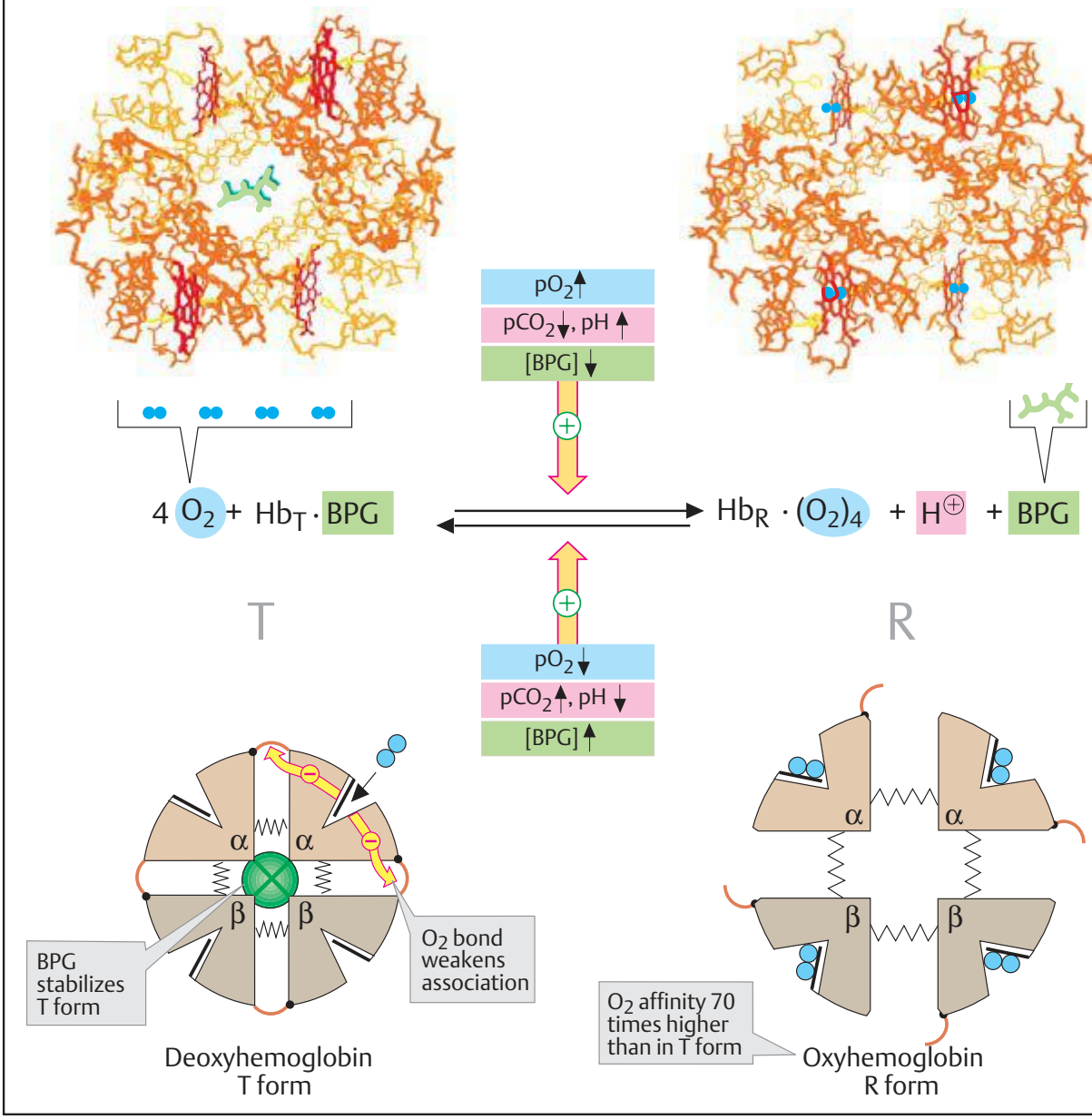
As mentioned above, hemoglobin in adults consists of two α- and two β-chains. In addition to this main form (**HbA_1**, $\alpha_2\beta_2$), adult blood also contains small amounts of a second form with a higher O_2 af nity in which the β-chains are replaced by δ-chains (**HbA_2**, $\alpha_2\delta_2$). Two other forms occur during embryonic and fetal development. In the first three months, **embryonic hemoglobins** are formed, with the structure $\zeta_2\varepsilon_2$ and $\alpha_2\varepsilon_2$. Up to the time of birth, **fetal hemoglobin** then predominates (HbF, $\alpha_2\gamma_2$), and it is gradually replaced by HbA during the first few months of life. Embryonic and fetal hemoglobins have higher O_2 af nities than HbA, as they have to take up oxygen from the maternal circulation.

A. Hemoglobin: structure



Hemoglobin A $(\alpha_2 \beta_2)$ M: 65 kDa

B. Hemoglobin: allosteric effects



Gas transport

Most tissues are constantly dependent on a supply of molecular oxygen (O_2) to maintain their oxidative metabolism. Due to its poor solubility, O_2 is bound to hemoglobin for transport in the blood (see p. 280). This not only increases the oxygen transport capacity, but also allows regulation of O_2 uptake in the lungs and O_2 release into tissues.

A. Regulation of O_2 transport ◑

When an enzyme reacts to effectors (substrates, activators, or inhibitors) with conformational changes that increase or reduce its activity, it is said to show *allosteric behavior* (see p. 116). Allosteric enzymes are usually oligomers with several subunits that mutually influence each other.

Although **hemoglobin** is not an enzyme (it releases the bound oxygen without changing it), it has all the characteristics of an allosteric protein. Its effectors include oxygen, which as a *positive homotropic effector* promotes its own binding. The **O_2 saturation curve** of hemoglobin is therefore markedly sigmoidal in shape (**2**, curve 2). The non-sigmoidal saturation curve of the muscular protein **myoglobin** is shown for comparison (curve 1). The structure of myoglobin (see p. 336) is similar to that of a subunit of hemoglobin, but as a monomer it does not exhibit any allosteric behavior.

CO_2, H^+, and a special metabolite of erythrocytes—**2,3-bisphosphoglycerate (BPG)**—act as *heterotropic effectors* of hemoglobin. BPG is synthesized from *1,3–bisphosphoglycerate,* an intermediate of glycolysis (see p. 150), and it can be returned to glycolysis again by breakdown into 2–phosphoglycerate (**1**), with loss of an ATP.

BPG binds selectively to *deoxy–Hb,* thereby increasing its amount of equilibrium. The result is *increased O_2 release* at constant pO_2. In the diagram, this corresponds to a **right shift** of the saturation curve (**2**, curve 3). CO_2 and H^+ act in the same direction as BPG. Their influence on the position of the curve has long been known as the **Bohr effect**.

The effects of CO_2 and BPG are *additive.* In the presence of both effectors, the saturation curve of isolated Hb is similar to that of whole blood (curve 4).

B. Hemoglobin and CO_2 transport ◑

Hemoglobin is also decisively involved in the transport of carbon dioxide (CO_2) from the tissues to the lungs.

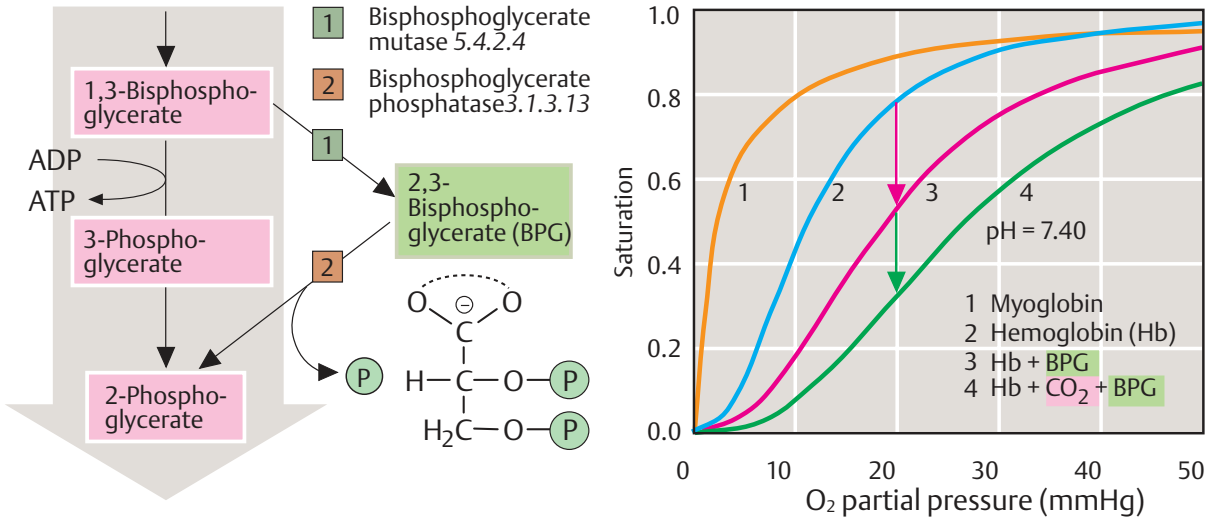
Some 5% of the CO_2 arising in the tissues is covalently bound to the N terminus of hemoglobin and transported as *carbaminohemoglobin* (not shown). About 90% of the CO_2 is first converted in the periphery into *hydrogen carbonate* (HCO_3^-), which is more soluble (bottom). In the lungs (top), CO_2 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 therefore binds additional protons (about 0.7 H^+ per tetramer), which promotes the formation of HCO_3^- from CO_2 in the peripheral tissues. The resulting HCO_3^- is released into the plasma via an antiporter in the erythrocyte membrane in exchange for Cl^-, and passes from the plasma to the lungs. In the lungs, the reactions described above then proceed in reverse order: deoxy-Hb is oxygenated and releases protons. The protons shift the HCO_3/CO_2 equilibrium to the left and thereby promote CO_2 release.

O_2 binding to Hb is regulated by H^+ ions (i.e., by the pH value) via the same mechanism. High concentrations of CO_2 such as those in tissues with intensive metabolism locally increase the H^+ concentration and thereby reduce hemoglobin's O_2 af nity (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 the erythrocytes by *carbonate dehydratase* (carbonic anhydrase) [1])—an enzyme that occurs in high concentrations in the erythrocytes.

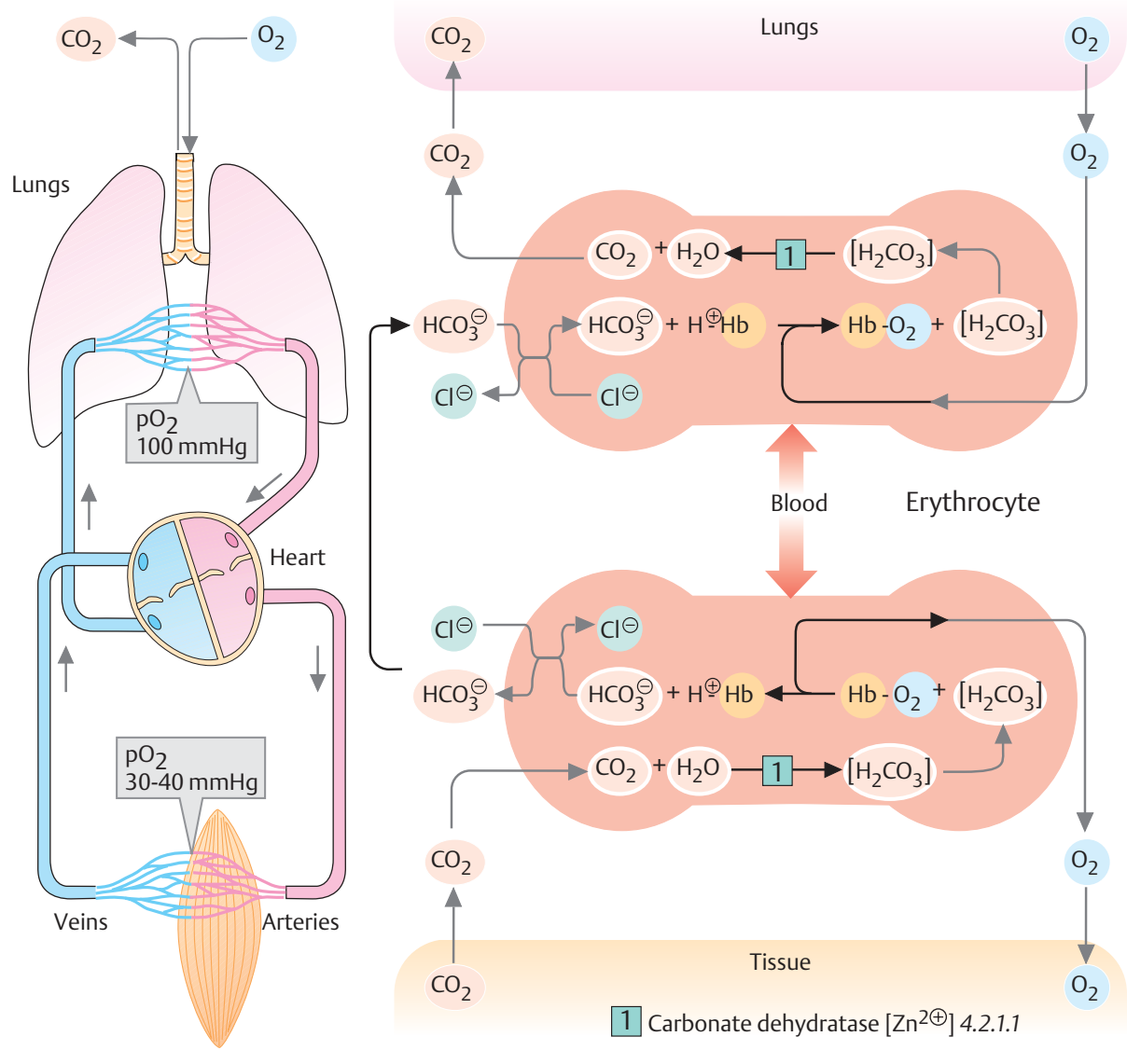
A. Regulation of O_2 transport



1. BPG metabolism

2. Saturation curves

D. Hemoglobin and CO_2 transport



Erythrocyte metabolism

Cells living in aerobic conditions are dependent on molecular oxygen for energy production. 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* (see p. 32), which damage cellular structures 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 dioxygen 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** (O_2^-) arises. Another reduction step (**b**) leads to the **peroxide anion** (O_2^{2-}), which easily binds protons and thus becomes **hydrogen peroxide** (H_2O_2). Inclusion 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** (OH). A fourth electron transfer and subsequent protonation also convert O^- into water.

The synthesis of ROS can be catalyzed by iron ions, for example. Reaction of O_2 with FMN or FAD (see p. 32) also constantly produces ROS. By contrast, reduction of O_2 by *cytochrome c-oxidase* (see p. 140) is "clean," as the enzyme does not release the intermediates. In addition to antioxidants (**B**), **enzymes** also provide protection against ROS: *superoxide dismutase* [1] breaks down ("disproportionates") two superoxide molecules 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].

B. Biological antioxidants ◑

To protect them against ROS and other radicals, all cells contain **antioxidants**. These are *reducing agents* that react easily with oxidative substances and thus protect more important molecules from oxidation. Biological antioxidants include vitamins C and E (see pp. 364, 368), coenzyme Q (see p. 104), and several carotenoids (see pp. 132, 364). Bilirubin, which is formed during heme degradation (see p. 194), also serves for protection against oxidation.

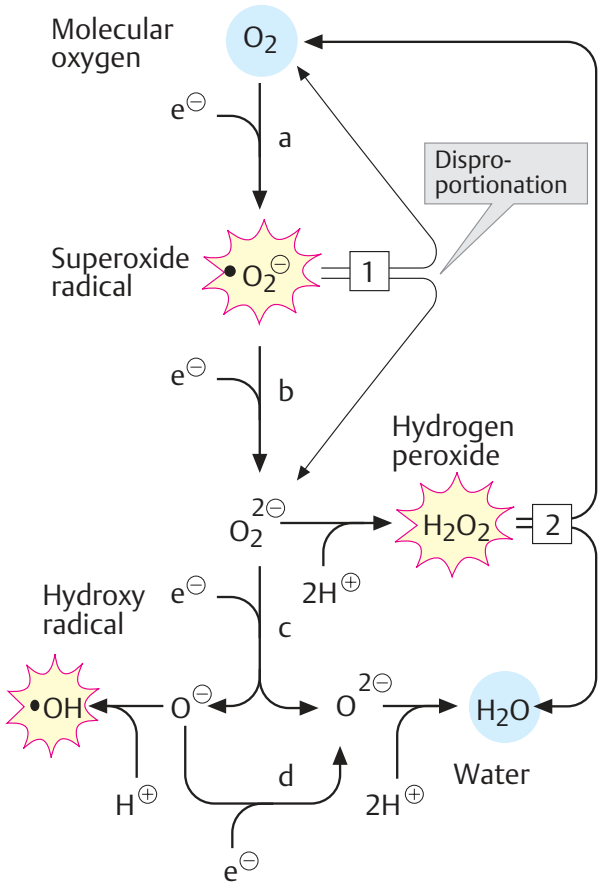
Glutathione, a tripeptide that occurs in high concentrations in almost all cells, is particularly important. Glutathione (sequence: Glu–Cys–Gly) contains an atypical γ-peptide bond between Glu and Cys. The thiol group of the cysteine residue is redox-active. Two molecules of the reduced form (GSH, top) are bound to the disulfide (GSSG, bottom) during oxidation.

C. Erythrocyte metabolism ◑

Erythrocytes also have systems that can inactivate ROS (superoxide dismutase, catalase, GSH). They are also able to repair damage caused by ROS. This requires products that are supplied by the erythrocytes' **maintenance metabolism**, which basically only involves anaerobic glycolysis (see p. 150) and the pentose phosphate pathway (PPP; see p. 152).

The **ATP** formed during glycolysis serves mainly to supply *Na^+/K^+-ATPase,* which maintains the erythrocytes' membrane potential. The allosteric effector **2,3-BPG** (see p. 282) is also derived from glycolysis. The PPP supplies **$NADPH+H^+$**, which is needed to regenerate **glutathione** (GSH) from GSSG with the help of *glutathione reductase* [3]. GSH, the most important antioxidant in the erythrocytes, serves as a coenzyme for *glutathione peroxidase* [5]. This selenium-containing enzyme detoxifies H_2O_2 and hydroperoxides, which arise during the reaction of ROS with unsaturated fatty acids in the erythrocyte membrane. The reduction of methemoglobin ($Hb\,Fe^{3+}$) to Hb ($Hb\,Fe^{2+}$, [4]) is carried out by GSH or ascorbate by a non-enzymatic pathway; however, there are also NAD(P)H-dependent Met-Hb reductases.

A. Reactive oxygen species



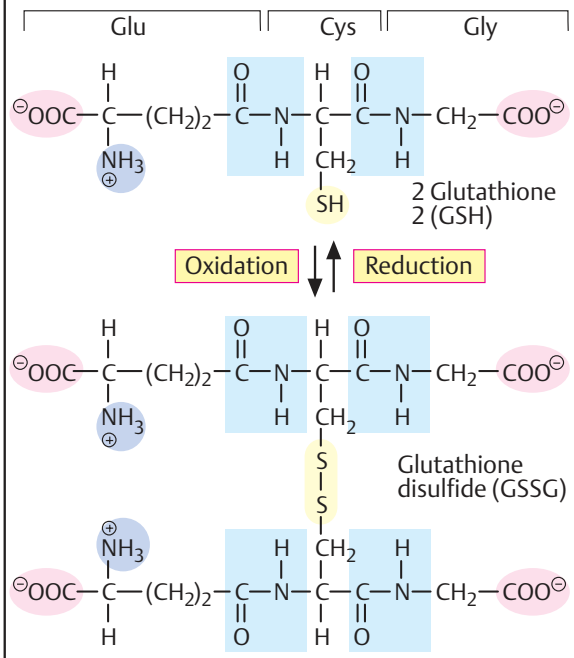
1 Superoxide dismutase *1.15.1.1*

2 Catalase *1.11.1.6*

B. Biological antioxidants

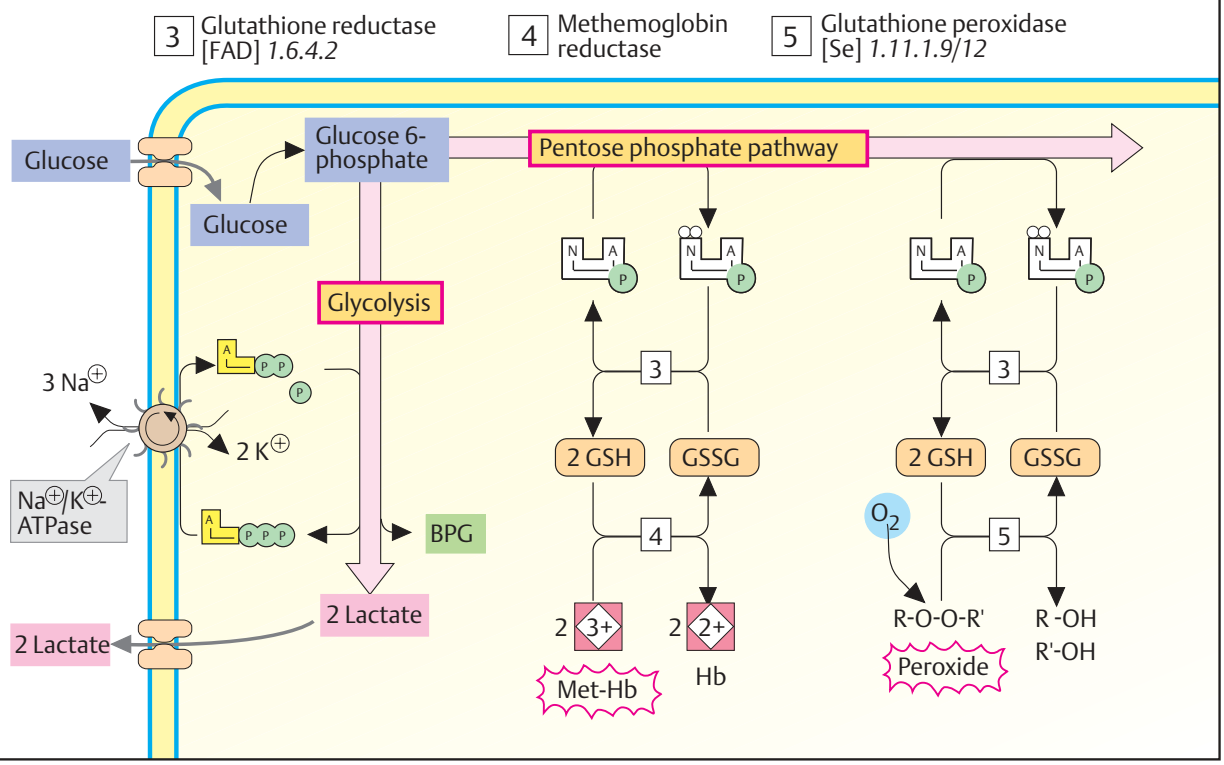
Quinols and enols	α-Tocopherol (vitamin E) Ubiquinol (coenzyme Q) Ascorbic acid (vitamin C)
Carotenoids	β-Carotin Lycopin
Others	Glutathione Bilirubin

1. Examples



2. Glutathione

C. Erythrocyte metabolism



Iron metabolism

A. Distribution of iron ◑

Iron (Fe) is quantitatively the most important trace element (see p. 362). The human body contains 4–5 g iron, which is almost exclusively present in protein-bound form. Approximately three-quarters of the total amount is found in **heme proteins** (see pp. 106, 192), mainly hemoglobin and myoglobin. About 1% of the iron is bound in **iron–sulfur clusters** (see p. 106), which function as cofactors in the respiratory chain, in photosynthesis, and in other redox chains. The remainder consists of iron in transport and storage proteins (transferrin, ferritin; see **B**).

B. Iron metabolism ◑

Iron can only be resorbed by the bowel in bivalent form (i. e., as Fe^{2+}). For this reason, reducing agents in food such as ascorbate (vitamin C; see p. 368) promote **iron uptake**. Via transporters on the luminal and basal side of the enterocytes, Fe^{2+} enters the blood, where it is bound by *transferrin*. Part of the iron that is taken up is stored in the bowel in the form of *ferritin* (see below). Heme groups can also be resorbed by the small intestine.

Most of the resorbed iron serves for the formation of red blood cells in the bone marrow (**erythropoiesis**, top). As discussed on p. 192, it is only in the final step of hem biosynthesis that Fe^{2+} is incorporated by *ferrochelatase* into the previously prepared tetrapyrrol framework.

In the blood, 2.5–3.0 g of hemoglobin iron circulates as a component of the erythrocytes (top right). Over the course of several months, the flexibility of the red blood cells constantly declines due to damage to the membrane and cytoskeleton. Old erythrocytes of this type are taken up by macrophages in the spleen and other organs and broken down. The organic part of the heme is oxidized into bilirubin (see p. 194), while the iron returns to the plasma pool. The quantity of heme iron recycled per day is much larger than the amount resorbed by the intestines.

Transferrin, a β-globulin with a mass of 80 kDa, serves to transport iron in the blood. This monomeric protein consists of two similar domains, each of which binds an Fe^{2+} ion very tightly. Similar iron transport proteins are found in secretions such as saliva, tears, and milk; these are known as lactoferrins (bottom right). Transferrin and the lactoferrins maintain the concentration of *free* iron in body fluids at values below 10^{-10} mol L^{-1}. This low level prevents bacteria that require free iron as an essential growth factor from proliferating in the body. Like LDLs (see p. 278), transferrin and the lactoferrins are taken up into cells by *receptor-mediated endocytosis*.

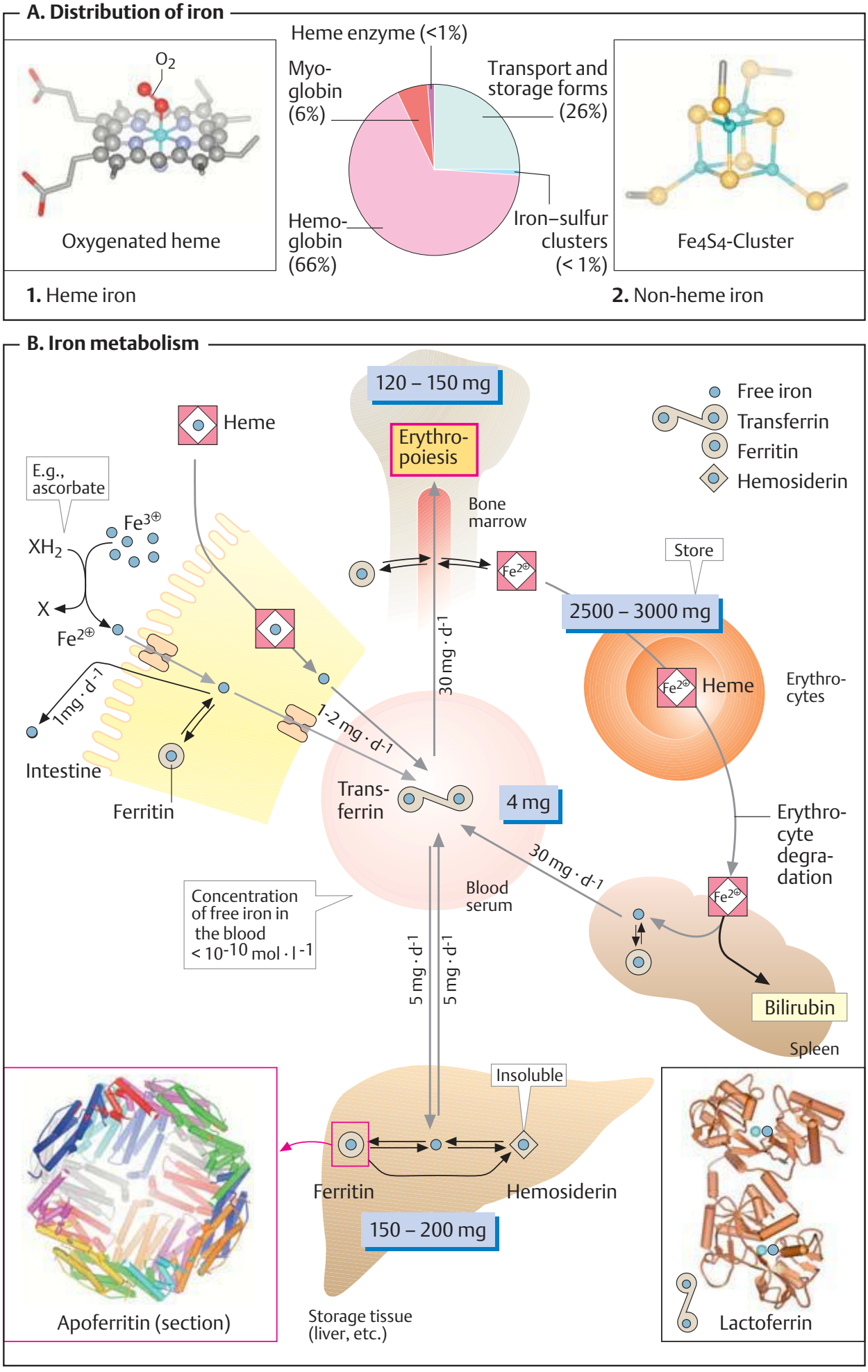
Excess iron is incorporated into **ferritin** and stored in this form in the liver and other organs. The ferritin molecule consists of 24 subunits and has the shape of a hollow sphere (bottom left). It takes up Fe^{2+} ions, which in the process are oxidized to Fe^{3+} and then deposited in the interior of the sphere as *ferrihydrate*. Each ferritin molecule is capable of storing several thousand iron ions in this way. In addition to ferritin, there is another storage form, **hemosiderin**, the function of which is not yet clear.

Further information

Disturbances of the iron metabolism are frequent and can lead to severe disease pictures.

Iron deficiency is usually due to blood loss, or more rarely to inadequate iron uptake. During pregnancy, increased demand can also cause iron deficiency states. In severe cases, reduced hemoglobin synthesis can lead to **anemia** ("iron-deficiency anemia"). In these patients, the erythrocytes are smaller and have less hemoglobin. As their membrane is also altered, they are prematurely eliminated in the spleen.

Disturbances resulting from raised iron concentrations are less frequent. Known as **hemochromatoses**, these conditions can have genetic causes, or may be due to repeated administration of blood transfusions. As the body has practically no means of excreting iron, more and more stored iron is deposited in the organs over time in patients with untreated hemochromatosis, ultimately leading to severe disturbances of organ function.



Acid–base balance

A. Hydrogen ion concentration in the blood plasma ◑

The H^+ concentration in the blood and extracellular space is approximately 40 nM ($4 \cdot 10^{-8}$ mol L^{-1}). This corresponds to a pH of 7.40. The body tries to keep this value constant, as large shifts in pH are incompatible with life.

The pH value is kept constant by **buffer systems** that cushion minor disturbances in the *acid–base balance* (**C**). In the longer term, the decisive aspect is maintaining a balanced equilibrium between H^+ production and uptake and H^+ release. If the blood's buffering capacity is not suf cient, 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—already 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 in the cells. Their S atoms are oxidized in the liver to form sulfuric acid, which supplies protons by dissociation into sulfate.

During anaerobic glycolysis in the muscles and erythrocytes, glucose is converted into **lactate**, releasing protons in the process (see p. 338). The synthesis of the **ketone bodies** acetoacetic acid and 3–hydroxybutyric acid in the liver (see p. 312) also releases protons. Normally, the amounts formed are small and of little influence on the proton balance. If acids are formed in large amounts, however (e. g., during *starvation* or in *diabetes mellitus;* see p. 160), they strain the buffer systems and can lead to a reduction in pH (**metabolic acidoses**; *lactacidosis* or *ketoacidosis*).

Only the **kidney** is capable of excreting protons in exchange for Na^+ ions (see p. 326). In the urine, the H^+ ions are buffered by NH_3 and phosphate.

C. Buffer systems in the plasma ◑

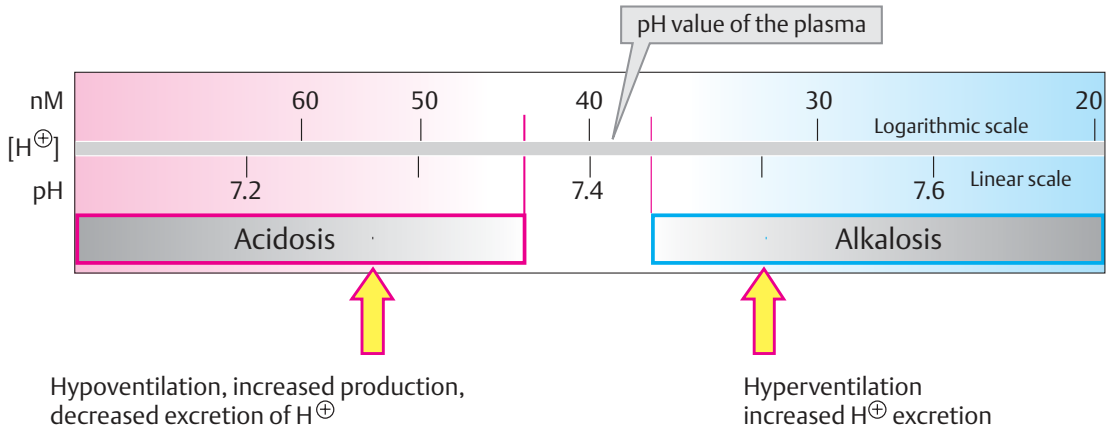
The **buffering capacity** of a buffer system depends on its concentration and its pK_a value. The strongest effect is achieved if the pH value corresponds to the buffer system's pK_a value (see p. 30). For this reason, weak acids with pK_a values of around 7 are best suited for buffering purposes in 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]; see also p. 282). 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 6.1. 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, it does *not* lead to the excretion of protons.

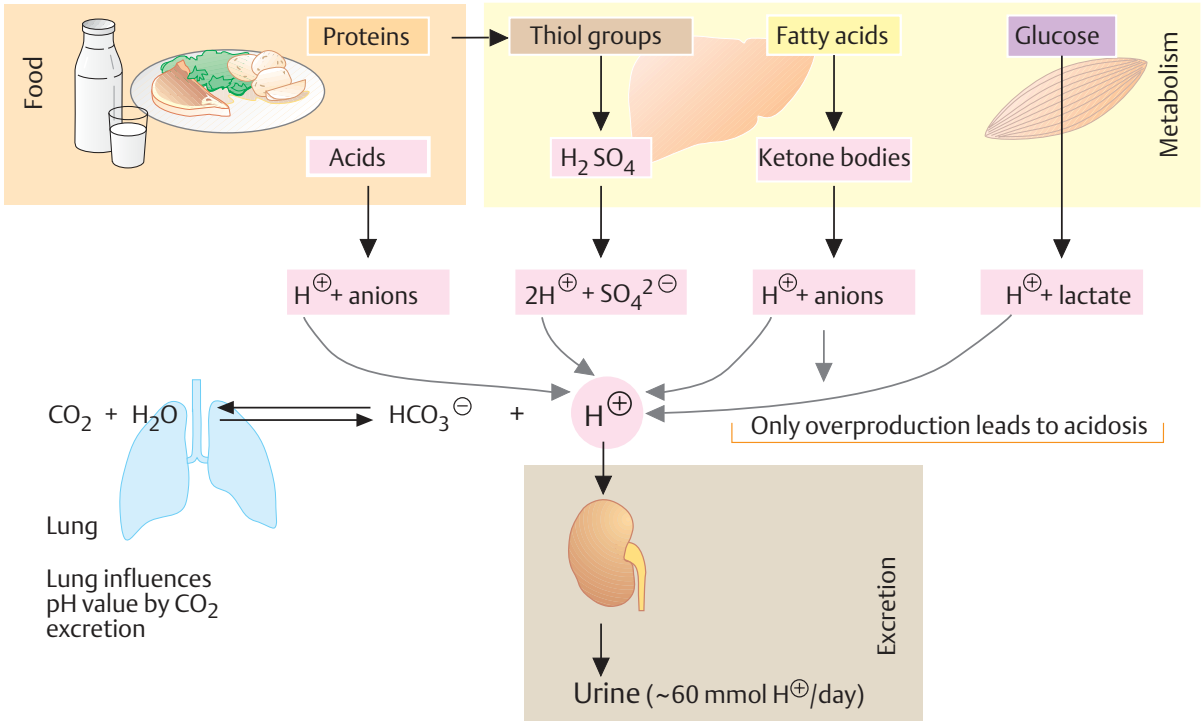
Due to their high concentration, **plasma proteins**—and **hemoglobin** in the erythrocytes in particular—provide about one-quarter of the blood's buffering capacity. The buffering effect of proteins involves contributions from all of the ionizable side chains. At the pH value of blood, the acidic amino acids (Asp, Glu) and histidine are particularly effective.

The second dissociation step in **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).

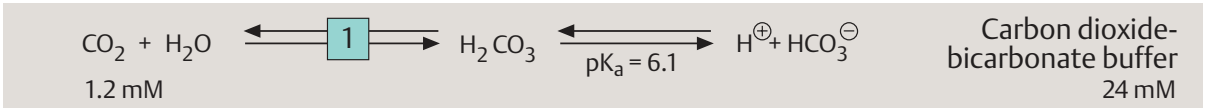
A. Hydrogen ion concentration in the blood plasma

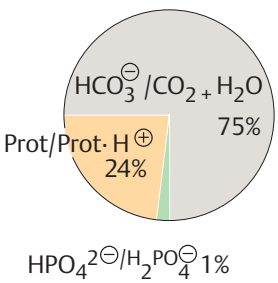


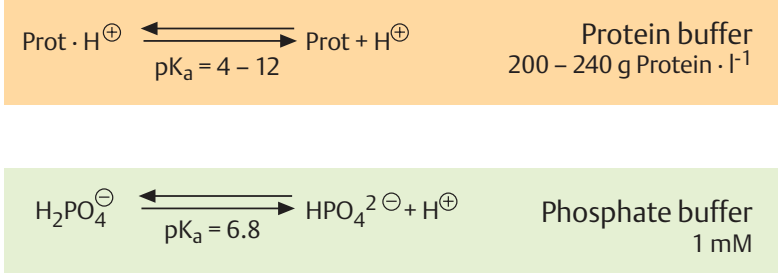
B. Acid–base balance



C. Buffer systems in the plasma







Blood clotting

Following injury to blood vessels, **hemostasis** ensures that blood loss is minimized. Initially, thrombocyte activation leads to contraction of the injured vessel and the formation of a loose clot consisting of thrombocytes (**hemostasis**). Slightly later, the action of the enzyme *thrombin* leads to the formation and deposition in the thrombus of polymeric fibrin (**coagulation, blood clotting**). The coagulation process is discussed here in detail.

A. Blood clotting ◑

The most important reaction in blood clotting is the conversion, catalyzed by *thrombin,* of the soluble plasma protein *fibrinogen* (factor I) into polymeric *fibrin,* which is deposited as a fibrous network in the primary thrombus. Thrombin (factor IIa) is a serine proteinase (see p. 176) 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 (see p. 270). 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 synthesis and also generates the active factors IXa and Xa from their precursors. With the aid of factor VIIIa, PL, and Ca^{2+}, factor IXa 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.

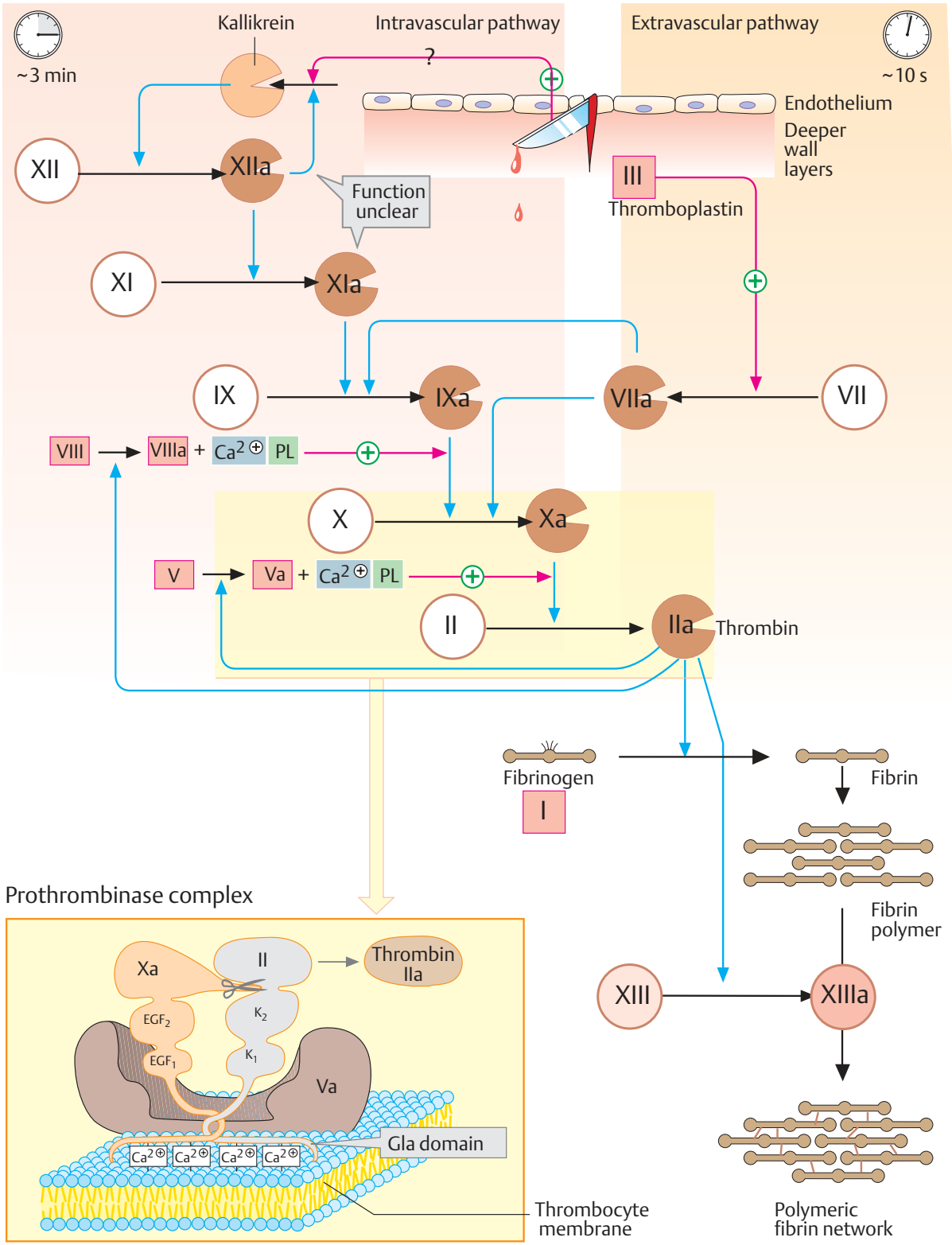
Both pathways depend on the presence of activated **thrombocytes**, on the surface of which several reactions take place. For example, the *prothrombinase complex* (left) forms when factors Xa and II, with the help of Va, bind via Ca^{2+} ions to anionic phospholipids in the thrombocyte membrane. For this to happen, factors II and X have to contain the nonproteinogenic amino acid **γ-carboxyglutamate** (Gla; see p. 62), which is formed in the liver by post-translational carboxylation of the factors. The Gla residues are found in groups in special domains that create contacts to the Ca^{2+} ions. Factors VII and IX are also linked to membrane phospholipids via Gla residues.

Substances that bind Ca^{2+} ions (e. g., *citrate*) prevent Gla-containing factors from attaching to the membrane and therefore inhibit coagulation. Antagonists of *vitamin K,* which is needed for synthesis of the Gla residues (see p. 364) also have anticoagulatory effects. These include dicumarol, for example.

Active **thrombin** not only converts fibrinogen into fibrin, but also indirectly promotes its own synthesis by catalyzing 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.

Regulation of blood clotting (not shown). To prevent the coagulation reaction from becoming excessive, the blood contains a number of anticoagulant substances, including highly effective proteinase inhibitors. For example, *antithrombin III* binds to various serine proteinases in the cascade and thereby inactivates them. *Heparin,* an anticoagulant glycosaminoglycan (see p. 346), potentiates the effect of antithrombin III. *Thrombomodulin,* which is located on the vascular endothelia, also inactivates thrombin. A glycoprotein known as *Protein C* ensures proteolytic degradation of factors V and VIII. As it is activated by thrombin, coagulation is shut down in this way.

A. Blood clotting



Coagulation factors

- I Fibrinogen
- ◆ II Prothrombin* *3.4.21.5*
- III Tissue factor/thromboplastin
- IV $Ca^{2\oplus}$
- V Proaccelerin
- VI Synonym for Va
- ◆ VII Proconvertin* *3.4.21.21*
- VIII Antihemophilic factor A
- ◆ IX Christmas factor* *3.4.21.22*
- ◆ X Stuart–Prower factor* *3.4.21.6*
- XI Plasma thromboplastin antecedent* (PTA) *3.4.21.27*
- XII Hageman factor* *3.4.21.38*
- XIII Fibrin-stabilizing factor* *2.3.2.13*

* Proenzyme
◆ Contains γ-carboxyglutamate

Fibrinolysis, blood groups

A. Fibrinolysis ◑

The fibrin thrombus resulting from blood clotting (see p. 290) is dissolved again by *plasmin,* a serine proteinase found in the blood plasma. For this purpose, the precursor *plasminogen* first has to be proteolytically activated by enzymes from various tissues. This group includes the *plasminogen activator* from the kidney (*urokinase*) and *tissue plasminogen activator* (t-PA) from vascular endothelia. 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 proteinase with similar activity, are used clinically to dissolve thrombi following *heart attacks.* All of these proteins are expressed recombinantly in bacteria (see p. 262).

B. Blood groups: the ABO system ◑

During blood transfusions, immune reactions can occur that destroy the erythrocytes transfused from the donor. These reactions result from the formation of antibodies (see p. 300) directed to certain surface structures on the erythrocytes. Known as **blood group antigens**, these are *proteins* or *oligosaccharides* 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.

In the **ABO system**, the carbohydrate parts of glycoproteins or glycolipids act as antigens. In this relatively simple system, there are four *blood groups* (A, B, AB, and 0). In individuals with blood groups A and B, the antigens consist of tetrasaccharides that only differ in their terminal sugar (galactose or *N*-acetylgalactosamine). Carriers of the AB blood group have both antigens (A *and* B). Blood group 0 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.

Antibodies are only formed against antigens that the individual concerned does *not* possess. For example, carriers of blood group A form antibodies against antigen B ("anti-B"), while carriers of group B form antibodies against antigen A ("anti-A"). Individuals with blood group 0 form both types, and those with blood group AB do not form any of these antibodies.

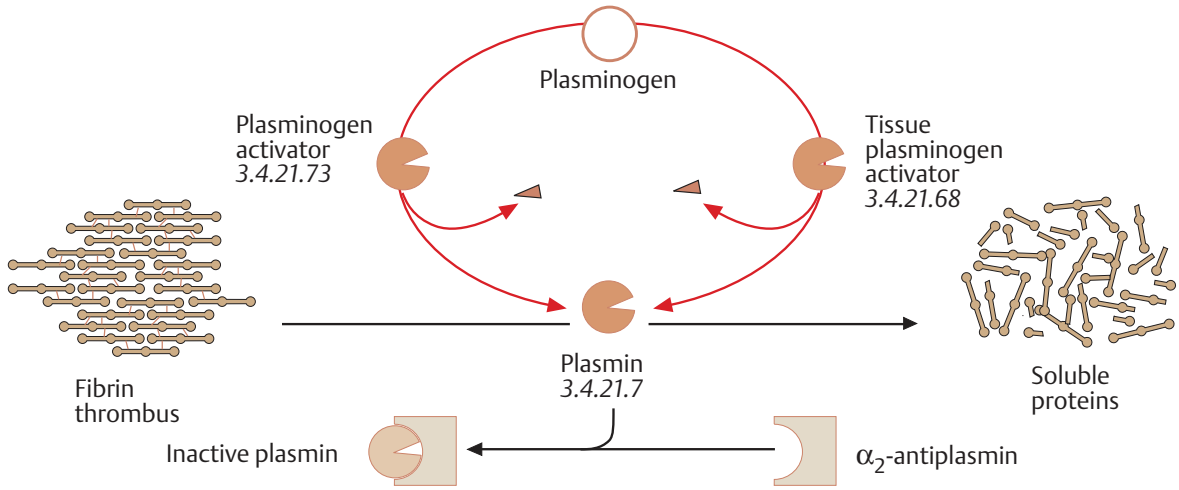
If blood from blood group A is transfused into the circulation of an individual with blood group B, for example, then the anti-A present there binds to the A antigens. The donor erythrocytes marked in this way are recognized and destroyed by the complement system (see p. 298). In the test tube, *agglutination* of the erythrocytes can be observed when donor and recipient blood are incompatible.

The recipient's serum should not contain any antibodies against the donor erythrocytes, and the donor serum should not contain any antibodies against the recipient's erythrocytes. Donor blood from blood group 0 is unproblematic, as its erythrocytes do not possess any antibodies and therefore do not react with anti-A or anti-B in the recipient's blood. Conversely, blood from the AB group can only be administered to recipients with the AB group, as these are the only ones without antibodies.

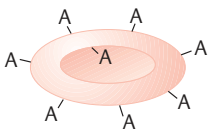
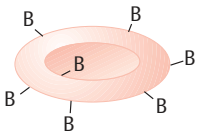
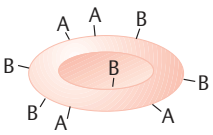
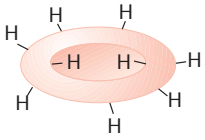
In the **Rh system** (not shown), proteins on the surface of the erythrocytes act as antigens. These are known as "rhesus factors," as the system was first discovered in rhesus monkeys.

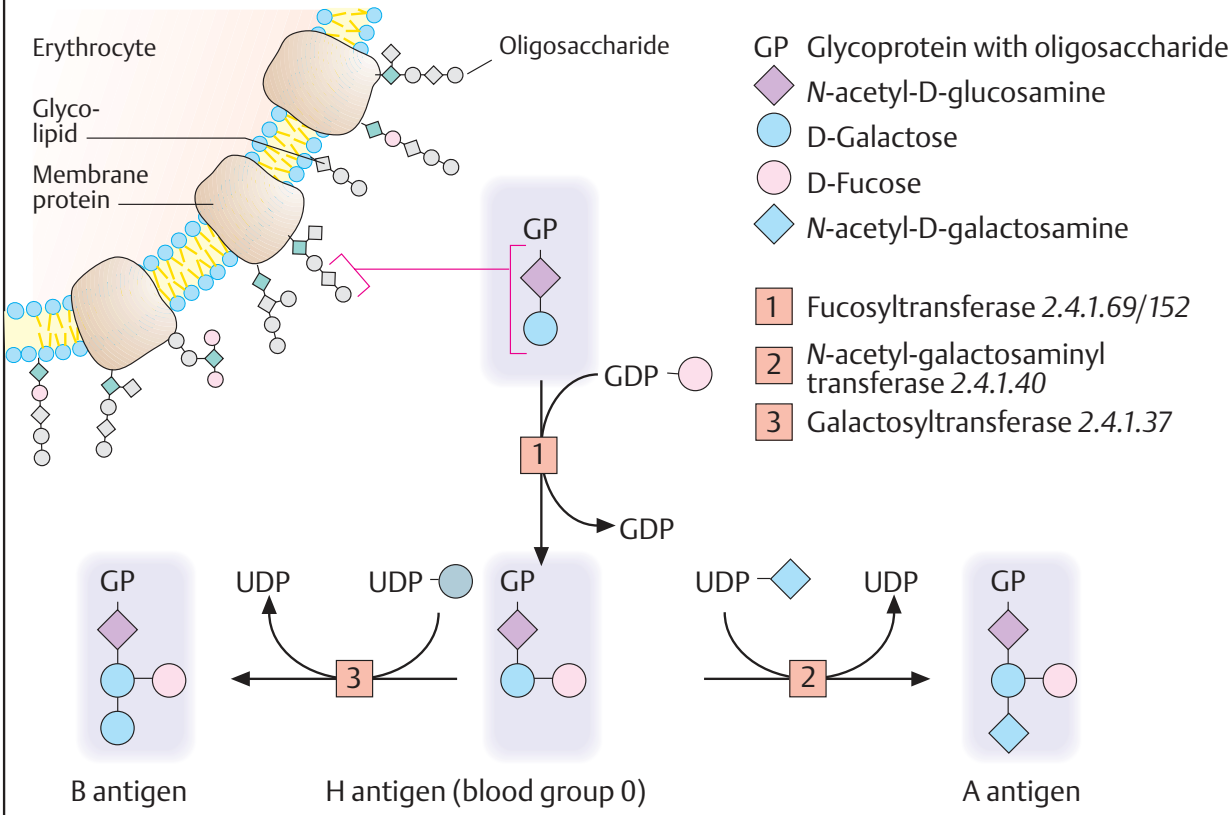
The rhesus D antigen occurs in 84% of all white individuals, who are therefore *"Rh-positive."* If an Rh-positive child is born to an Rh-negative mother, fetal erythrocytes can enter the mother's circulation during birth and lead to the formation of antibodies (IgG) against the D antigen. This initially 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*).

A. Fibrinolysis



B. Blood groups: the AB0 system

Blood group	A	B	AB	0
Genotypes	AA and A0	BB and B0	AB	00
Antigens				
Antibodies in blood	anti-B	anti-A	—	anti-A anti-B
Frequency in central Europe	40%	16%	4%	40%



Immune response

Viruses, bacteria, fungi, and parasites that enter the body of vertebrates of 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 makes it easier for the immune cells to reach the site of infection.

Two different systems are involved in the immune response. The **innate immune system** is based on receptors that can distinguish between bacterial and viral surface structures or foreign proteins (known as *antigens*) and those that are endogenous. With the help of these receptors, *phagocytes* bind to the pathogens, absorb them by endocytosis, and break them down. The complement system (see p. 298) is also part of the innate system.

The **acquired** (adaptive) **immune system** is based on the ability of the *lymphocytes* to form 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. Depending on their function, another distinction is made between *cytotoxic T cells* (green) and *helper T cells* (blue). **Humoral immunity** is based on the activity of the *B lymphocytes* (B cells, light brown), which mature in the bone marrow. After activation by T cells, B cells are able to release soluble forms of their specific antigen receptors, known as *antibodies* (see p. 300), 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 ◑

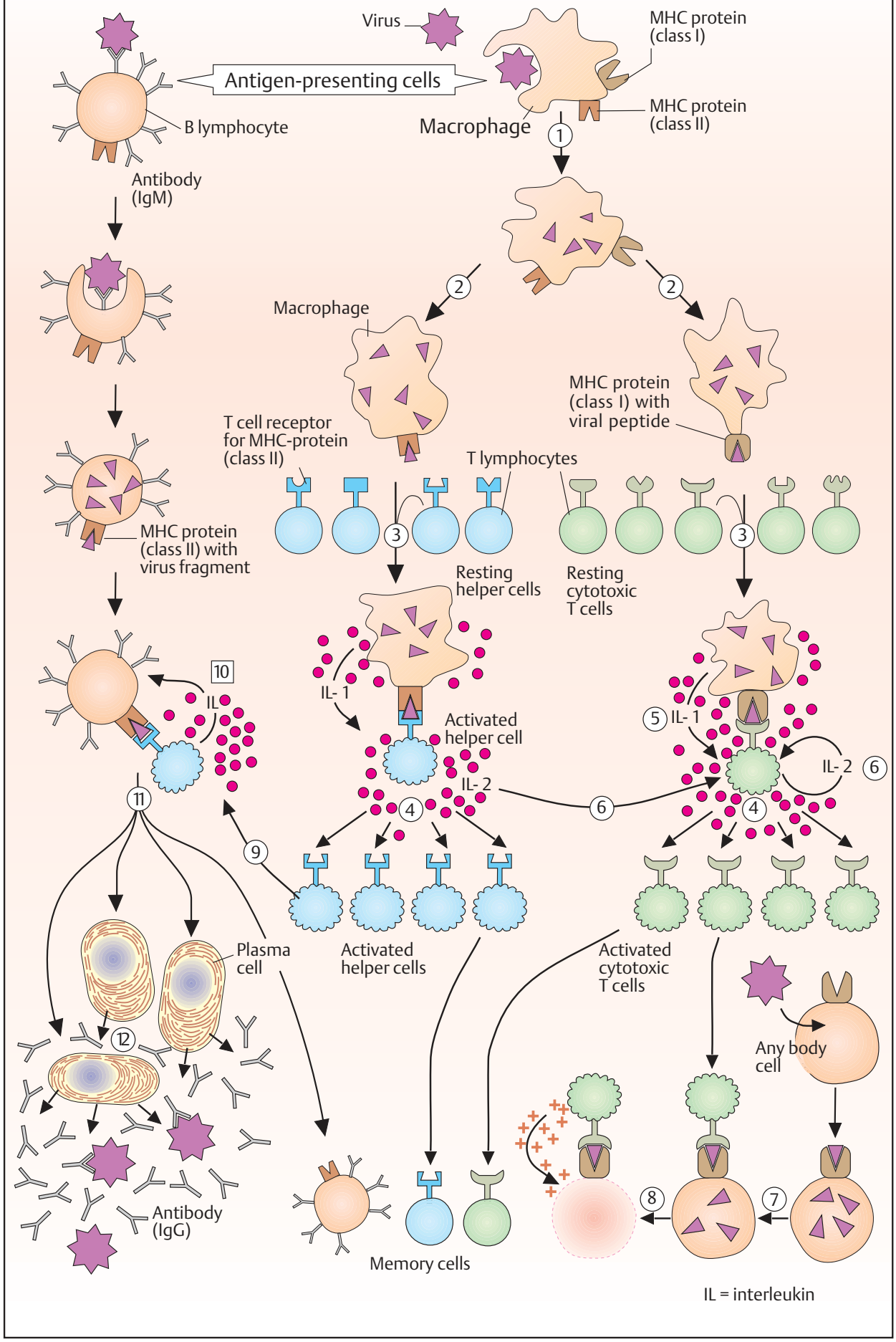
Pathogens that have entered the body—e. g., viruses (top)—are taken up by **antigen-presenting cells** (APCs) and proteolytically degraded (**1**). The viral fragments produced in this way are then presented on the surfaces of these cells with the help of special membrane proteins (MHC proteins; see p. 296) (**2**). The APCs include 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 that carry a receptor that matches the antigen ("T-cell receptors"; see p. 296) (**3**). Binding leads to activation of the T cell concerned and selective replication of it (**4**, *"clonal selection"*). The proliferation of immune cells is stimulated by *interleukins* (IL). These are a group of more than 20 signaling substances belonging to the cytokine family (see p. 392), with the help of 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 (see p. 396) or kill them with *perforin*, a protein that perforates the target cell's plasma membrane (**8**).

B lymphocytes, which as APCs present viral fragments on their surfaces, are recognized by **helper T cells** (blue) or their T cell receptors (**9**). Stimulated by interleukins, selective clonal replication then takes place of B cells that carry antigen receptors matching those of the pathogen (**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 (see p. 294), 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; see p. 300). 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 to the membranes. Intramolecular and intermolecular disulfide bonds are also usually found in proteins belonging to the Ig family.

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. T helper cells use **CD4** as a co-receptor instead (not shown). 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 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.

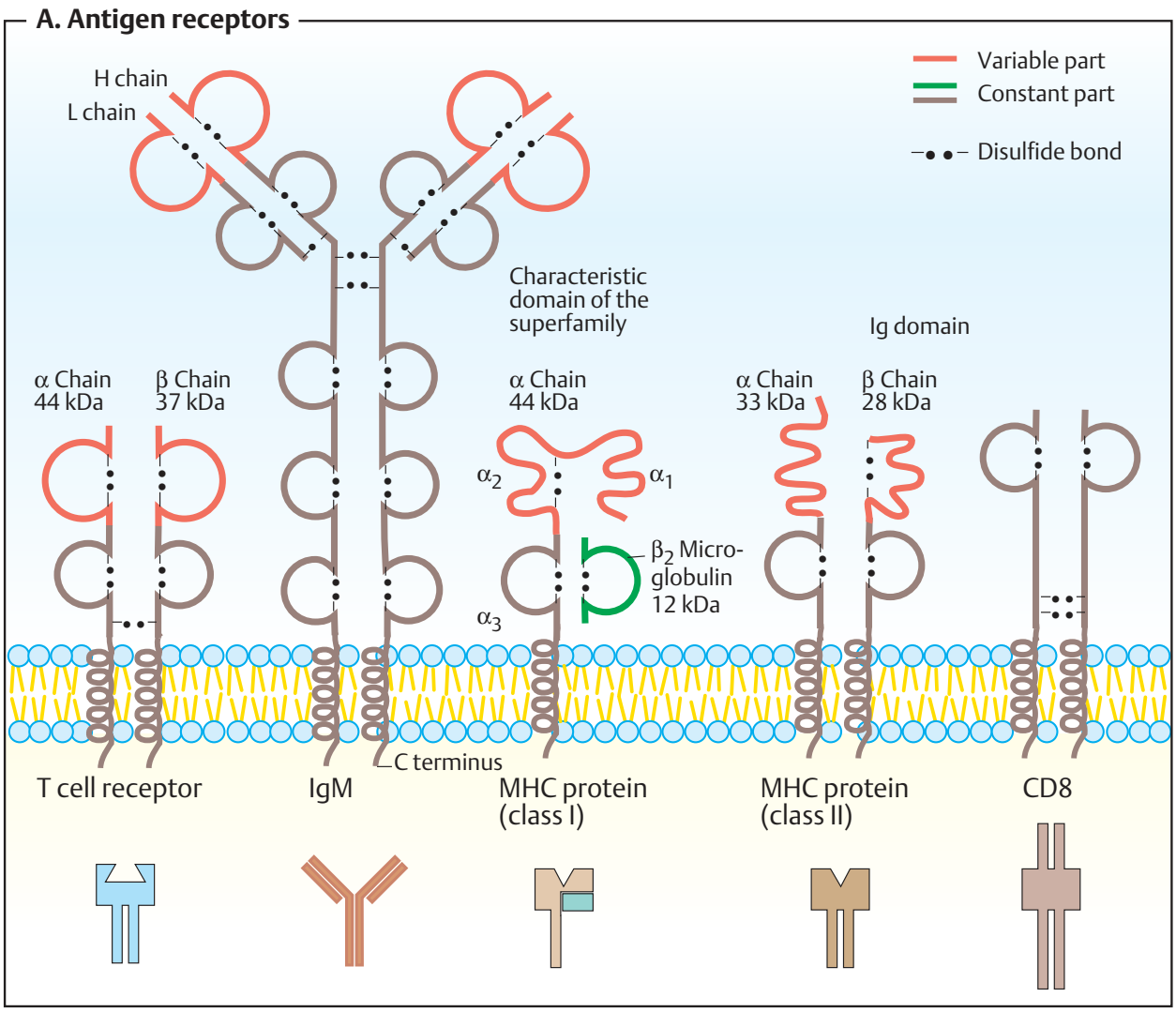
Class I MHC proteins occur in almost all nucleated cells. They mainly interact with cytotoxic T cells and are the reason for the rejection of transplanted organs. Class I MHC proteins are heterodimers ($\alpha\beta$). The β subunit is also known as β_2-microglobulin.

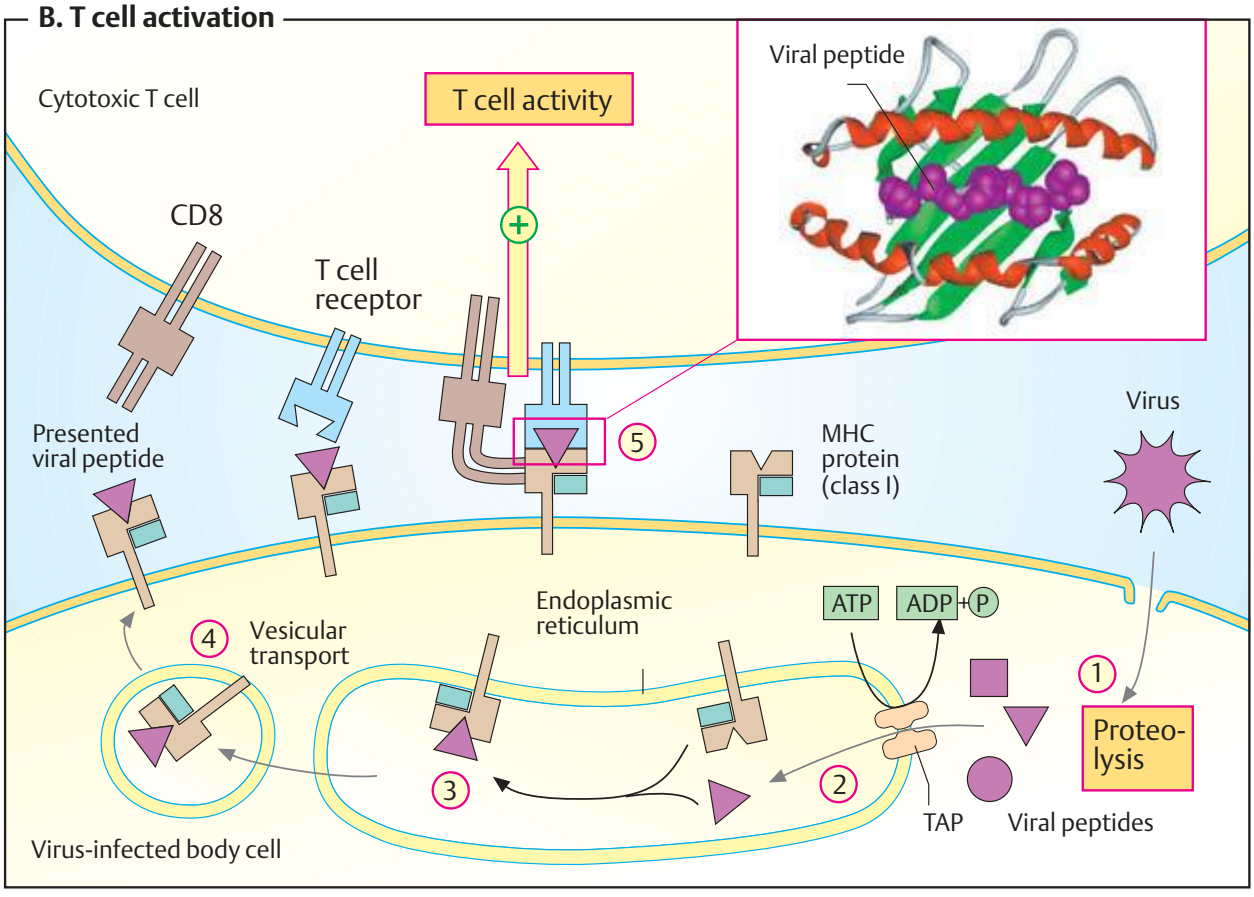
Class II MHC proteins also consist of two peptide chains, which are related to each other. MHC II molecules are found on all antigen-presenting cells in the immune system. They serve for interaction between these cells and CD4-carrying T helper cells.

B. T-cell activation ◑

The illustration shows an interaction between a virus-infected body cell (bottom) and a CD8-carrying cytotoxic T lymphocyte (top). The infected cell breaks down viral proteins in its cytoplasm (**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 one of the peptides (**3**) and then transferred to the cell surface by vesicular transport (**4**). The viral peptides are bound on the surface of the α_2 domain of the MHC protein in a depression formed by an insertion as a "floor" and two helices as "walls" (see smaller illustration).

Supported by CD8 and other co-receptors, a T cell with a matching T cell receptor binds to the MHC peptide complex (**5**; cf. p. 224). This binding activates protein kinases in the interior of the T cell, which trigger a chain of additional reactions (*signal transduction;* see p. 388). Finally, destruction of the virus-infected cell by the cytotoxic T lymphocytes takes place.





Complement system

The complement system is part of the innate immune system (see p. 294). It supports *nonspecific defense* against microorganisms. The system consists of some 30 different proteins, the *"complement factors,"* which are found in the blood and represent about 4% of all plasma proteins there. When inflammatory reactions occur, the complement factors enter the infected tissue and take effect there.

The complement system works in three different ways:

Chemotaxis. Various complement factors attract immune cells that can attack and phagocytose pathogens.

Opsonization. Certain complement factors ("opsonins") bind to the pathogens and thereby 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 pathogen (see below).

A. Complement activation ◑

The reactions that take place in the complement system can be initiated in several ways. During the early phase of infection, lipopolysaccharides and other structures on the surface of the pathogens trigger the *alternative pathway* (right). If antibodies against the pathogens become available later, the antigen–antibody complexes formed activate the *classic pathway* (left). Acute-phase proteins (see p. 276) are also able to start the complement cascade (*lectin pathway,* not shown).

Factors C1 to **C4** (for "complement") belong to the classic pathway, while **factors B** and **D** form the reactive components of the alternative pathway. Factors **C5** to **C9** are responsible for membrane attack. Other components not shown here regulate the system.

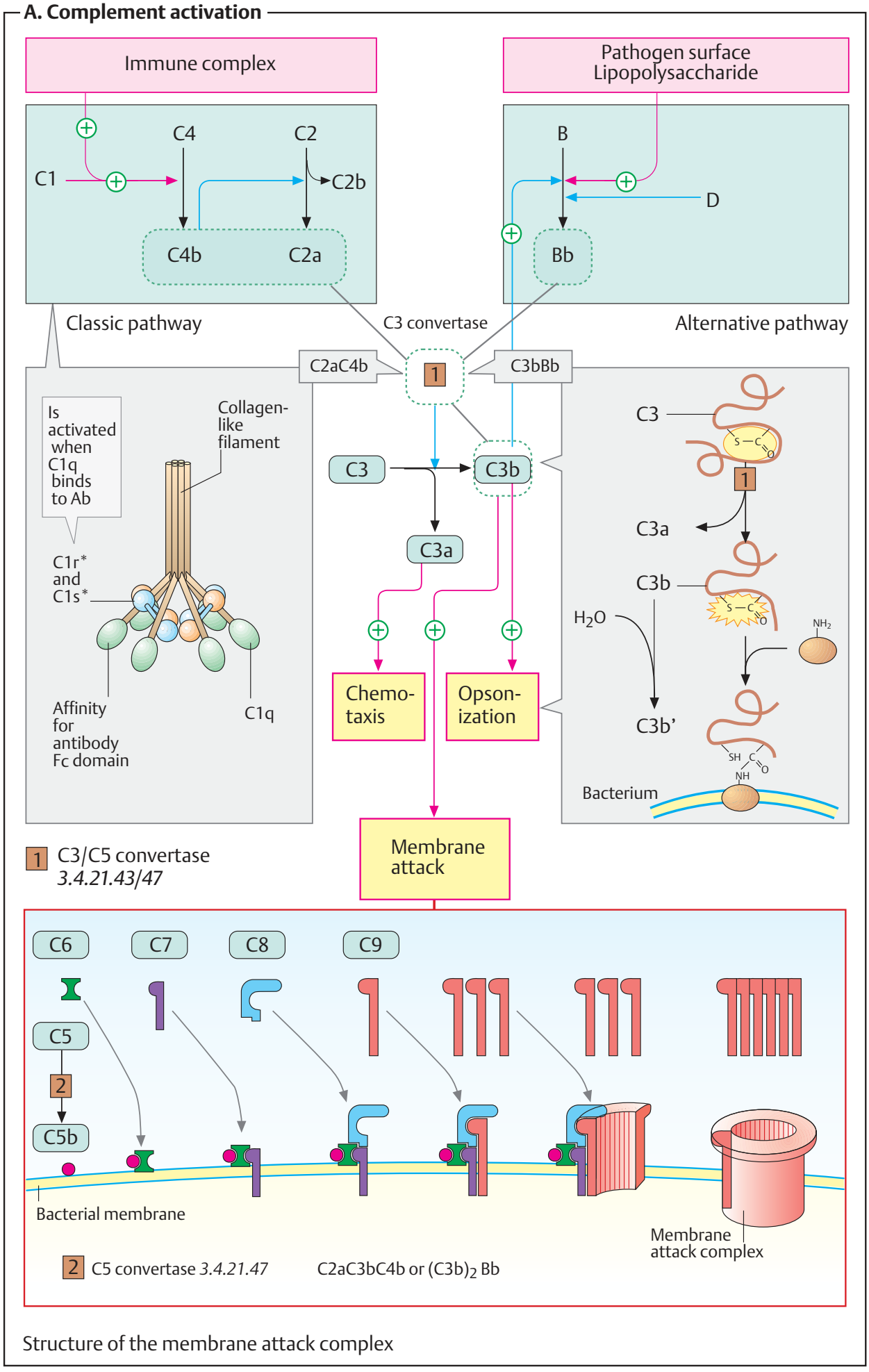
As in blood coagulation (see p. 290), the *early components* in the complement system are *serine proteinases,* which mutually activate each other through limited proteolysis. They create a self-reinforcing **enzyme cascade**. Factor **C3**, the products of which are involved in several functions, is central to the complement system.

The **classic pathway** is triggered by the formation of factor C1 at IgG or IgM on the surface of microorganisms (left). C1 is an 18-part molecular complex with three different components (C1q, C1r, and C1s). C1q is shaped like a bunch of tulips, the "flowers" of which bind to the F_c region of antibodies (left). This activates C1r, a *serine proteinase* that initiates the cascade of the classic pathway. First, C4 is proteolytically activated into C4b, which in turn cleaves C2 into C2a and C2b. C4B and C2a together form *C3 convertase* [1], which finally catalyzes the cleavage of C3 into C3a and C3b. Small amounts of C3b also arise from non-enzymatic hydrolysis of C3.

The **alternative pathway** starts with the binding of factors C3b and B to bacterial lipopolysaccharides (endotoxins). The formation of this complex allows cleavage of B by factor D, giving rise to a second form of *C3 convertase (C3bBb).*

Proteolytic cleavage of factor **C3** provides two components with different effects. The reaction exposes a highly *reactive thioester group* in C3b, which reacts with hydroxyl or amino groups. This allows C3b to bind covalently to molecules on the bacterial surface (*opsonization,* right). In addition, C3b initiates a chain of reactions leading to the formation of the *membrane attack complex* (see below). Together with C4a and C5a (see below), the smaller product C3a promotes the inflammatory reaction and has chemotactic effects.

The "late" factors C5 to C9 are responsible for the development of the **membrane attack complex** (bottom). 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 actual pore—then bind to this core.



Structure of the membrane attack complex

Antibodies

Soluble antigen receptors, which are formed by activated B cells (plasma cells; see p. 294) and released into the blood, are known as **antibodies**. They are also members of the immunoglobulin family (Ig; see p. 296). 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 (*neutralization;* see p. 404, for example).

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 (see p. 298) and thereby promote the innate immune defense system (*opsonization*).

In addition, antibodies have become indispensable aids in medical and biological diagnosis (see p. 304).

A. Domain structure of immunoglobulin G ◑

Type G immunoglobulins (**IgG**) are quantitatively the most important antibodies in the blood, where they form the fraction of γ-globulins (see p. 276). IgGs (mass 150 kDa) are tetramers with two **heavy chains** (H chains; red or orange) and two **light chains** (L chains; yellow). Both H chains are glycosylated (violet; see also p. 43).

The proteinase *papain* cleaves IgG into two F_{ab} fragments and one F_c fragment. The **F_{ab}** ("antigen-binding") fragments, which each consist of one L chain and the N-terminal part of an H chain, are 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 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 in Ω form). 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.

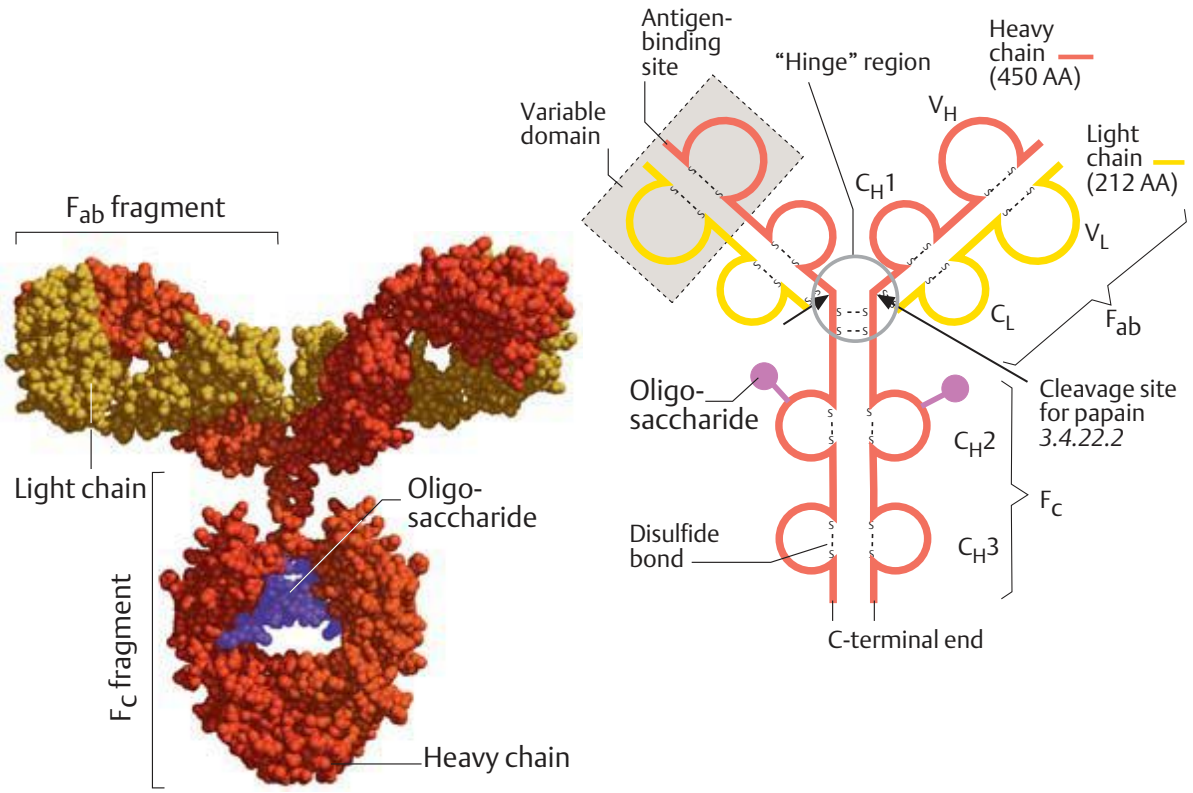
Disulfide bonds link the two heavy chains to each other and also link the heavy chains to the light chains. Inside the domains, there are also disulfide bonds that stabilize the tertiary structure. The domains are approximately 110 amino acids (AA) long and are homologous with each other. The antibody structure evidently developed as a result of gene duplication. In its central region, known as the "hinge" region, the antibodies are highly mobile.

B. Classes of immunoglobulins ◑

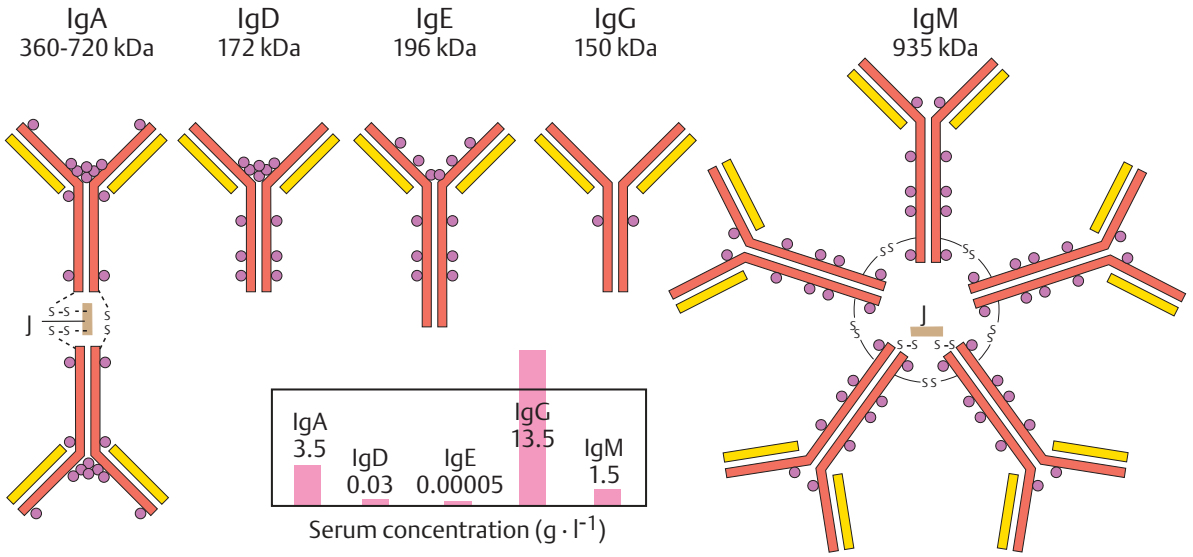
Human immunoglobulins are divided into five classes. **IgA** (with two subgroups), **IgD, IgE, IgG** (with four subgroups), and **IgM** are defined by their H chains, which are designated by the Greek letters α, δ, ε, γ, and μ. By contrast, there are only two types of **L chain** (κ and λ). IgD and IgE (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 antibodies have different tasks. **IgMs** are the first immunoglobulins formed after contact with a foreign antigen. Their early forms are located on the surface of B cells (see p. 296), while the later forms are secreted from plasma cells as pentamers. Their action targets microorganisms in particular. Quantitatively, **IgGs** are the most important immunoglobulins (see the table showing serum concentrations). They occur in the blood and interstitial fluid. As they can pass the placenta with the help of receptors, they can be transferred from mother to fetus. **IgAs** mainly occur in the intestinal tract and in body secretions. **IgEs** are found in low concentrations in the blood. As they can trigger degranulation of mast cells (see p. 380), they play an important role in allergic reactions. The function of **IgDs** is still unexplained. Their plasma concentration is also very low.

A. Domain structure of immunoglobulin G



B. Classes of immunoglobulins



Chain:					
H	α	δ	ε	γ	μ
L	κ or λ	κ or λ	κ or λ	κ or λ	κ or λ
Structure:					
	$(\alpha_2 \kappa_2)_n J$	$\delta_2 \kappa_2$	$\varepsilon_2 \kappa_2$	$\gamma_2 \kappa_2$	$(\mu_2 \kappa_2)_5 J$
	$(\alpha_2 \lambda_2)_n J$	$\delta_2 \lambda_2$	$\varepsilon_2 \lambda_2$	$\gamma_2 \lambda_2$	$(\mu_2 \lambda_2)_5 J$
	n = 1, 2 or 3				

Antibody biosynthesis

The acquired (adaptive) immune system (see p. 294) is based on the ability of the lymphocytes to keep an extremely large repertoire of antigen receptors and soluble antibodies ready for use, so that even infections involving new types of pathogen can be combated. The wide range of immunoglobulins (Ig) are produced by genetic recombination and additional mutations during the development and maturation of the individual lymphocytes.

A. Variability of immunoglobulins ◑

It is estimated that more than 10^8 different antibody variants occur in every human being. This **variability** affects both the heavy and the light chains of immunoglobulins.

There are five different types of heavy (H) chain, according to which the antibody classes are defined (α, δ, ε, γ, μ), and two types of light (L) chain (κ and λ; see p. 300). The various Ig types that arise from combinations of these chains are known as **isotypes**. During immunoglobulin biosynthesis, plasma cells can switch from one isotype to another ("gene switch"). **Allotypic variation** is based on the existence of various alleles of the same gene—i. e., genetic differences between individuals. The term **idiotypic variation** refers to the fact that the antigen binding sites in the F_{ab} fragments can be highly variable. Idiotypic variation affects the *variable domains* (shown here in pink) of the light and heavy chains. At certain sites—known as the *hypervariable regions* (shown here in red)—variation is particularly wide; these sequences are directly involved in the binding of the antigen.

B. Causes of antibody variety ◑

There are three reasons for the extremely wide variability of antibodies:

1. **Multiple genes.** Various genes are available to code for the variable protein domains. Only one gene from among these is selected and expressed.

2. **Somatic recombination.** The genes are divided into several segments, of which there are various versions. Various ("untidy") combinations of the segments during lymphocyte maturation give rise to randomly combined new genes ("mosaic genes").

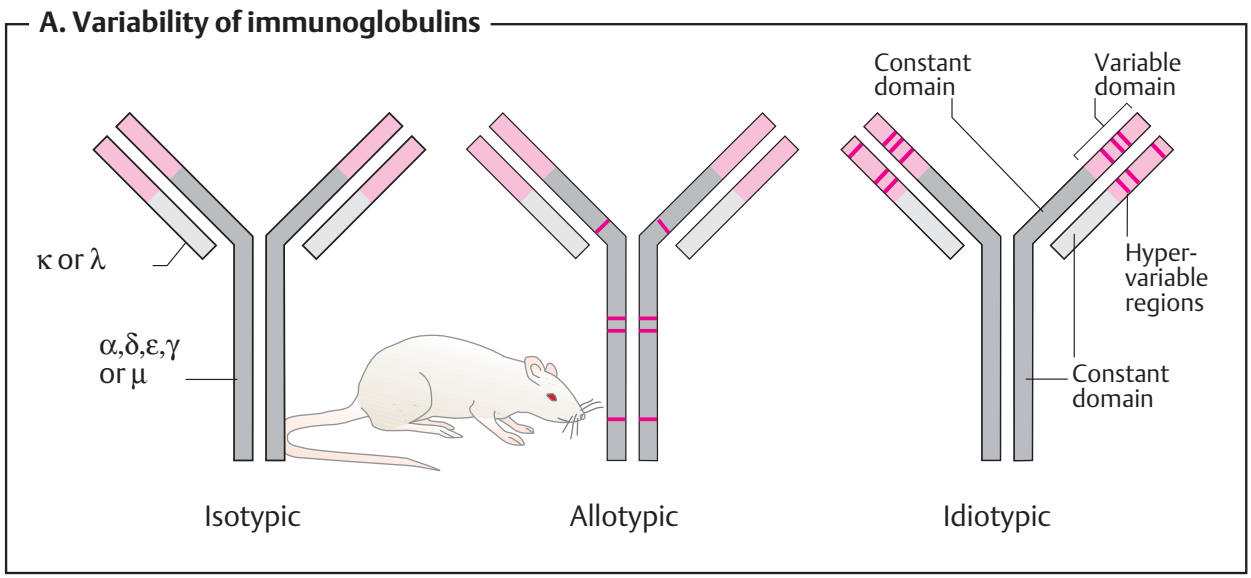
3. **Somatic mutation.** During differentiation of B cells into plasma cells, the coding genes mutate. In this way, the "primordial" *germline genes* can become different *somatic genes* in the individual B cell clones.

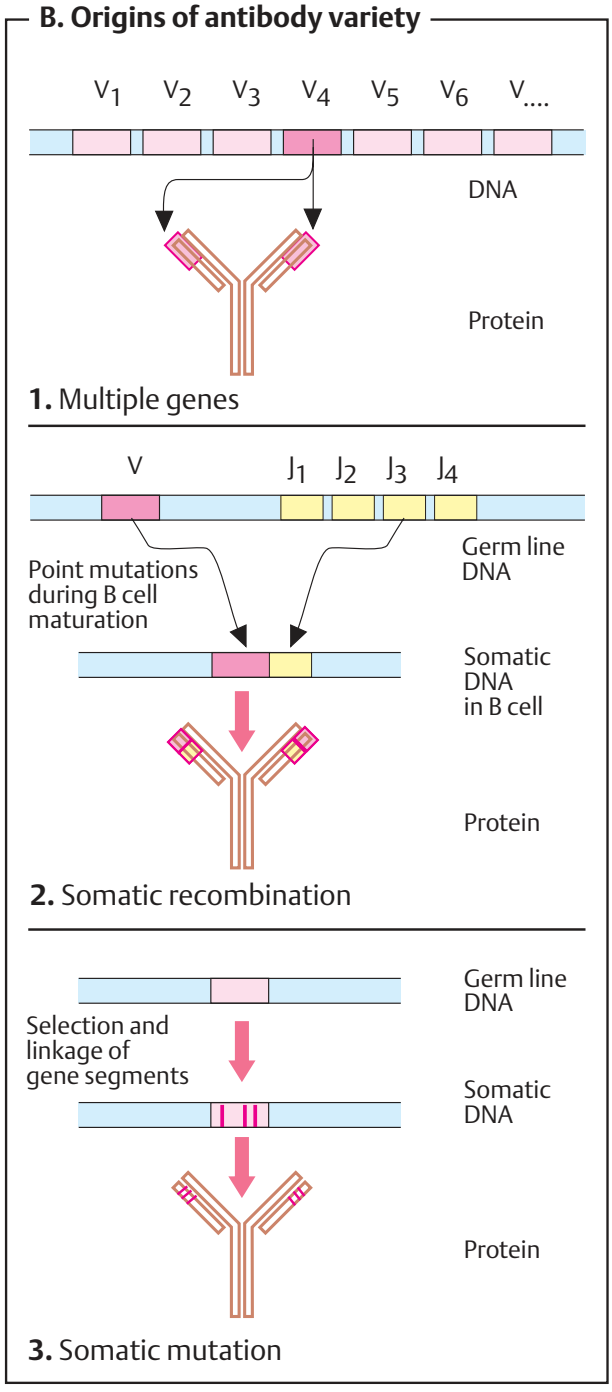
C. Biosynthesis of a light chain ○

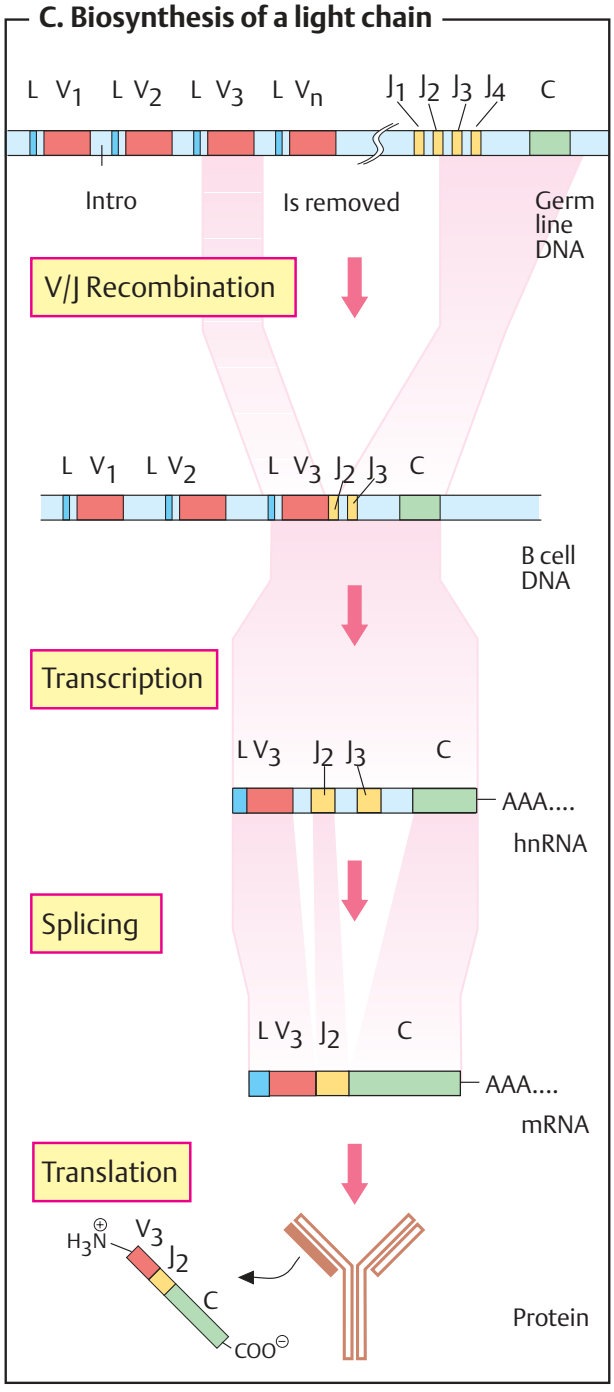
We can look at the basic features of the genetic organization and synthesis of immunoglobulins using the biosynthesis of a mouse κ chain as an example. The gene segments for this light chain are designated L, V, J, and C. They are located on chromosome 6 in the **germ-line DNA** (on chromosome 2 in humans) and are separated from one another by introns (see p. 242) of different lengths.

Some 150 identical **L segments** code for the signal peptide ("leader sequence," 17–20 amino acids) for secretion of the product (see p. 230). The **V segments**, of which there are 150 different variants, code for most of the variable domains (95 of the 108 amino acids). L and V segments always occur in pairs—in tandem, so to speak. By contrast, there are only five variants of the **J segments** (joining segments) at most. These code for a peptide with 13 amino acids that links the variable part of the κ chains to the constant part. A single **C segment** codes for the constant part of the light chain (84 amino acids).

During the differentiation of B lymphocytes, individual *V/J combinations* arise in each B cell. One of the 150 L/V tandem segments is selected and linked to one of the five J segments. This gives rise to a *somatic gene* that is much smaller than the germline gene. Transcription of this gene leads to the formation of the **hnRNA** for the κ chain, from which introns and surplus J segments are removed by splicing (see p. 246). Finally, the completed **mRNA** still contains one each of the L–V–J–C segments and after being transported into the cytoplasm is available for translation. The subsequent steps in Ig biosynthesis follow the rules for the synthesis of membrane-bound or secretory proteins (see p. 230).







Monoclonal antibodies, immunoassay

A. Monoclonal antibodies ○

Monoclonal antibodies (MABs) are secreted by immune cells that derive from a single antibody-forming cell (from a single cell *clone*). This is why each MAB is directed against only one specific *epitope* of an immunogenic substance, known as an *"antigenic determinant."* Large molecules contain several epitopes, against which various antibodies are formed by various B cells. An antiserum containing a mixture of all of these antibodies is described as being *polyclonal.*

To obtain MABs, **lymphocytes** isolated from the spleen of immunized mice (**1**) are fused with mouse tumor cells (**myeloma cells**, **2**). This is necessary because antibody-secreting lymphocytes in culture have a lifespan of only a few weeks. Fusion of lymphocytes with tumor cells gives rise to cell hybrids, known as **hybridomas,** which are potentially immortal.

Successful *fusion* (**2**) is a rare event, but the frequency can be improved by adding polyethylene glycol (PEG). To obtain only successfully fused cells, incubation is required for an extended period in a **primary culture** with *HAT medium* (**3**), which contains hypoxanthine, aminopterin, and thymidine. *Aminopterin*, an analogue of dihydrofolic acid, competitively inhibits *dihydrofolate reductase* and thus inhibits the synthesis of dTMP (see p. 402). As dTMP is essential for DNA synthesis, myeloma cells cannot survive in the presence of aminopterin. Although spleen cells are able to circumvent the inhibitory effect of aminopterin by using *hypoxanthine* and *thymidine,* they have a limited lifespan and die. Only hybridomas survive culture in HAT medium, because they possess both the immortality of the myeloma cells and the spleen cells' metabolic side pathway.

Only a few fused cells actually produce antibodies. To identify these cells, the hybridomas have to be isolated and replicated by **cloning** (**4**). After the clones have been tested for antibody formation, positive cultures are picked out and selected by further cloning (**5**). This results in hybridomas that synthesize *monoclonal antibodies.* Finally, MAB production is carried out in vitro using a bioreactor, or in vivo by producing ascites fluid in mice (**6**).

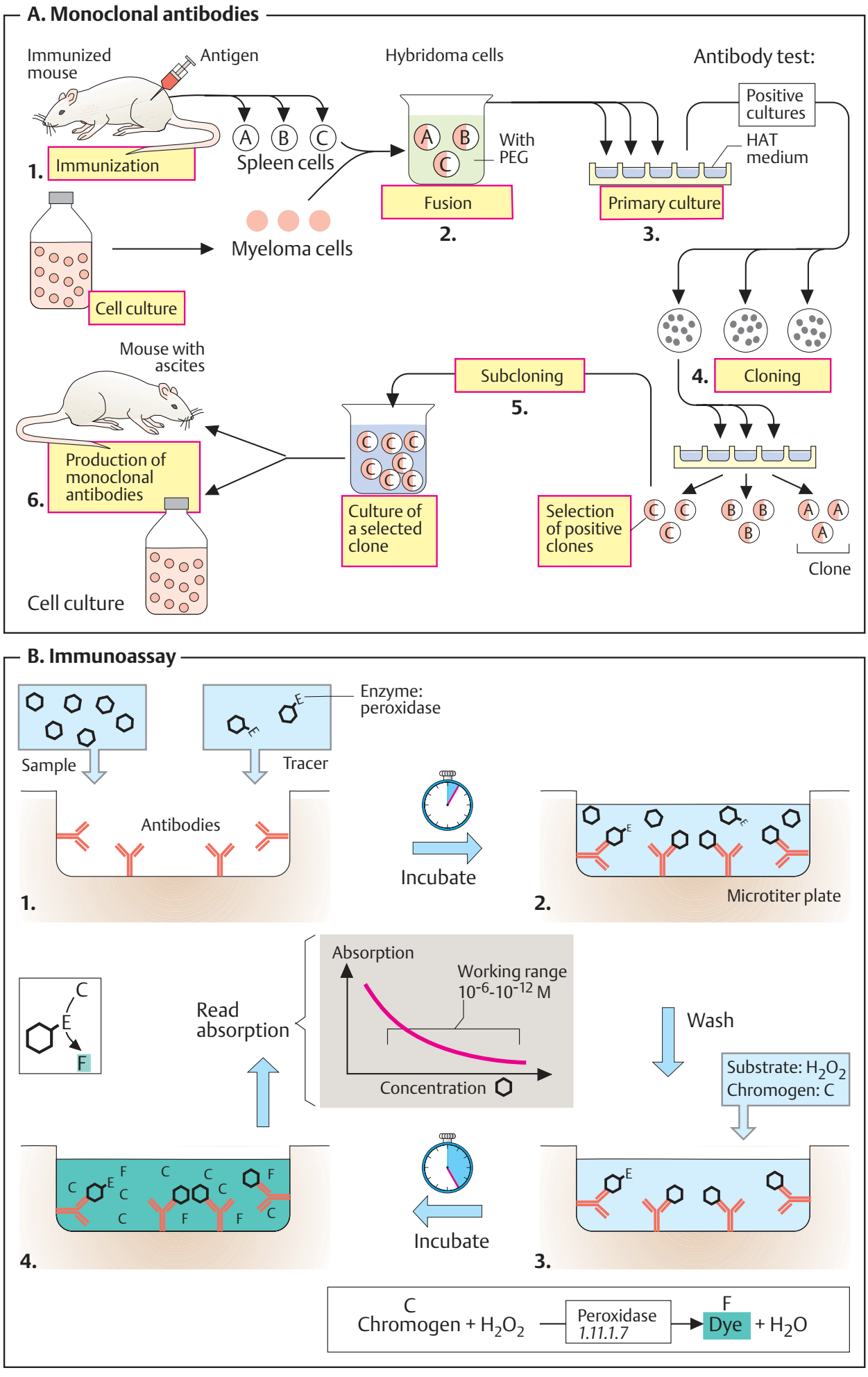
B. Immunoassay ○

Immunoassays are semiquantitative procedures for assessing substances with low concentrations. In principle, immunoassays can be used to assess any compound against which antibodies are formed.

The basis for this procedure is the *antigen–antibody "reaction"*—i. e., specific binding of an antibody to the molecule being assayed. Among the many different immunoassay techniques that have been developed—e. g., *radioimmunoassay* (RIA), and *chemoluminescence immunoassay* (CIA)—a version of the **enzyme-linked immunoassay** (EIA) is shown here.

The substance to be assayed—e. g., the hormone thyroxine in a serum sample—is pipetted into a microtiter plate (**1**), the walls of which are coated with *antibodies* that specifically bind the hormone. At the same time, a small amount of thyroxine is added to the incubation to which an enzyme known as the *"tracer"* (**1**) has been chemically coupled. The tracer and the hormone being assayed compete for the small number of antibody binding sites available. After binding has taken place (**2**), all of the unbound molecules are rinsed out. The addition of a substrate solution for the enzyme (a *chromogenic solution*) then triggers an indicator reaction (**3**), the products of which can be assessed using photometry (**4**).

The larger the amount of enzyme that can bind to the antibodies on the container's walls, the larger the amount of dye that is produced. Conversely, the larger the amount of the substance being assayed that is present in the sample, the *smaller* the amount of tracer that can be bound by the antibodies. Quantitative analysis can be carried out through parallel measurement using standards with a known concentration.



Liver: 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. Diagram of a hepatocyte ◑

The 3 10^{11} cells in the liver—particularly the **hepatocytes**, which make up 90% of the cell mass—are the central location for the body's *intermediary metabolism.* They are in close contact with the blood, which enters the liver from the portal vein and the hepatic arteries, flows through capillary vessels known as sinusoids, and is collected again in the central veins of the hepatic lobes. Hepatocytes are particularly rich in endoplasmic reticulum, as they carry out intensive protein and lipid synthesis. The cytoplasm contains granules of insoluble glycogen. Between the hepatocytes, there are bile capillaries through which bile components are excreted.

B. 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. Biosynthesis of endogenous compounds and storage, conversion, and degradation of them into excretable molecules (**metabolism**). 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.
4. **Detoxification** of toxic compounds by biotransformation.
5. **Excretion** of substances with the bile.

C. 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, in order 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 (see p. 310).

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's special ability to convert fatty acids into *ketone bodies* and to release these again is also important (see p. 312).

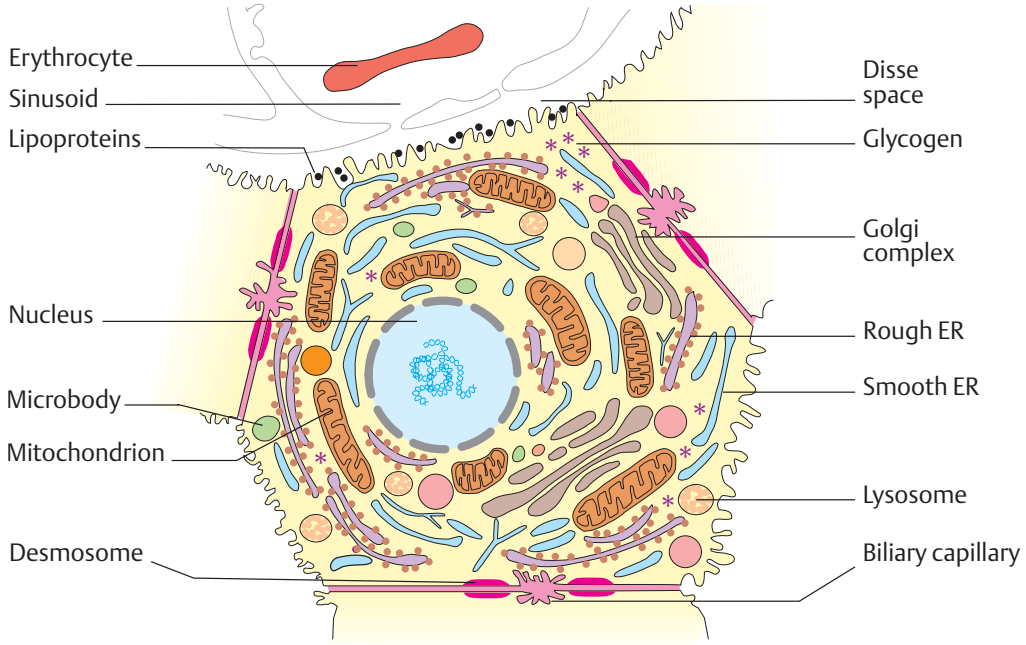
Like other organs, the liver also synthesizes cholesterol, which is transported to other tissues as a component of lipoproteins. Excess cholesterol is converted into bile acids in the liver or directly excreted with the bile (see p. 314).

Amino acid and protein metabolism. The liver controls the plasma levels of the amino acids. Excess amino acids are broken down. With the help of the urea cycle (see p. 182), 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 broken down in the liver (see p. 276).

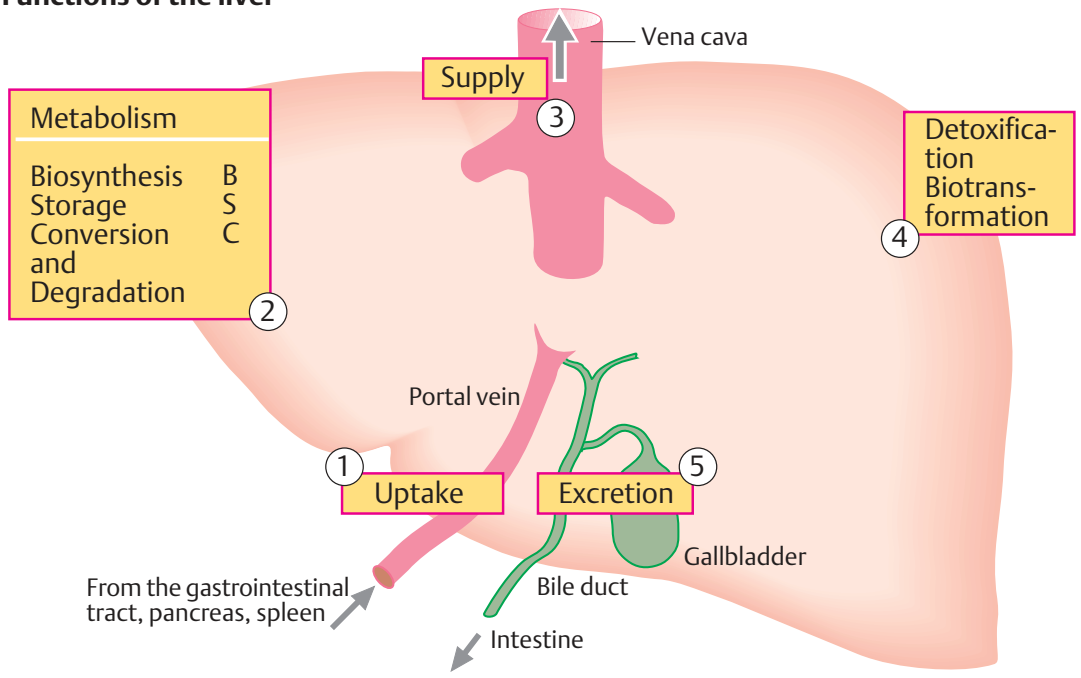
Biotransformation. Steroid hormones and bilirubin, as well as drugs, ethanol, and other xenobiotics are taken up by the liver and inactivated and converted into highly polar metabolites by conversion reactions (see p. 316).

Storage. The liver not only stores energy reserves and nutrients for the body, but also certain mineral substances, trace elements, and vitamins, including iron, retinol, and vitamins A, D, K, folic acid, and B_{12}.

A. Diagram of a hepatocyte



B. Functions of the liver



C. Liver metabolism

Carbohydrate metabolism	
Glucose	BSC
Galactose	C
Fructose	C
Mannose	C
Pentoses	BC
Lactate	C
Glycerol	BC
Glycogen	BSC

Lipid metabolism	
Fatty acids	BC
Fats	BC
Ketone bodies	B
Cholesterol	BEC
Bile acids	BE
Vitamins	SC

Amino acid metabolism	
Amino acids	C
Urea	B

Plasma proteins	
Lipoproteins	BC
Albumin	BC
Coagulation factors	BC
Hormones	C
Enzymes	BC

Biotransformation	
Steroid hormones	EC
Bile pigments	EC
Ethanol	C
Drugs	EC

B	Biosynthesis
C	Conversion and degradation
E	Excretion
S	Storage

Buffer function in organ metabolism

All of the body's tissues have a constant requirement for energy substrates and nutrients. The body receives these metabolites with food, but the supply is irregular and in varying amounts. The liver acts here along with other organs, particularly adipose tissue, as a balancing *buffer* and *storage organ.*

In the metabolism, a distinction is made between the *absorptive state (well-fed state)* immediately after a meal and the *postabsorbtive state (state of starvation)*, which starts later and can merge into hunger. The switching of the organ metabolism between the two phases depends on the concentration of energy-bearing metabolites in the blood (plasma level). This is regulated jointly by hormones and by the autonomic nervous system.

A. Absorptive state ◑

The absorptive state continues for 2–4 hours after food intake. As a result of food digestion, the plasma levels of glucose, amino acids, and fats (triacylglycerols) temporarily increase.

The endocrine **pancreas** responds to this by altering its hormone release—there is an increase in *insulin* secretion and a reduction in *glucagon* secretion. The increase in the insulin/glucagon quotient and the availability of substrates trigger an *anabolic phase* in the tissues—particularly liver, muscle, and adipose tissues.

The **liver** forms increased amounts of glycogen and fats from the substrates supplied. Glycogen is stored, and the fat is released into the blood in very low density lipoproteins (VLDLs).

Muscle also refills its glycogen store and synthesizes proteins from the amino acids supplied.

Adipose tissue removes free fatty acids from the lipoproteins, synthesizes triacylglycerols from them again, and stores these in the form of insoluble droplets.

During the absorptive state, the **heart** and **neural tissue** mainly use glucose as an energy source, but they are unable to establish any substantial energy stores. Heart muscle cells are in a sense "omnivorous," as they can also use other substances to produce energy (fatty acids, ketone bodies). By contrast, the central nervous system (CNS) is dependent on glucose. It is only able to utilize ketone bodies after a prolonged phase of hunger (**B**).

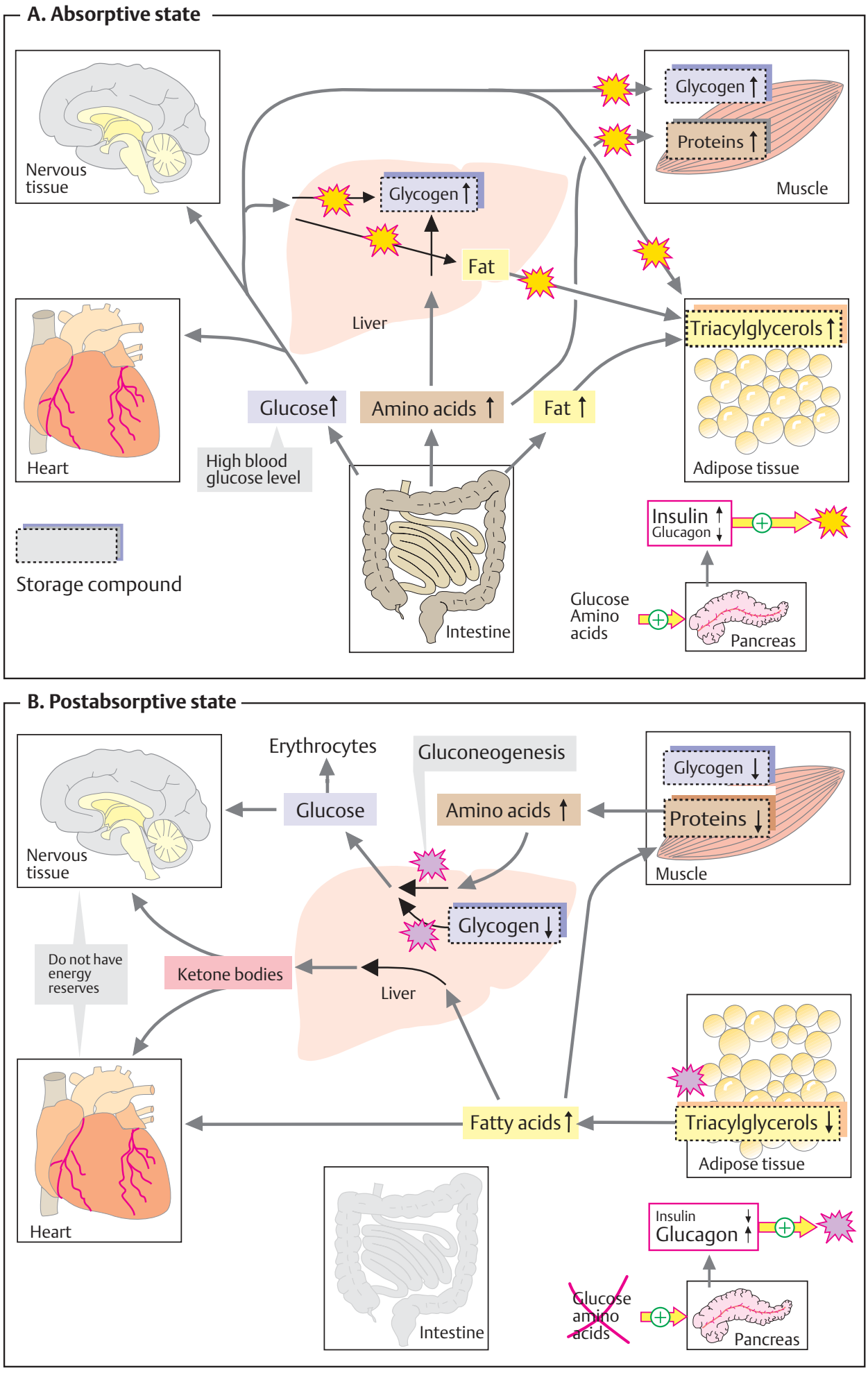
B. Postabsorptive state ◑

When the food supply is interrupted, the postabsorbtive state quickly sets in. The pancreatic A cells now release increased amounts of *glucagon,* while the B cells reduce the amount of *insulin* they secrete. The reduced insulin/glucagon quotient leads to switching of the intermediary metabolism. The body now falls back on its energy reserves. To do this, it breaks down *storage substances* (glycogen, fats, and proteins) and shifts energy-supplying metabolites between the organs.

The **liver** first empties its glycogen store (*glycogenolysis;* see p. 156). It does not use the released glucose itself, however, but supplies the other tissues with it. In particular, the brain, adrenal gland medulla, and erythrocytes depend on a constant supply of glucose, as they have no substantial glucose reserves themselves. When the liver's glycogen reserves are exhausted after 12–24 hours, *gluconeogenesis* begins (see p. 154). The precursors for this are derived from the musculature (amino acids) and adipose tissue (glycerol from fat degradation). From the fatty acids that are released (see below), the liver starts to form ketone bodies (*ketogenesis;* see p. 312). These are released into the blood and serve as important energy suppliers during the hunger phase. After 1–2 weeks, the CNS also starts to use ketone bodies to supply part of its energy requirements, in order to save glucose.

In **muscle**, the extensive glycogen reserves are exclusively used for the muscles' own requirements (see p. 320). The slowly initiated protein breakdown in muscle supplies amino acids for gluconeogenesis in the liver.

In **adipose tissue**, glucagon triggers *lipolysis,* releasing fatty acids and glycerol. The fatty acids are used as energy suppliers by many types of tissue (with the exception of brain and erythrocytes). An important recipient of the fatty acids is the liver, which uses them for ketogenesis.



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 glucosesupplying and glucose-utilizing processes. Glucose suppliers include the intestines (glucose from food), liver, and kidneys. The liver plays the role of a "glucostat" (see p. 308).

The liver is also capable of forming glucose by converting other sugars—e. g., *fructose* and *galactose*—or by synthesizing from other metabolites. The conversion of lactate to glucose in the *Cori cycle* (see p. 338) and the conversion of alanine to glucose with the help of the *alanine cycle* (see p. 338) are particularly important for the supply of erythrocytes and muscle cells.

Transporters in the plasma membrane of hepatocytes allow insulin-independent transport of glucose and other sugars in both directions. In contrast to muscle, the liver possesses the enzyme *glucose-6-phosphatase,* which can release glucose from glucose-6-phosphate.

A. Gluconeogenesis: overview ◑

Regeneration of glucose (up to 250 g per day) mainly takes place in the liver. The tubule cells of the kidney are also capable of carrying out gluconeogenesis, but due to their much smaller mass, their contribution only represents around 10% of total glucose formation. Gluconeogenesis is regulated by hormones. *Cortisol, glucagon,* and *epinephrine* promote gluconeogenesis, while *insulin* inhibits it (see pp. 158, 244).

The main precursors of gluconeogenesis in the liver are *lactate* from anaerobically working muscle cells and from erythrocytes, glucogenic *amino acids* from the digestive tract and muscles (mainly alanine), and *glycerol* from adipose tissue. The kidney mainly uses amino acids for gluconeogenesis (Glu, Gln; see p. 328).

In mammals, fatty acids and other suppliers of acetyl CoA are not capable of being used for gluconeogenesis, as the acetyl residues formed during β-oxidation in the tricarboxylic acid cycle (see p. 132) are oxidized to CO_2 and therefore cannot be converted into oxaloacetic acid, the precursor for gluconeogenesis.

B. Fructose and galactose metabolism ◑

Fructose is mainly metabolized by the liver, which channels it into glycolysis (left half of the illustration).

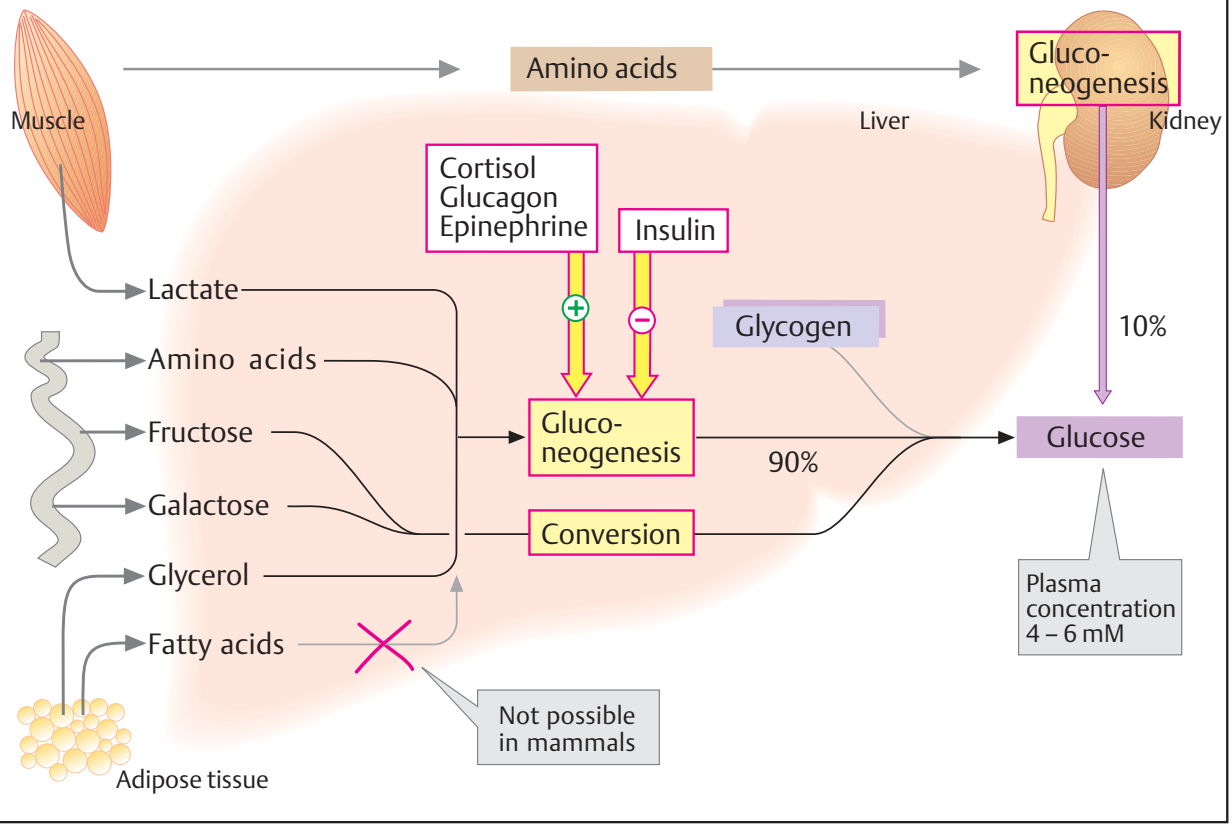
A special *ketohexokinase* [1] initially phosphorylates fructose into **fructose 1-phosphate**. This is then cleaved by an *aldolase* [2], which is also fructose-specific, to yield **glycerone 3-phosphate** (dihydroxyacetone phosphate) and **glyceraldehyde**. Glycerone 3-phosphate is already an intermediate of glycolysis (center), while glyceraldehyde can be phosphorylated into glyceraldehyde 3-phosphate by *triokinase* [3].

To a smaller extent, glyceraldehyde is also reduced to glycerol [4] or oxidized to glycerate, which can be channeled into glycolysis following phosphorylation (not shown). The reduction of glyceraldehyde [4] 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 (see p. 320).

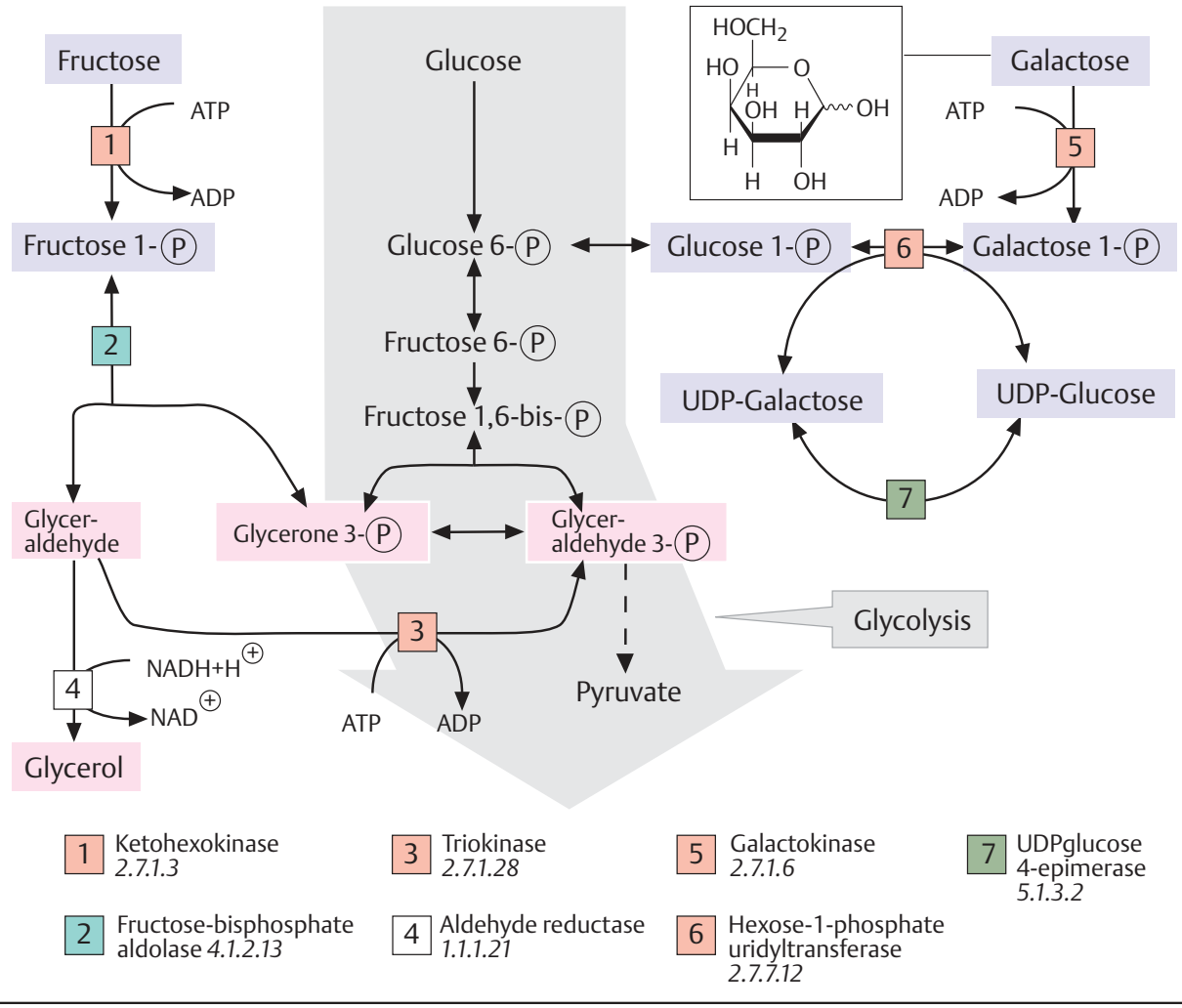
Outside of the liver, fructose is channeled into the sugar metabolism by reduction at C-2 to yield sorbitol and subsequent dehydration at C-1 to yield glucose (the *polyol pathway;* not shown).

Galactose is also 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** [5]. 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* [6] transfers a uridine 5′-monophosphate (UMP) residue from uridine diphosphoglucose (UDPglucose) to galactose 1-phosphate. This releases glucose 1-phosphate, while galactose 1-phosphate is converted into uridine diphosphogalactose (UDPgalactose). This then is isomerized into UDPglucose. The *biosynthesis* of galactose also follows this reaction pathway, which is freely reversible up to reaction [5]. Genetic defects of enzymes [5] or [6] can lead to the clinical picture of *galactosemia.*

A. Gluconeogenesis: overview



B. Fructose and galactose metabolism



Lipid metabolism

The liver is the most important site for the formation of *fatty acids, fats (triacylglycerols), 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 the carbohydrate and amino acid metabolism. When there is a good supply of nutrients in the *resorptive (wellfed) state* (see p. 308), the liver converts glucose 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 (see p. 162). Fatty acids from both sources are converted into **fats** and **phospholipids**. Together with apoproteins, they are packed into very-low-density lipoproteins (**VLDLs**; see p. 278) and then released into the blood by exocytosis. The VLDLs supply extrahepatic tissue, particularly adipose tissue and muscle.

In the *postresorptive state* (see p. 292)—particularly during fasting and starvation—the lipid metabolism is readjusted and the organism falls back on its own reserves. In these conditions, adipose tissue 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** (see p. 314). In addition, it serves as a building block for cell membranes (see p. 216), 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; see p. 278).

B. Biosynthesis of ketone bodies ◑

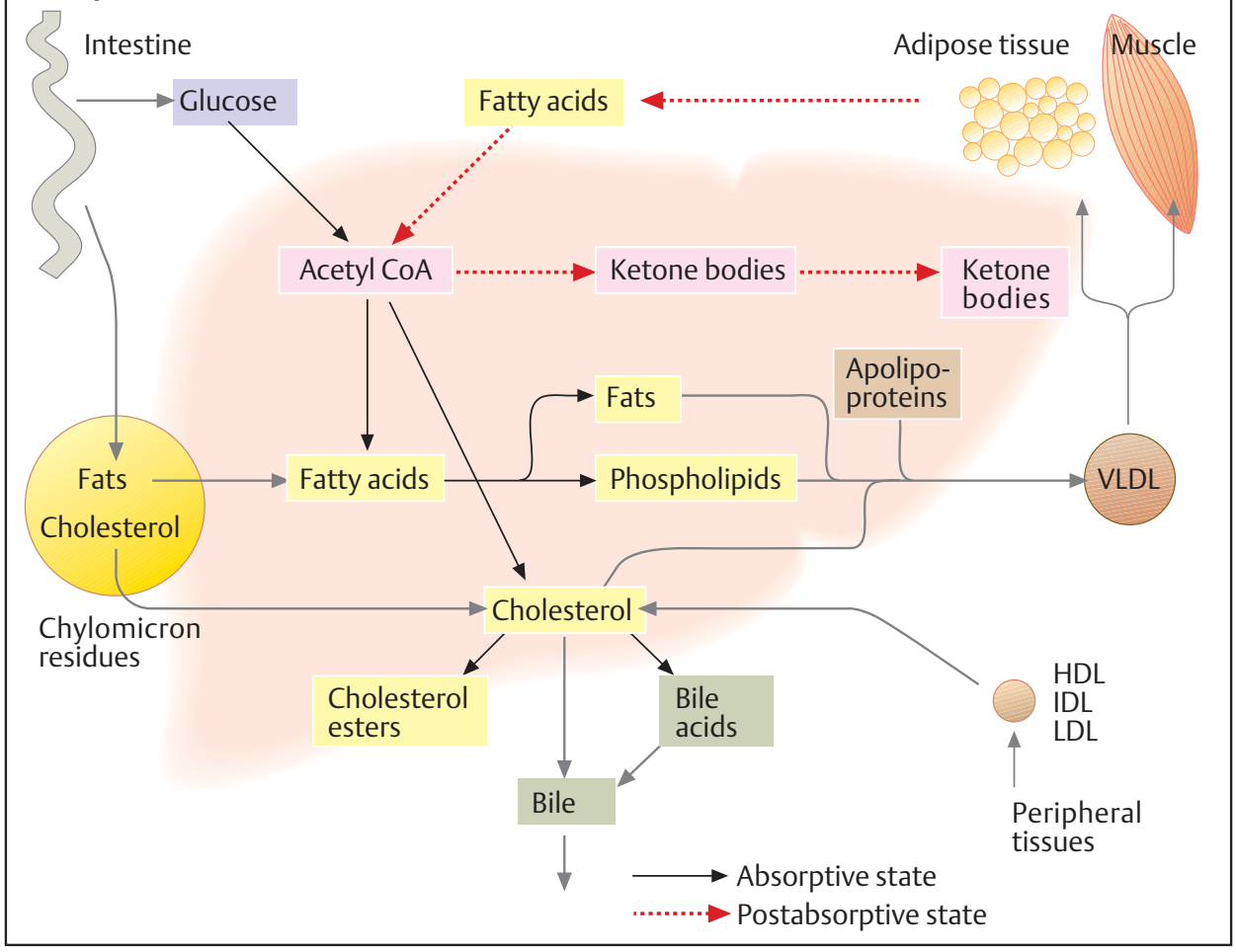
At high concentrations of acetyl-CoA in the liver mitochondria, two molecules condense to form **acetoacetyl CoA** [1]. The transfer of another acetyl group [2] gives rise to 3-hydroxy-3-methylglutaryl-CoA (**HMG CoA**), which after release of acetyl CoA [3] yields free **acetoacetate** (*Lynen cycle*). Acetoacetate can be converted to **3-hydroxybutyrate** by reduction [4], or can pass into **acetone** by nonenzymatic 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 (see p. 288).

The ketone bodies are released by the liver into the blood, in which they are easily soluble. Blood levels of ketone bodies therefore rise during periods of hunger. Together with free fatty acids, 3-hydroxybutyrate and acetoacetate are then the most important energy suppliers in many tissues (including heart muscle). Acetone cannot be metabolized and is exhaled via the lungs or excreted with urine.

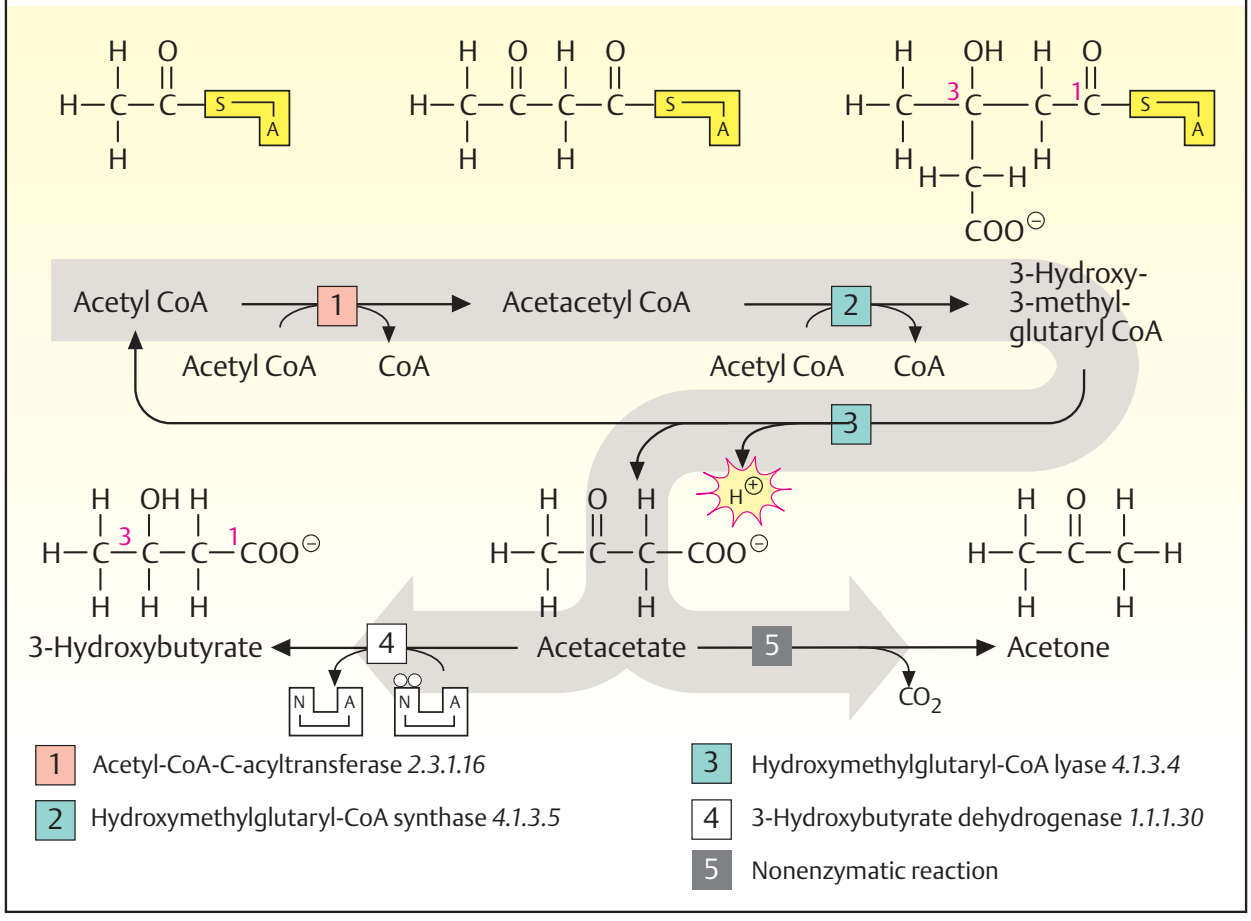
To channel ketone bodies into the energy metabolism, acetoacetate is converted with the help of succinyl CoA into succinic acid and acetoacetyl CoA, which is broken down by β-oxidation into acetyl CoA (not shown; see p. 180).

If the production of ketone bodies exceeds the demand for them outside the liver, 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 *diabetes mellitus.* Severe ketonuria with ketoacidosis can cause electrolyte shifts and loss of consciousness, and is therefore life-threatening (*ketoacidotic coma*).

A. Lipid metabolism



A. Biosynthesis of ketone bodies



Bile acids

Bile is an important product released by the hepatocytes. It promotes the digestion of fats from food by *emulsifying* them in the small intestine (see p. 2770). 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 (see p. 312).

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 via an extensive reaction pathway (top). Cytochrome P450 enzymes in the sER of hepatocytes are involved in many of the steps (seep. 318). Initially, the cholesterol double bond is removed. Monooxygenases then introduce one or two additional OH groups into the sterane framework. Finally, 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 (see p. 54). The result of this is that all of the hydrophilic groups in the bile acids lie on one side of the molecule. Cholesterol, which is weakly amphipathic (top), has a small polar "head" and an extended apolar "tail." By contrast, the much more strongly amphipathic bile acid molecules (bottom) resemble disks with polar top sides and apolar bottom sides. At physiological pH values, the carboxyl groups are almost completely dissociated and therefore negatively charged.

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 non-proteinogenic amino acid *taurine* (see p. 62). The acid amides formed in this way are known as **conjugated bile acids** or **bile salts**. They are even more amphipathic than the primary products.

Deoxycholic acid and *lithocholic acid* are only formed in the intestine 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 (see **A**). 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*). In this way, the bile acids present in the liver regulate the rate of cholesterol utilization.

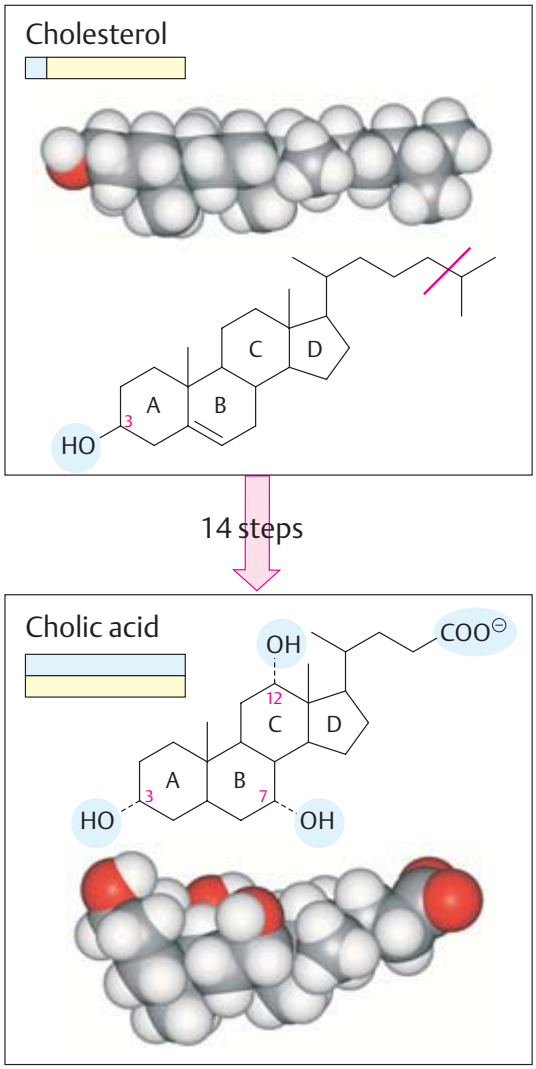
Before leaving the liver, a large proportion of the bile acids are activated with CoA and then conjugated with the amino acids *glycine* or *taurine* (**2**; cf. **A**). In this way, cholic acid gives rise to *glycocholic acid* and *taurocholic acid.* The *liver bile* secreted by the liver becomes denser 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 are resorbed again 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.

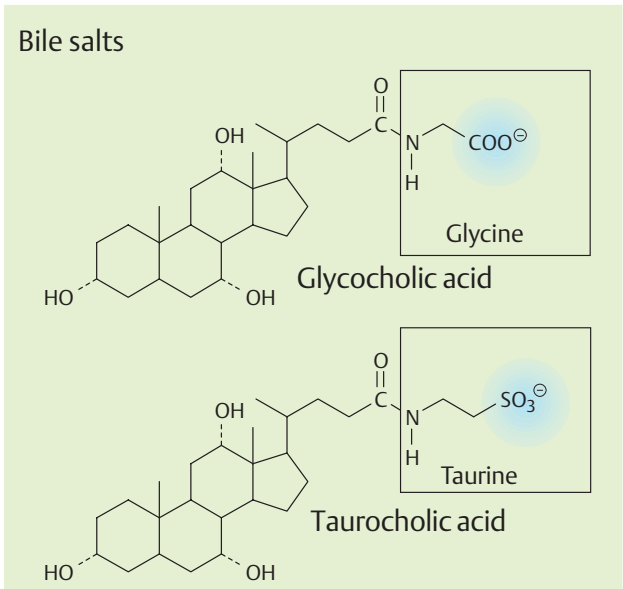
Further information

The cholesterol excreted with the bile is poorly water-soluble. Together with phospholipids and bile acids, it forms micelles (see p. 270), 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 Ca^{2+} salts of bile acids and bile pigments (pigment stones).

A. Bile acids and bile salts

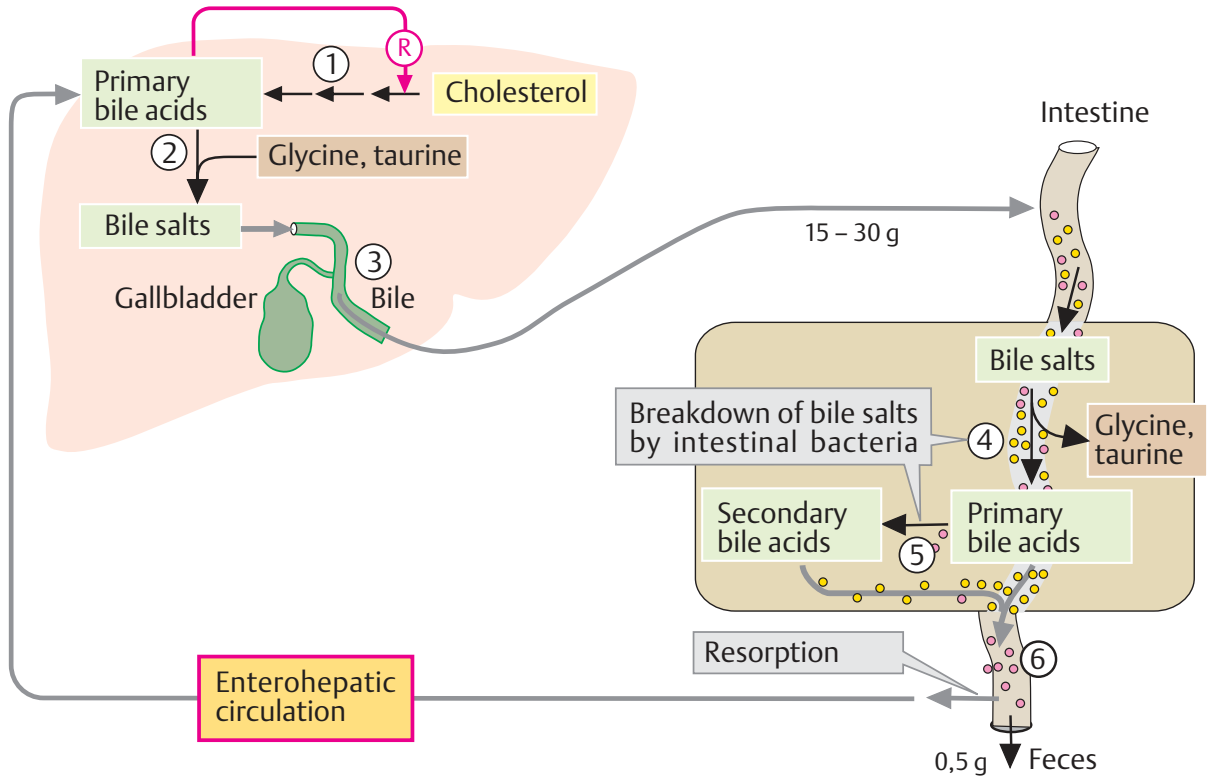


Bile acids	HO– in position		
Primary bile acids			
— Cholic acid	C-3	C-7	C-12
— Chenodeoxycholic acid	C-3	C-7	-
Secondary bile acids			
— Deoxycholic acid	C-3	-	C-12
— Lithocholic acid	C-3	-	-



Bile salts = conjugated bile acids

B. Metabolism of bile salts



Biotransformations

The body is constantly taking up **foreign substances** (= *xenobiotics*) from food or through contact with the environment, via the skin and lungs. These substances can be natural in origin, or may have been synthetically produced by humans. Many 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 enzymatically converted. Biotransformations mainly take place in the *liver.*

A. Biotransformations ◑

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, for example, benzo[*a*]pyrene, p. 256) 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, epoxide formation, sulfoxide formation, dealkylation, deamination. For example, benzene is oxidized into phenol, and toluene (methylbenzene) is oxidized into benzoic acid.
- **Reductions.** Reduction of carbonyl, azo-, or nitro- compounds, dehalogenation.
- **Methylations.** Example (**2**) illustrates the inactivation of the catecholamine *norepinephrine* by methylation of a phenolic OH group (see p. 334).
- **Desulfurations.** The reactions take place in the hepatocytes on the smooth endoplasmic reticulum.

  Most oxidation reactions are catalyzed by **cytochrome P450 systems** (see p. 318). These monooxygenases are induced by their substrates and show wide specificity. The substrate-specific enzymes of the steroid metabolism (see p. 376) are exceptions to this.

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 uridine diphosphate glucuronate, the *"active glucuronate"* (see p. 110). Coupling with the polar glucuronate makes an apolar (hydrophobic) molecule more strongly polar, and it becomes suf ciently water-soluble and capable of being excreted. Example (**3**) shows the glucuronidation of *tetrahydrocortisol,* a metabolite of the glucocorticoid cortisol (see p. 374).

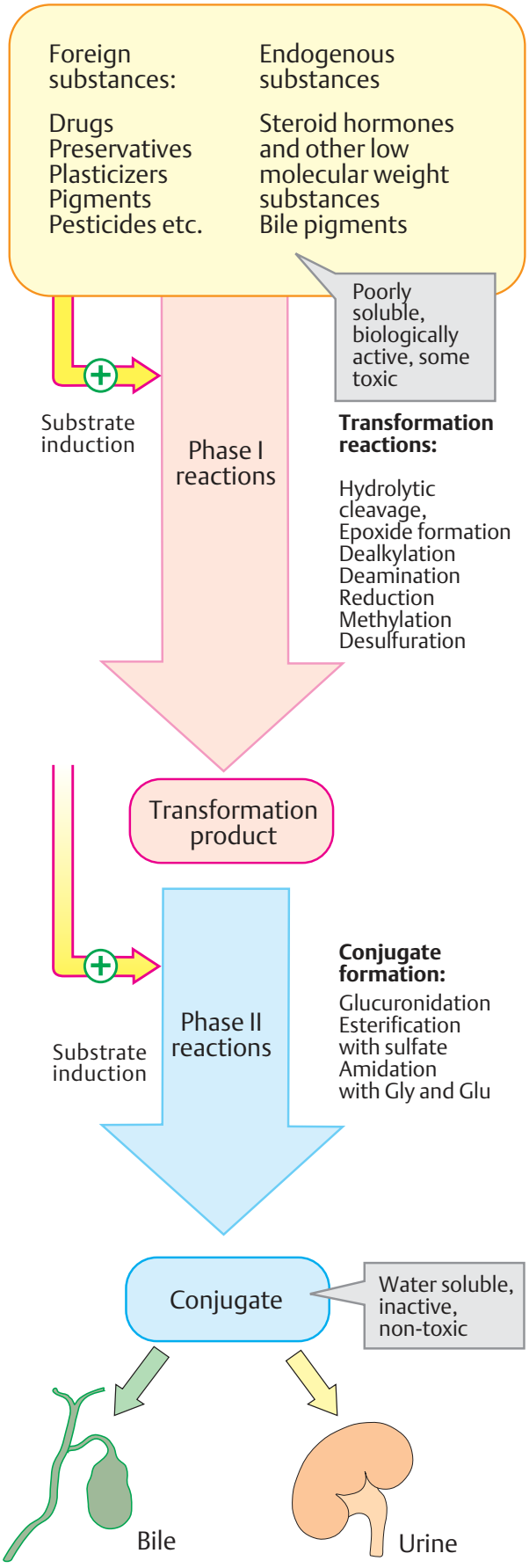
The biosynthesis of sulfate esters with the help of *phosphoadenosine phosphosulfate* (PAPS), the *"active sulfate",* (see p. 110) and amide formation with *glycine* and *glutamine* also play a role in conjugation. For example, benzoic acid is conjugated with glycine to form the more soluble and less toxic *hippuric acid* (*N*-benzoylglycine; see p. 324).

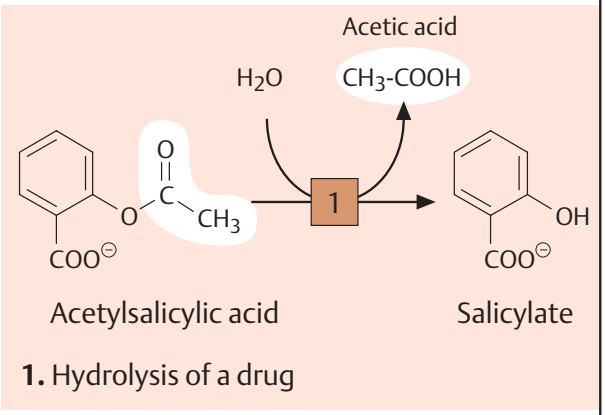
In contrast with unconjugated compounds, the conjugates are much more water-soluble and capable of being excreted. The conjugates are eliminated from the liver either by the *biliary* route—i. e., by receptor-mediated excretion into the bile—or by the *renal* route, via the blood and kidneys by filtration.

Further information

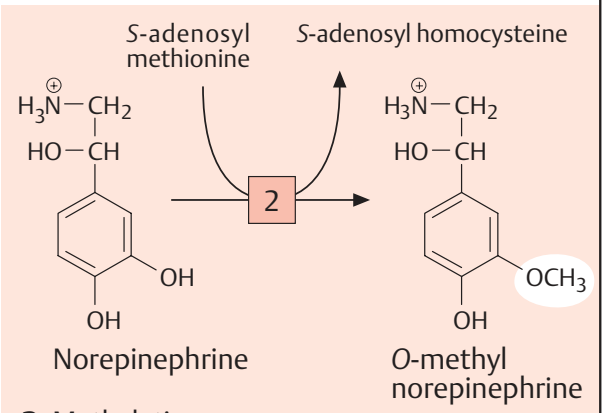
To detoxify **heavy metals**, the liver contains *metallothioneins,* a group of cysteine-rich proteins with a high af nity for divalent metal ions such as Cd^{2+}, Cu^{2+}, Hg^{2+}, and Zn^{2+}. These metal ions also induce the formation of metallothioneins via a special metal-regulating element (MRE) in the gene's promoter (see p. 244).

A. Biotransformations

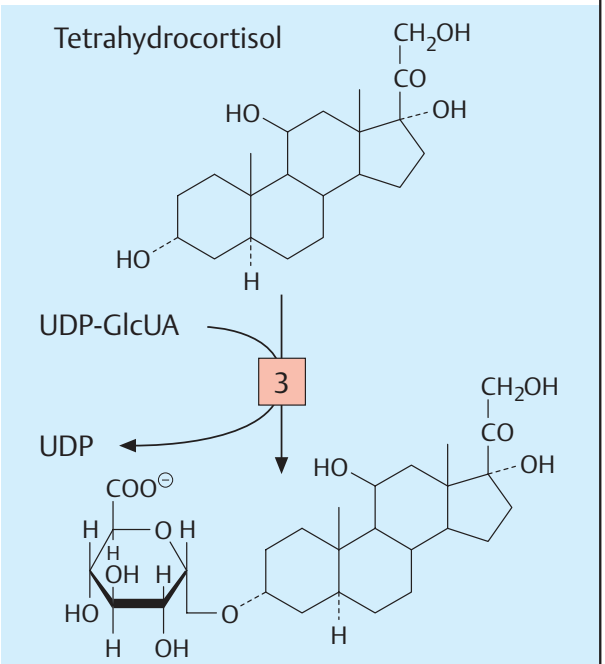




1. Hydrolysis of a drug



2. Methylation of a hormone/neurotransmitter



3. Glucuronidation of a hormone

1 Arylesterase *3.1.1.2*
2 Catechol *O*-methyl-transferase *2.1.1.6*
3 Glucuronosyltransferase *2.4.1.17*

Cytochrome P450 systems

During the first phase of biotransformation in the liver, compounds that are weakly chemically reactive are enzymatically hydroxylated (see p. 316). This makes it possible for them to be conjugated with polar substances. The hydroxylating enzymes are generally *monooxygenases* that contain a **heme** as the redox-active coenzyme (see p. 106). In the reduced form, the heme can bind carbon monoxide (CO), and it then shows characteristic light absorption at 450 nm. This was what led to this enzyme group being termed **cytochrome P450** (Cyt P450).

Cyt P450 systems are also involved in many other metabolic processes—e. g., the biosynthesis of steroid hormones (see p. 172), bile acids (see p. 314), and eicosanoids (see p. 390), as well as the formation of unsaturated fatty acids (see p. 409). The liver's reddish-brown color is mainly due to the large amounts of P450 enzymes it contains.

A. Cytochrome P450-dependent mono oxygenases: reactions ◑

Cyt P450-dependent monooxygenases catalyze reductive cleavage of molecular oxygen (O_2). One of the two oxygen atoms is transferred to the substrate, while the other is released as a water molecule. The necessary reducing equivalents are transferred to the actual monooxygenase by an FAD-containing auxiliary enzyme from the coenzyme $NADPH+H^+$.

Cyt P450 enzymes occur in numerous forms in the liver, steroid-producing glands, and other organs. The substrate specificity of liver enzymes is low. Apolar compounds containing aliphatic or aromatic rings are particularly easily converted. These include endogenous substances such as steroid hormones, as well as medical drugs, which are inactivated by phase I reactions. This is why Cyt P450 enzymes are of particular interest in pharmacology. The degradation of ethanol in the liver is also partly catalyzed by Cyt P450 enzymes (the "microsomal ethanol-oxidizing system"; see p. 304). As alcohol and drugs are broken down by the same enzyme system, the effects of alcoholic drinks and medical drugs can sometimes be mutually enhancing—even sometimes to the extent of becoming life-threatening.

Only a few examples of the numerous Cyt P450-dependent reactions are shown here. *Hydroxylation* of aromatic rings (**a**) plays a central part in the metabolism of medicines and steroids. Aliphatic methyl groups can also be oxidized to hydroxyl groups (**b**). *Epoxidation* of aromatics (**c**) by Cyt P450 yields products that are highly reactive and often toxic. For example, the mutagenic effect of benzo[*a*]pyrene (see p. 244) is based on this type of interconversion in the liver. In Cyt P450 dependent *dealkylations* (**d**), alkyl substituents of *O, N,* or *S* atoms are released as aldehydes.

B. Reaction mechanism ○

The course of Cyt P450 catalysis is in principle well understood. The most important function of the *heme group* consists of converting molecular oxygen into an especially reactive atomic form, which is responsible for all of the reactions described above.

[1] In the resting state, the heme iron is trivalent. Initially, the substrate binds near the heme group.

[2] Transfer of an electron from $FADH_2$ reduces the iron to the divalent form that is able to bind an O_2 molecule (2).

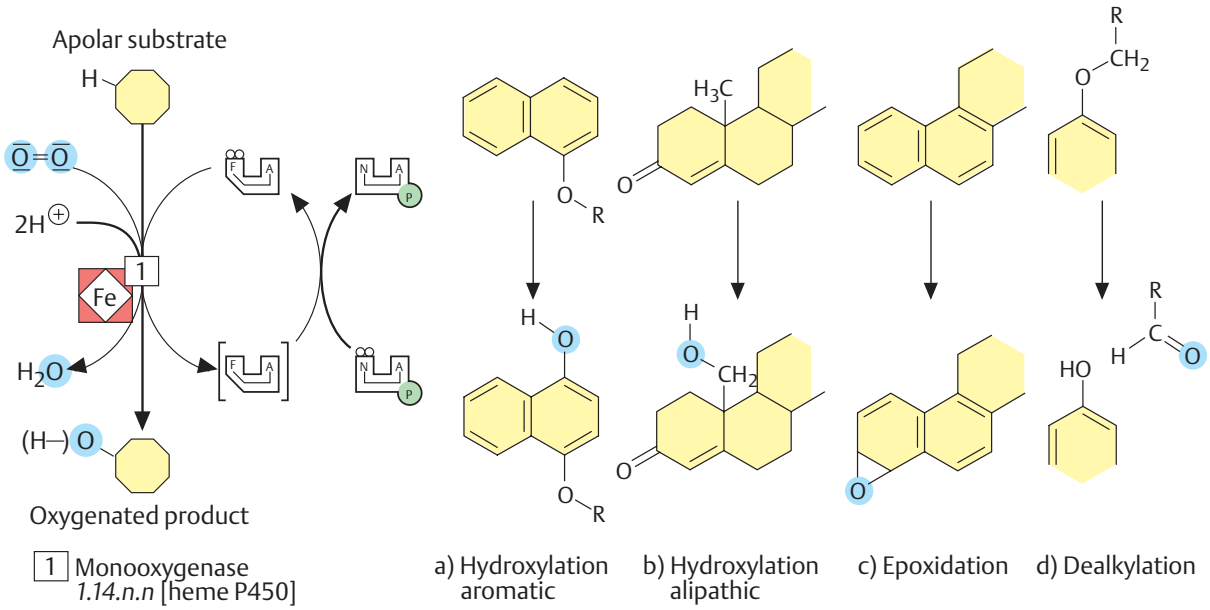
[3] Transfer of a second electron and a change in the valence of the iron reduce the bound O_2 to the peroxide.

[4] A hydroxyl ion is now cleaved from this intermediate. Uptake of a proton gives rise to H_2O and the reactive form of oxygen mentioned above. In this ferryl radical, the iron is formally tetravalent.

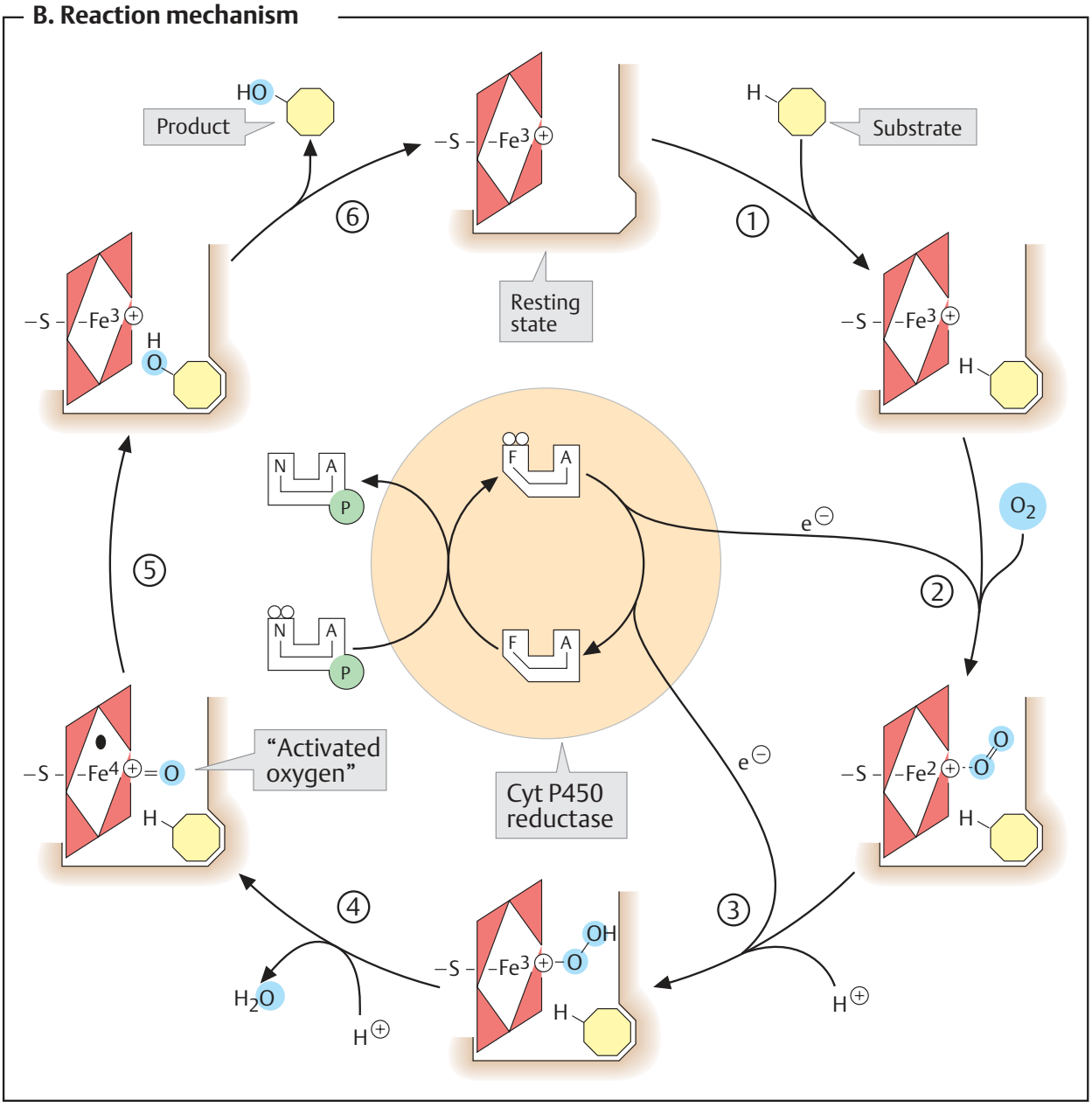
[5] The activated oxygen atom inserts itself into a C–H bond in the substrate, thereby forming an OH group.

[6] Dissociation of the product returns the enzyme to its initial state.

A. Cytochrome P450-dependent monooxygenases: reactions



B. Reaction mechanism



Ethanol metabolism

A. Blood ethanol level ◑

Ethanol (EtOH, "alcohol") naturally occurs in fruit in small quantities. Alcoholic drinks contain much higher concentrations. Their alcohol content is usually given as percent 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 kg 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 resorbed. The maximum blood level is already reached within 60–90 min after drinking. The *resorption 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 resorption, 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 resorption of the ethanol contained in one bottle of beer (16 g) by a person weighing 70 kg (distribution in 70 kg 70/100 = 49 kg) leads to a blood alcohol level of 0.33 per thousand (7.2 mM). The lethal concentration of alcohol is approximately 3.5 per thousand (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* to form **ethanal** (acetaldehyde). A further oxidization, catalyzed by *aldehyde dehydrogenase,* leads to acetate. Acetate is then converted with the help of *acetate-CoA ligase* to form **acetyl CoA**, using ATP and providing a link to the intermediary metabolism. In addition to cytoplasmic alcohol dehydrogenase, *catalase* and inducible *microsomal alcohol oxidase* ("MEOS"; see p. 318) also contribute to a lesser extent to ethanol degradation. Many of the enzymes mentioned above are induced by ethanol.

The rate of ethanol degradation in the liver is limited by alcohol dehydrogenase activity. The amount of NAD^+ available is the limiting factor. As the maximum degradation rate 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 kJ g^{-1}. Alcoholic drinks—particularly in alcoholics—can therefore represent a substantial proportion of dietary energy intake.

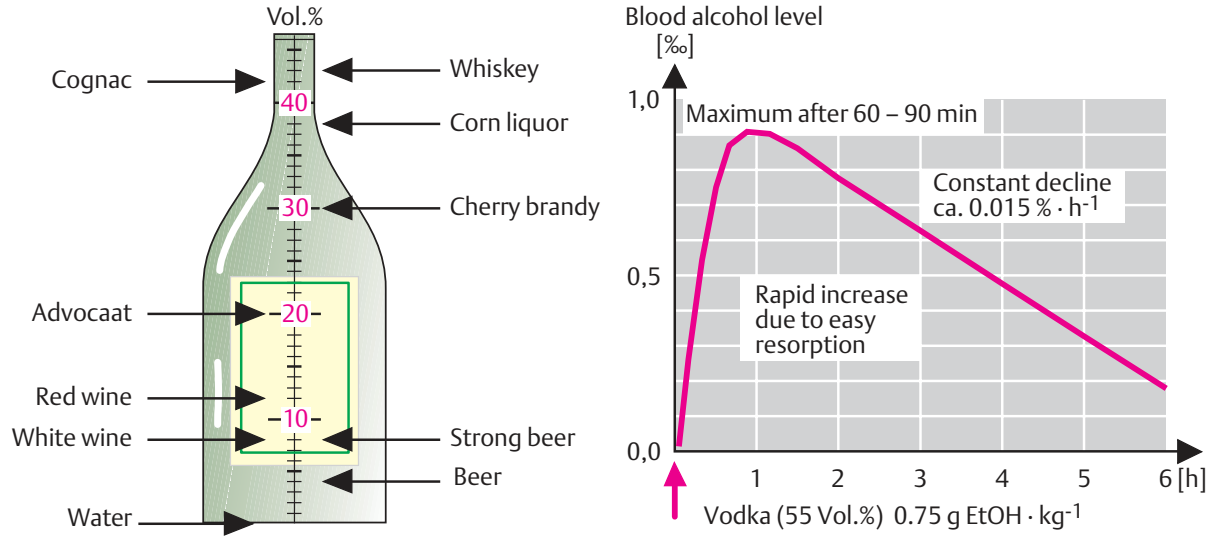
C. Liver damage due to alcohol ◑

Alcohol is a socially accepted drug of abuse in Western countries. Due to the high potential for addiction to develop, however, it is actually a "hard" drug and has a much larger number of victims than the opiate drugs, for example. In the brain, ethanol is deposited in membranes due to its amphipathic properties, and it influences receptors for neurotransmitters (see p. 352). The effect of GABA is enhanced, while that of glutamate declines.

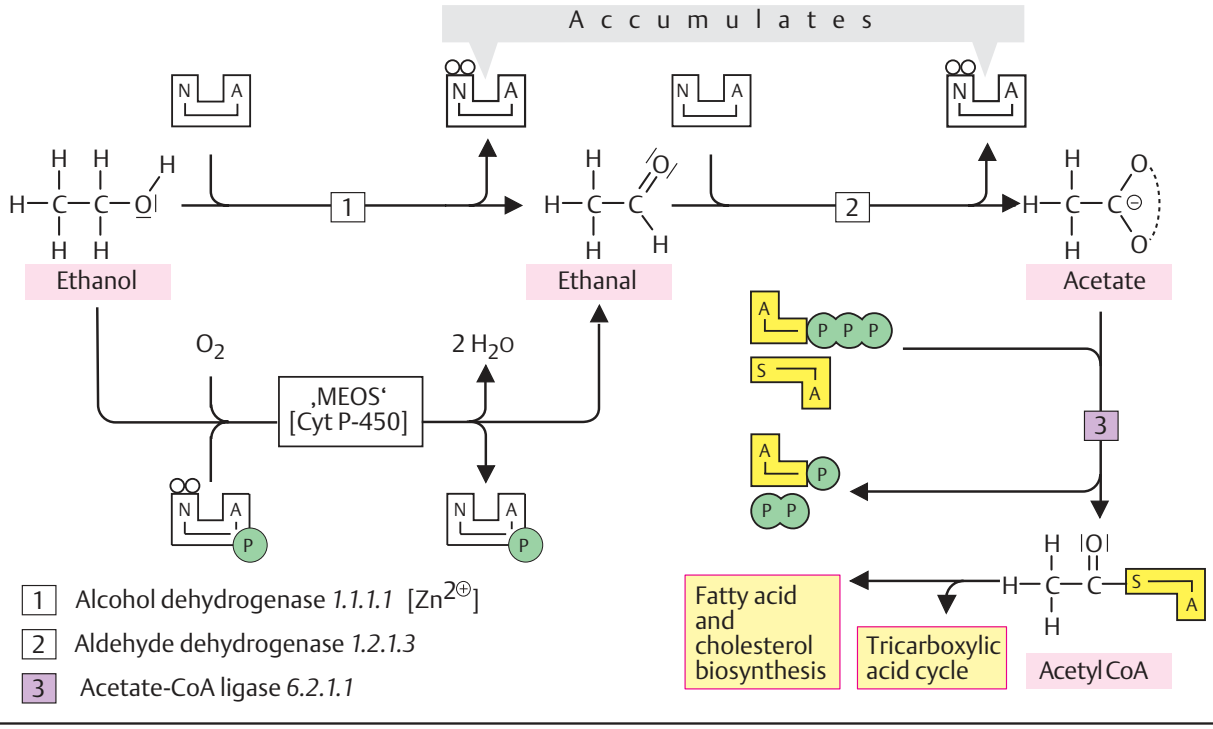
High ethanol consumption over many years leads to liver damage. For a healthy man, the limit is about 60 g per day, and for a woman about 50 g. However, these values are strongly dependent on body weight, health status, and other factors.

Ethanol-related high levels of $NADH+H^+$ and acetyl-CoA in the liver lead to increased synthesis of neutral fats and cholesterol. However, since the export of these in the form of VLDLs (see p. 278) is reduced due to alcohol, storage of lipids occurs (**fatty liver**). This increase in the fat content of the liver (from less than 5% to more than 50% of the dry weight) is initially reversible. However, in chronic alcoholism the hepatocytes are increasingly replaced by connective tissue. When **liver cirrhosis** occurs, the damage to the liver finally reaches an irreversible stage, characterized by progressive loss of liver functions.

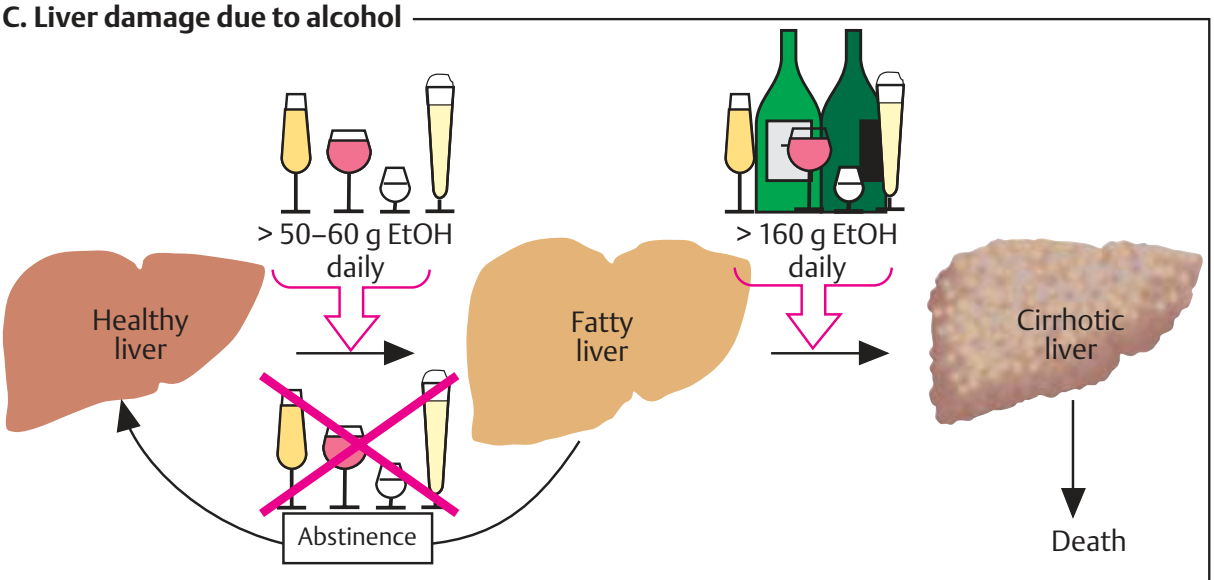
A. Blood ethanol level



B. Ethanol metabolism



C. Liver damage due to alcohol



Kidney: functions

A. Functions of the kidneys ●

The kidneys' main function is **excretion** of water and water-soluble substances (**1**). This is closely associated with their role in regulating the body's electrolyte and acid–base balance (**homeostasis, 2**; see pp. 326 and 328). Both excretion and homeostasis are subject to hormonal control. The kidneys are also involved in synthesizing several **hormones** (**3**; see p. 315). Finally, the kidneys also play a role in the **intermediary metabolism** (**4**), particularly in amino acid degradation and gluconeogenesis (see p. 154).

The kidneys are extremely well-perfused organs, with about 1500 L of blood flowing through them every day. Approximately 180 L of primary urine is filtered out of this. Removal of water leads to extreme concentration of the primary urine (to approximately one-hundredth of the initial volume). As a result, only a volume of 0.5–2.0 L of **final urine** is excreted per day.

B. Urine formation ◑

The functional unit of the kidney is the **nephron**. It is made up of the Malpighian bodies or renal corpuscles (consisting of Bowman's capsules and the glomerulus), the proximal tubule, Henle's 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.

Ultrafiltration. 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, which are made up of type IV collagen (see p. 344), have an effective mean diameter of 2.9 nm. This allows all plasma components with a molecular mass of up to about 15 kDa to pass through unhindered. At increasing masses, molecules are progressively held back; at masses greater than 65 kDa, they are completely unable to enter the primary urine. This applies to almost all plasma proteins—which in addition, being anions, are repelled by the negative charge in the basal membrane.

Resorption. All low-molecular weight plasma components enter the primary urine via glomerular filtration. Most of these are transported back into the blood by resorption, to prevent losses of valuable metabolites and electrolytes. In the proximal tubule, organic metabolites (e.g., glucose and other sugars, amino acids, lactate, and ketone bodies) are recovered by secondary active transport (see p. 220). There are several group-specific transport systems for resorbing amino acids, with which hereditary diseases can be associated (e.g., *cystinuria, glycinuria,* and *Hartnup's disease*). HCO_3^-, Na^+, phosphate, and sulfate are also resorbed by ATP-dependent (active) mechanisms in the proximal tubule. The later sections of the nephron mainly serve for additional water recovery and regulated resorption of Na^+ and Cl^- (see pp. 326, 328). These processes are controlled by hormones (aldosterone, vasopressin).

Secretion. Some excretable substances are released into the urine by *active transport* in the renal tubules. These substances include H^+ and K^+ ions, urea, and creatinine, as well as drugs such as penicillin.

Clearance. Renal clearance is used as a quantitative measure of renal function. It is defined as the plasma volume cleared of a given substance per unit of time. *Inulin,* a fructose polysaccharide with a mass of ca. 6 kDa (see p. 40) that is neither actively excreted nor resorbed but is freely filtered, has a clearance of 120 mL min^{-1} in healthy individuals.

Further information

Concentrating urine and transporting it through membranes are processes that require large amounts of energy. The kidneys therefore have very high energy demands. In the proximal tubule, the ATP needed is obtained from oxidative metabolism of *fatty acids, ketone bodies,* and several *amino acids.* To a lesser extent, lactate, glycerol, and citric acid are also used. In the distal tubule and Henle's loop, *glucose* is the main substrate for the energy metabolism. The endothelial cells in the proximal tubule are also capable of *gluconeogenesis.* The substrates for this are mainly the carbohydrate skeletons of amino acids. Their amino groups are used as ammonia for buffering urine (see p. 311). Enzymes for peptide degradation and the amino acid metabolism occur in the kidneys at high levels of activity (e.g., amino acid oxidases, amine oxidases, glutaminase).

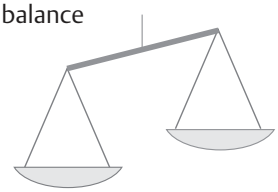
A. Functions of the kidneys

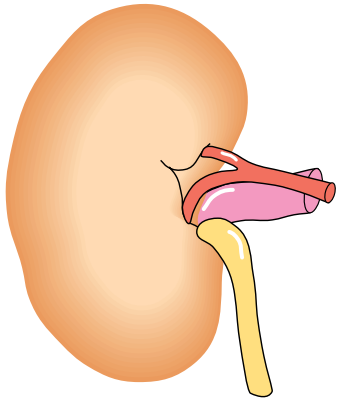
1. Excretion

Water
Salts
Metabolic wastes
Foreign substances

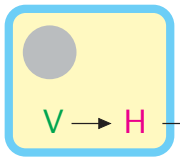
2. Homeostasis

Acid-base balance
Electrolyte balance





3. Hormone synthesis



→ Erythropoietin
Calcitriol

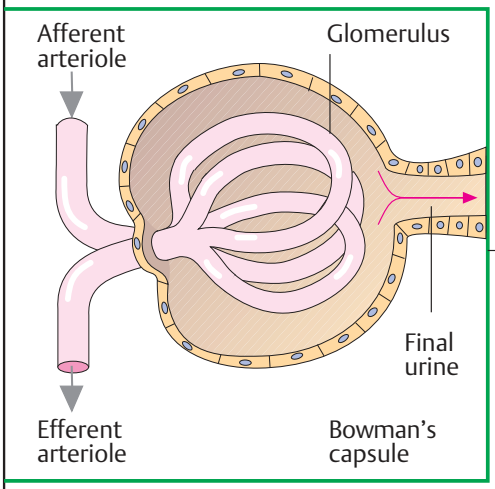
4. Metabolism

Glucose → Blood

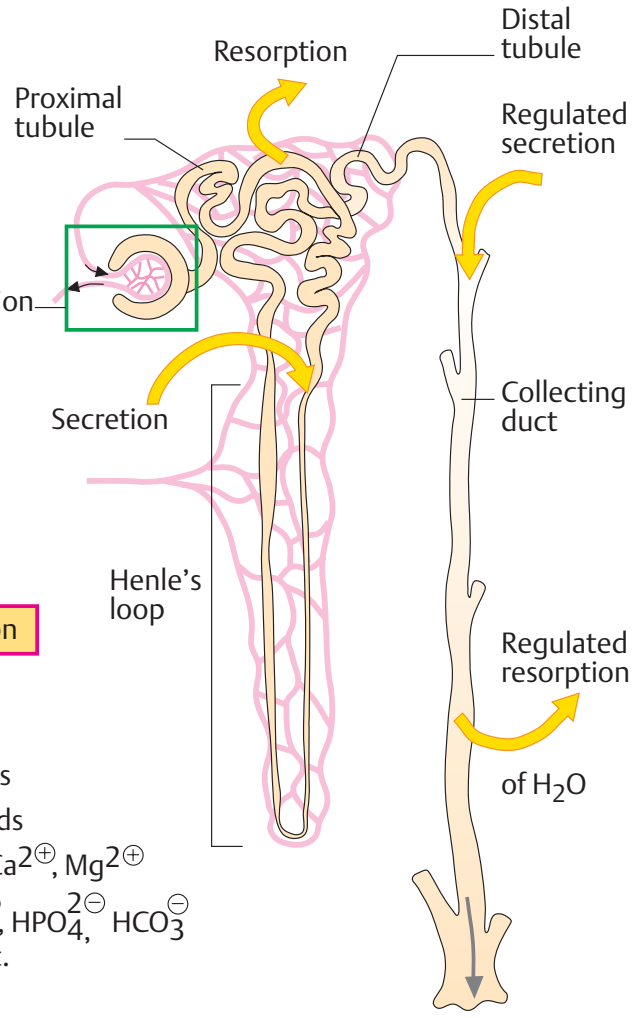
Amino acids → Gluconeo-genesis

NH_3 → Urine

B. Urine formation



Renal corpuscle



Ultrafiltration	Secretion	Resorption
All solute plasma components smaller than 3 nm = 15 kDa	$H^{\oplus}$	Glucose
	$K^{\oplus}$	Lactate
	Drugs	2-Oxoacids
	Uric acid	Amino acids
	Creatinine	$Na^{\oplus}$, $K^{\oplus}$, $Ca^{2\oplus}$, $Mg^{2\oplus}$
		$Cl^{\ominus}$, $SO_4^{2\ominus}$, $HPO_4^{2\ominus}$, $HCO_3^{\ominus}$
		Water, etc.

Urine

A. Urine ◑

Water and water-soluble compounds are excreted with the urine. The volume and composition of urine are subject to wide variation and depend on food intake, body weight, age, sex, and living conditions such as temperature, humidity, physical activity, and health status. As there is a marked circadian rhythm in urine excretion, the amount of urine and its composition are usually given relative to a 24-hour period.

A human adult produces 0.5–2.0 L urine per day, around 95% of which consists of water. The urine usually has a slightly acidic pH value (around 5.8). However, the pH value of urine is strongly affected by metabolic status. After ingestion of large amounts of plant food, it can increase to over 7.

B. Organic components ◑

Nitrogen-containing compounds are among the most important organic components of urine. **Urea,** which is mainly synthesized in the liver (urea cycle; see p. 182), is the form in which nitrogen atoms from amino acids are excreted. Breakdown of pyrimidine bases also produces a certain amount of urea (see p. 190). When the nitrogen balance is constant, as much nitrogen is excreted as is taken up (see p. 174), and the amount of urea in the urine therefore reflects protein degradation: 70 g protein in food yields approximately 30 g urea in the urine.

Uric acid is the end product of the purine metabolism. When uric acid excretion via the kidneys is disturbed, gout can develop (see p. 190). **Creatinine** is derived from the muscle metabolism, where it arises spontaneously and irreversibly by cyclization of creatine and creatine phosphate (see p. 336). Since the amount of creatinine an individual excretes per day is constant (it is directly proportional to muscle mass), creatinine as an endogenous substance can be used to measure the glomerular filtration rate. The amount of **amino acids** excreted in free form is strongly dependent on the diet and on the ef ciency of liver function. Amino acid derivatives are also found in the urine (e. g., **hippurate**, a detoxification product of benzoic acid). Modified amino acids, which occur in special proteins such as *hydroxyproline* in collagen and *3-methylhistidine* in actin and myosin, can be used as indicators of the degradation of these proteins.

Other components of the urine are conjugates with sulfuric acid, glucuronic acid, glycine, and other polar compounds that are synthesized in the liver by biotransformation (see p. 316). In addition, metabolites of many hormones (catecholamines, steroids, serotonin) also appear in the urine and can provide information about hormone production. The proteohormone *chorionic gonadotropin* (hCG, mass ca. 36 kDa), which is formed at the onset of pregnancy, appears in the urine due to its relatively small size. Evidence of hCG in the urine provides the basis for an immunological *pregnancy test.*

The yellow color of urine is due to *urochromes,* which are related to the bile pigments produced by hemoglobin degradation (see p. 194). If urine is left to stand long enough, oxidation of the urochromes may lead to a darkening in color.

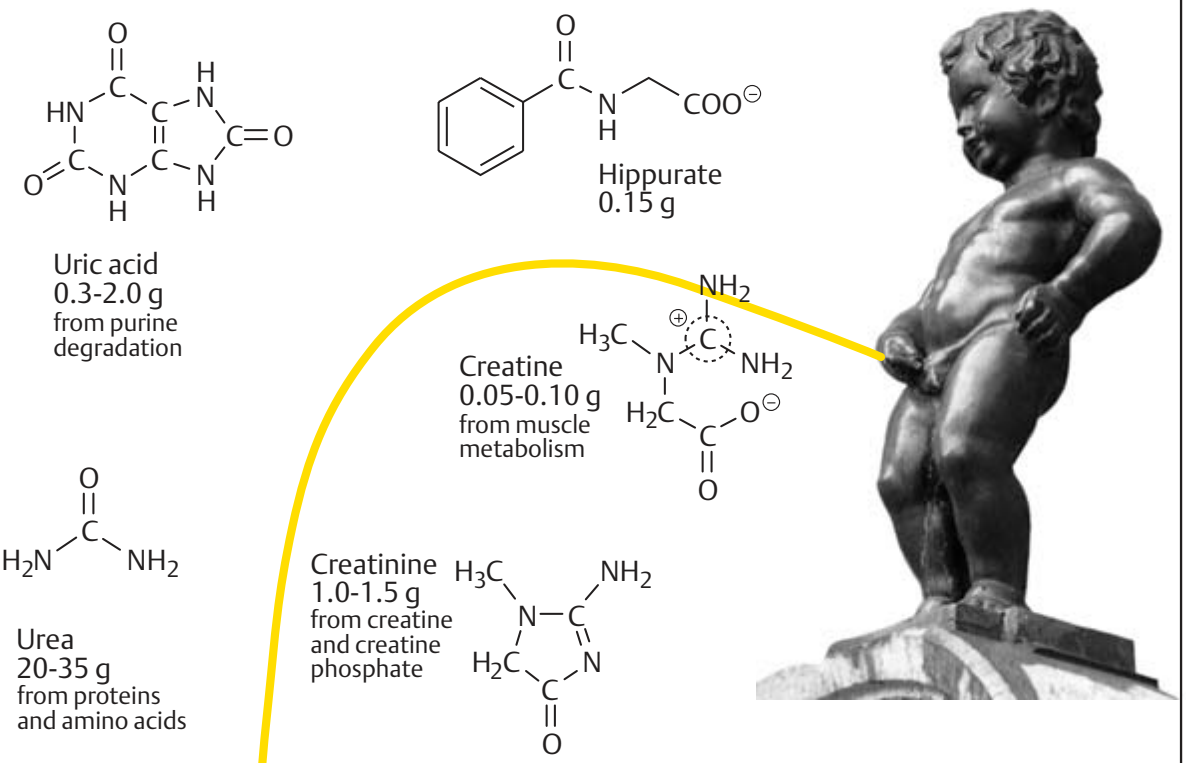
C. Inorganic components ◑

The main inorganic components of the urine are the *cations* Na^+, K^+, Ca^{2+}, Mg^{2+}, and NH_4^+ and the *anions* Cl^-, SO_4^{2-}, and HPO_4^{2-}, as well as traces of other ions. In total, Na^+ and Cl^- represent about two-thirds of all the electrolytes in the final urine. Calcium and magnesium occur in the feces in even larger quantities. The amounts of the various inorganic components of the urine also depend on the composition of the diet. For example, in acidosis there can be a marked increase in the excretion of ammonia (see p. 326). Excretion of Na^+, K^+, Ca^{2+}, and phosphate via the kidneys is subject to hormonal regulation (see p. 330).

Further information

Shifts in the concentrations of the *physiological components* of the urine and the appearance of *pathological urine components* can be used to diagnose diseases. Important examples are glucose and ketone bodies, which are excreted to a greater extent in *diabetes mellitus* (see p. 160).

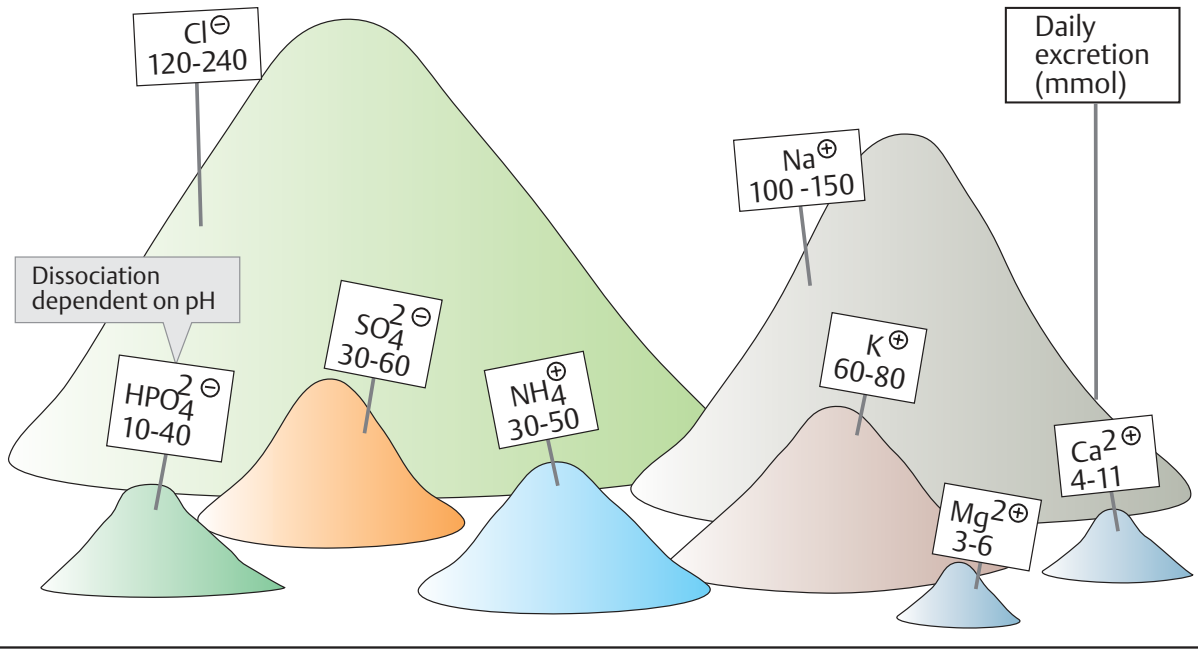
A. Urine



B. Organic constituents

Glucose < 0.16 g	Ketone bodies < 3 g
Proteins < 0.15 g	Amino acids 1-3 g

C. Inorganic constituents



Functions in the acid–base balance

Along with the lungs, the kidneys are particularly involved in keeping the pH value of the extracellular fluid constant (see p. 288). The contribution made by the kidneys particularly involves resorbing HCO_3^- and actively excreting protons.

A. Proton excretion ◑

The renal tubule cells are capable of secreting protons (H^+) from the blood into the urine against a concentration gradient, despite the fact that the H^+ concentration in the urine is up to a thousand times higher than in the blood. To achieve this, carbon dioxide (CO_2) is taken up from the blood and—together with water (H_2O) and with the help of *carbonate dehydratase* (carbonic anhydrase, [1])—converted into hydrogen carbonate ("bicarbonate," HCO_3^-) and one H^+. Formally, this yields carbonic acid H_2CO_3 as an intermediate, but it is not released during the reaction.

The hydrogen carbonate formed in carbonic anhydrase returns to the plasma, where it contributes to the blood's base reserve. The proton is exported into the urine by *secondary active transport* in antiport for Na^+ (bottom right). The driving force for **proton excretion**, as in other secondary active processes, is the Na^+ gradient established by the ATPase involved in the *Na^+/K^+* exchange ("Na^+/K^+ ATPase", see p. 220). This integral membrane protein on the basal side (towards the blood) of tubule cells keeps the Na^+ concentration in the tubule cell low, thereby maintaining Na^+ inflow. In addition to this secondary active H^+ transport mechanism, there is a V-type H^+-transporting ATPase in the distal tubule and collecting duct (see p. 220).

An important function of the secreted H^+ ions is to promote **HCO_3^- resorption** (top right). Hydrogen carbonate, the most important buffering base in the blood, passes into the primary urine quantitatively, like all ions. In the primary urine, HCO_3^- reacts with H^+ ions to form water and CO_2, which returns by free diffusion to the tubule cells and from there into the blood. In this way, the kidneys also influence the CO_2/HCO_3^- buffering balance in the plasma.

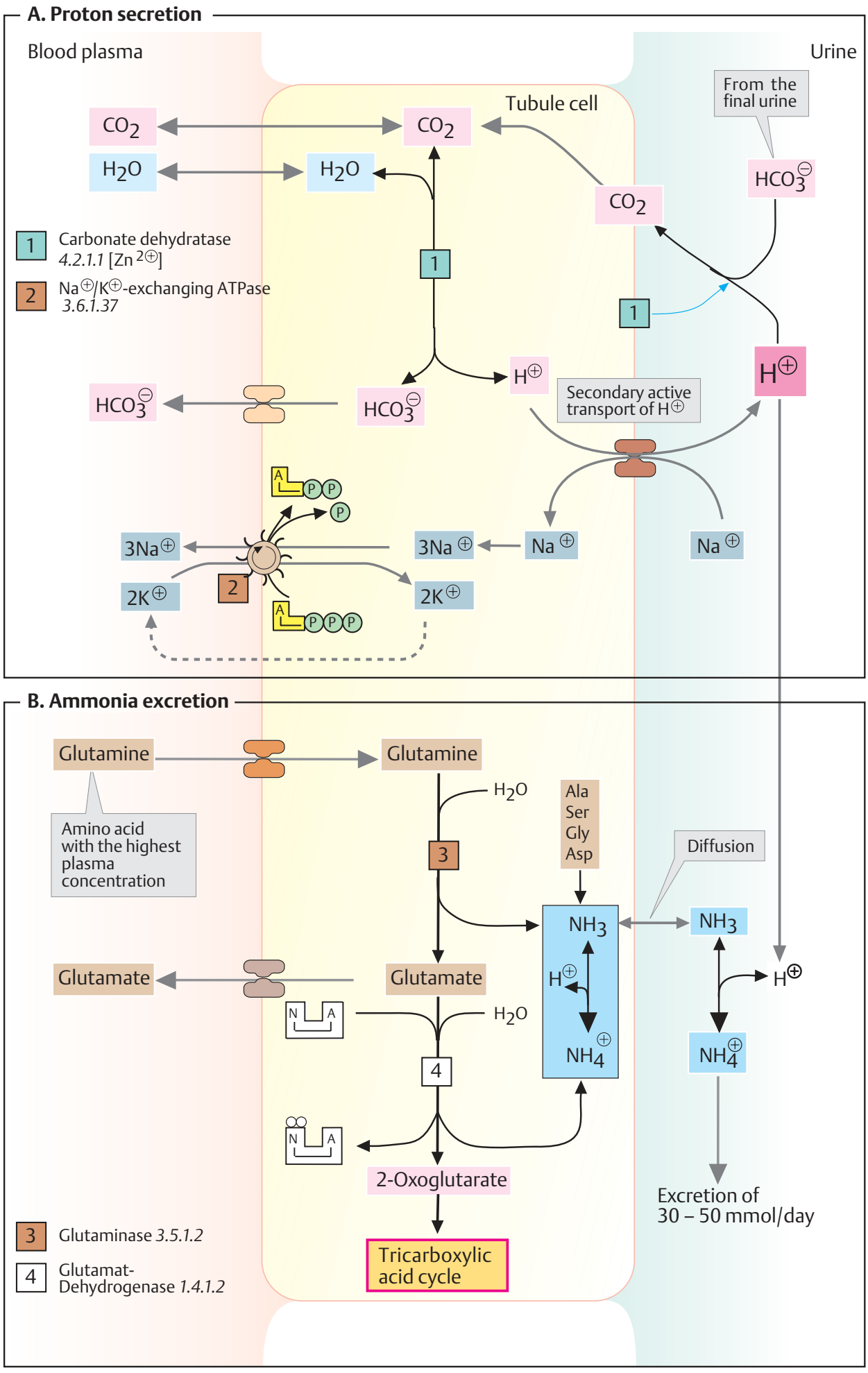
B. Ammonia excretion ◑

Approximately 60 mmol of protons are excreted with the urine every day. Buffering systems in the urine catch a large proportion of the H^+ ions, so that the urine only becomes weakly acidic (down to about pH 4.8).

An important buffer in the urine is the *hydrogen phosphate/dihydrogen phosphate system* ($HPO_4^{2-}/H_2PO_4^-$). In addition, *ammonia* also makes a vital contribution to buffering the secreted protons.

Since plasma concentrations of *free* ammonia are low, the kidneys release NH_3 from **glutamine** and other amino acids. At 0.5–0.7 mM, glutamine is the most important amino acid in the plasma and is the preferred form for ammonia transport in the blood. The kidneys take up glutamine, and with the help of *glutaminase* [4], initially release NH_3 from the amide bond hydrolytically. From the **glutamate** formed, a second molecule of NH_3 can be obtained by oxidative deamination with the help of *glutamate dehydrogenase* [5] (see p. 178). The resulting **2-oxoglutarate** is further metabolized in the tricarboxylic acid cycle. Several other amino acids—*alanine* in particular, as well as *serine, glycine,* and *aspartate*—can also serve as suppliers of ammonia.

Ammonia can diffuse freely into the urine through the tubule membrane, while the **ammonium ions** that are formed in the urine are charged and can no longer return to the cell. Acidic urine therefore promotes ammonia excretion, which is normally 30–50 mmol per day. In metabolic **acidosis** (e.g., during fasting or in *diabetes mellitus*), after a certain time increased *induction of glutaminase* occurs in the kidneys, resulting in increased NH_3 excretion. This in turn promotes H^+ release and thus counteracts the acidosis. By contrast, when the plasma pH value shifts towards alkaline values (*alkalosis*), renal excretion of ammonia is reduced.



Electrolyte and water recycling

A. Electrolyte and water recycling ◑

Electrolytes and other plasma components with low molecular weights enter the *primary urine* by ultrafiltration (right). Most of these substances are recovered by energy-dependent resorption (see p. 322). The extent of the resorption determines the amount that ultimately reaches the final urine and is excreted. The illustration does not take into account the *zoning* of transport processes in the kidney (physiology textbooks may be referred to for further details).

Calcium and phosphate ions. Calcium (Ca^{2+}) and phosphate ions are almost completely resorbed from the primary urine by active transport (i.e., in an ATP-dependent fashion). The proportion of Ca^{2+} resorbed is over 99%, while for phosphate the figure is 80–90%. The extent to which these two electrolytes are resorbed is regulated by the three hormones parathyrin, calcitonin, and calcitriol.

The peptide hormone *parathyrin* (PTH), which is produced by the parathyroid gland, stimulates Ca^{2+} resorption in the kidneys and at the same time inhibits the resorption of phosphate. In conjunction with the effects of this hormone in the bones and intestines (see p. 344), this leads to an *increase in the plasma level of* Ca^{2+} and a reduction in the level of phosphate ions.

Calcitonin, a peptide produced in the C cells of the thyroid gland, inhibits the resorption of both calcium and phosphate ions. The result is an overall *reduction in the plasma level* of both ions. Calcitonin is thus a parathyrin antagonist relative to Ca^{2+}.

The steroid hormone *calcitriol,* which is formed in the kidneys (see p. 304), stimulates the resorption of both calcium and phosphate ions and thus increases the plasma level of both ions.

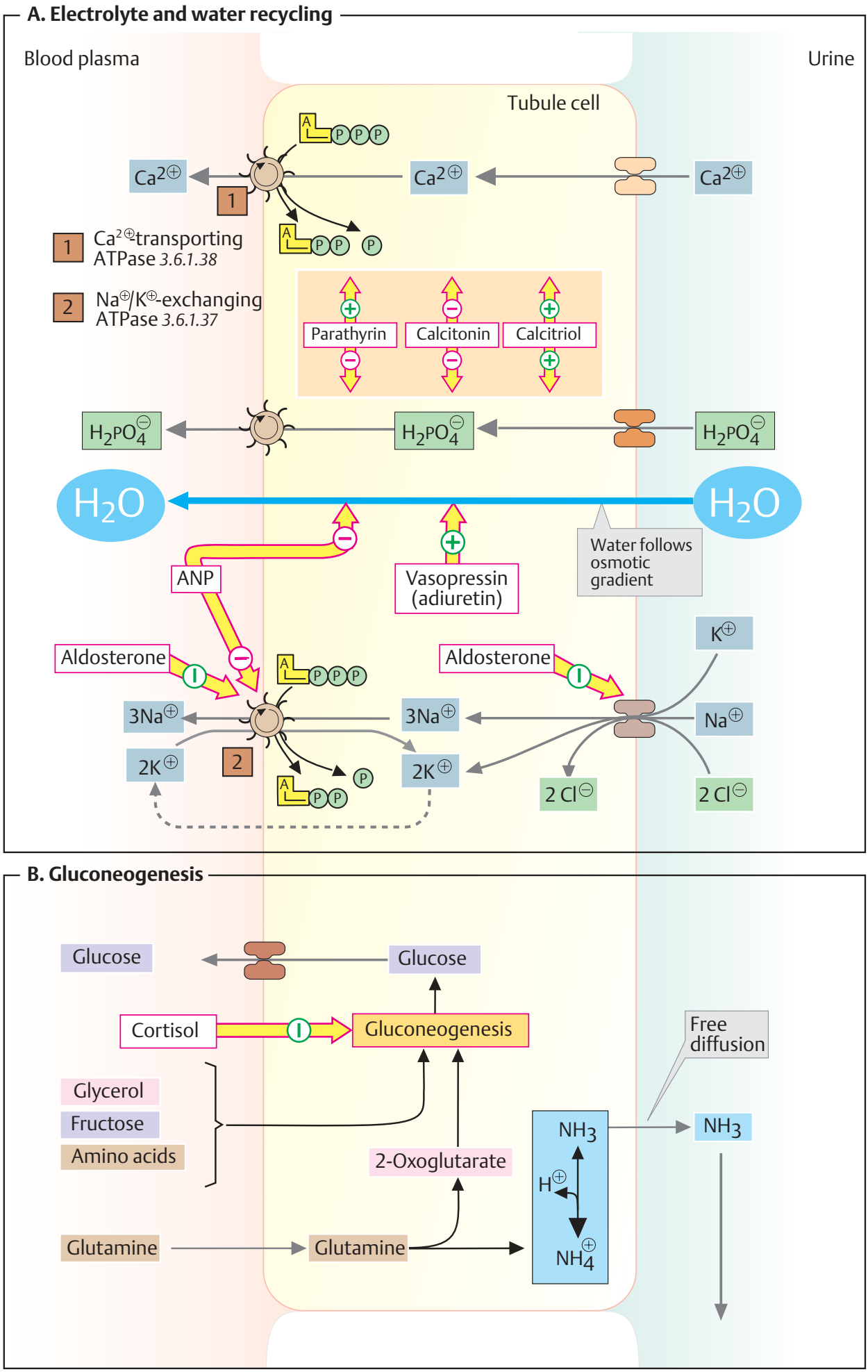
Sodium ions. Controlled resorption of Na^+ from the primary urine is one of the most important functions of the kidney. Na^+ resorption is highly effective, with more than 97% being resorbed. Several mechanisms are involved: some of the Na^+ is taken up passively in the proximal tubule through the junctions between the cells (paracellularly). In addition, there is secondary active transport together with glucose and amino acids (see p. 322). These two pathways are responsible for 60–70% of total Na^+ resorption. In the ascending part of Henle's loop, there is another transporter (shown at the bottom right), which functions electroneutrally and takes up one Na^+ ion and one K^+ ion together with two Cl^- ions. This symport is also dependent on the activity of Na^+/K^+ ATPase [2], which pumps the Na^+ resorbed from the primary urine back into the plasma in exchange for K^+.

The steroid hormone *aldosterone* (see p. 55) increases Na^+ reuptake, particularly in the distal tubule, while *atrial natriuretic peptide* (ANP) originating from the cardiac atrium reduces it. Among other effects, aldosterone induces Na^+/K^+ ATPase and various Na^+ transporters on the luminal side of the cells.

Water. Water resorption in the proximal tubule is a *passive process* in which water follows the osmotically active particles, particularly the Na^+ ions. Fine regulation of water excretion (**diuresis**) takes place in the collecting ducts, where the peptide hormone *vasopressin* (antidiuretic hormone, ADH) operates. This promotes recovery of water by stimulating the transfer of aquaporins (see p. 220) into the plasma membrane of the tubule cells via V_2 receptors. A lack of ADH leads to the disease picture of *diabetes insipidus,* in which up to 30 L of final urine is produced per day.

B. Gluconeogenesis ◑

Apart from the liver, the kidneys are the only organs capable of producing glucose by neosynthesis *(gluconeogenesis;* see p. 154). The main substrate for gluconeogenesis in the cells of the proximal tubule is **glutamine**. In addition, other amino acids and also **lactate**, **glycerol**, and **fructose** can be used as precursors. As in the liver, the key enzymes for gluconeogenesis are induced by *cortisol* (see p. 374). Since the kidneys also have a high level of glucose consumption, they only release very little glucose into the blood.



Renal hormones

A. Renal hormones ◑

In addition to their involvement in excretion and metabolism, the kidneys also have endocrine functions. They produce the hormones **erythropoietin** and **calcitriol** and play a decisive part in producing the hormone **angiotensin II** by releasing the enzyme *renin.* Renal prostaglandins (see p. 390) have a local effect on Na^+ resorption.

Calcitriol (vitamin D hormone, 1α,25-dihydroxycholecalciferol) is a hormone closely related to the steroids that is involved in Ca^{2+} homeostasis (see p. 342). In the kidney, it is formed from calcidiol by hydroxylation at C-1. The activity of *calcidiol-1-monooxygenase* [1] is enhanced by the hormone *parathyrin* (PTH).

Erythropoietin is a peptide hormone that is formed predominantly by the kidneys, but also by the liver. Together with other factors known as *"colony-stimulating factors"* (CSF; see p. 392), it regulates the differentiation of stem cells in the bone marrow.

Erythropoietin release is stimulated by hypoxia (low pO_2). Within hours, the hormone ensures that erythrocyte precursor cells in the bone marrow are converted to erythrocytes, so that their numbers in the blood increase. Renal damage leads to reduced erythropoietin release, which in turn results in *anemia.* Forms of anemia with renal causes can now be successfully treated using erythropoietin produced by genetic engineering techniques. The hormone is also administered to dialysis patients. Among athletes and sports professionals, there have been repeated cases of erythropoietin being misused for doping purposes.

B. Renin–angiotensin system ◑

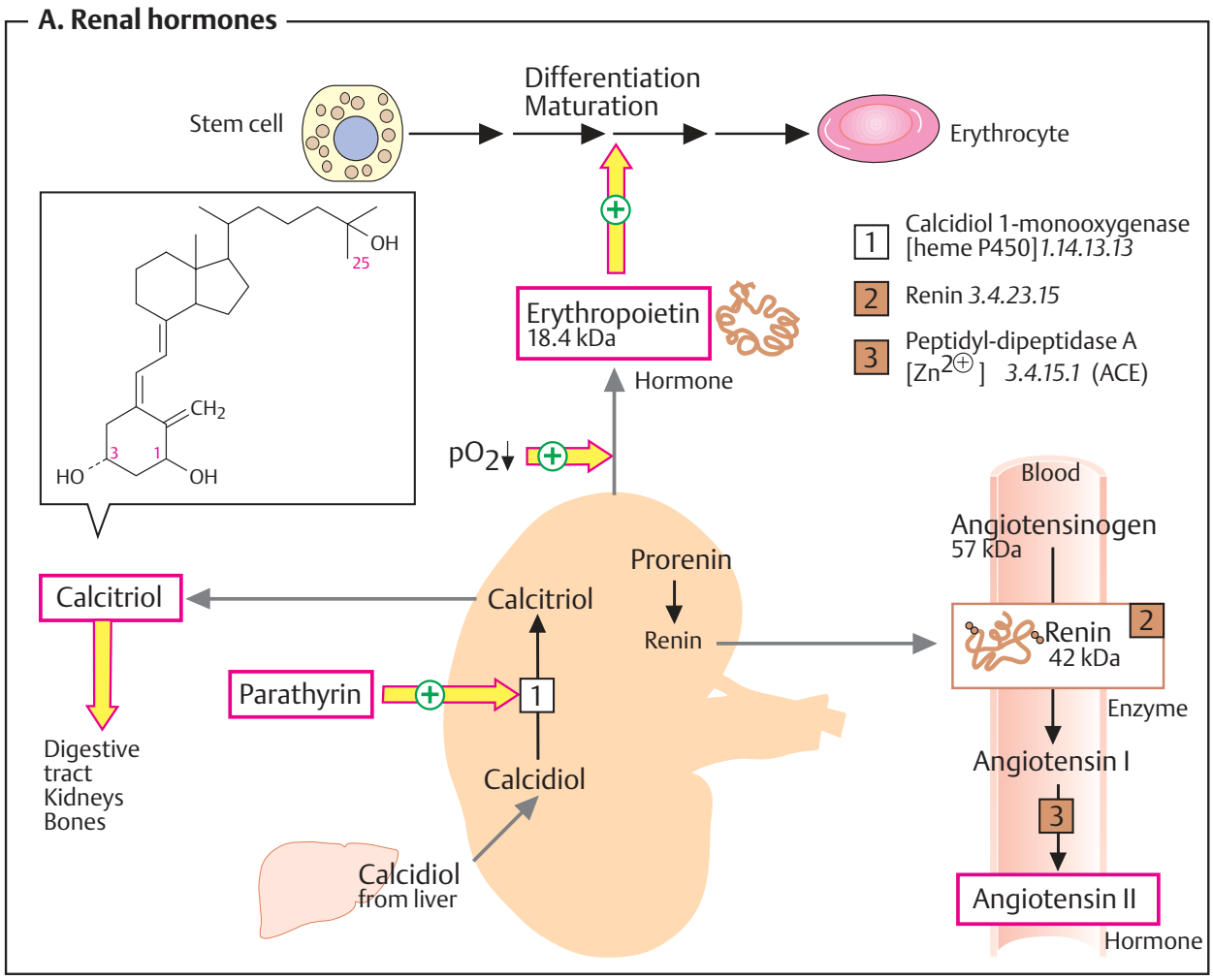
The peptide hormone angiotensin II is not synthesized in a hormonal gland, but in the blood. The kidneys take part in this process by releasing the enzyme renin.

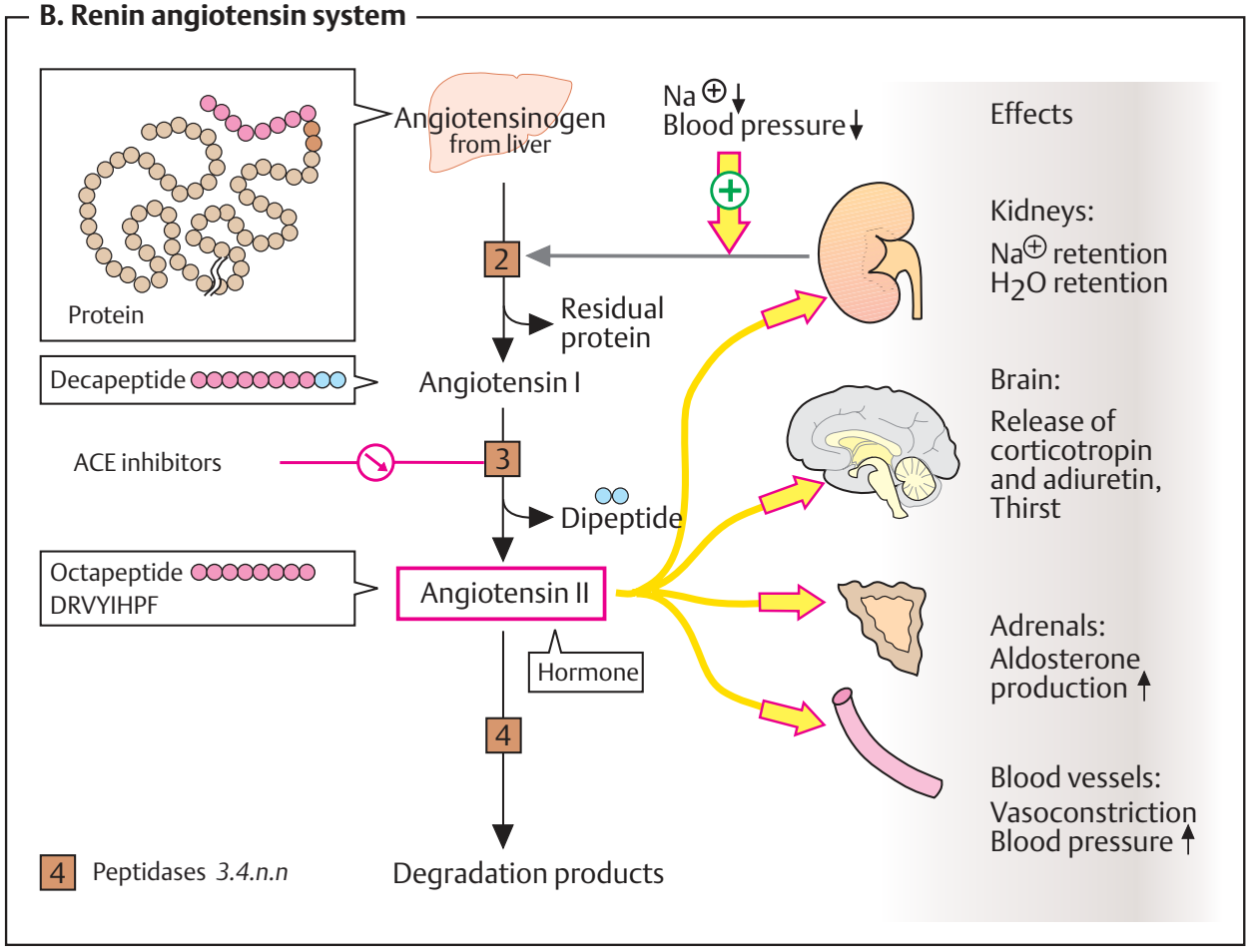
Renin [2] is an aspartate proteinase (see p. 176). It is formed by the kidneys as a precursor (prorenin), which is proteolytically activated into renin and released into the blood. In the blood plasma, renin acts on **angiotensinogen,** a plasma glycoprotein in the α_2-globulin group (see p. 276), which like almost all plasma proteins is synthesized in the liver. The decapeptide cleaved off by renin is called **angiotensin I.** Further cleavage by *peptidyl dipeptidase A* (*angiotensin-converting enzyme,* ACE), a membrane enzyme located on the vascular endothelium in the lungs and other tissues, gives rise to the octapeptide **angiotensin II** [3], which acts as a hormone and neurotransmitter. The lifespan of angiotensin II in the plasma is only a few minutes, as it is rapidly broken down by other peptidases (angiotensinases [4]), which occur in many different tissues.

The plasma level of angiotensin II is mainly determined by the rate at which renin is released by the kidneys. Renin is synthesized by juxtaglomerular cells, which release it when sodium levels decline or there is a fall in blood pressure.

Effects of angiotensin II. Angiotensin II has effects on the kidneys, brain stem, pituitary gland, adrenal cortex, blood vessel walls, and heart via membrane-located receptors. It increases blood pressure by triggering *vasoconstriction* (narrowing of the blood vessels). In the kidneys, it promotes the *retention of* Na^+ and water and reduces potassium secretion. In the brain stem and at nerve endings in the sympathetic nervous system, the effects of angiotensin II lead to increased tonicity (neurotransmitter effect). In addition, it triggers the *sensation of thirst.* In the pituitary gland, angiotensin II stimulates *vasopressin release* (antidiuretic hormone) and corticotropin (ACTH) release. In the adrenal cortex, it increases the *biosynthesis and release of aldosterone,* which promotes sodium and water retention in the kidneys. All of the effects of angiotensin II lead directly or indirectly to *increased blood pressure,* as well as increased *sodium and water retention.* This important hormonal system for blood pressure regulation can be pharmacologically influenced by *inhibitors* at various points:

- Using angiotensinogen analogs that inhibit renin.
- Using angiotensin I analogs that competitively inhibit the enzyme ACE [3].
- Using hormone antagonists that block the binding of angiotensin II to its receptors.





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 an 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** (see p. 204) 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 size 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). 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 amino end, 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 (see p. 204). This filamentous polymer is held 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 rod-like dimer and connects approximately seven actin units with each other. The heterotrimer **troponin** (78 kDa) is bound to one end of tropomyosin.

In addition to the above proteins, a number of other proteins are also typical of muscle—including *titin* (the largest known protein), *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:

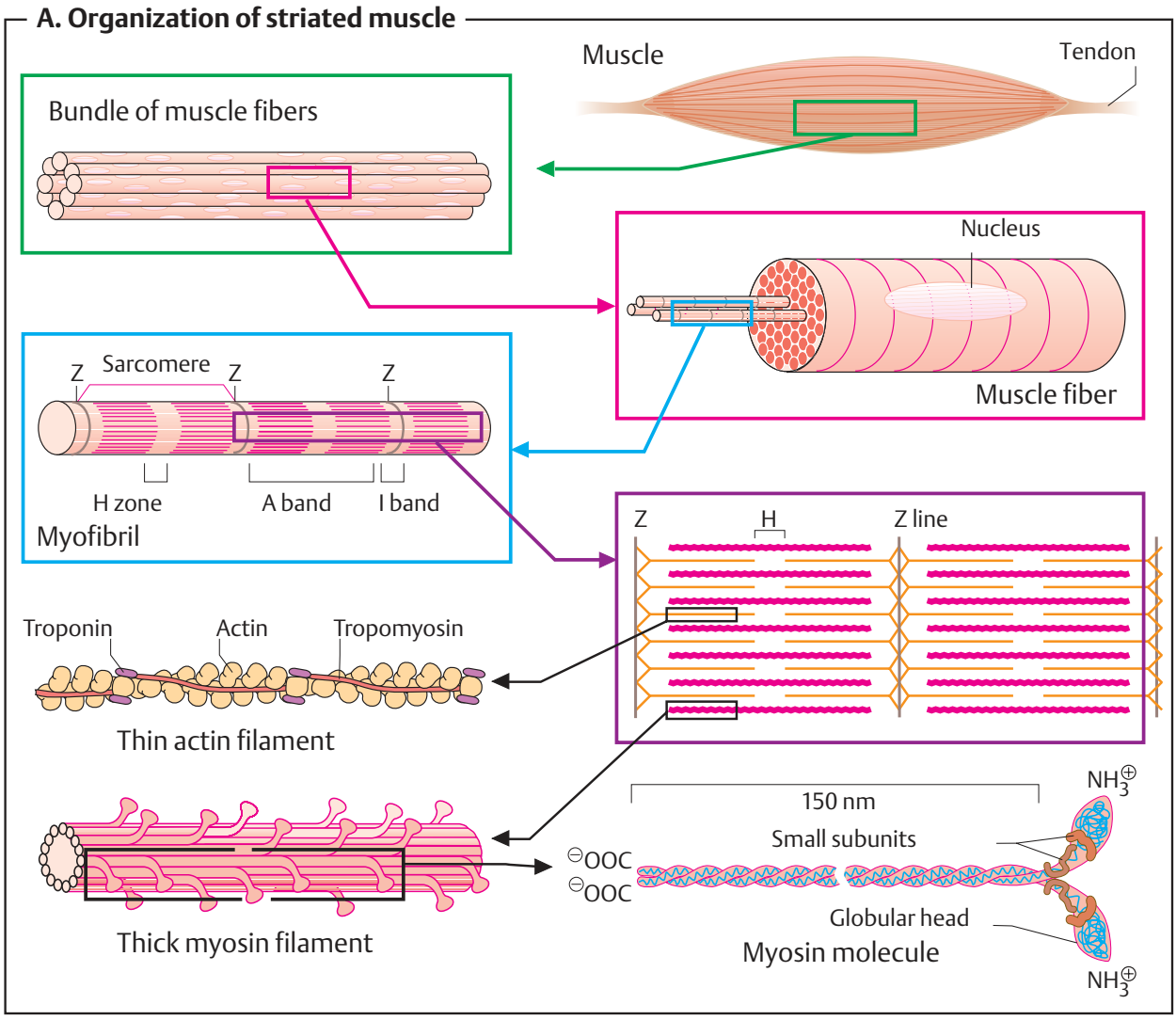
[**1**] In the initial state, the myosin heads are attached to actin. When ATP is bound, the heads detach themselves from the actin (the "plasticizing" effect of ATP).

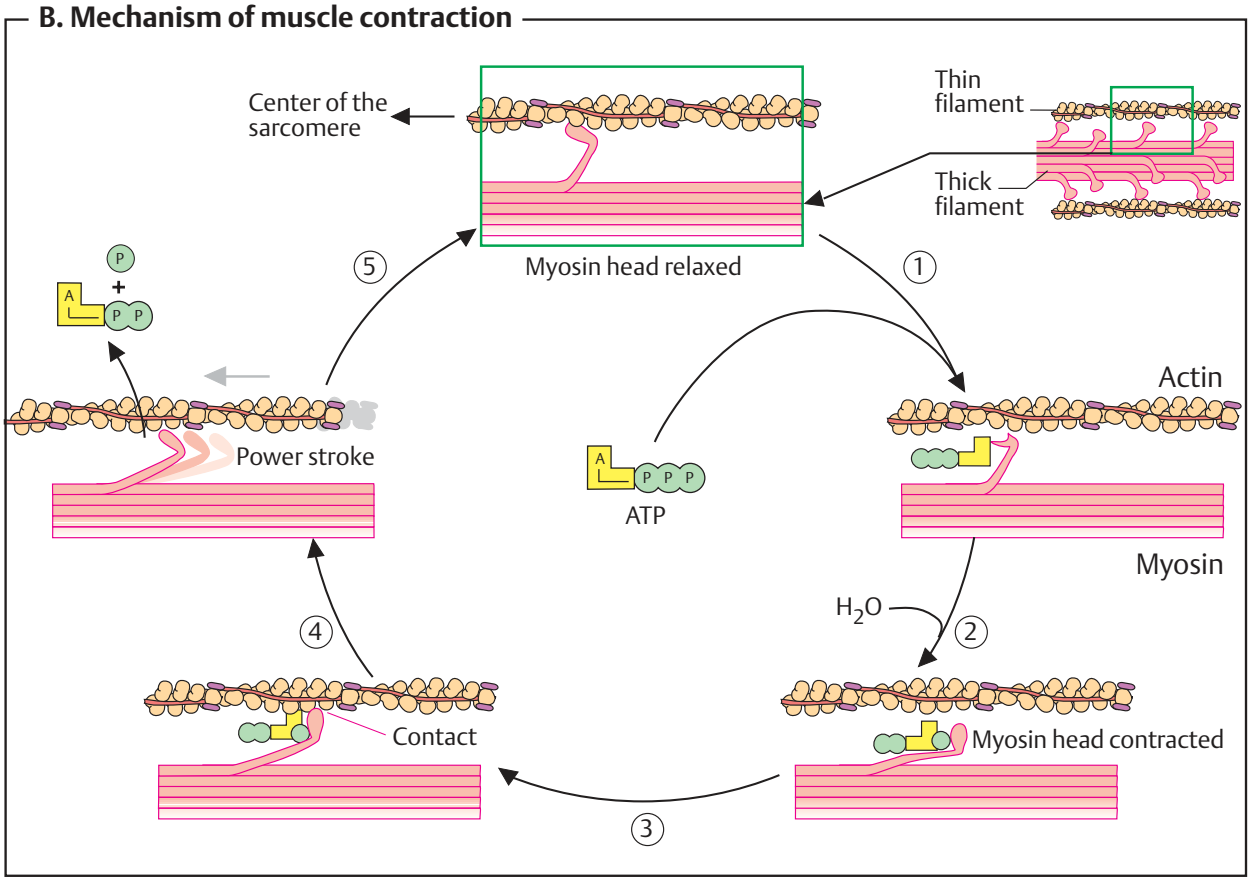
[**2**] The myosin head hydrolyzes the bound ATP to ADP and P_i, but initially withholds the two reaction products. ATP cleavage leads to allosteric tension in the myosin head.

[**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 afterwards 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.

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.





Control of muscle contraction

A. Neuromuscular junction ◑

Muscle contraction is triggered by *motor neurons* that release the neurotransmitter *acetylcholine* (see p. 352). The transmitter 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 (see p. 222). This leads to an inflow of Na^+, which triggers an *action potential* (see p. 350) 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 fiber.

B. Sarcoplasmic reticulum (SR) ◑

The action potential (**A**) 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} M). By contrast, the **sarcoplasmic reticulum** (SR), which corresponds to the ER, contains Ca^{2+} ions at a concentration of about 10^{-3} M. 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 (see p. 220). In addition, the SR also contains *calsequestrin,* a protein (55 kDa) 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. There is a structure involved in the contact between the T tubule and the SR that was formerly known as the "SR foot" (it involves parts of the *ryanodine receptor;* see p. 386).

At the point of contact with the SR, the action potential triggers the opening of the *Ca^{2+} channels* on the surface of the sarcolemma. Calcium ions then leave the SR and enter the sarcoplasm, where they lead to a rapid increase in Ca^{2+} concentrations. This in turn causes the myofibrils to contract (**C**).

C. Regulation by calcium ions ◑

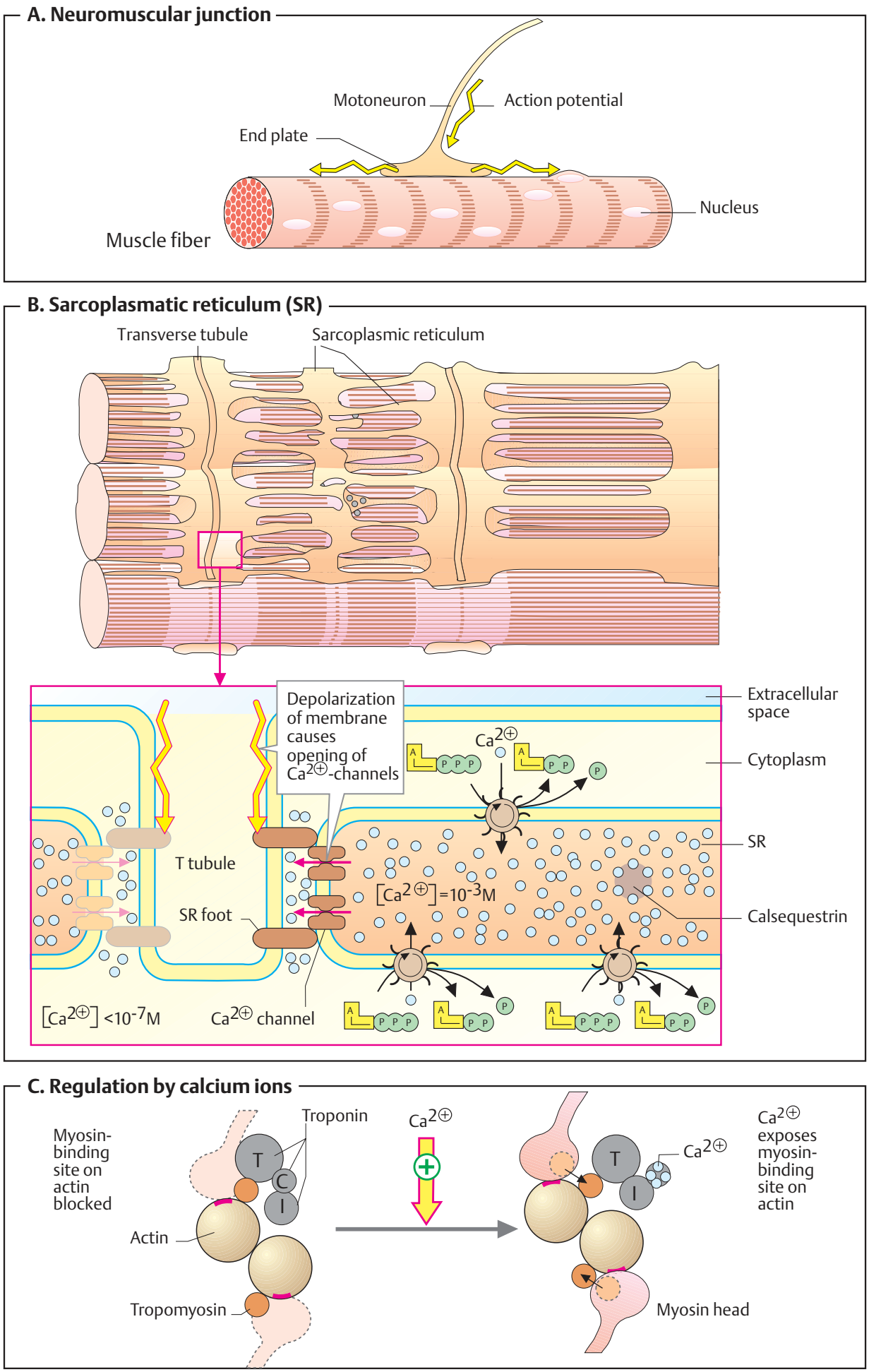
In relaxed skeletal muscle, the complex consisting of **troponin** and **tropomyosin** blocks the access of the myosin heads to actin (see p. 332). Troponin consists of three different subunits (**T**, **C**, and **I**). The rapid increase in cytoplasmic Ca^{2+} concentrations caused by opening of the calcium channels in the SR leads to binding of Ca^{2+} to the C subunit of troponin, which closely resembles calmodulin (see p. 386). This produces a conformational change in troponin that causes the whole troponin–tropomyosin complex to slip slightly and expose a binding site for myosin (red). This initiates the contraction cycle. After contraction, the sarcoplasmic Ca^{2+} concentration is quickly reduced again by active transport back into the SR. This results in troponin losing the bound Ca^{2+} ions and returning to the initial state, in which the binding site for myosin on actin is blocked. It is not yet clear whether the mechanism described above is the only one that triggers binding of myosin to actin.

When **triggering of contraction** in striated muscle occurs, the following sequence of processes thus takes place:

1. The sarcolemma is depolarized.
2. The action potential is signaled to Ca^{2+} channels in the SR.
3. The Ca^{2+} channels open and the Ca^{2+} level in the sarcoplasm increases.
4. Ca^{2+} binds to troponin C and triggers a conformational change.
5. Troponin causes tropomyosin to slip, and the myosin heads bind to actin.
6. The actin–myosin cycle takes place and the muscle fibers contract.

Conversely, at the **end of contraction**, the following processes take place:

1. The Ca^{2+} level in the sarcoplasm declines due to transport of Ca^{2+} back into the SR.
2. Troponin C loses Ca^{2+} and tropomyosin returns to its original position on the actin molecule.
3. The actin–myosin cycle stops and the muscle relaxes.



Muscle metabolism I

Muscle contraction is associated with a high level of ATP consumption (see p. 332). Without constant resynthesis, the amount of ATP available in the resting state would be used up in less than 1 s of contraction.

A. Energy metabolism in the white and red muscle fibers ◑

Muscles contain two types of fibers, the proportions of which vary from one type of muscle to another. **Red fibers** (type I fibers) are suitable for prolonged effort. Their metabolism is mainly aerobic and therefore depends on an adequate supply of O_2. **White fibers** (type II fibers) are better suited for fast, strong contractions. These fibers are able to form suf cient ATP even when there is little O_2 available. With appropriate training, athletes and sports participants are able to change the proportions of the two fiber types in the musculature and thereby prepare themselves for the physiological demands of their disciplines in a targeted fashion. The expression of functional muscle proteins can also change during the course of training.

Red fibers provide for their ATP requirements mainly (but not exclusively) from **fatty acids**, which are broken down via β-oxidation, the tricarboxylic acid cycle, and the respiratory chain (right part of the illustration). The red color in these fibers is due to the monomeric heme protein **myoglobin**, which they use as an O_2 reserve. Myoglobin has a much higher af nity for O_2 than hemoglobin and therefore only releases its O_2 when there is a severe drop in O_2 partial pressure (cf. p. 282).

At a high level of muscular effort—e. g., during weightlifting or in very fast contractions such as those carried out by the eye muscles—the O_2 supply from the blood quickly becomes inadequate to maintain the aerobic metabolism. White fibers (left part of the illustration) therefore mainly obtain ATP from **anaerobic glycolysis**. They have supplies of **glycogen** from which they can quickly release glucose-1-phosphate when needed (see p. 156). By isomerization, this gives rise to glucose-6-phosphate, the substrate for glycolysis. The $NADH+H^+$ formed during glycolysis has to be reoxidized into NAD^+ in order to maintain glucose degradation and thus ATP formation. If there is a lack of O_2, this is achieved by the formation of **lactate**, which is released into the blood and is resynthesized into glucose in the liver (Cori cycle; see p. 338).

Muscle-specific auxiliary reactions for ATP synthesis exist in order to provide additional ATP in case of emergency. **Creatine phosphate** (see **B**) acts as a buffer for the ATP level. Another ATP-supplying reaction is catalyzed by *adenylate kinase* [1] (see also p. 72). This disproportionates two molecules of ADP into ATP and AMP. The AMP is deaminated into IMP in a subsequent reaction [2] in order to shift the balance of the reversible reaction [1] in the direction of ATP formation.

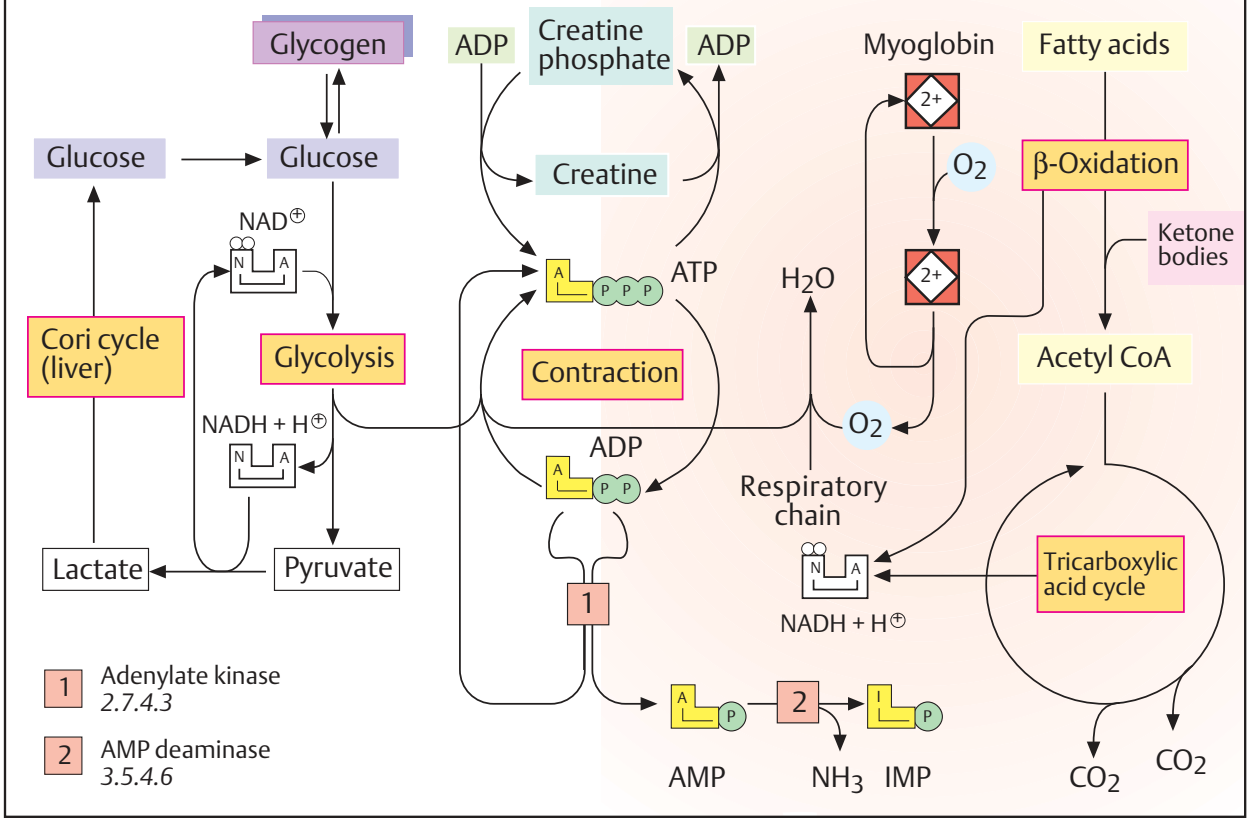
B. Creatine metabolism ◑

Creatine (*N*-methylguanidoacetic acid) and its phosphorylated form **creatine phosphate** (a guanidophosphate) serve as an ATP buffer in muscle metabolism. In creatine phosphate, the phosphate residue is at a similarly high chemical potential as in ATP and is therefore easily transferred to ADP. Conversely, when there is an excess of ATP, creatine phosphate can arise from ATP and creatine. Both processes are catalyzed by *creatine kinase* [5].

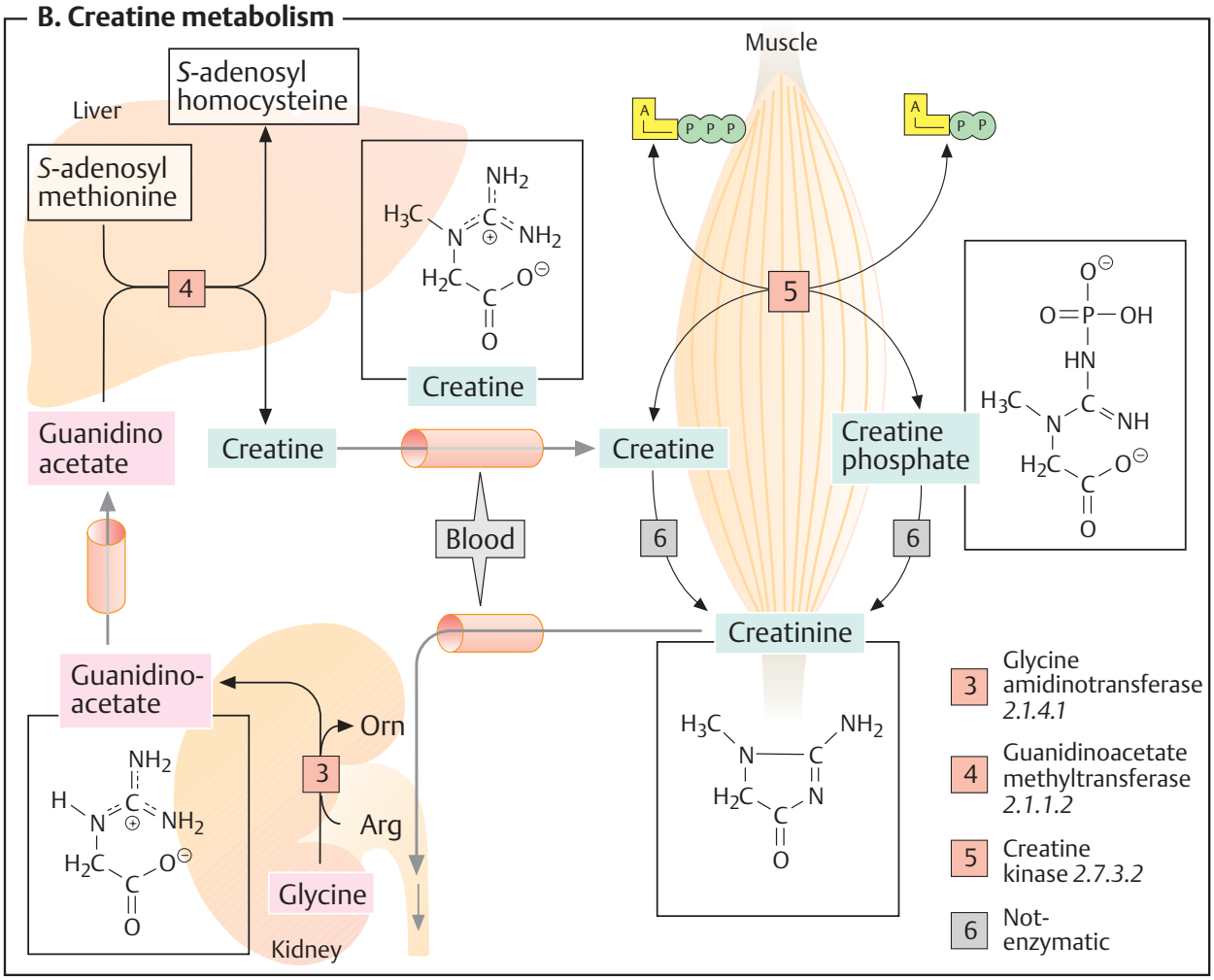
In resting muscle, creatine phosphate forms due to the high level of ATP. If there is a risk of a severe drop in the ATP level during contraction, the level can be maintained for a short time by synthesis of ATP from creatine phosphate and ADP. In a nonenzymatic reaction [6], small amounts of creatine and creatine phosphate cyclize constantly to form **creatinine**, which can no longer be phosphorylated and is therefore excreted with the urine (see p. 324).

Creatine does not derive from the muscles themselves, but is synthesized in two steps in the kidneys and liver (left part of the illustration). Initially, the guanidino group of arginine is transferred to glycine in the kidneys, yielding **guanidino acetate** [3]. In the liver, *N*-methylation of guanidino acetate leads to the formation of creatine from this [4]. The coenzyme in this reaction is *S-adenosyl methionine* (SAM; see p. 110).

A. Energy metabolism in the white and red muscle fibers



B. Creatine metabolism



Muscle metabolism II

A. Cori and alanine cycle ◑

White muscle fibers (see p. 336) mainly obtain ATP from **anaerobic glycolysis**—i. e., they convert glucose into lactate. The **lactate** arising in muscle and, in smaller quantities, its precursor **pyruvate** are released into the blood and transported to the liver, where lactate and pyruvate are resynthesized into glucose again via *gluconeogenesis,* with ATP being consumed in the process (see p. 154). The glucose newly formed by the liver returns via the blood to the muscles, where it can be used as an energy source again. This circulation system is called the **Cori cycle**, after the researchers who first discovered it. There is also a very similar cycle for erythrocytes, which do not have mitochondria and therefore produce ATP by anaerobic glycolysis (see p. 284).

The muscles themselves are not capable of gluconeogenesis. Nor would this be useful, as gluconeogenesis requires much more ATP than is supplied by glycolysis. As O_2 deficiencies do not arise in the liver even during intensive muscle work, there is always suf - cient energy there available for gluconeogenesis.

There is also a corresponding circulation system for the amino acid alanine. The **alanine cycle** in the liver not only provides alanine as a precursor for gluconeogenesis, but also transports to the liver the amino nitrogen arising in muscles during protein degradation. In the liver, it is incorporated into urea for excretion.

Most of the amino acids that arise in muscle during proteolysis are converted into glutamate and 2-oxo acids by *transamination* (not shown; cf. p. 180). Again by transamination, glutamate and pyruvate give rise to alanine, which after glutamine is the second important form of transport for amino nitrogen in the blood. In the liver, alanine and 2-oxoglutarate are resynthesized into pyruvate and glutamate (see p. 178). Glutamate supplies the urea cycle (see p. 182), while pyruvate is available for gluconeogenesis.

B. Protein and amino acid metabolism ◑

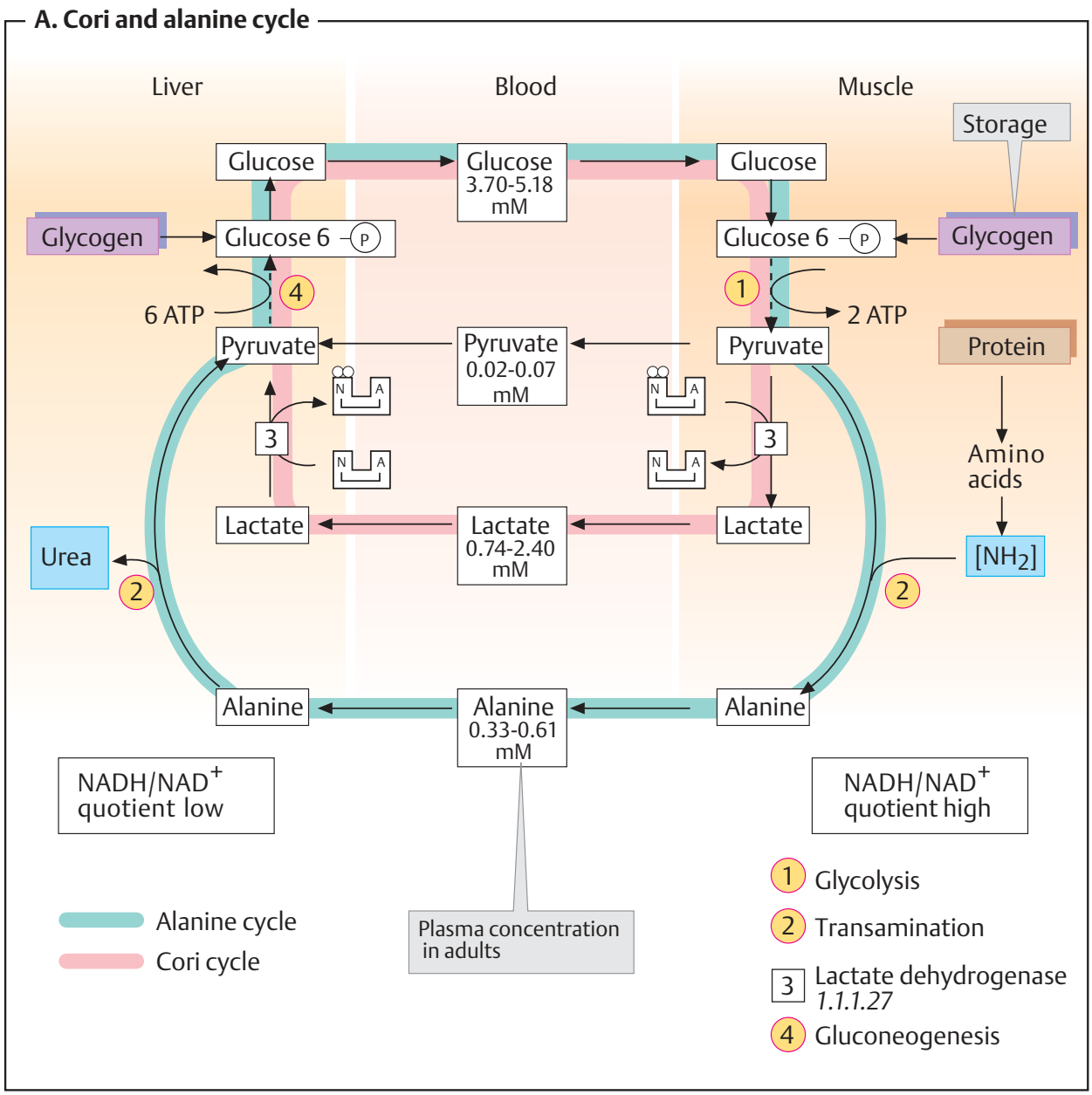
The skeletal muscle is the most important site for degradation of the *branched-chain amino acids* (Val, Leu, Ile; see p. 414), but other amino acids are also broken down in the muscles. **Alanine** and **glutamine** are resynthesized from the components and released into the blood. They transport the nitrogen that arises during amino acid breakdown to the liver (*alanine cycle;* see above) and to the kidneys (see p. 328).

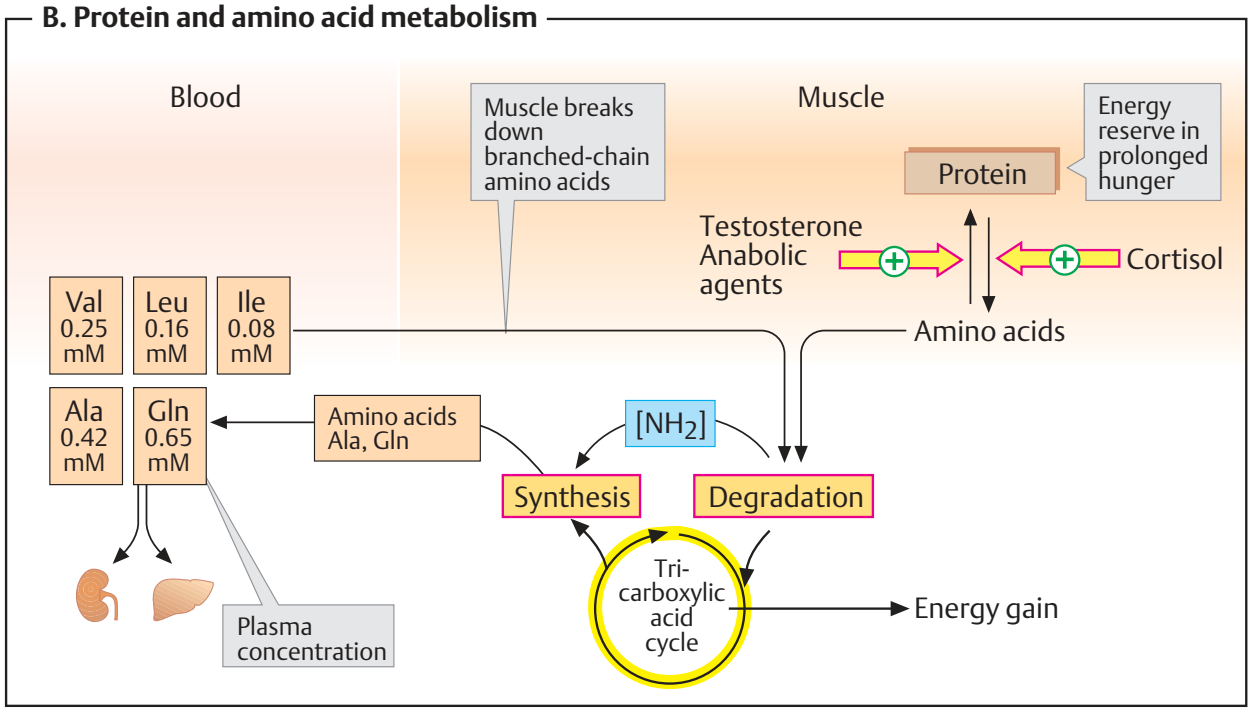
During periods of hunger, **muscle proteins** serve as an energy reserve for the body. They are broken down into amino acids, which are transported to the liver. In the liver, the carbon skeletons of the amino acids are converted into intermediates in the tricarboxylic acid cycle or into acetoacetyl-CoA (see p. 175). These amphibolic metabolites are then available to the energy metabolism and for gluconeogenesis. After prolonged starvation, the brain switches to using ketone bodies in order to save muscle protein (see p. 356).

The synthesis and degradation of muscle proteins are regulated by hormones. **Cortisol** leads to muscle degradation, while **testosterone** stimulates protein formation. Synthetic **anabolics** with a testosterone-like effect have repeatedly been used for doping purposes or for intensive muscle-building.

Further information

Smooth muscle differs from skeletal muscle in various ways. Smooth muscles—which are found, for example, in blood vessel walls and in the walls of the intestines—do not contain any muscle fibers. In smooth-muscle cells, which are usually spindle-shaped, the contractile proteins are arranged in a less regular pattern than in striated muscle. Contraction in this type of muscle is usually not stimulated by nerve impulses, but occurs in a largely spontaneous way. Ca^{2+} (in the form of Ca^{2+}-calmodulin; see p. 386) also activates contraction in smooth muscle; in this case, however, it does not affect troponin, but activates a protein kinase that phosphorylates the light chains in myosin and thereby increases myosin's ATPase activity. Hormones such as epinephrine and angiotensin II (see p. 330) are able to influence vascular tonicity in this way, for example.





Bone and teeth

The family of connective-tissue cells includes *fibroblasts, chondrocytes* (cartilage cells), and *osteoblasts* (bone-forming cells). They are specialized to secrete extracellular proteins, particularly collagens, and mineral substances, which they use to build up the *extracellular matrix* (see p. 346). By contrast, *osteoclasts* dissolve bone matter again by secreting H^+ and collagenases (see p. 342).

A. Bone ◑

Bone is an extremely dense, specialized form of connective tissue. In addition to its supportive function, it serves to store calcium and phosphate ions. In addition, blood cells are formed in the bone marrow. The most important mineral component of bone is **apatite**, a form of crystalline *calcium phosphate.*

Apatites are complexes of cationic Ca^{2+} 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* $Ca_{10}(PO_4)_6CO_3$, as *hydroxyapatite* $Ca_{10}(PO_4)_6(OH)_2$, or *fluoroapatite* $Ca_{10}(PO_4)_6F_2$. 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 (for details, see p. 342).

The most important *organic components* of bone are **collagens** (mainly type I; see p. 344) and **proteoglycans** (see p. 346). These form the extracellular matrix into which the apatite crystals are deposited (*biomineralization*). Various proteins are involved in this not yet fully understood process of bone formation, including collagens and phosphatases. *Alkaline phosphatase* is found in osteoblasts and *acid phosphatase* in osteoclasts. Both of these enzymes serve as *marker enzymes* for bone cells.

B. Teeth ◑

The illustration shows a longitudinal section through an incisor, one of the 32 permanent teeth in humans. The majority of the tooth consists of **dentine**. The crown of the tooth extends beyond the gums, and it is covered in **enamel**. By contrast, the root of the tooth is coated in dental **cement**.

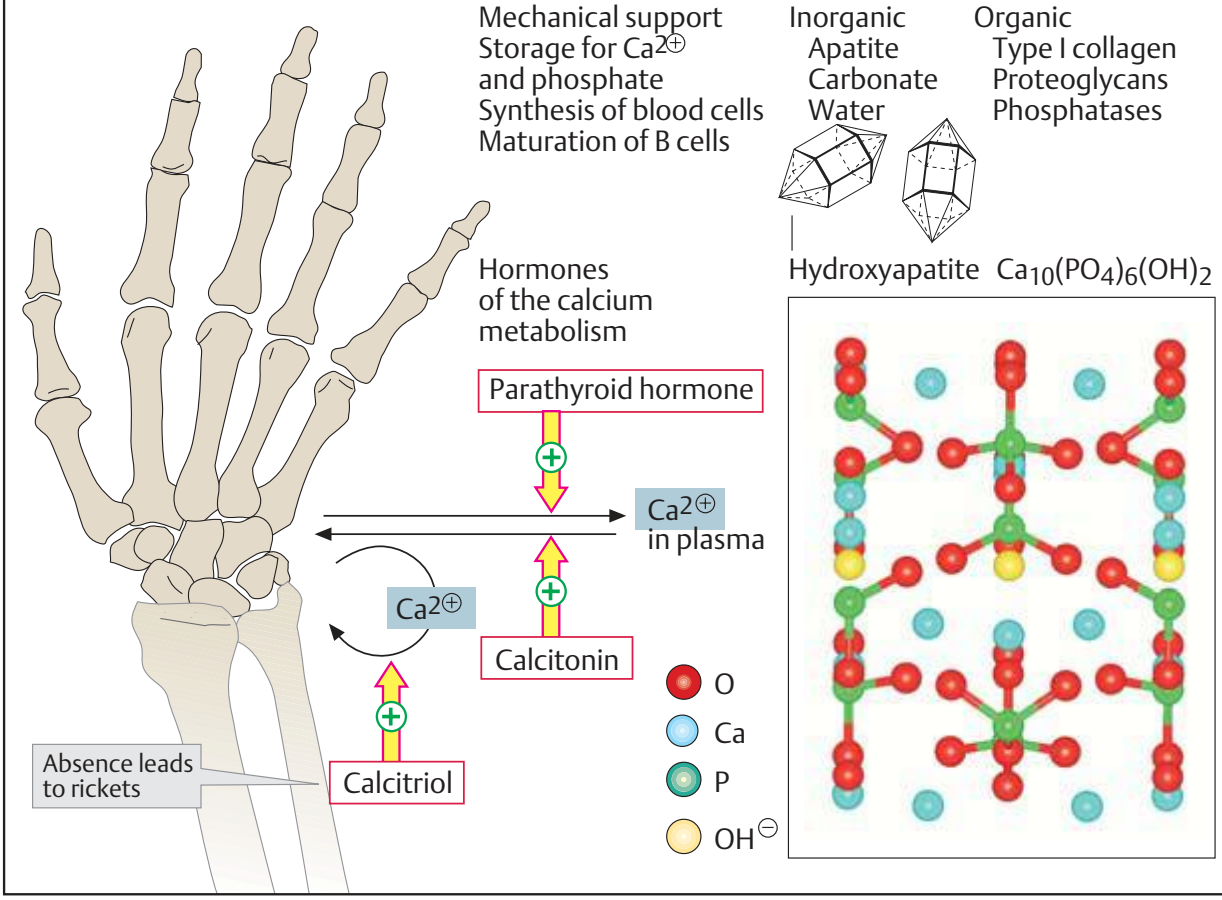
Cement, dentin, and enamel are bone-like substances. The high proportion of inorganic matter they contain (about 97% in the dental enamel) gives them their characteristic hardness. The organic components of cement, dentin, and enamel mainly consist of *collagens* and *proteoglycans;* their most important 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 (see **A**). Acids occur in food, or are produced by microorganisms that live on the surfaces of the teeth (e. g., *Streptococcus mutans*).

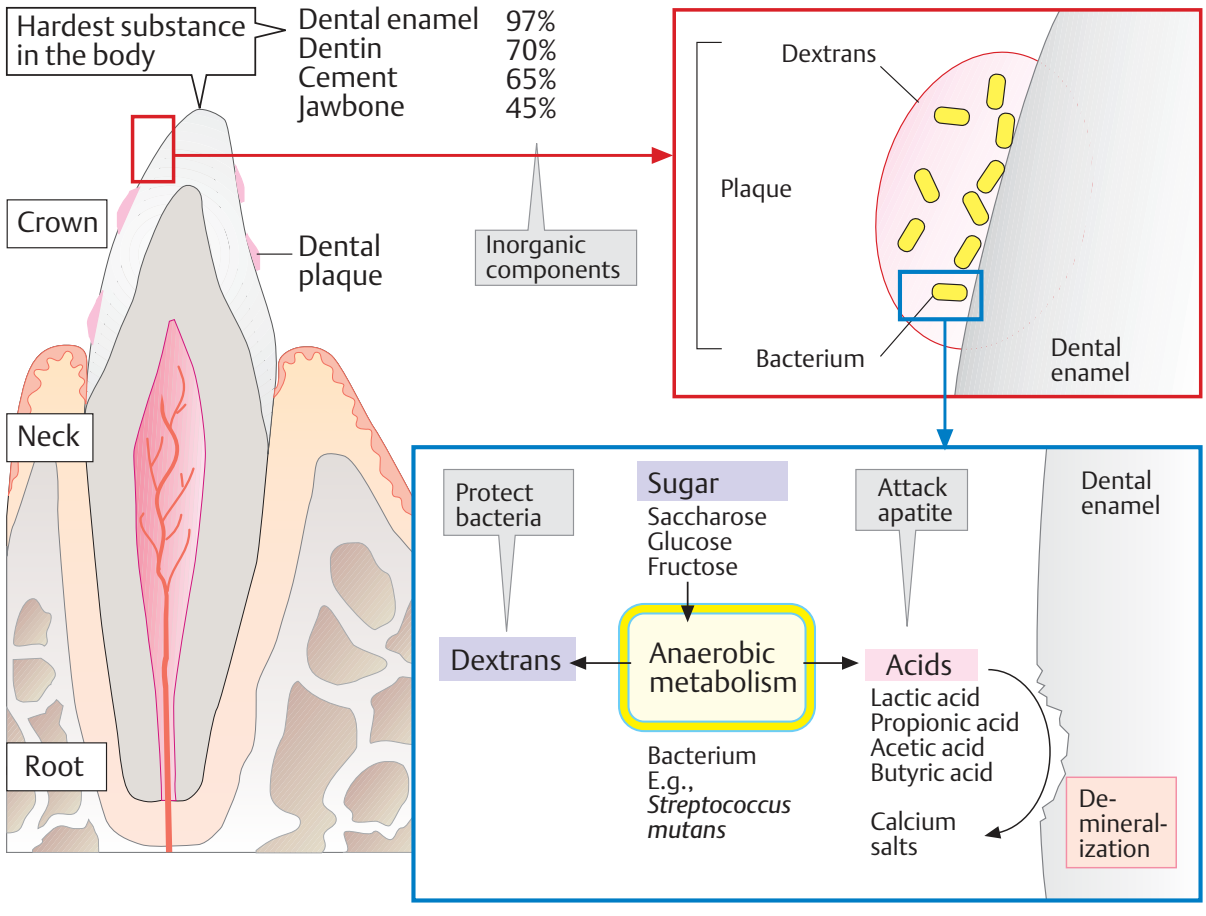
The main product of anaerobic degradation of sugars by these organisms is lactic acid. Other products of bacterial carbohydrate metabolism include extracellular dextrans (see p. 40)—insoluble polymers of glucose that help bacteria to protect themselves from their environment. Bacteria and dextrans are components of *dental plaque,* which forms on inadequately cleaned teeth. When 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 saccharose, glucose, and fructose). Small children in particular should not have very sweet drinks freely available to them. Regular removal of plaque by cleaning the teeth and hardening of the dental enamel by fluoridization are also important. Fluoride has a protective effect because fluoroapatite (see **A**) is particularly resistant to acids.

A. Bone



B. Teeth



Calcium metabolism

A. Functions of calcium ●

The human body contains 1–1.5 kg Ca^{2+}, most of which (about 98%) is located in the mineral substance of bone (see p. 362).

In addition to its role as a **bone component**, calcium functions as a **signaling substance**. Ca^{2+} ions act as *second messengers* in signal transduction pathways (see p. 386), they trigger *exocytosis* (see p. 228) and *muscle contraction* (see p. 334), and they are indispensable as cofactors in *blood coagulation* (see p. 290). Many *enzymes* also require Ca^{2+} for their activity. The intracellular and extracellular concentrations of Ca^{2+} are strictly regulated in order to make these functions possible (see **B**, **C**, and p. 388).

Proteins bind Ca^{2+} via oxygen ligands, particularly carboxylate groups and carbonyl groups of peptide bonds. This also applies to the structure illustrated here, in which a Ca^{2+} ion is coordinated by the oxygen atoms of carboxylate and acid amide groups.

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 (top) deposit collagen, as well as Ca^{2+} and phosphate, and thereby create new bone matter, while **osteoclasts** (bottom) secrete H^+ ions and collagenases that locally dissolve bone (*bone remodeling*). Osteoblasts and osteoclasts mutually activate each other by releasing **cytokines** (see p. 392) and **growth factors**. This helps keep bone formation and bone breakdown in balance.

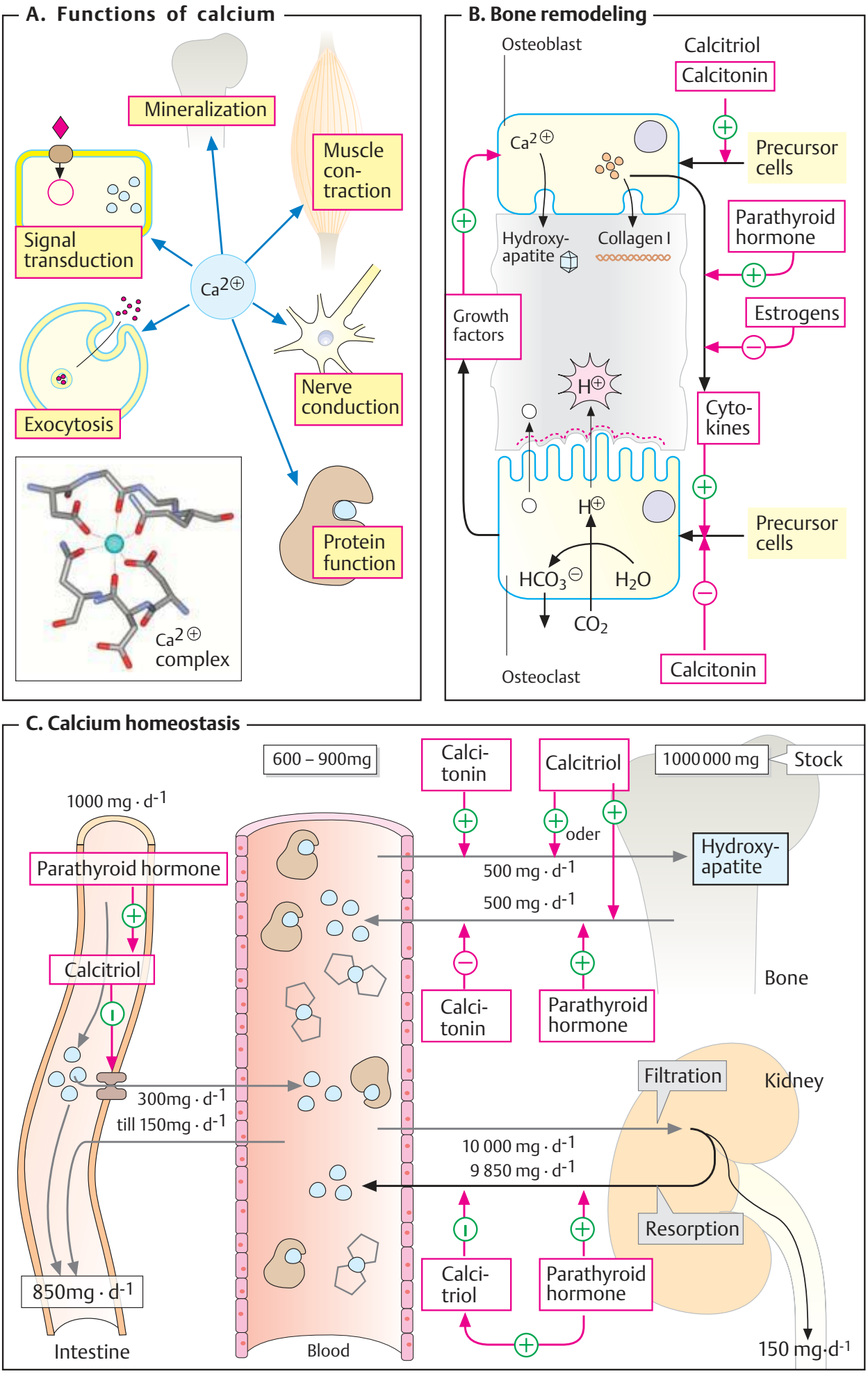
The Ca^{2+}-selective hormones calcitriol, parathyroid hormone, and calcitonin influence this interaction in the bone cells. **Parathyroid hormone** promotes Ca^{2+} release by promoting the release of cytokines by osteoblasts. In turn, the cytokines stimulate the development of mature osteoclasts from precursor cells (bottom). **Calcitonin** inhibits this process. At the same time, it promotes the development of osteoblasts (top). *Osteoporosis,* which mainly occurs in women following the menopause, is based (at least in part) on a reduction in **estrogen** levels. Estrogens normally inhibit the stimulation of osteoclast differentiation by osteoblasts. If the effects of estrogen decline, the osteoclasts predominate and excess bone removal occurs.

The effects of the steroid hormone **calcitriol** (see p. 330) 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;* see p. 364). 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;* see p. 364).

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 resorbed. The same amount is also excreted again. The amounts of Ca^{2+} released from bone and deposited in it per day are much smaller. 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 mg 2.0–2.6 mM). The peptide **parathyroid hormone** (PTH; 84 AA) and the steroid **calcitriol** (see p. 374) promote direct or indirect processes that raise the Ca^{2+} level in blood. Calcitriol increases Ca^{2+} resorption in the intestines and kidneys by inducing transporters. Parathyroid hormone supports these processes by stimulating calcitriol biosynthesis in the kidneys (see p. 330). In addition, it directly promotes resorption of Ca^{2+} in the kidneys (see p. 328) and Ca^{2+} release from bone (see **B**). The PTH antagonist **calcitonin** (32 AA) counteracts these processes.



Collagens

Collagens are quantitatively the most abundant of animal proteins, representing 25% of the total. They form insoluble tensile fibers that occur as structural elements of the extracellular matrix and connective tissue throughout the body. Their name (which literally means "glue-producers") is derived from the gelatins that appear as a decomposition product when collagen is boiled.

A. Structure of collagens ◑

Nineteen different collagens are now known, and they are distinguished using roman numerals. They mostly consist of a dextrorotatory **triple helix** made up of three polypeptides (α-chains) (see p. 70).

The triplet **Gly-X-Y** is constantly repeated in the sequence of the triple-helical regions—i. e., every third amino acid in such sequences is a *glycine*. *Proline* (Pro) is frequently found in positions X or Y; the Y position is often occupied by *4-hydroxyproline* (4Hyp), although *3-hydroxyproline* (3Hyp) and *5-hydroxylysine* (5Hyl) also occur. These hydroxylated amino acids are characteristic components of collagen. They are only produced after protein biosynthesis by **hydroxylation** of the amino acids in the peptide chain (see p. 62).

The formation of Hyp and Hyl residues in procollagen is catalyzed by iron-containing *oxygenases* ("proline and lysine hydroxylase," *EC 1.14.11.1/2*). *Ascorbate* is required to maintain their function. Most of the symptoms of the vitamin C deficiency disease *scurvy* (see p. 368) 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 consist of different combinations of α-chains (α1 to α3 and other subtypes). Types I, II, and III represent 90% of collagens. The **type I** collagen shown here has the structure $[\alpha1(I)]_2\alpha2(1)$.

Numerous **tropocollagen** molecules (mass 285 kDa, length 400 nm) aggregate extracellularly into a defined arrangement, forming cylindrical **fibrils** (20–500 nm in diameter). Under the electron microscope, these fibrils are seen to have a characteristic banding pattern of elements that are repeated every 64–67 nm.

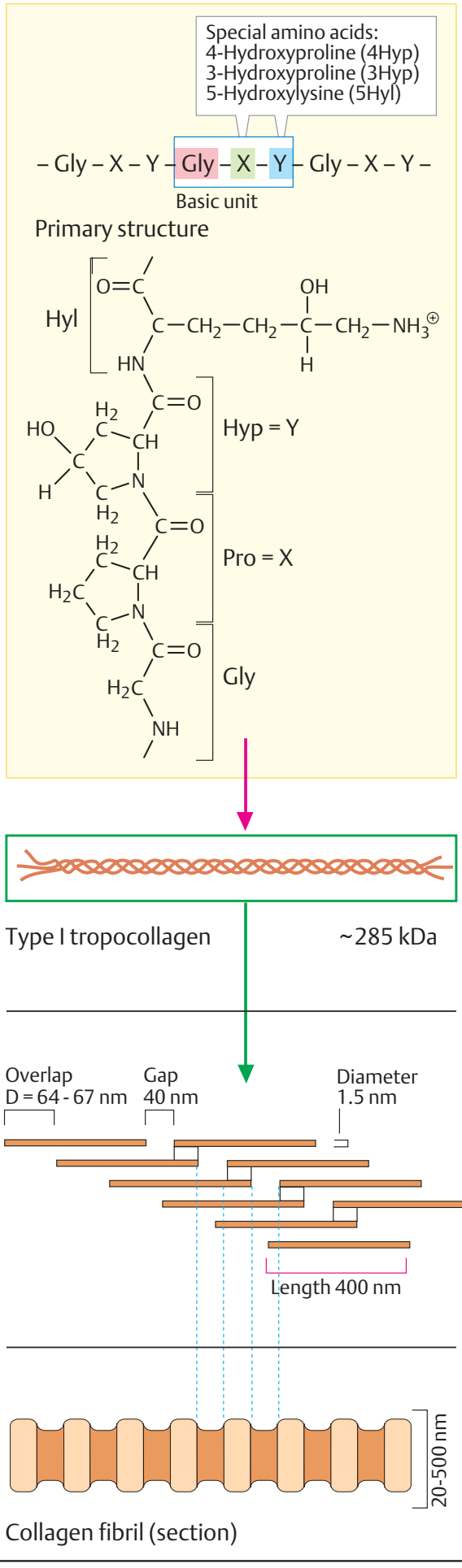
Tropocollagen molecules are firmly linked together, particularly at their ends, by covalent networks of altered lysine side chains. The number of these links increases with age. **Type IV** collagens form networks with a defined mesh size. The size-selective filtering effect of the basal membranes in the renal glomeruli is based on this type of structure (see p. 322).

B. Biosynthesis ◑

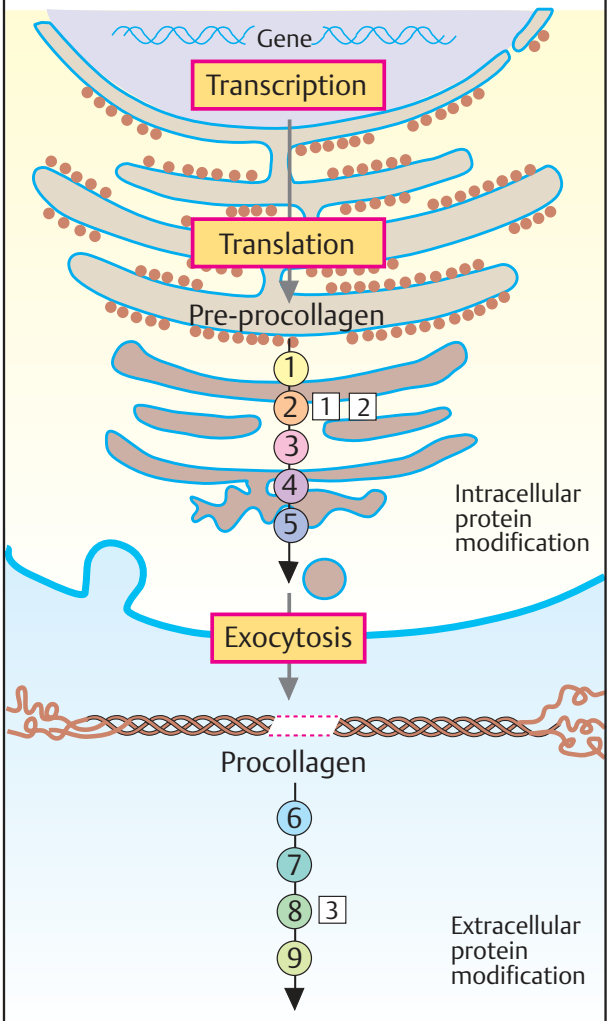
The precursor molecule of collagen (*preprocollagen*), formed in the rER, is subject to extensive **post-translational modifications** (see p. 232) in the ER and Golgi apparatus.

Cleavage of the signal peptide gives rise to **procollagen**, which still carries large *propeptides* at each end [1]. During this phase, most proline residues and some lysine residues of procollagen are hydroxylated [2]. The procollagen is then glycosylated at hydroxylysine residues [3]. Intramolecular and intermolecular disulfide bonds form in 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 secreted into the extracellular space by exocytosis. This is where the *N*- and *C*-terminal propeptides are removed proteolytically [6], allowing the staggered aggregation of the **tropocollagen** molecules to form fibrils [7]. Finally, several ε-amino groups in lysine residues are oxidatively converted into aldehyde groups [8]. Covalent links between the molecules then form as a result of condensation [9]. In this way, the fibrils reach their final structure, which is characterized by its high *tensile strength* and *proteinase resistance.*

A. Structure of collagens



B. Biosynthesis



1. Removal of the prepeptide
2. Hydroxylation of Pro and Lys residues
3. Glycosylation of 5Hyl and Asn
4. Oxidation of Cys in propeptides
5. Assemblage to form triple helix
6. Removal of the propeptide
7. Staggered deposition to form fibrils
8. Oxidation of Lys and 5Hyl to aldehydes
9. Cross-linking to form supramolecules

[1] Procollagen-proline 4-dioxygenase *1.14.11.2* [ascorbate, Fe]
[2] Procollagen-lysine 5-dioxygenase *1.14.11.4* [ascorbate, Fe]
[3] Protein-lysine 6-oxidase *1.4.3.13* [Cu]

Extracellular matrix

A. Extracellular matrix ◑

The space between the cells (the interstitium) is occupied by a substance with a complex composition known as 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. In *connective tissue, cartilage,* and *bone,* the ECM is particularly strongly marked and is actually the functional part of the tissue (see p. 340). The illustration shows the three main constituents of the extracellular matrix in a highly schematic way: collagen fibers, network-forming adhesive proteins, and space-filling proteoglycans.

The ECM has a very wide variety of functions: it establishes mechanical connections between cells; it creates structures with special mechanical properties (as in bone, cartilage, tendons, and joints); it creates filters (e.g., in the basal membrane in the renal corpuscles; see p. 322); 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.

Collagens (see p. 344), of which there are at least 19 different varieties, form fibers, fibrils, networks, and ligaments. Their characteristic properties are *tensile strength* and *flexibility*. **Elastin** is a fiber protein with a high degree of elasticity.

Adhesive proteins provide the connections between the various components of the extracellular matrix. Important representatives include **laminin** and **fibronectin** (see **B**). These multifunctional proteins simultaneously bind to several other types of matrix component. Cells attach to the cell surface receptors in the ECM with the help of the adhesive proteins.

Due to their polarity and negative charge, **proteoglycans** (see **C**) bind water molecules and cations. As a homogeneous "cement," they fill the gaps between the ECM fibers.

B. Fibronectins ○

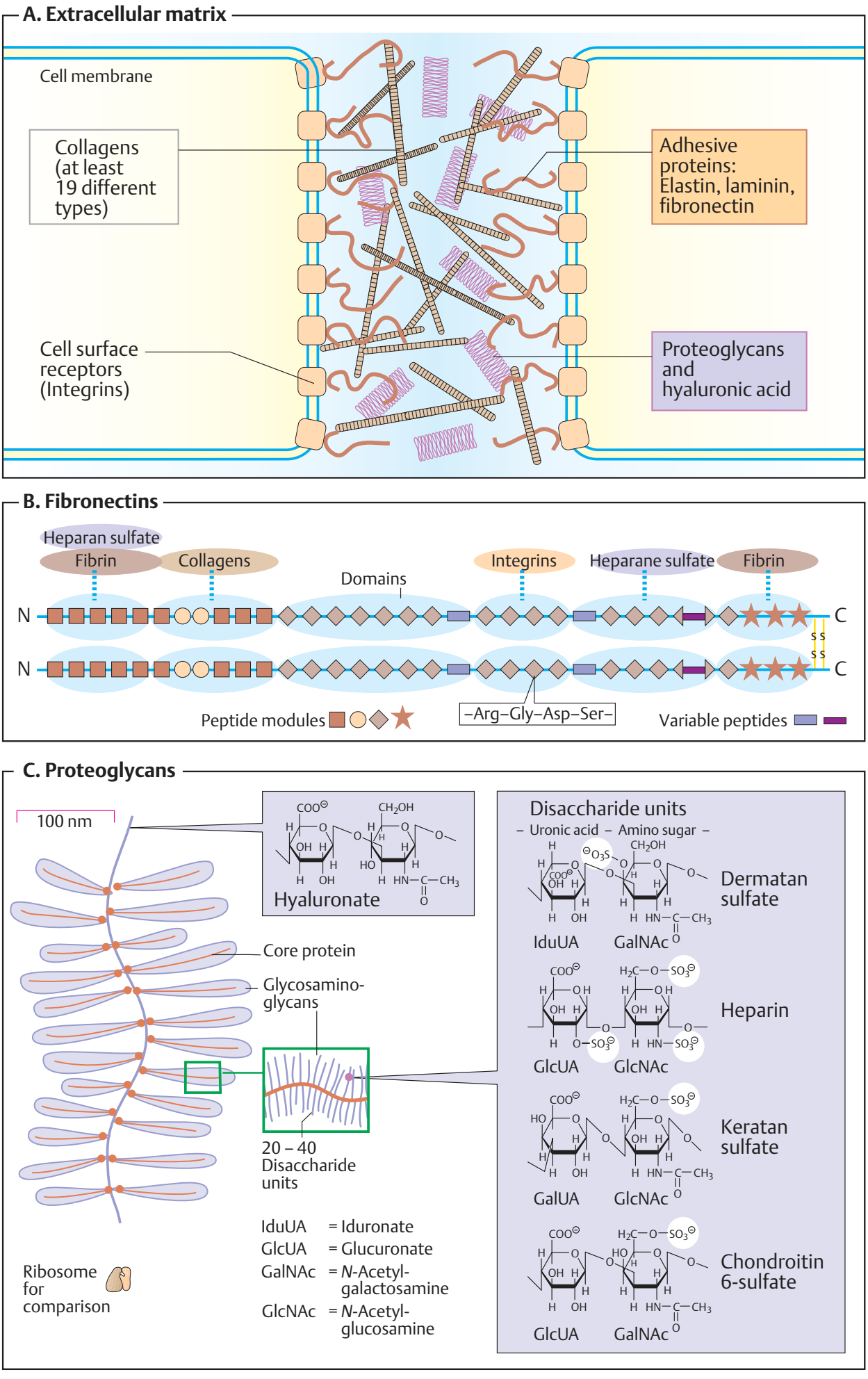
Fibronectins are typical representatives of adhesive proteins. They are filamentous dimers consisting of two related peptide chains (each with a mass of 250 kDa) linked to each other by disulfide bonds. The fibronectin molecules are divided into different *domains,* which bind to cell-surface receptors, 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* (see p. 246) of the hnRNA transcript of the fibronectin gene leads to fibronectins with different compositions. The module that causes adhesion to cells contains the characteristic amino acid sequence –Arg–Gly–Asp–Ser–. It is these residues that enable fibronectin to bind to cell-surface receptors, known as **integrins**.

C. Proteoglycans ◑

Proteoglycans are giant molecule complexes consisting of carbohydrates (95%) and proteins (5%), with masses of up to 2 10^6 Da. Their bottlebrush-shaped structure is produced by an axis consisting of **hyaluronate**. This thread-like polysaccharide (see p. 44) has **proteins** attached to it, from which in turn long polysaccharide chains emerge. Like the central hyaluronate, these terminal polysaccharides belong to the glycosaminoglycan group (see p. 44).

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) (see p. 38). Many of the amino sugars are also esterified with sulfuric acid (sulfated), further increasing their polarity. The proteoglycans bind large amounts of water and fill the gaps between the fibrillar components of the ECM in the form of a hydrated gel. This inhibits the spread of pathogens in the ECM, for example.



Signal transmission in the CNS

A. Structure of nerve cells ●

Nerve cells (neurons) are easily 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* (see p. 352) are stored in the axonal nerve endings. These signaling substances are released in response to electrical signals in order to excite neighboring neurons (or muscle cells). It is estimated that each neuron in the brain is in contact via synapses with approximately 10 000 other neurons.

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 phospholipids, glycolipids, and sphingolipids (see p. 216).

B. Neurotransmitters and neurohormones ◑

Neurosecretions are classed into two groups: **neurotransmitters** are released into the *synaptic cleft* in order 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** (see p. 350) reaches the presynaptic membrane, *voltage-gated Ca^{2+} channels* integrated into the membrane open and trigger **exocytosis** of the neurotransmitter stored in the presynaptic cell (for details, see p. 228).

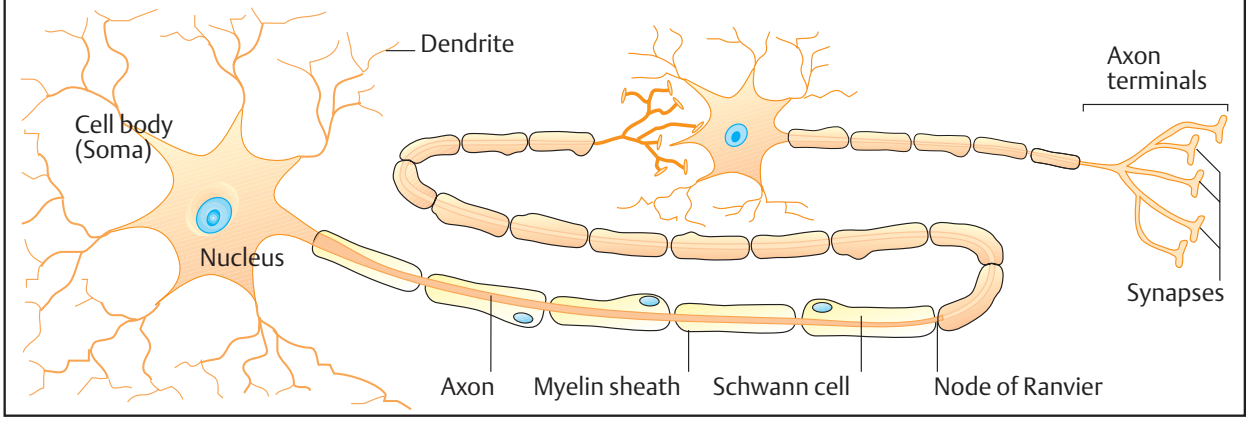
Each neuron usually releases *only one type* of **neurotransmitter**. Neurons that release dopamine are referred to as "dopaminergic," for example, while those that release acetylcholine are "cholinergic," etc. The transmitters that are released diffuse through the synaptic cleft and bind on the other side to **receptors** on the postsynaptic membrane. These receptors are integral membrane proteins that have binding sites for neurotransmitters on their exterior (see p. 224).

The receptors for neurotransmitters are divided into two large groups according to the effect produced by binding of the transmitter (for details, see p. 354).

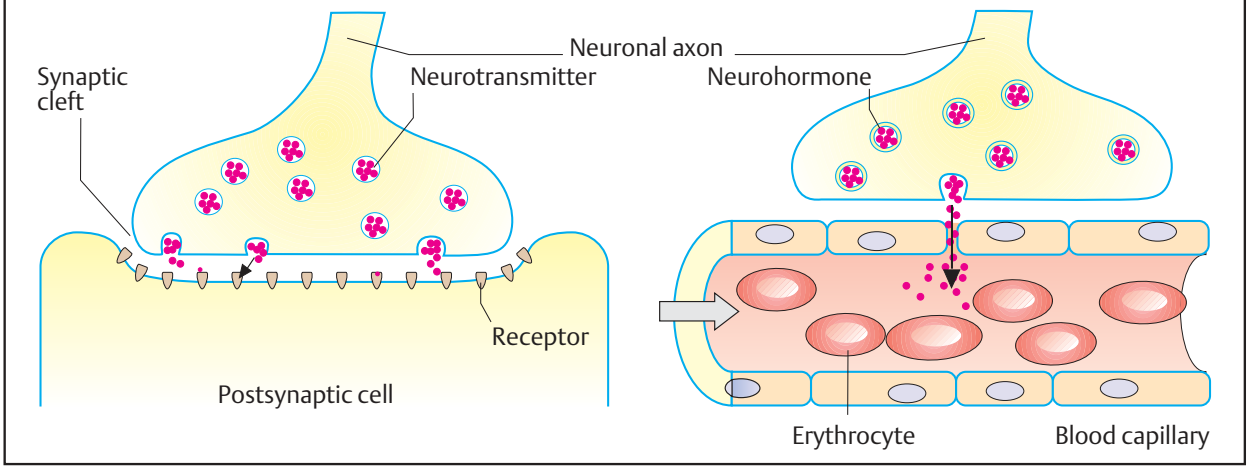
Ionotropic receptors (bottom left) are *ligand-gated ion channels.* When they open as a result of the transmitter's influence, ions flow in due to the membrane potential (see p. 126). If the inflowing ions are cations (Na^+, K^+, Ca^{2+}), **depolarization** of the membrane occurs and an action potential is triggered on the surface of the postsynaptic cell. 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 dif cult. 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** (bottom right). After binding of the transmitter, these interact on the inside of the postsynaptic membrane with *G proteins* (see p. 384), 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;* see p. 386).

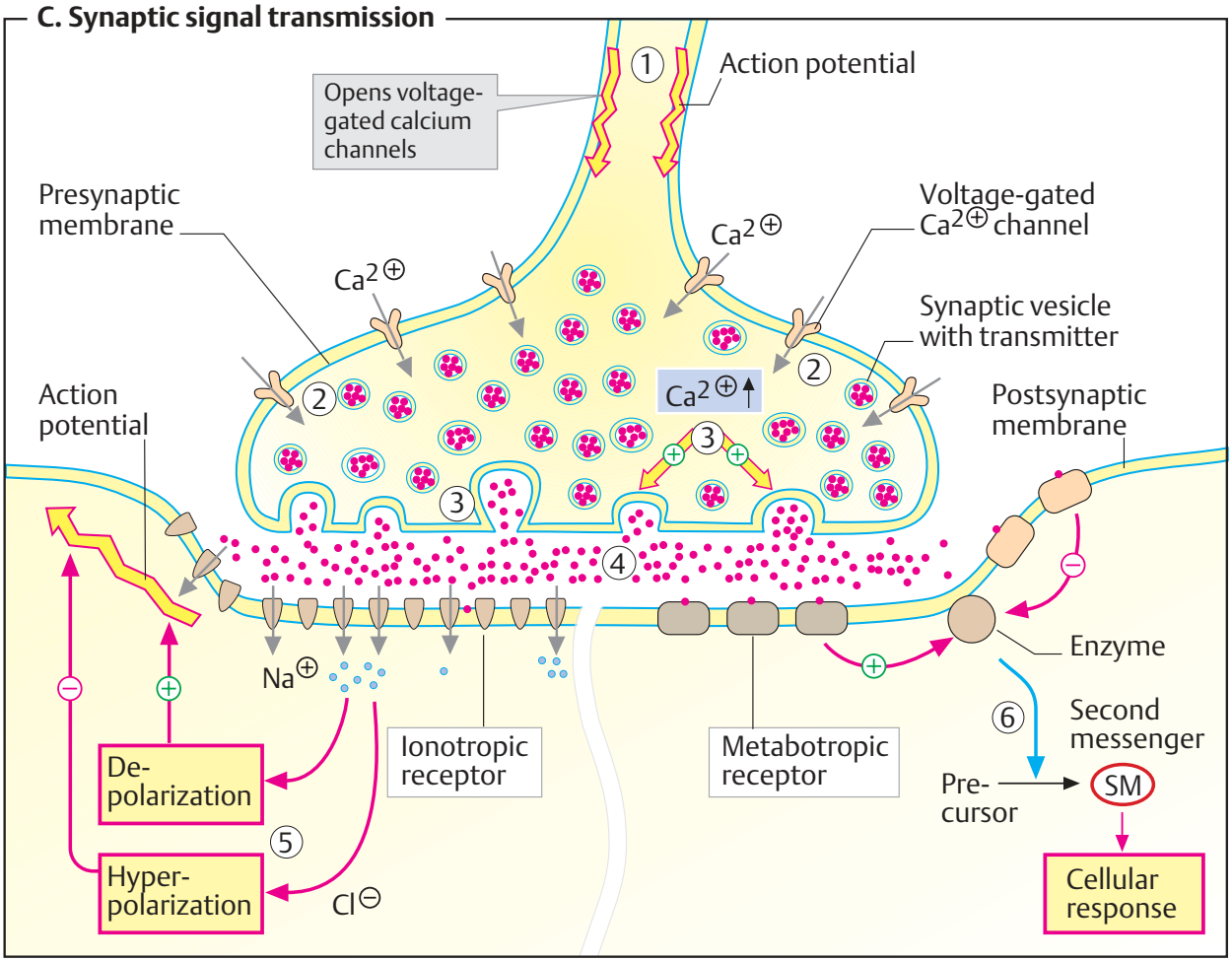
A. Structure of nerve cells



A. 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 positively and negatively charged ions on the inside and outside of the plasma membrane. This gives rise to a **membrane potential** (see p. 126)—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 in most cells is –60 to –90 mV. It mainly arises from the activity of *Na^+/K^+ transporting ATPase* ("Na^+/K^+ ATPase"), which occurs on practically all animal cells. Using up ATP, this P-type enzyme (see p. 220) "pumps" three Na^+ ions out of the cell in exchange for two 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 anions predominate inside it.

An **equilibrium potential** 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 Ca^{2+}. 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; see p. 222) and those regulated by ligands *("ligand-gated"*—e. g., nicotinic acetylcholine receptors; see p. 222).

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). Binding of a neurotransmitter to an ionotropic receptor results in a brief local increase in the membrane potential from –60 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.

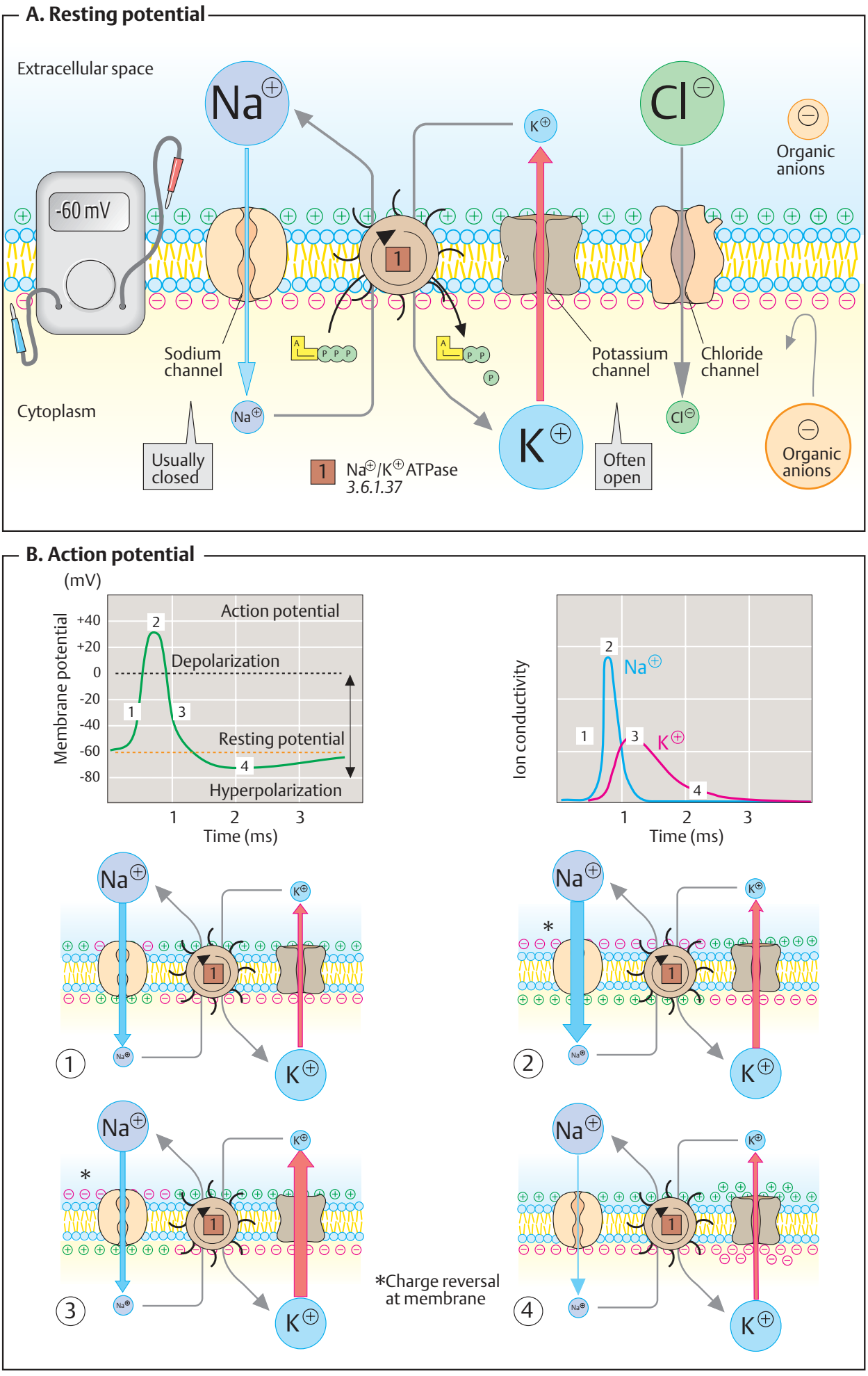
[1] The process starts with the opening of voltage-gated Na^+ channels (see p. 222). Due to their high equilibrium potential (see **A**), Na^+ ions flow into the cell and reverse the local membrane potential (**depolarization**).

[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 (see **A**) 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 again after a short refractory period, 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*.



Neurotransmitters

Neurotransmitters in the strict sense are substances 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.

A. Important neurotransmitters ◑

Neurotransmitters can be classified into several groups according to their chemical structure. The table lists the most important representatives of this family, which has more than 100 members.

Acetylcholine, the acetic acid ester of the cationic alcohol choline (see p. 50) acts at neuromuscular junctions, where it triggers muscle contraction (see p. 334), and in certain parts of the brain and in the autonomous nervous system.

Several proteinogenic **amino acids** (see p. 60) have neurotransmitter effects. A particularly important one is *glutamate,* which acts as a stimulatory transmitter in the CNS. More than half of the synapses in the brain are glutaminergic. The metabolism of glutamate and that of the amine GABA synthesized from it (see below) are discussed in more detail on p. 356. *Glycine* is an inhibitory neurotransmitter with effects in the spinal cord and in parts of the brain.

Biogenic amines arise from amino acids by decarboxylation (see p. 62). This group includes *4-aminobutyrate* (γ-aminobutyric acid, GABA), which is formed from glutamate and is the most important inhibitory transmitter in the CNS. The *catecholamines* norepinephrine and epinephrine (see **B**), *serotonin,* which is derived from tryptophan, and *histamine* also belong to the biogenic amine group. All of them additionally act as hormones or mediators (see p. 380).

Peptides make up the largest group among the neurosecretions. Many peptide hormones—e. g., thyroliberin (TRH) and angiotensin II—simultaneously act as transmitters. Most neuropeptides are small (3–15 AA). 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 acid amide ($-NH_2$). This provides better protection against breakdown by peptidases.

Endorphins, dynorphins, and *enkephalins* are a particularly interesting group of neuropeptides. They act as "endogenous opiates" by producing analgetic, sedative, and euphoriant effects in extreme situations. Drugs such as morphine and heroin activate the receptors for these peptides (see p. 354).

Purine derivatives with neurotransmitter 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* is mainly based on the fact that it binds to adenosine receptors.

B. Biosynthesis of catecholamines ◑

The catecholamines are *biogenic amines* that have a catechol group. Their biosynthesis in the adrenal cortex and CNS starts from **tyrosine**.

[1] Hydroxylation of the aromatic ring initially produces **dopa** (3,4-dihydroxyphenylalanine). This reaction uses the unusual coenzyme *tetrahydrobiopterin* (THB). Dopa (cf. p. 6) is also used in the treatment of Parkinson's disease.

[2] Decarboxylation of dopa yields **dopamine**, an important transmitter in the CNS. In dopaminergic neurons, catecholamine synthesis stops at this point.

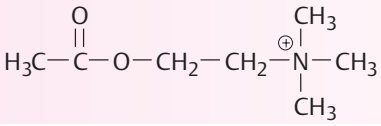
[3] The adrenal gland and adrenergic neurons continue the synthesis by hydroxylating dopamine into **norepinephrine** (noradrenaline). *Ascorbic acid* (vitamin C; see p. 368) acts as a hydrogen-transferring coenzyme here.

[4] Finally, *N*-methylation of norepinephrine yields **epinephrine** (adrenaline). The coenzyme for this reaction is *S*-adenosylmethionine (SAM; see p. 110).

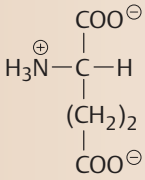
The physiological effects of the catecholamines are mediated by a large number of different receptors that are of particular interest in pharmacology. Norepinephrine acts in the autonomic nervous system and certain areas of the brain. Epinephrine is also used as a transmitter by some neurons.

A. Important neurotransmitters

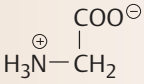
Acetylcholine



Amino acids: Glutamate, Glycine, Dopa

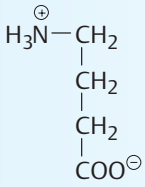


Glutamate

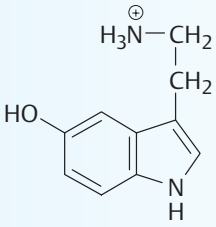


Glycine

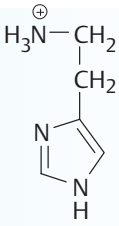
Biogenic amines: γ-Aminobutyrate (GABA), Dopa, Dopamine, Norepinephrine, Epinephrine, Serotonine, Histamine



GABA



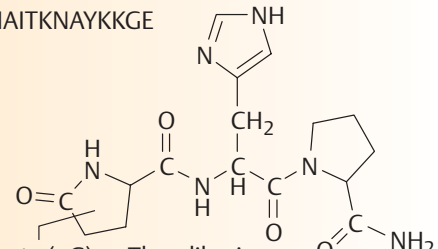
Serotonin



Histamine

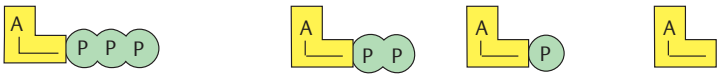
Peptides:

β-Endorphin	YGGFMTSEKSQTPLVTLFKNAITKNAYKKGE
Met- and Leu-enkephalin	YGGFM und YGGFL
Thyroliberin (TRH)	<GHP-NH_2
Gonadoliberin (GnRH)	<GHWSYGLRPG-NH_2
Substance P	RPKPQQFFGLM
Somatostatin	AGCKNFFWKTFTSC
Angiotensin II	DRVYIHPF
Cholecystokinin (CCK-4)	WMDF-NH_2
and many others	



Pyroglutamate (<G) Thyroliberin

Purine derivatives: ATP, ADP, AMP, Adenosine



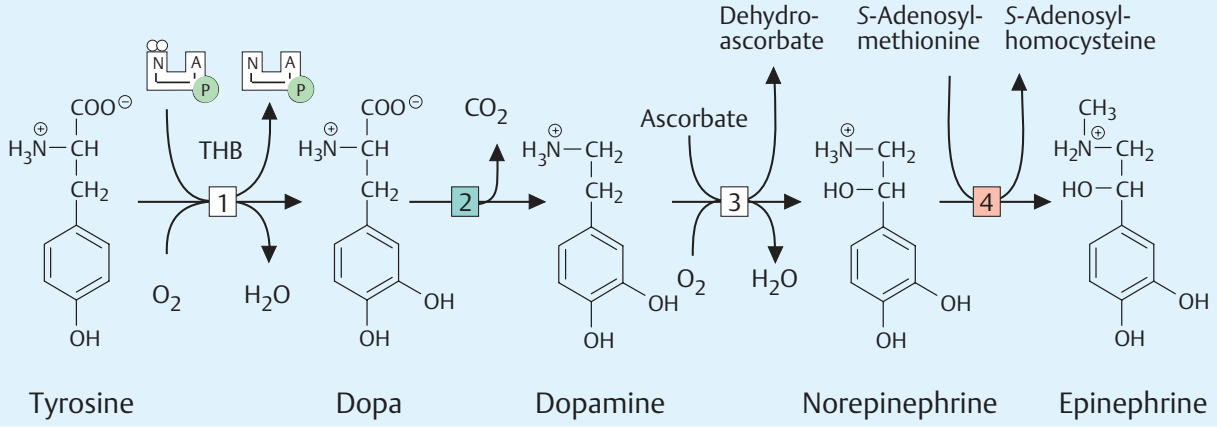
B. Biosynthesis of the catecholamines

1 Tyrosine 3-monooxygenase [$Fe^{2\oplus}$,THB] *1.14.16.2*

2 Aromatic-L-amino-acid decarboxylase (Dopa decarboxylase) [PLP] *4.1.1.28*

3 Dopamine β-monooxygenase[Cu] *1.14.17.1*

4 Phenylethanolamine *N*-methyltransferase *2.1.1.28*



Receptors for neurotransmitters

Like all signaling substances, neurotransmitters (see p. 352) act via receptor proteins. The receptors for neurotransmitters are integrated into the membrane of the postsynaptic cell, where they trigger ion inflow or signal transduction processes (see p. 348).

A. Receptors for neurotransmitters ◑

A considerable number of receptors for neurotransmitters are already known and new ones are continuing to be discovered. The table only lists the most 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). The receptors for stimulatory transmitters (indicated in the table by a ⊕) mediate the inflow of cations (mainly Na^+). When these open after binding of the transmitter, local *depolarization* of the postsynaptic membrane occurs. By contrast, inhibitory neurotransmitters (GABA and glycine) allow Cl^- to flow in. This increases the membrane's negative resting potential and hinders the action of stimulatory transmitters (*hyperpolarization,* Θ).

Metabotropic receptors (right half of the table) are coupled to G proteins (see p. 386), through which they influence the *synthesis of second messengers.* Receptors that work with type G_s proteins (see p. 386) increase the cAMP level in the postsynaptic cell ([cAMP] ↑), while those that activate G_i proteins reduce it ([cAMP] ↓). Via type G_q proteins, other receptors increase the intracellular Ca^{2+} concentration ($[Ca^{2+}]$ ↑).

There are several **receptor subtypes** for most neurotransmitters. These are distinguished numerically (e.g., D_1 to D_5) or are named after their agonists—i.e., after molecules experimentally found to activate the receptor. For example, one specific subtype of glutamate receptors reacts to NMDA (*N*-methyl-D-aspartate), while another subtype reacts to the compound AMPA, etc.

B. Acetylcholine receptors ◑

Acetylcholine (ACh) was the neurotransmitter first discovered, at the beginning of the last century. It binds to two types of receptor.

The **nicotinic ACh receptor** responds to the alkaloid *nicotine* contained in tobacco (many of the physiological effects of nicotine are based on this). The nicotinic receptor is ionotropic. Its properties are discussed in greater detail on p. 222.

The **muscarinic ACh receptors** (of which there are at least five subtypes) 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 at the receptor, but in contrast to ACh (see **C**), it is not broken down and therefore causes permanent stimulation of muscle.

The muscarinic ACh receptors influence the cAMP level in the postsynaptic cells (M_1, M_3 and M_5 increase it, while subtypes M_2 and M_4 reduce it).

C. Metabolism of acetylcholine ◑

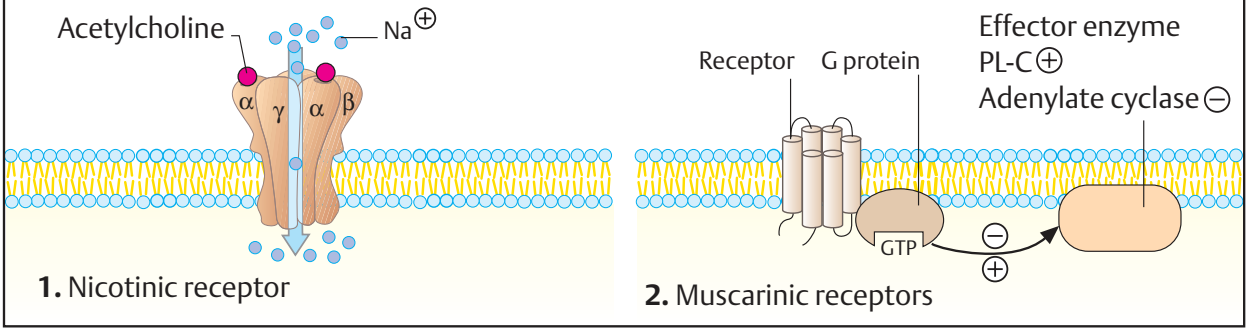
Acetylcholine is synthesized from acetyl-CoA and choline in the cytoplasm of the presynaptic axon [1] and is stored in **synaptic vesicles**, each of which contains around 1000–10 000 ACh molecules. After it is released by exocytosis (see p. 228), the transmitter travels by diffusion to the receptors on the postsynaptic membrane. Catalyzed by *acetylcholinesterase,* hydrolysis of ACh to acetate and choline immediately starts in the synaptic cleft [2], and within a few milliseconds, the ACh released has been eliminated again. The cleavage products **choline** and **acetate** are taken up again by the presynaptic neuron and reused for acetylcholine synthesis [3].

Substances that block the serine residue in the active center of acetylcholinesterase [2]—e.g., the neurotoxin E605 and other *organophosphates*—prevent ACh degradation and thus cause prolonged stimulation of the postsynaptic cell. This impairs nerve conduction and muscle contraction. *Curare,* a paralyzing arrow-poison used by South American Indians, competitively inhibits binding of ACh to its receptor.

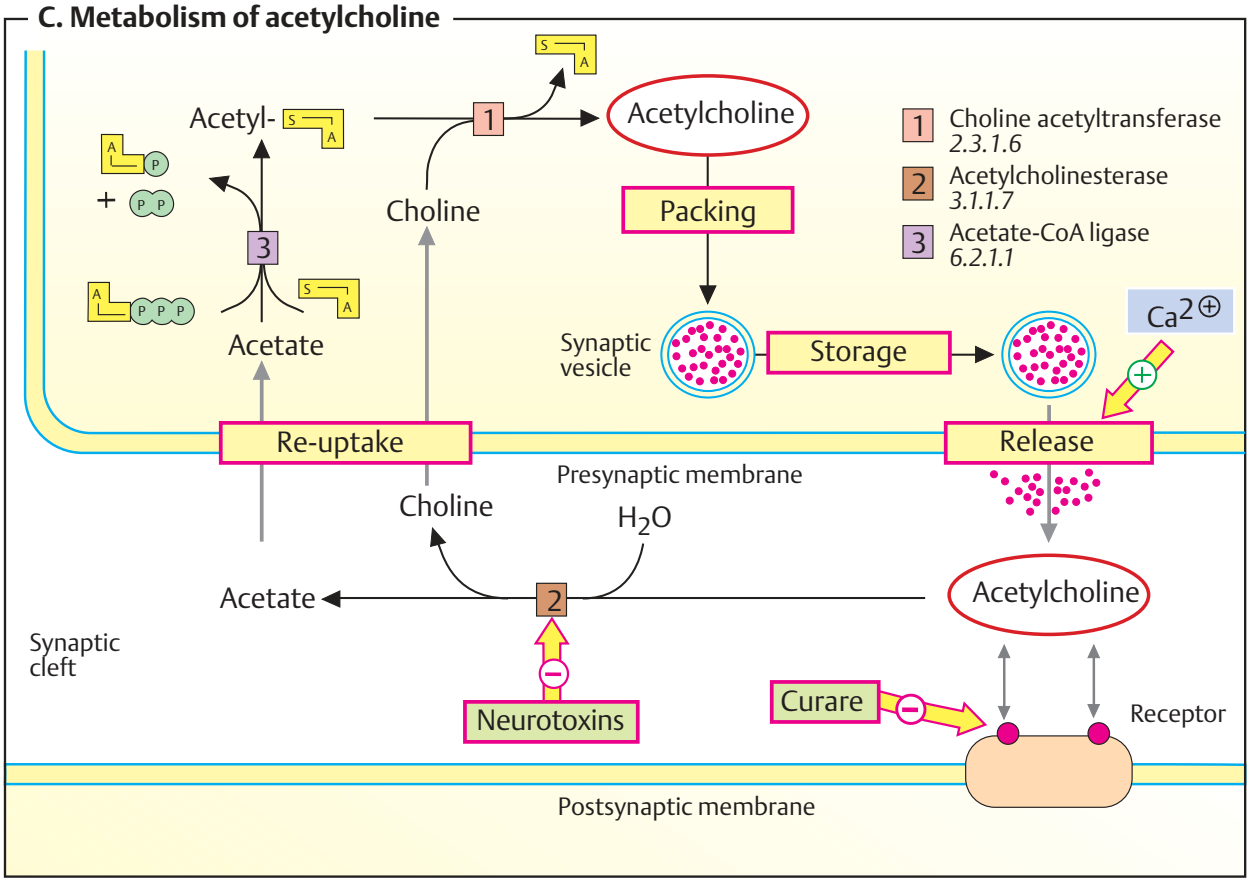
A. Receptors for neurotransmitters

Ionotropic				Metabotropic		
Receptor	**Transmitter**	**Ion(s)**	**Effect**	**Receptor**	**Transmitter**	**Effect**
Acetyl-choline (nicotinic)	Acetyl-choline	$Na^{\oplus}$	$\oplus$	Acetylcholine (muscarinic) M1, M3, M5, M2, M4	Acetylcholine	$[Ca^{2\oplus}]\uparrow$ $[cAMP]\downarrow$
5HT3	Serotonin	$Na^{\oplus}$	$\oplus$	$5HT_1$ $5HT_2$ $5HT_4$	Serotonin „ „	$[Ca^{2\oplus}]\uparrow$ $[cAMP]\uparrow$ $[cAMP]\downarrow$
GABAa	GABA	$Cl^{\ominus}$	$\ominus$	α_1 α_2 $\beta_1, \beta_2, \beta_3$	Norepinephrine „ „	$[Ca^{2\oplus}]\uparrow$ $[cAMP]\uparrow$ $[cAMP]\downarrow$
Glycine	Glycine	$Cl^{\ominus}$	$\ominus$			
AMPA NMDA Kainate	Glutamate Glutamate Glutamate	$Na^{\oplus}$ $K^{\oplus}$ $Na^{\oplus}$ $K^{\oplus}$ $Ca^{2\oplus}$ $Na^{\oplus}$ $K^{\oplus}$	$\oplus$ $\oplus$ $\oplus$	D_1, D_5 D_2, D_3, D_4	Dopamine „	$[cAMP]\uparrow$ $[cAMP]\downarrow$
				δ,κ,μ	Opioids	$[cAMP]\downarrow$

B. Acetylcholine receptors



C. Metabolism of acetylcholine



Metabolism

The brain and other areas of the central nervous system (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 (see p. 350).

A. Energy metabolism of the brain ●

Glucose is normally the only metabolite from which the brain is able to obtain adequate amounts of ATP through aerobic glycolysis and subsequent terminal oxidation to CO_2 and H_2O. Lipids are unable to pass the **blood–brain barrier**, and amino acids are also only available in the brain in limited quantities (see **B**). As neurons 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—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) also fint affects the brain. 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").

During periods of starvation, the brain after a certain time acquires the ability to use **ketone bodies** (see p. 312) in addition to glucose to form ATP. In the first weeks of a starvation period, there is a strong increase in the activities of the enzymes required for this in the brain. The degradation of ketone bodies in the CNS saves glucose and thereby reduces the breakdown of muscle protein that maintains gluconeogenesis in the liver during starvation. After a few weeks, the extent of muscle breakdown therefore declines to one-third of the initial value.

B. Glutamate, glutamine, and GABA ◑

The proteinogenic amino acid **glutamate** (Glu) and the biogenic amine **4-aminobutyrate** derived from it are among the most important neurotransmitters in the brain (see p. 352). They are both synthesized in the brain itself. In addition to the neurons, which use Glu or GABA as transmitters, *neuroglia* 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 "glutaminergic" and "GABAergic" neurons with the precursor **glutamine** (Gln), which they produce from glutamate with the help of *glutamine synthetase* [1].

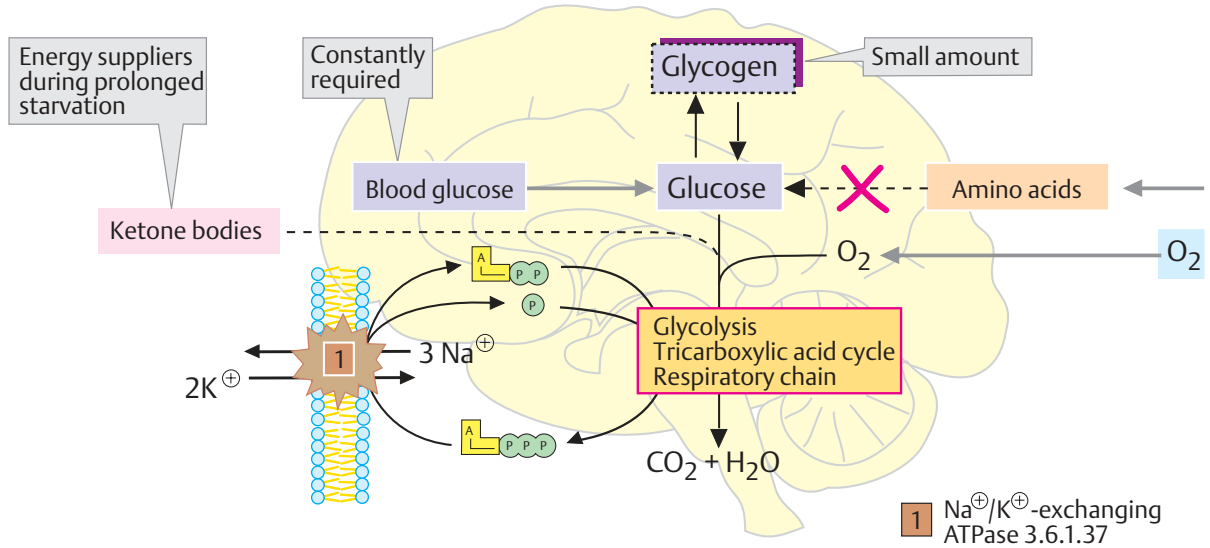
GABA neurons (left) and glutamate neurons (right) initially hydrolyze glutamine with the help of *glutaminase* [1] to form glutamate again. The glutamate neurons store this in vesicles and release it when stimulated. The GABA neurons continue the degradation process by using *glutamate decarboxylase* [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.

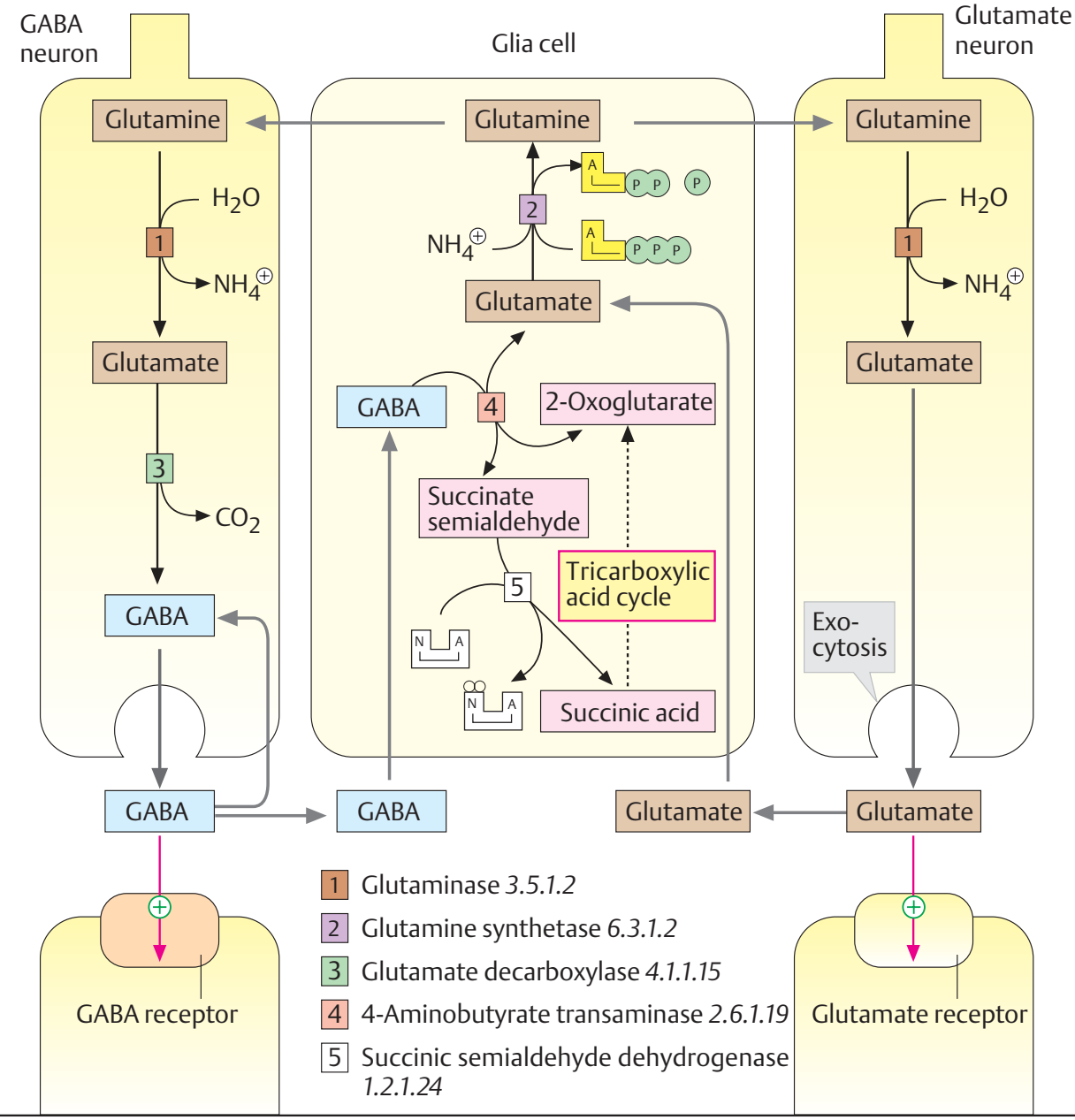
Glutamate can also be produced again from GABA. The reaction sequence needed for this, known as the **GABA shunt**, is characteristic of the CNS. A *transaminase* [4] first converts GABA and 2-oxoglutarate into glutamate and succinate semialdehyde ($-OOC-CH_2-CH_2-CHO$). In an NAD^+-dependent reaction, the aldehyde is oxidized to succinic acid [5], from which 2-oxoglutarate can be regenerated again via tricarboxylic acid 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." In sensitive individuals, the monosodium glutamate used as a flavor enhancer in Chinese cooking can raise the glutamate level in the brain to such an extent that transient mild neurological disturbances can occur (dizziness, etc.).

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.* Rods are sensitive to low levels of light, while the cones are responsible for color vision at higher light intensities.

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

A. Photoreceptor ◑

The cell illustrated opposite, a **rod**, has a structure divided by membrane discs into which the 7-helix receptor **rhodopsin** is integrated (see p. 224). In contrast to other receptors in the 7-helix class (see p. 384), rhodopsin is a light-sensitive *chromoprotein.* Its protein part, **opsin**, contains the aldehyde **retinal** (see p. 364)—an isoprenoid which is bound to the ε-amino group of a lysine residue as an *aldimine*.

The light absorption of rhodopsin is in the visible range, with a maximum at about 500 nm. The absorption properties of the visual pigment are thus optimally adjusted to the spectral distribution of sunlight.

Absorption of a photon triggers isomerization from the 11-*cis* form of retinal to all-*trans*-retinal (top right). Within milliseconds, this *photochemical process* leads to an allosteric conformational change in rhodopsin. The active conformation (**rhodopsin***) binds and activates the G protein **transducin**. The *signal cascade* (**B**) that now follows causes the rod cells to release less neurotransmitter (glutamate) at their synapses. The adjoining bipolar neurons register this change and transmit it to the brain as a signal for light.

There are several different rhodopsins in the **cones**. All of them contain retinal molecules as light-sensitive components, the absorption properties of which are modulated by the different proportions of opsin they contain in such a way that colors can also be perceived.

B. Signal cascade ◑

Dark (bottom left). Rod cells that are not exposed to light contain relatively high concentrations (70 μM) of the cyclic nucleotide **cGMP** (3′,5′-cycloGMP; cf. cAMP, p. 386), which is synthesized by a *guanylate cyclase* ([2], see p. 388). 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 (see p. 356).

Light (bottom right). When the G protein transducin binds to light-activated rhodopsin* (see **A**, on the structure of the complex; see p. 224), it leads to the GDP that is bound to the transducin being exchanged for GTP. In transducin* that has been activated in this way, the GTP-containing α-subunit breaks off from the rest of the molecule and in turn activates a membrane *cGMP phosphodiesterase* [1]. This hydrolyzes cGMP to GMP and thus reduces the level of free cGMP within milliseconds. As a consequence, the cGMP bound at the ion channel dissociates off and the channel closes. As cations are constantly being pumped out of the cell, the membrane potential falls and **hyperpolarization** of the cell occurs, which interrupts glutamate release.

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

1. The α-subunit of transducin* inactivates itself by GTP hydrolysis and thus terminates the activation of cGMP esterase.
2. The reduced Ca^{2+} concentration causes activation of guanylate cyclase, which increases the cGMP level until the cation channels reopen.
3. An isomerase [3] transfers all-*trans* -retinal to the 11-*cis* -form, in which it is available for the next cycle. A dehydrogenase [4] can also allow retinal to be supplied from vitamin A (retinol).